

THE ROLE OF SELF-ASSEMBLED MONOLAYERS OF THE PURINE AND PYRIMIDINE BASES IN THE EMERGENCE OF LIFE

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Abstract. The experimental evidence for the spontaneous formation and structure determination of two-dimensional monolayers of the purine and pyrimidine bases is examined. The plausibility of such structures forming spontaneously at the solid-liquid interface following their prebiotic synthesis suggests a functional role for them in the emergence of life. It is proposed that prebiotic interactions of enantiomorphous monolayers of mixed base composition with racemic amino acids might be implicated in a simultaneous origin of a primitive genetic coding mechanism and biomolecular homochirality. The interactions of these monolayers with carbohydrates and other derivatives is also discussed.

1. Introduction

Models for a heterotrophic origin of life are supported from several lines of evidence. These include experimental chemical reactions designed to simulate the earth's early atmosphere (Miller, 1953), analysis of compounds found on extraterrestrial debris such as meteorites (Kvenvolden *et al.*, 1970) and the spectroscopic analysis of interstellar gases (Mann and Williams, 1980). The evidence strongly supports the prebiotic availability of several classes of organic compounds, particularly those resulting from hydrogen cyanide based chemistries, namely α and β amino acids, carbodiimide condensing agents and derivatives of the purine and pyrimidine bases (Ferris and Hagen, 1984). Plausible routes for potentially prebiotic syntheses of the purines, adenine, guanine, hypoxanthine, xanthine and diaminourine are well characterized. They proceed via hydrogen cyanide oligomerization, as do the assembly of the pyrimidines, cytosine and uracil, which are synthesized in much smaller yields (Ferris and Hagen 1984), although, alternative routes to these and other substituted pyrimidines are available (Robertson and Miller, 1995). It is attractive to invoke these classes of organic molecules in models for the origin of life because of their present day roles in biology with purine and pyrimidine derivatives being responsible for coding of genetic information through polymeric nucleic acids, and biochemical catalysis predominantly controlled by polymeric amino acids. It has, however, been experimentally difficult to demonstrate processes subsequent to simple chemical syntheses that may be implicated in the formation of functional polymeric compounds. It seems plausible that in the absence of the cellular catalytic machinery, some form of molecular self-assembly was responsible for the formation of higher-ordered classes of organic compounds. It has

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previously been suggested that the ordering of monomers at the solid-liquid interface may have played a role in this process (Bernal, 1951; Cairns-Smith, 1982; Wächtershäuser, 1988; Russell *et al.*, 1989). Recently, studies of the spontaneous formation of monolayers of purine and pyrimidine bases at the solid-liquid interface (Allen *et al.*, 1991; Heckl *et al.*, 1991; Srinivasan *et al.*, 1991; Srinivasan and Murphy, 1992; Heckl, 1993; Srinivasan and Gopalan, 1993; Tao *et al.*, 1993; Boland and Ratner, 1994; Tao and Shi, 1994; Tao and Shi, 1994; Sowerby, 1995; Sowerby *et al.*, 1996; Sowerby and Petersen, 1997) has provoked consideration for this process in the origin of life (Sowerby, 1995; Sowerby *et al.*, 1996). Here we examine the supporting experimental evidence for the formation of these monolayers and explore the possible consequences of such phenomena in a prebiotic context.

2. Biological Function of the Purines and Pyrimidines

Deoxyribose phosphate derivatives of the two purine bases, adenine and guanine, and the two pyrimidine bases, cytosine and thymine encode cellular genetic information by way of their consecutive order in the linear polymer, DNA. Ribose phosphate derivatives of these base molecules are also polymerized into linear molecules (RNA) except that the pyrimidine base uracil, replaces the position occupied by thymine. These polymers also encode cellular genetic information but can additionally function with structural and catalytic capabilities (Cech, 1986). This together with the co-factor functions of purine ribosides and the ability of RNA to undergo Darwinian-like evolution (Beaudry and Joyce, 1992) supports an evolutionary role for these derivatives within the RNA world hypothesis for the origin of life (Gilbert, 1986).

In DNA and RNA polymers, the deoxyribose- and ribose-phosphate moieties are involved in the polymeric bonding of adjacent monomers via phosphodiester linkages but have no coding function. The base moieties that distinguish each monomer are capable of direct interaction with other base derivatives through intermolecular hydrogen bonding. The interaction is specific with adenine interacting with thymine (or uracil) and guanine interacting with cytosine. These interactions can occur between the base moieties within the same polymer strand or between different strands and give rise to the well characterized structural and functional configurations of the nucleic acids. They are also responsible for the mechanism of heredity where one polymer can act as the template for the synthesis of daughter strands.

The hydrogen bonding capability of the nucleic acid purine and pyrimidine bases has been well established through x-ray crystallography of the pure bases and of complexes involving the bases (Saenger, 1984) and these capabilities have often been invoked in the structural interpretation of the two-dimensional monolayers formed from these compounds (Saffarian *et al.*, 1987; Heckl *et al.*, 1991, 1993; de

Levie and Wandlowski, 1994; Sowerby, 1995; Sowerby *et al.*, 1996; Dretschkow *et al.*, 1997; Freund *et al.*, 1997; Sowerby and Petersen, 1997).

3. Two-dimensional Monolayers of the Purines and Pyrimidines

Some of the first evidence for the two-dimensional condensation of organic molecules was demonstrated electrochemically for nanoic acid at the (liquid) mercury-water interface (Lorenz, 1958). Vetterl subsequently demonstrated the ability of the nucleic acid purines and pyrimidines to form such condensed monolayers by careful measurements of the changing interfacial capacitance (Vetterl, 1965, 1966). Plots of interfacial capacitance versus the applied electrode potential of these systems showed that the electrode exhibited reduced capacitance within certain potential ranges. The so called 'capacitance pits' also showed hysteresis as the potential was slowly swept into and out of the pit. These observations were interpreted in terms of phase transitions between a normal physisorbed state and a condensed monolayer state. These, and subsequent studies, have shown that the observed phase transitions could be controlled by electrode potential, temperature, electrolyte composition, and adsorbate and electrolyte concentration and could be well described by both thermodynamics and statistical mechanics expressions. A comprehensive review of the published literature on the experimental and theoretical aspects of these systems has been published (de Levie, 1988).

Although detailed electrochemical analysis has revealed both thermodynamic and kinetic information on the monolayer formation and dissolution at the mercury-water interface, investigation of the monolayer structure could only be addressed indirectly by techniques which measured thermodynamic properties such as interfacial tension. These studies typically resulted in numerical values for molecular areas which allowed prediction of the molecular orientation at the interface (Brabec *et al.*, 1978; Saffarian *et al.*, 1987). Data obtained for the condensed monolayer structure of the 2,4-dioxypyrimidines (uracils) were first interpreted in terms of base stacking interactions with the plane of the molecules oriented perpendicular to the interface (Brabec *et al.*, 1978). Subsequent studies indicated that these molecules might be oriented in a planar configuration stabilized by intermolecular hydrogen bonding, analogous with their arrangement in the bulk crystal structure (Saffarian *et al.*, 1987).

More recently, electrochemical studies of purine and pyrimidine two-dimensional condensation have been extended to solid electrodes with well defined crystallographic orientations and include thymine on cadmium (001) (Popov *et al.*, 1992) and silver (111) (Hölzle *et al.*, 1995) and uracil on gold (111) and (100) (Hölzle *et al.*, 1995), and silver (111) and (100) (Wandlowski, 1995; Wandlowski and Hölzle, 1996; Wandlowski *et al.*, 1996). Similarly, capacitance pits with the characteristic potential dependent hysteresis were observed for these systems and suggested processes comparable with those observed on the mercury electrode.

Real space analysis of crystalline surfaces with near field techniques, namely scanning tunneling microscopy (STM), which is capable of resolving, with electronic contrast, the structural features of surfaces with atomic detail (Binnig *et al.*, 1982), and atomic force microscopy (AFM), which resolves these features topographically (Binnig *et al.*, 1986), have been used in conjunction with electrochemical techniques to study the potential controlled formation of base monolayers on solid electrodes. These include guanine in NaCl electrolyte on highly oriented pyrolytic graphite (HOPG) (Srinivasan *et al.*, 1991), adenine in NaCl on HOPG (Srinivasan and Murphy, 1992), adenine in NaCl, NaBr and NaI on HOPG (Srinivasan and Gopalan, 1993), adenine, guanine, cytosine and thymine in NaClO₄ on gold (111) (Tao *et al.*, 1993), adenine and guanine in NaCl on HOPG (Tao and Shi, 1994), xanthine in NaCl on HOPG (Tao and Shi, 1994), cytosine in KClO₄ on gold (111) (Wandlowski *et al.*, 1996), thymine on gold (111), (100) and (210) (Roelfs *et al.*, 1997), uracil in KClO₄ on gold (111) (Hölzle *et al.*, 1995), and uracil in H₂SO₄ on gold (111) and (100) (Dretschkow *et al.*, 1997).

