J Mol Evol (1996) 43:419–424

JOURNAL OF MOLECULAR EVOLUTION

Chiral Symmetry Breaking During the Self-Assembly of Monolayers from Achiral Purine Molecules

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Received: 4 March 1996 / Accepted: 6 May 1996

Abstract. Scanning tunneling microscopy was used to investigate the structure of the two-dimensional adsorbate formed by molecular self-assembly of the purine base, adenine, on the surfaces of the naturally occurring mineral molybdenite and the synthetic crystal highly oriented pyrolytic graphite. Although formed from adenine, which is achiral, the observed adsorbate surface structures were enantiomorphic on molybdenite. This phenomenon suggests a mechanism for the introduction of a localized chiral symmetry break by the spontaneous crystallization of these prebiotically available molecules on inorganic surfaces and may have some role in the origin of biomolecular optical asymmetry. The possibility that purine-pyrimidine arrays assembled on naturally occurring mineral surfaces might act as possible templates for biomolecular assembly is discussed.

Key words: Chiral symmetry breaking — Molecular self-assembly — Origin of life — Purine Bases — Scanning tunneling microscopy — Two-dimensional arrays

Introduction

The results of electric discharge experiments (Miller 1987) and other physicochemical reconstructions of the prebiosphere (Ferris and Hagan 1984; Hennet et al. 1992; Shock 1990) suggest that many biomolecule precursors could have accumulated at some stage during the prebiotic history of earth and that heterotrophic life may have subsequently evolved from simple chemical precursors. A plausible route for the prebiotic synthesis of the common nucleic acid purine base, adenine, proceeds via hydrogen cyanide oligomerization. Similar reactions leading to the formation of the other purines, guanine, xanthine, hypoxanthine, and diaminopurine, as well as the pyrimidines, cytosine and uracil, have also been studied (Ferris and Hagan 1984). Alternative pathways can lead to the formation of 5-substituted uracils (Robertson and Miller 1995). Amino acids can also be synthesized via similar chemical pathways (Ferris and Hagan 1984) and under the redox conditions imposed by the mineral assemblages found in submarine hydrothermal vents (Hennet et al. 1992; Shock 1990).

The principal polymers of life are composed of chiral stereoisomers. Amino acids of the L-configuration are polymerized into proteins, and the carbohydrate component of nucleic acids is of the D-configuration. Chiral homogeneity in biology is a consequence of biosynthetic pathways which employ asymmetric enzyme catalysts with chiral binding sites. It is both theoretically and experimentally difficult to envisage how biomolecular asymmetry could have originated in the absence of such catalysts (Bonner 1991). Although the mechanisms by which the first catalytically active polymers of homochiral material were assembled are not known, resolution of chiral stereoisomers from racemic mixtures has been

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suggested as a functional requirement for the origin of life (Avetisov and Goldanskii 1993).

Scanning tunneling microscopy (STM) (Binnig et al. 1982) allows real space-structure determination of surfaces, with atomic resolution. STM has previously been used to study the condensation of some purine and pyrimidine bases following the thermal evaporation of their aqueous solutions on the surfaces of the synthetic crystal, highly oriented pyrolytic graphite (HOPG) (Allen et al. 1992; Heckl 1993; Heckl et al. 1991; Sowerby 1995) and the naturally occurring mineral molybdenite (MoS_2) (Heckl 1993; Heckl et al. 1991; Sowerby 1995). The assembly of purine and pyrimidine structures, deposited under electrochemical control and imaged by electrochemical STM, has also been demonstrated on HOPG (Srinivasan et al. 1991; Tao and Shi 1994) and on crystalline gold surfaces (Boland and Ratner 1994; Tao et al. 1993). The spontaneous condensation of some purines from saline solution onto HOPG has been observed *in situ* by electrochemical STM imaging and atomic force microscopy (Tao and Shi 1994).

Crystallization has been suggested as a mechanism for the prebiotic fractionation and concentration of organic molecules (Bernal 1951) and for the resolution of chiral stereoisomers (Bonner 1991). Analysis of the STM images of two-dimensional crystals of adenine molecules self-assembled onto the surface of the basal plane of molybdenite shown that the structures of the adsorbates are enantiomorphic. We suggest that the thermodynamically favorable formation of chiral structures through the self-assembly of achiral prebiotically available molecules onto mineral surfaces which show no asymmetry may have had some significance for the development of biomolecular homochirality in the early stages of the origin of life.

Results

The STM analysis of adenine absorbed onto molybdenite typically resulted in images of adenine molecules as irregularly shaped objects extending in adjacent parallel rows (Fig. 1A). Comparison of the adsorbate image with the underlying substrate image (Fig. 1B) revealed that the observed periodicities occurred on equivalent locations of the molybdenite lattice. Several images of adsorbates were compared with their corresponding images of the underlying substrate and showed consistent adsorbate unit mesh dimensions. Studies of images obtained in a series of experiments revealed the existence of a second species of STM image of adenine on molybdenite (Fig. 1C). Comparison of these two oblique adsorbate lattice structures indicates that they are related to each other by a mirror reflection perpendicular to the plane of the substrate. The lattice structures of the adsorbate unit mesh that are coincident with the underlying substrate are represented as general parallelograms on both of the adsorbate STM images (Fig. 1A and C).

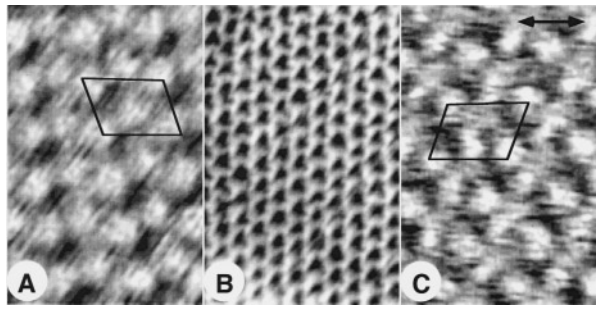

Fig. 1. STM images of adenine on molybdenite and the underlying molybdenite. The adsorbate structures were prepared by the ''sizzling'' technique (Allen et al. 1991; Heckl et al. 1991). STM measurements were performed using a Nanoscope II STM (Digital Instruments, Inc., Santa Barbara, CA, U.S.A.) using electrochemically etched tungsten tips (4 M-NaOH, 10 V AC) at ambient conditions. The measurements were made at bias voltages of 200 mV to 1,500 mV (sample to tip) and tunneling currents between 30 pA and 100 pA. Nonlinearities of the piezoelectric scanner were corrected for by assuming a perfect threefold symmetry for the atoms of the underlying crystal substrate surface which were imaged by piercing the tip through the adsorbate crystal after reducing the gap resistance (Heckl et al. 1991). Geometric corrections were performed on the STM images using the Microscopy Image Processing System version 2.2.1 software package. **A** STM image of an ordered array of adenine molecules adsorbed to the molybdenite surface (imaging parameters; bias voltage = 1,464 mV, tunnel current = 100 pA). The *black parallelogram* describes the adsorbate unit mesh that is coincident with the underlying substrate. **B** The image of the underlying molybdenite substrate beneath **A** showing the uppermost sulfur atoms, 3.16 Å apart (imaging parameters; bias voltage = 335 mV, tunnel current = 100 pA). **C** A second STM image of adenine molecules adsorbed to the molybdenite surface that is a mirror reflection