The early studies employing *in situ* STM techniques confirmed the presence of the condensed two-dimensional phases by comparing images of the electrode surface obtained with the applied electrode potential both inside and outside the capacitance pit (Srinivasan *et al.*, 1991; Srinivasan and Murphy, 1992). These investigations resulted in STM images of the adsorbed layers as periodic structures and their interpretation was in terms of the available crystal structures of the purine hydrates and chlorides. The electrochemical behavior of these species at the HOPG-water interface also showed that molecular adsorption occurred at the interface when the electrode was at the potential of zero charge, an observation originally established in the first investigations of the condensation of the purine and pyrimidine bases at the mercury-water interface (Vetterl, 1965, 1966).

Confirmation by STM in air on HOPG surfaces (Heckl 1993), by *in situ* STM in ethanol on gold (111) (Boland and Ratner, 1994) and by *in situ* ECSTM in NaClO₄ electrolyte on gold (111) (Tao *et al.*, 1993) that all four DNA bases can spontaneously self-assemble into condensed two-dimensional monolayers is supported by studies of individual purine and pyrimidine derivatives. These include, in addition to the *in situ* electrochemical studies, the examination adenine and thymine on HOPG by STM in air (Allen *et al.*, 1991, 1992), guanine (Heckl *et al.*, 1991), adenine (Sowerby *et al.*, 1996) and the nucleic acid 2,4-dioxypyrimidines (Sowerby and Petersen, 1997) on HOPG and molybdenum disulfide (MoS₂) by STM in air and of guanine monolayers on HOPG by scanning thermopower microscopy (Poler *et al.*, 1995).

The studies of the purine and pyrimidine monolayers by STM in air was first reported simultaneously, but independently, by both Allen *et al.* (1991) and Heckl *et al.* (1991). The monolayer formation was mediated at moderate temperatures (80–120 °C) by the evaporation of aqueous solutions of the bases, adenine and thymine, on the freshly cleaved surfaces HOPG (Allen *et al.*, 1991; Allen *et al.*, 1992) and by the evaporation of aqueous solutions of the base, guanine, on both HOPG and

the naturally occurring mineral, MoS₂ (Heckl *et al.*, 1991). The resolution afforded by STM in air allowed lattice models of the adsorbed monolayers to be determined from the real-space images of both the adsorbed layer and the underlying substrate crystal lattice. These studies suggested that the base molecules heteroepitaxially registered on the substrate crystal with their planar rings lying flat on the surface. The base molecules within the guanine lattice models were stabilized laterally by intermolecular hydrogen bonding (Heckl *et al.*, 1991). This was supported by real-time STM observations of individual molecular events recorded by video which showed lateral stability for individual molecules at the adsorbate edge, but only in specific lattice directions (Heckl, 1993). The characterization of the guanine monolayers made by Heckl *et al.* showed the electronic effect of the two substrates on the STM contrast of the adsorbate. It was suggested that on HOPG, the electronic coupling of molecular states between the HOPG and the adsorbate molecules played an important role in the STM contrast mechanism. On MoS₂, however, the adsorbate image was composed predominantly of isolated adsorbate molecular states. These differences enabled a comparative analysis of both the molecular and sub-molecular structure of the guanine monolayer. Almost identical configurations of the guanine monolayer formed on both surfaces but the structure of the underlying crystal subtly affected the adsorbate lattice dimensions. Similar monolayer studies have also addressed the mechanism of STM image contrast on HOPG and MoS₂ substrates (Smith *et al.*, 1992; Fisher and Blöchl, 1993; Ludwig *et al.*, 1994), and theoretical interpretations of the image contrast of guanine (Wang *et al.*, 1996) and adenine (Ou-Yang *et al.*, 1994) on HOPG have been applied using a quantum chemical approach.

The effect of adsorbate-substrate interactions on adsorbate lattice structure was highlighted further with the STM analysis in air of adenine monolayers prepared by evaporation on HOPG and MoS₂ (Sowerby *et al.*, 1996). The lattice models presented for the adenine adsorbates on both substrate lattices also presented a planar arrangement of molecules. These were stabilized by intermolecular hydrogen bonding and van der Waals packing constraints but showed the formation of two possible structures for the adenine adsorbate on MoS₂ and only one on HOPG. Although adenine is achiral, the two structures that were observed on MoS₂ were enantiomorphic and suggested a mechanism for a prebiotic symmetry breaking event (Sowerby *et al.*, 1996). The occurrence of two different packing structures for the adenine monolayer on both HOPG and MoS₂ was attributed to the combination of different adsorbate-substrate interactions and two lattice configurations which are possible from the intermolecular hydrogen bonding capabilities of the adenine molecules. The spontaneous condensation of adenine and guanine in a NaCl electrolyte into monolayers on HOPG has also been studied by *in situ* STM and *in situ* AFM techniques (Tao and Shi, 1994). The guanine monolayer studies suggested the presence of two condensed states on the basis of adsorbate lattice dimensions whereas the observed adenine lattice was invariant. Structural lattice models were presented for these systems based on comparison of the adsorbate with

the underlying substrate images and intermolecular hydrogen bonds were proposed to account for lateral stability. Spontaneous adenine and guanine adsorbate formation and potential controlled dissolution was imaged in real-time by both STM and AFM in situ (Tao and Shi, 1994). The anisotropic line tensions observed for guanine monolayer growth and dissolution on HOPG supported the proposed lattice models (Heckl *et al.*, 1991; Tao and Shi, 1994) which placed intermolecular hydrogen bonds in only one lattice direction. Potential controlled formation of both adenine and guanine lattices on HOPG showed no observable change in the adsorbate lattice structure from that of spontaneous formation although a potential dependent contrast was observed in the STM images (Tao and Shi, 1994). Comparison of the published adenine structures (Allen *et al.*, 1991; Tao and Shi, 1994; Sowerby *et al.*, 1996) showed consistent lattice dimensions and suggests a common structure with alternative interpretations. The application of low energy electron diffraction (LEED) and molecular mechanics to ultra high vacuum (UHV)STM studies had previously been applied to organic molecules (Seidel *et al.*, 1997). Similarly, the structure of adenine monolayers prepared by molecular beam deposition on native graphite crystals was determined (Freund *et al.*, 1997). This study also confirmed the previously observed lattice dimensions of adenine on HOPG but an alternative structure was proposed based on the symmetry constraints determined by the reciprocal space analysis using LEED.

The effects of substrate influence on adsorbate structure was also demonstrated for the 2,4-dioxypyrimidines thymine and uracil prepared on both HOPG and MoS₂ surfaces by evaporation and investigated by STM in air (Sowerby and Petersen, 1997). The models proposed for these structures were based on the comparative analysis of adsorbate and substrate images and on the hydrogen bonded arrangement seen in the plane-parallel sheets of the three-dimensional crystals. These configurations required some skewing so as to accommodate the observed substrate dependent lattice dimensions, but the extent of the skewing was minor and allowed by the flexibility of hydrogen bonding interactions. The STM images of uracil on MoS₂ showed a herring bone pattern which presumably resulted from the hydrogen bonded dimer formation as seen in the solid state (Figure 1a). Calculation of the molecular areas for the pyrimidines was based on the proposed lattice models of planar arranged uracil and thymine molecules. The values determined for each molecule on HOPG and MoS₂ surfaces showed close agreement with each other respectively, from those values determined electrochemically at the mercury-liquid interface and determined from the x-ray crystal structures of their three-dimensional solids. This suggested that for both of these pyrimidines, the same hydrogen bonded planar configuration of molecules seen in the solid state was also present on HOPG and MoS₂ surfaces and at the mercury-liquid interface (Sowerby and Petersen, 1997). Molecular mechanics calculations have also been applied to the proposed configuration of the uracil adsorbate on MoS₂. The results of this calculation reasonably simulated the observed lattice structure (Figure 1a) and is shown in Figure 1b.

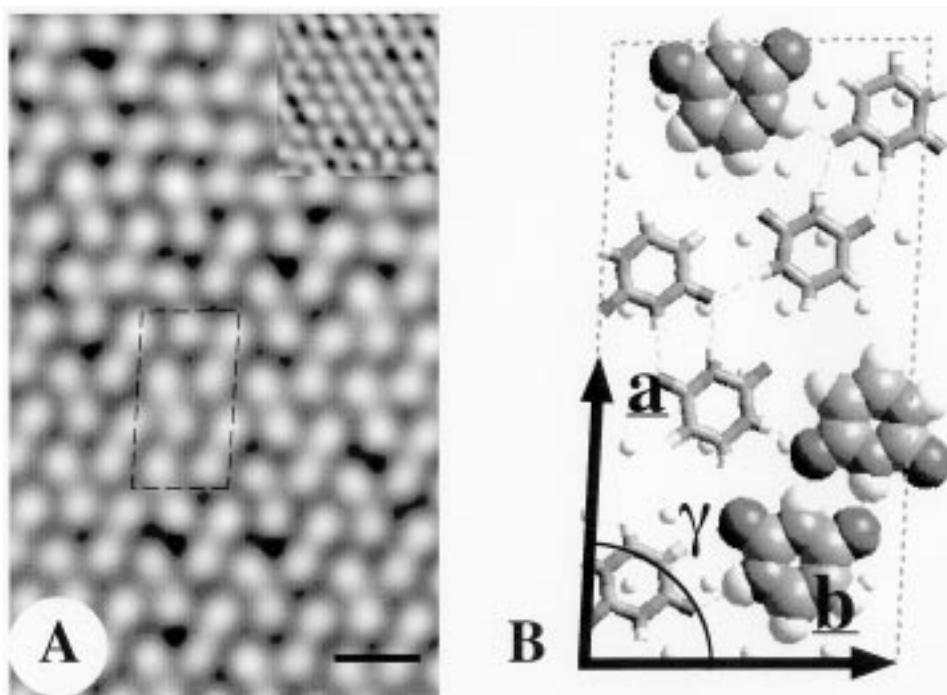


Figure 1. Uracil monolayers. (A) STM image of uracil on MoS₂ and inset the underlying MoS₂, for details see (Sowerby and Petersen, 1997). The commensurate adsorbate mesh is outlined by the black dashed parallelogram. The STM images were lowpass filtered in their respective Fourier space and the black scale bar represents 1 nm. (B) An energy minimized model of the structure and molecular arrangement of uracil molecules on the underlying MoS₂. The lattice vectors *a* and *b* describe the primitive unit mesh of the adsorbate system and are 1.27 and 1.23 nm respectively. The angle that separates them (γ) is 86.3°. Putative intermolecular hydrogen bonds are shown by the dashed lines. For details of the minimization calculation see Appendix A.

The *in situ* electrochemical STM studies of uracil adsorption on gold (111) and (100) surfaces also showed substrate dependent effects on the structure of the uracil adsorbate (Dretschkow *et al.*, 1997). The uracil system has been well studied electrochemically at both the mercury-water interface and on reconstructed gold and silver electrodes. Wandlowski and co-workers have undertaken a systematic study on the effect of substrate structure on uracil condensed monolayer formation and stability. Their analysis showed the following stability sequence for the physisorbed uracil monolayers, gold (100) > gold (111) > silver (100) > silver (111) > mercury. (Wandlowski *et al.*, 1992; Hölzle *et al.*, 1995; Wandlowski, 1995; Wandlowski and Hölzle, 1996). At large electrode potentials, however, the evidence suggested deprotonation of uracils and a subsequent chemisorbed state with high temperature stability.

Similarly, electrochemical studies have established that ions present in the electrolyte can sometimes affect the formation of the condensed monolayers or can

even be coadsorbed within the monolayer structure (Kontoyannis *et al.*, 1987). The influence of the supporting electrolyte anion on the formation and stability of adenine monolayers at the mercury-water interface was examined by testing the effect of changing NaCl, NaBr and NaI concentrations (Vetterl and de Levie, 1991). This study suggested the presence of two condensed monolayer phases at least one of which had incorporated bromide ions. Conditions were also obtained that allowed a metastable coexistence of the two observed states. These effects were the subject of an investigation by *in situ* STM of adenine on HOPG but the analysis was unable to confirm the presence or absence of anions in the monolayer structure (Srinivasan and Gopalan, 1993).

The proposed models to account for the observed STM images of the purine and pyrimidines in air (Heckl *et al.*, 1991; Sowerby *et al.*, 1996; Sowerby and Petersen, 1997) in solution (Tao and Shi, 1994; Dretschkow *et al.*, 1997) or in UHV (Freund *et al.*, 1997), have invoked close packed structures held together by lateral interactions such as intermolecular hydrogen bonding and van der Waals packing constraints. These models excluded water and electrolyte ions. The observed stability of the hydrogen bonded purine and pyrimidine monolayers is probably derived from centrosymmetric dimer formation and co-operative cyclic and caternary hydrogen bonding interactions. In the three-dimensional crystal structures of purine and pyrimidine derivatives, hydrogen bonded homobase pairs with centrosymmetrical configurations are observed (Jeffrey and Saenger, 1994). These structural motifs have also been invoked in the interpretation of the monolayers formed by the bases (Heckl *et al.*, 1991, 1993; Tao and Shi, 1994; Sowerby, 1995; Sowerby *et al.*, 1996; Dretschkow *et al.*, 1997; Freund *et al.*, 1997; Sowerby and Petersen, 1997). The formation of the centrosymmetric motif results in the effective cancellation of the large dipole moments of the base molecules by their antiparallel configurations in dimers. These dimers are stabilized by cyclic π co-operativity interactions which enhance hydrogen bond strength by resonance assisted electron contributions to the cyclic hydrogen bond structure (Jeffrey and Saenger, 1994). Adjacent dimers are stabilized in the monolayer by additional hydrogen bonding interactions and van der Waals packing forces.

The exclusion of water from the interface had previously been established on the basis of electrochemical studies (de Levie, 1988, and references therein). The potential of zero charge is where interfacial capacitance measurements show maximal adsorption for the purines at the mercury-water interface (Vetterl, 1966). The polar nature of water molecules would ensure an electrostatic component to their adsorption only on the charged electrode surface. A mechanism for the assembly of neutral base molecules into hydrogen bonded monolayers has been proposed by de Levie and Wandlowski (1994). They have suggested, that at the interface the availability of the aqueous solvent is spatially restricted and adsorbate-adsorbate hydrogen bonding interactions are favored. However, in the bulk solution, the base molecules are surrounded by water molecules and remain dissolved.

Similarly, both heat and salt would also function in reducing water concentration at the interface.

Electrochemical studies have allowed the deterministic and stochastic behavior of monolayer structure formation and dissolution on the mercury electrode to be studied. These data have allowed mechanistic models of the two-dimensional systems to be applied and have been used to deduce nucleation times and growth rates (de Levie, 1988, and references therein).

4. Bulk Adsorption Studies on Mineral Surfaces

Although the purine and pyrimidine monolayer structures have been investigated on HOPG, MoS₂ and metal surfaces, the prebiotic availability of other plausible substrate surfaces is extensive. The aforementioned substrate surfaces were used because of their well defined crystallographic orientations and their convenience in electrochemical and scanning probe microscopy studies. Other examples of crystalline surfaces of prebiotic relevance may include metal sulfides, quartzes and clays etc.

Evidence from bulk adsorption studies of purines, pyrimidines, their derivatives and precursors on sodium-montmorillonite clays, shows compelling evidence for the affinity of these compounds for organic molecules. Winter and Zubay (1995) have demonstrated a high affinity for adenine, hypoxanthine and uracil on clay following incubation in buffered saline solutions. Nucleoside and nucleotide derivatives of adenine weakly adsorbed to the clays but the free bases bound much more strongly. The observed linear response of adsorbate concentration to adsorbate binding, suggested non co-operative binding interactions. The authors suggested that the planar molecules might lie flat on the surface of the clay and that electrostatic interactions with positive charges on the surfaces could account for the observed affinities. It was proposed that reduced binding of the nucleoside and nucleotide derivatives was a function of steric hindrance on the bases by the carbohydrate components making it difficult for them to achieve a flat conformation. Co-operative binding interactions were proposed to account for the sigmoidal response of adsorbate concentration to adsorbate binding of uracil and hypoxanthine adsorption when mixed with adenine. Intermolecular hydrogen bonding interactions between planar adsorbed species were proposed to account for this observation. Analysis of these binding interactions as a function of pH showed maximal affinities around neutral conditions in aqueous NaCl. These observations suggested to us, that hydrogen bond mediated self-assembly of the purine and pyrimidine monolayers of the type observed on HOPG, MoS₂ and some metal surfaces may have been the predominant mechanism for adsorption on these clay surfaces rather than electrostatic interactions.



Figure 2. Reflectance polarization light micrograph (approximately 300x magnification) of uracil crystals on the surface of MoS₂. The three-fold symmetry of the underlying substrate is represented in the adsorbate crystals (indicated by the white lines) and shows heteroepitaxial registration of the adsorbate on the substrate.

5. Prebiotic Relevance of Self-assembled Monolayers of Purine and Pyrimidine Bases

The prebiotic relevance of self-assembled monolayers of purine and pyrimidine bases is one of conjecture. The results of accumulated studies show the preponderance for spontaneous self-assembly phenomena of these molecules at the solid-liquid interface and suggests the prebiotic availability of such structures. The observations that spontaneous monolayer formation can occur as a function of heat or salinity in the aqueous environment suggests plausible locations for such phenomena that extend from submarine hydrothermal vents, terrestrial and subterranean areas of geothermal activity, to drying marine or non-marine pool systems. The spontaneous formation of purine and pyrimidine monolayers suggests an energy minimizing process and could act as a mechanism for the concentration and purification of these organic species. Crystallization is an example of non-equilibrium thermodynamics as it favors the synthesis of the reaction product by removing it from the chemical equilibrium expression. This would push prebiotic

Table I
Observed unit mesh parameters for self-assembled monolayers of the nucleic acid bases spontaneously formed at the solid-liquid interface

Base	Substrate	Adsorbate mesh parameters			Oblique
		a (nm)	b (nm)	γ ($^{\circ}$)	
Adenine	HOPG ¹	2.21	0.85	90.0	no
	HOPG ²	2.13	0.98	90.0	no
	MoS ₂ ²	1.10	0.96	85.0	yes
	MoS ₂ ³	1.09	0.84	70.0	yes
Guanine	HOPG ⁴	1.07	1.97	90.0	no
	HOPG ⁵	0.85	1.15	90.0	no
	MoS ₂ ^{2,4}	1.11	1.92	95.0	yes
Cytosine	HOPG ²	1.27	0.85	120.0	yes
	Au (111) ⁶	1.05	0.95	102.0	yes
Thymine	HOPG ^{2a}	0.48	0.35	101.0	yes
	HOPG ⁷	0.65	1.37	88.1	yes
	MoS ₂ ⁷	0.69	1.38	89.0	yes
	Au (111) ⁶	0.65	0.71	105.0	yes
Uracil	HOPG ⁷	1.18	1.24	89.6	no
	MoS ₂ ⁷	1.23	1.27	86.3	yes
	Au (111) ^{8b}	1.22	1.51	90.0	no
	Au (100) ^{8b}	1.26	1.55	90.0	no
	-(hex)				
	Au (100) ^{8b}	1.32	1.52	85.0	yes
-(1x1)					
Xanthine	HOPG ⁹	0.90	9.80	90.0	no

¹ (Allen *et al.*, 1991; Tao and Shi, 1994; Sowerby *et al.*, 1996; Freund *et al.*, 1997); ² (Heckl, 1993); ³ (Sowerby *et al.*, 1996); ⁴ (Heckl *et al.*, 1991); ⁵ (Tao and Shi, 1994); ⁶ (Tao *et al.*, 1993); ⁷ (Sowerby and Petersen, 1997); ⁸ (Dretschkow *et al.*, 1997); ⁹ (Tao and Shi, 1994).

^a Molecules laying edge on.

^b Formed electrochemically at negative electrode charge densities.

reactions in the direction of purine and pyrimidine base synthesis and allow the accumulation of these molecular species even from low yielding reactions. Optical examination by reflectance polarization light microscopy of the base structures prepared by the evaporation technique, often revealed the presence of large planar crystals (Heckl, 1993) which showed Newton interference patterns and sometimes displayed 3-fold lattice registration with the substrate (Figure 2). Examination of

these structures and STM observations showed the presence of multiple layered adsorbates. Poly-layer formation has also been confirmed electrochemically but the evidence suggests that the structure of subsequent layers may be quite different from that most adjacent to the underlying substrate (de Levie, 1988, and references therein). These observations pose questions concerning the prebiotic availability of bulk crystals of purine and pyrimidine derivatives. However, we have favored a prebiotic role for the monolayers formed at the solid-liquid interface over the bulk crystals for primarily two reasons. Firstly, the number of prebiotically available substrate surfaces would confer a larger number of adsorbate structures than the bulk crystal structures. This is because the substrate dependent registration of the bases by heteroepitaxy on different surfaces, confers different (substrate dependent) adsorbate structures (Table I). This may have some advantages for the monolayer structures if they were to subsequently have some functional role that was dependent on their spatial configurations. Secondly, the preparation of bulk purine and pyrimidine crystals and their hydrates and chlorides, which are suitable for x-ray crystallography studies has been achieved through a variety of processes. These include, the preparation of cytosine monohydrate by evaporation of dilute aqueous solution (Jeffrey and Kinoshita, 1963), adenine hydrochloride from hydrochloric acid (Broomhead, 1948, 1951; Cochrane, 1951), guanine monohydrate from dimethylamine (Thewalt *et al.*, 1971), and uracil by sublimation (Parry, 1953). This suggests that a common mechanism for bulk crystal preparation is not likely. The observation that monolayers of the purines and pyrimidines might have been prebiotically available via the same mechanism of formation would suggest that they would be more prevalent on the prebiotic earth.

6. Enantiomorphism in Purine and Pyrimidine Monolayers

A summary of the published purine and pyrimidine adsorbates lattice parameters is shown in Table I. In addition to showing the effect of varying the substrate structure on the adsorbate lattice, the mesh structures are categorized according to whether they form an oblique lattice. The observed structure of the adenine monolayer on MoS_2 was that of an oblique lattice ($a \neq b, \gamma \neq 90^\circ$). This clearly suggested the possibility of a non-superimposable mirror image structure which was subsequently observed (Sowerby *et al.*, 1996). The occurrence of these two structures suggested a symmetry breaking event by the formation of localized enantiomorphic domains. The cause of this was not completely obvious as adenine is achiral. The proposed mechanism to account for this phenomenon was based on adsorbate-substrate registration requirements and the allowed intermolecular hydrogen bonding configurations of adenine. The adsorption of an adenine molecule at the solid-liquid interface results in the loss of a rotational degree of freedom for that molecule restricting movement to within the plane of the surface. The two-fold symmetry element through the plane of the molecule allows for only two configurations

of adsorption. As monolayer formation is a nucleation and growth process (de Levie, 1988, and references therein) subsequent adsorbate-substrate registration requirements and hydrogen bonding configurations allow for only molecules of the same configuration to bond with the growing adsorbate crystal edge on MoS₂. This results in the formation of two possible lattice structures. This effect was not seen on HOPG because the substrate registration requirements could accommodate both enantiomeric forms of adenine adsorption in the one lattice structure (Sowerby *et al.*, 1996). On the graphite surface, LEED was used to determine the p2gg symmetry of the adenine adsorbate (Freund *et al.*, 1997) in which adjacent pairs of centrosymmetric dimers were related by mirror reflection and placed in a rectangular unit mesh. This motif has also been observed for guanine (Heckl *et al.*, 1991) and uracil (Sowerby and Petersen, 1997) on HOPG. Examination of the published lattice structures of purine and pyrimidine monolayers (Table I) shows the presence of other oblique adsorbate lattices which suggests that these would also show enantiomorphism.

It was not clear whether there was an enantiomeric bias in monolayer formation (Sowerby *et al.*, 1996). Both HOPG and MoS₂ have completely symmetrical surface structures and would not be expected to influence enantiomeric bias. The *in situ* studies of adenine and guanine growth on HOPG, however, showed that monolayer growth can initiate at grain boundaries of the substrate crystal lattice (Tao and Shi, 1994). This suggests a possible mechanism for creating a localized enantiomeric bias, if the grain boundary or other high energy surface site could impart some type of asymmetry at the interface. Alternatively, the substrate registration requirements of other crystalline surfaces that allow the formation of purine and pyrimidine monolayers, might directly influence enantiomeric bias of monolayer formation if the substrate crystal surface itself was enantiomeric such as the iron sulfides or quartzes. Although this phenomenon does not solve the origin of biomolecular homochirality, the formation of localized domains of enantiomeric structure suggests an attractive mechanism for implementing a localized chiral symmetry break.

It has previously been shown that chiral surfaces can selectively adsorb chiral stereoisomers of predominantly one configuration (Bonner, 1991, and references therein). The possibility that only one stereoisomer of chiral racemate could interact with an enantiomeric two-dimensional monolayer of base molecules has been proposed (Sowerby *et al.*, 1996) and suggested the possibility of direct interaction between surface immobilized base molecules and other molecules of prebiotic origin.

7. Aperiodic Purine and Pyrimidine Monolayers

The well characterized ability of the nucleic acid purines and pyrimidines to form intermolecular hydrogen bonds suggested the possibility of monolayers of mixed

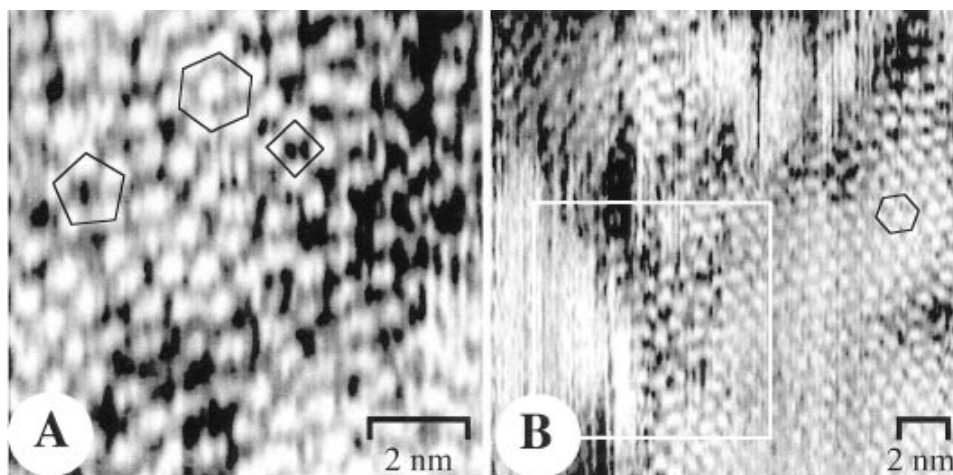


Figure 3. STM images of a monolayer prepared on MoS₂ by evaporation of an aqueous solution of a mixture of uracil and guanine (30:1) on the heated MoS₂ surface (80 °C) (A) A region of quasicrystalline aperiodicity showing four, five and six fold symmetry elements within close proximity. (B) A subsequent STM scan of the same area showing the remaining stable ordered phase which resembles that of the pure uracil monolayers. The white square shows the area corresponding to (A). The high background noise in the images was presumably a function of displaced adsorbate molecules interfering with data acquisition at the STM probe-surface interface.

composition stabilized by heteromolecular hydrogen bonding interactions. STM investigations of monolayers prepared from mixtures of bases have not been examined extensively because the preliminary investigations had focused in understanding the structure and formation of monolayers of the pure component systems. Electrochemical studies of adenine monolayer formation on mercury electrodes suggest the possibility of a heterogeneous mixed film as one condensed monolayer film undergoes a metastable transition into a more stable phase through a transition with patches of one film embedded in the other (Vetterl and de Levie, 1991). Our preliminary investigations of mixtures prepared by the evaporation procedure have indicated that domains of pure composition occur as a result of phase separation during self-assembly at the solid-liquid interface. Occasionally, however, the observed lattice structure deviated from the known periodicities of the pure component systems and localized regions of aperiodicity were observed. Figure 3A shows a region of aperiodic structure observed in a monolayer prepared on the surface of MoS₂ from a solution of uracil containing a small guanine impurity. It is difficult at this stage to give an unambiguous interpretation of such images except to say that they were inconsistent with images of either uracil or guanine alone. STM investigations of these aperiodic systems were hampered by the reduced stability of the mixed monolayer systems. This was demonstrated in a subsequent image (Figure 3B) obtained in the same location to that of Figure 3A and showed that the forces exerted by the STM probe had swept the disordered aperiodic structure to leave only the stable ordered structure. This is consistent with images of the pure

uracil monolayer on MoS₂. The co-operative binding affinities observed for mixtures of bases on clays also supported the possibility of heteromolecular hydrogen bonding interactions between purine and pyrimidine base molecules (Winter and Zubay, 1995).

In monolayers of mixed composition, the localized picture of the surface adsorbate would be one of an aperiodic quasi-crystalline monolayer with different combinations of base molecules lying juxtaposed on the substrate crystal surface. The prebiotic availability of aperiodic structures composed of components of the modern genetic machinery is an exciting prospect and it is attractive to speculate about possible functional consequences of such structures.

In the remaining stages of this communication, we suggest possible functions for aperiodic purine and pyrimidine monolayers in the prebiotic context with the express view of examining possible scenarios which might have been applicable in the development of autocatalytic self-replicating systems that may have led to the origin of life.

8. Interaction of Purine and Pyrimidine Monolayers with other Molecules of Prebiotic Significance

8.1. AMINO ACIDS

The possibility that amino acids could adsorb onto a purine-pyrimidine monolayer by stereo-selective hydrogen bonding has been proposed (Sowerby *et al.*, 1996). The proton accepting groups of the purine and pyrimidine bases, namely the free ring nitrogens and the keto substituents, are still accessible to the liquid medium at the solid-liquid interface. These are consequently accessible to the proton donors of the amino acids, namely the zwitterionic ammonium group and some of the amino acid side chain substituents. It is attractive to suggest an interaction between the purine and pyrimidine monolayers and amino acids as the physicochemical hypothesis for origin of the genetic code is based on a direct interaction of the triplet or doublet component of the 3 base genetic codon or anti codon, with its corresponding amino acid (Crick, 1968; Lacey and Mullins, 1983; Lacey *et al.*, 1992; Mellersh, 1993; Di Giulio *et al.*, 1994). Hydrophobicity correlations between dinucleoside monophosphates and amino acids suggested that there may be an anticodonic physicochemical relationship between nucleoside doublets and their amino acids (Lacey and Mullins, 1983; Lacey *et al.*, 1992). It has been suggested that surface adsorbed RNAs could directly code for amino acids through a lock and key relationship between codonic triplet base clefts in the RNA and the amino acid side chains (Mellersh, 1993). It is conceivable, that different combinations of base molecules in aperiodic monolayers could code for specific amino acids by way of direct interaction because physicochemical differences would occur where different combinations of bases lie juxtaposed on the surface.

Following amino acid adsorption onto the purine and pyrimidine monolayer, polymerization of amino acids might be achieved in condensation reactions between sterically positioned ammonium and carboxylate groups of adjacent molecules. For such reactions to occur, the surfaces which allow the formation of purine and pyrimidine monolayers, would also need to impose the appropriate structural configurations on the monolayers to allow the adsorption of amino acids in the correct spatial orientations for the condensation reactions to proceed. Because of the variability in the physical length of the amino acid side chains, it would seem likely that amino acids should interact with the monolayer with their carboxylate and ammonium substituents. The side chain would be oriented away from the surface. The x-ray crystal analysis of protein-nucleic acid complexes showed that specific interactions involved in molecular recognition processes, were predominantly hydrogen bonding interactions between the bases and the peptide functional groups. The interactions that occurred between the nucleic acids and the amino acid residue side chains are typically through interactions with the carbohydrate and phosphate components (Saenger, 1984).

The application of molecular mechanics calculations to organic monolayer systems has previously been used to distinguish between competing structures proposed for the interpretation of STM and low energy diffraction data (Freund *et al.*, 1997; Seidel *et al.*, 1997). An additional motivation for modeling these systems is to provide starting structures for molecular dynamics simulations and property prediction. The ability of molecular mechanics calculations to reasonably simulate the monolayers of the purine and pyrimidine bases motivates us to perform predictive calculations on these systems with a relative degree of confidence. In one such experiment, we have used molecular mechanics simulations to test for an interaction between the monolayer of uracil adsorbed to the surface of MoS₂ and the amino acid, glycine. We have applied molecular mechanics energy minimization algorithms to the proposed structure of the uracil monolayer adsorbed to the surface of MoS₂ (Sowerby and Petersen, 1997). The resulting energy minimized model is shown in Figure 1b. In a theoretical amino acid binding experiment, an energy minimized model of glycine was placed in closed proximity to the surface of the monolayer model. Several configurations of these were generated as starting models. The start configurations for the experiment were all generated by a series of rotations of the amino acid shown in Figure 4a and described in more precision in the figure legend. A subsequent energy minimization was performed on each system (Appendix A). The conditions of the minimization and choice of parameters were identical to those chosen for the simulation of the monolayer alone. The corresponding end configurations for each of the minimizations are shown in Figure 4b–h. The results show that local minima were reached within the potential energy hypersurface that satisfied the termination criteria for all configurations except Figures 4c and 4f which could not escape a local minima barrier of high energy. The energy minimized structures consistently suggested that the glycine molecule could interact with the monolayer through a linear hydrogen bond (Fig-

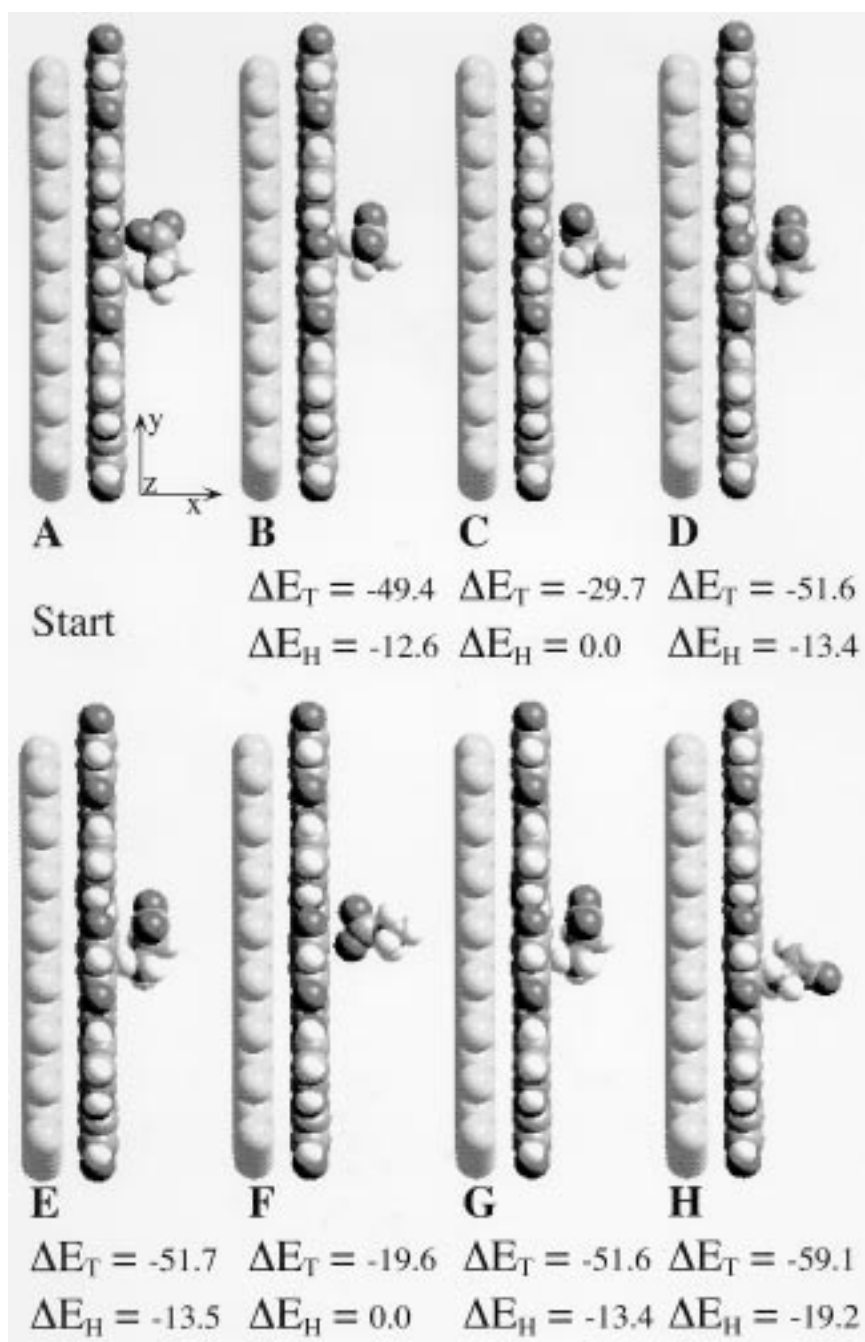


Figure 4. Start (A) and end (B-H) configurations of molecular mechanics simulations of glycine adsorption on the uracil-MoS₂ monolayer system. Geometric translations about the chiral carbon of (A) generated the start configurations for calculations: (B) unchanged; (C) y axis rotation 90°; (D) y axis rotation 180°; (E) y axis rotation 270°; (F) z axis rotation 90°; (G) z axis rotation 180°; (H) z axis rotation 270°. The calculated energies of simulated glycine adsorption ΔE_T , and the hydrogen bond component of that, ΔE_H , are given in kJ mol⁻¹. For details of the minimization calculation see Appendix A.

ure 4b, d, e and g). This was between the protonated ammonium of the glycine and a keto group within the monolayer. Relative interaction energies could be determined for the simulated adsorption of the amino acid by calculating the difference in energy between the minimized structure with and without the glycine retracted from the surface. The component of this which contributed to the hydrogen bonding in the simulation was also determined. These values are shown below their corresponding models in Figure 4. The hydrogen bond component of the interaction energies were in the order of 12.6 to 13.5 kJ mol⁻¹ and compare well with that determined for the hydrogen bond between the carbonyl and the protonated nitrogen of formamide (14.6 kJ mol⁻¹) (Jeffrey and Saenger, 1994). The interaction of glycine through two hydrogen bonds was also observed (Figure 4h). This was also represented by the hydrogen bond energy (19.2 kJ mol⁻¹). These hydrogen bonds, however, were not linear and could account for the hydrogen bond energy term not being simply a multiple of the linear hydrogen bond component. We point out at this stage, however, that neglect of solvent, ion and charge effects in the calculation procedure may test the confidence of such an approach. A more in-depth analysis of these and other simulated interactions of amino acids with monolayer systems will be treated in a separate publication. However, based on the satisfactory simulation of the monolayer systems alone, the proposed interaction of the amino acids through the hydrogen bonding described, seems reasonably feasible.

For amino acid polymerization to occur on a purine-pyrimidine monolayer, the reactive moieties of each amino acid must first be brought within close proximity. In the zwitterionic form, the positively charged ammonium group of an amino acid must be placed juxtaposed to the negatively charged carboxylate group of an adjacent amino acid. Energy minimization calculations of multiple amino acid adsorbates (Figure 5) support the possibility of hydrogen bonding between adjacent amino acids. This may facilitate amino acid adsorption and impose the spatial orientation required for subsequent dehydration reactions.

In the multiple adsorption example shown (Figure 5), the proton donor capability of the positively charged ammonium groups of the amino acids, interact through single hydrogen bonds with the proton accepting (keto) groups on the monolayer. Free rotation of the amino acids about this keto group inscribes a circle. We have drawn a circle (0.32 nm) to represent the geometric path available to the mobile carboxylate group, excluding steric considerations. This circle closely borders on three other proton accepting sites which are in the adjacent uracil molecules. The lateral distance between the proton accepting groups of hydrogen bonded uracil dimers corresponds, approximately, to the axial distance of 0.35 nm between the extended amino acid residues of the β -sheet polypeptide (Stryer, 1988). This is also a first order approximation for the distance required for peptide bond formation between adjacent amino acids. Adsorption of an amino acid on each of the other proton accepting groups in the monolayer, through the same hydrogen bonding interaction, would place three positively charged ammonium groups within the geometric accessibility of the negatively charged carboxylate substituent. This could

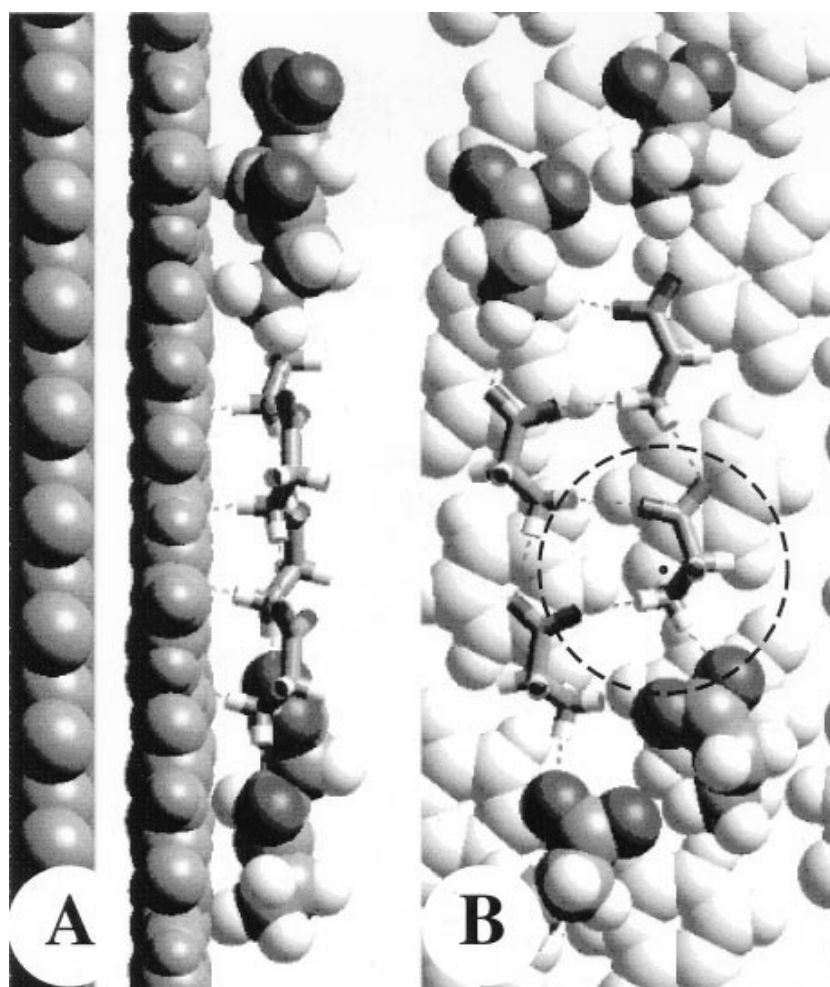


Figure 5. Molecular mechanics energy minimization of multiple glycine molecules on the uracil-MoS₂ monolayer system. The start configuration was generated by arbitrary placement of glycine molecules in the configuration of Figure 4d on the uracil surface. (A) Side view and (B) plan view showing the predicted interactions of the glycine molecules both with the uracil monolayer, and with adjacent glycines. Putative intermolecular hydrogen bonds are shown by the dashed lines. The dashed line circle represents the geometric path of the carboxylate group of a single ammonium proton immobilized glycine. The rotational center is placed on a keto substituent within the monolayer. The line intersects three other keto groups, in adjacent molecules. For details of the minimization calculation see Appendix A.

enable the carboxylate moieties to be brought within close proximity of one of the ammonium groups of the adjacent molecules. The charge difference between these two groups suggests that electrostatic effects could drive carboxylate-ammonium interactions. Of the three donor properties of the ammonium group, one proton would interact with the monolayer. The other two could each be satisfied by an

interaction with the carboxylates of adjacent amino acid residues. The tetrahedral configuration of the protons from the amino acid ammonium group would place one hydrogen bonding interaction pointing into the monolayer, the other two close to the plane of the monolayer with the side chain oriented away from the surface. The geometric feasibility of such a scenario may provide a mechanism for orienting amino acids in the correct spatial configuration for subsequent condensation reactions.

Inspection of other homo- and hetero-nucleic acid base dimers (Saenger, 1984) shows the same configuration of proton accepting groups between adjacent hydrogen bonded purines and pyrimidines. This consistent geometry suggests the possibility of a generalized mechanism for amino acid interaction with purine and pyrimidine monolayers. Non-coded protein synthesis has been proposed as a predecessor to the modern genetic machinery (Orgel, 1987). Polypeptide synthesis of this nature might be facilitated on purine-pyrimidine monolayers through non-specific interactions. It is conceivable, however, that physicochemical differences between different dimers could provide a mechanism for primitive genetic coding by way of direct discrimination between different amino acids.

The examination of the three-center hydrogen bonds formed between the ammonium and carboxylate groups of the zwitterionic crystal structures of the amino acids, showed considerable variation in the symmetry and the geometry of the intramolecular zwitterion bridge. Although these differences were thought to primarily arise from the packing constraints imposed by the crystal lattice (Jeffrey and Saenger, 1994), the influence of the side chain on the geometry and electronic character of this region of the amino acids, may provide the corresponding variation on the amino acids for specific interactions with purine-pyrimidine dimers through hydrogen bonding as described.

Primitive genetic coding might not necessarily resemble that of the modern biochemical apparatus but just act to provide a mechanism for the introduction of variation into nascent polypeptides. The formation of both homo- and hetero-dimers between the purine and pyrimidine bases suggests a mechanism for providing variation to nascent peptide molecules by way of aperiodic monolayer formation from juxtaposed hydrogen bonded dimers. The subsequent ordering of adsorbed amino acids would consequently be directed by the pattern of purine and pyrimidine aperiodicities in the monolayer. In Figure 5, a possible linear arrangement of amino acids on the uracil monolayer can be seen. A large variety of combinations of linear polymers could result from a two-dimensional mixed system because of the alternative dimer options within geometric accessibility of each adsorbate residue.

The formation of a peptide bond is thermodynamically unfavorable. The energy of FeS_2 formation from FeS and H_2S ($E^\circ \text{FeS}/\text{H}_2\text{S} = -620 \text{ mV}$ (Blöchl *et al.*, 1992)) has been demonstrated to provide the energy for such condensation reactions (Keller *et al.*, 1994). Alternatively, the energy deficit might be offset by the activation of the amino acids with hydrogen cyanide derived condensing agents for which there are well established prebiotic pathways (Ferris and Hagen, 1984) and which have

also been employed in spontaneous amino acid condensation reactions (Brack, 1993 and references therein). Similarly, polymerization reactions of amino acid derivatives has been facilitated on clays, in surfactant aggregates, in micelles, and in oriented monolayers (Brack, 1993, and references therein).

It is not conceptually difficult to envisage that the synthesis of peptides composed of amino acids of the same enantiomeric configuration might be achieved on enantiomorphic monolayers. A stereospecific interaction of the enantiomorphic monolayers with the asymmetric zwitterionic component about the chiral centers of the amino acids could facilitate selective adsorption of only one configuration of stereoisomer. The possibility of non-chiral organic substances crystallizing randomly into enantiomorphic crystals followed by asymmetric solid state synthesis, has previously been proposed as a prebiotic mechanism for chiral symmetry breaking (Bonner, 1995, and references therein). However, the experimental evidence to date suggested that esoteric compounds and carefully contrived chemical conditions were required for such processes to proceed. The formation of enantiomorphic monolayer structures of the purines and pyrimidines seems, at least, prebiotically feasible and could provide the asymmetry required for subsequent syntheses.

The synthesis of homochiral polymers could result in chiral peptide catalysts. Desorption of these peptide molecules from the solid-liquid interface might proceed as a consequence of conformational changes following peptide bond formation. Desorption would allow the active peptide molecules to catalyze other chemical reactions and homochiral peptide catalysts would be expected to act asymmetrically on their substrates. Such a process would suggest a mechanism for taking chiral surface information from a monolayer at the solid-liquid interface and amplifying it into the available solution chemistry. Such processes might act autocatalytically and even be capable of synthesizing more complex biomolecule precursors such as enantiomerically pure carbohydrates.

In such a scenario, the monolayer would act as a catalyst for peptide bond formation and it would be attractive to suggest that the origin of primitive genetic coding and the origin of biomolecular optical asymmetry were simultaneously established via the same peptide synthesizing mechanism.

Similarly, the interaction of amino acids with the purine and pyrimidine monolayers suggests the possibility of chemisorption of the amino acids via chemical bonds to the immobilized bases. Subsequent polymerization of these compounds might also occur at the interface and result in a peptide nucleic acid-like molecule which has been proposed as a predecessor of modern nucleic acids (Egholm *et al.*, 1992).

8.2. CARBOHYDRATES

Although the plausible prebiotic availability of carbohydrates has been questioned (Schwartz and de Graaf, 1993), early work on the prebiotic synthesis of purine and pyrimidine ribosides achieved significant products only by drying reactions.

Solutions of the bases were evaporated and then dried at moderate temperatures in the presence of ribose and inorganic salts and were referred to as solid phase reactions (Orgel and Lohrmann, 1974). It is not conceptually difficult to imagine that monolayers of the bases may have formed at the solid-liquid interface and could have stereochemically directed the carbohydrates into the appropriate orientation for β -riboside formation. The solid phase reactions were extended to phosphorylation, activation and polymerization reactions. Although these results were not convincing arguments for the prebiotic appearance of RNA (Joyce, 1989), it is conceivable that the transition from simple prebiotic organic syntheses to a more complex nucleic acid based chemistry was mediated via surface directed interactions following the appearance of carbohydrates. Similar to the interaction of base monolayers with racemic amino acids, it has been suggested that enantiomorphic monolayers of purines and pyrimidines might also act specifically with a racemic mixture of carbohydrates (Sowerby, 1995). Chemisorption of the carbohydrates via a glycosidic linkage to a base would form the nucleoside. In the presence of phosphate derivatives and other activating agents, polymerization reactions might also occur. Subsequent desorption from the surface might provide a mechanism for taking the aperiodic surface structure into solution as a linear polymer with nucleic acid-like character. It is also conceivable that nucleoside derivatives of the purine and pyrimidines could themselves interact with the base monolayers via intermolecular hydrogen bonding interactions, or even that nucleoside derivatives themselves could form condensed monolayers, for which there is electrochemical evidence for potential controlled formation at the mercury-liquid interface (Vetterl, 1966) and on gold (111) surfaces (Scharfe *et al.*, 1995). Boland and Ratner (1994) have suggested that the self-assembly of purine and pyrimidine monolayers at the solid liquid interface may help explain the polymerization of unblocked adenosine monomers on sodium-montmorillonite clays (Ferris and Ertem, 1992).

9. Summary

Although the plausible prebiotic availability of certain classes of organic molecules has been questioned (Schwartz and de Graaf, 1993; Shapiro, 1995), or even the nature of the chemistries involved in their syntheses (Wächtershäuser, 1988), their central role in modern biological systems necessitates their appearance at some stage during the development of life-like self-replicating systems and justifies their examination in the prebiotic context.

The examination of monolayers of the purine and pyrimidine bases has shown that they can spontaneously self-assemble at the solid-liquid interface and this together with their plausible prebiotic synthesis, suggests that these structures may have had prebiotic significance. We have proposed that these structures may be involved in subsequent interactions with other molecules of prebiotic relevance and that the monolayers may act as a template for the selection and assembly of larger

biologically relevant molecules. Although the plausible synthesis of simple organic molecules into aqueous environments has been well examined in the laboratory, a major experimental problem has been the synthesis of higher order polymers. These reactions require the formation of peptide bonds in proteins and phosphodiester bonds in nucleic acids. Both of these processes require the removal of water in their respective dehydration reactions, however, in the aqueous environment, hydrolysis and not dehydration is thermodynamically favored. The mechanism proposed to account for the self-assembly of purine and pyrimidine monolayers invokes a steric inaccessibility of water at the solid-liquid interface. This would also suggest that the solid-liquid interface is a plausible location for the polymerization reactions to occur.

The variability of the observed purine and pyrimidine adsorbate lattice structures appears to be constrained by four physical parameters. These are, the van der Waals packing constraints of the adsorbate molecules, the possible adsorbate lattice configurations resulting from intermolecular hydrogen bonding capabilities and substrate registry, the flexibility of hydrogen bonding interactions, and the number and variability of the available substrate surfaces. The availability of monolayers of mixed composition would result in extensive combinations of aperiodic configurations and suggests a physicochemically diverse array of monolayer structures. The aperiodicity of mixed composition monolayers of purine and pyrimidine bases provides an attractive alternative to bare inorganic surfaces (Bernal, 1951; Cairns-Smith, 1982; Wächtershäuser, 1988; Russell *et al.*, 1993) as it uses organic molecules of genetic interest. The possibility that a primitive genetic coding mechanism and homochiral purity may have arisen simultaneously in a peptide synthesizing process on nucleic acid base monolayers is also attractive, because polypeptide synthesis of chiral enzyme catalysts is now achieved by chiral selection and nucleic acid directed polymerization, in the cellular biochemical machinery. The degeneracy of the third 'wobble' position of the genetic code supports the hypothesis that the primitive genetic code may have been allocated primarily by a doublet coding system (Lacey and Mullins, 1983). The specific interaction of amino acids with purine-pyrimidine dimers would also support these proposals. It is, however, difficult at this stage to envisage how a monolayer based coding system could be subjected to the pressures of Darwinian-like evolution and then in that sense, genetic coding would be a very 'loose' description for template directed polypeptide synthesis. Similarly, the demonstration of templating activity of these monolayers may provide applications for these modified surfaces in separation science and supramolecular chemistry.

Examination of naturally occurring MoS₂ samples by STM suggested the presence of nanoscale inclusion compounds (Heckl *et al.*, 1991). Mass spectroscopic analysis of these samples indicated that biogenically derived carbon compounds were present and suggested that nanoscale features may be part of the fossil record. Examination of the fossil record, geochemically plausible mineral samples or even

extra terrestrial debris with the near field STM/AFM, may lead to the identification of prebiotically relevant systems.

A key point of this communication emphasizes the experimental evidence from which these proposals have been drawn. Our model for the implication of monolayers of the purine and pyrimidine bases in the origin of life is experimentally testable. Preliminary investigations have been performed on the monolayers formed from highly purified preparations of the nucleic acid bases. These artificial systems should not distract attention from the milieu of organic products available at the solid-liquid interface as a result of prebiotic chemistries, but do provide a useful starting point. Future investigations of the purine and pyrimidine monolayer systems and their interactions with other organic molecules may verify or discount some of the proposals made here. Currently available surface science, electrochemical and bulk adsorption techniques provide viable methods for examining these systems. Of particular interest are the near field microscopy techniques of STM and AFM, which allow the real-space investigation of surface structures and their adsorbates.

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Appendix A

Molecular Mechanics was applied to the proposed model of uracil on MoS₂ (Sowerby and Petersen, 1997) with the program Cerius² running on a Silicon Graphics Indigo II workstation. For the energy minimization calculations, we used the Dreiding II force field (Mayo *et al.*, 1990) which has been parametrized for organic, biological and main group inorganic molecules and has an explicit hydrogen bonding term. The potential energy (E_T) of the adsorbate monolayer system is the sum of the two- three- and four- body terms in the energy expression which is implemented by Cerius²,

$$E_T = E_{\text{bond}} + E_{\text{angle}} + E_{\text{torsion}} + E_{\text{inversion}} + E_{\text{vdW}} + E_{\text{Coulombic}} + E_H .$$

The expression can be divided into two terms. The valence terms, E_{bond} (bond stretching), E_{angle} (angular distortions), E_{torsion} (dihedral angle torsions) and $E_{\text{inversion}}$ (umbrella inversions) which relate to specific bond and atomic orientations of the molecular structure and were modeled by Newtonian mechanics expressions fitted to reproduce crystallographic and spectroscopic data. The non-bond terms, E_{vdW} (van der Waals) $E_{\text{Coulombic}}$ (Electrostatic) and E_H (hydrogen

bonding) relate to intermolecular interactions. The van der Waals term was modeled using the Lennard-Jones 12–6 potential used in Cerius² whereas the hydrogen bond applies the Lennard-Jones 12–10 potential. Electrostatic interactions took the form of a Coulombic expression between atom centered point charges.

The two-dimensional monolayer systems were modeled using periodic boundary conditions in a three-dimensional crystal model space as previously described (Freund *et al.*, 1997; Seidel *et al.*, 1997). The substrate model surface was the (0001) surface of MoS₂ which was the top layer of a six layer slab of MoS₂ bulk crystal model. The MoS₂ surface mesh dimensions were that of the adsorbate coincident mesh. The symmetry of the model space was reduced from R3m to P1. The length of the cell perpendicular to the surface was extended to 150 Å to ensure no long range interactions between the layers of adjacent cells. Individual uracil models were placed manually on the substrate model surface to generate plausible start configurations. Partial atomic charges were assigned to the atoms of the adsorbate molecules by the charge equilibration method (Rappé and Goddard, 1991). The charges of the MoS₂ were set to zero.

The energy minimization algorithms were applied to proposed start configurations. During the minimizations, atomic motion constraints were applied only to the atoms of the MoS₂ substrate and the partial atomic charges were re-calculated every 50 cycles. The minimizations were terminated when the total energy of the potential energy hypersurface reached an RMS force gradient of 0.03 kcal mol⁻¹ Å⁻¹. The model presented (Figure 1b) shows only the uppermost layer of sulfur atoms of the MoS₂.

The application of molecular mechanics to the proposed interaction of the amino acid glycine with the simulated monolayer was performed in an identical manner to that of the monolayer alone. The start configurations for the energy minimizations were generated by a series of geometric rotations of the glycine from a defined model (Figure 4a). The unit mesh was doubled in the plane of the surface for these systems to reduce the effect of ghost glycines generated by the periodic boundary conditions. The simulated adsorption energy ΔE_T was calculated by determining the difference in the E_T of the minimized structure, and that with the glycine removed 50 Å from the surface, but still within the model space. Similarly, the proposed interaction of multiple amino acids with the simulated monolayer was performed in an identical manner to that of the monolayer alone. The start configuration was generated by arbitrary placement of glycine molecules in the configuration of Figure 4d on the uracil surface.

Note added in proof

Since submission of the original manuscript, additional material concerning the structure and analysis of the two-dimensional monolayers of the nucleic acid purines and pyrimidines has been obtained. A more in-depth application of molec-

ular mechanics simulations has been applied to the LEED data obtained for adenine monolayers on graphite (Edelwirth *et al.*, 1997). The refined adsorbate structure placed the adenine molecules lying flat on the surface with a maximized van der Waals interaction and realized the maximum number of possible intermolecular hydrogen bonds between adjacent molecules. The adsorbate substrate interaction was determined to be predominantly of the van der Waals type and the electrostatic component was negligible. The calculated adsorption energy compared well to that determined by thermal desorption spectroscopy measurements. A re-examination of the adenine adsorbate on MoS₂ showed the effect of changing the scan direction of the STM tip on the adsorbate image contrast (Sowerby *et al.*, 1997). Molecular mechanics was also applied to this system and showed that the packing configuration determined by the LEED and molecular mechanics studies for adenine on graphite was most likely whereas all previously proposed structures for adenine on graphite and MoS₂ were energetically unrealistic. The refined lattice dimensions for this system ($a = 2.28$ nm, $b = 0.84$ nm, $\gamma = 93^\circ$) contradicted those previously determined (Heckl, 1993; Sowerby *et al.*, 1996). While the specific arguments presented for enantiomorphism as a consequence of the adsorbate molecular packing may no longer be applicable, the study did again show the enantiomorphic character of the oblique mesh with both mirror structures presented. This supports a possible prebiotic function for enantiomorphic monolayers in localized chiral symmetry breaking. The anisotropic response of the STM images of adenine on MoS₂ to changing scan rotation was interpreted in terms of 'conduction bands' formed by the concatenation of organic heterocycles by the cyclic hydrogen bonds. It was proposed that the network of hydrogen bonded adenines could have electronic energy transduction properties and that this may have some prebiotic relevance (Sowerby *et al.*, 1997). Molecular mechanics simulations of the type presented here were also applied to the uracil monolayer structures predicted by STM analysis on both graphite and MoS₂ surfaces (Sowerby and Petersen, 1997). These minimized well and supported the previous STM examination (Sowerby *et al.*, 1997).

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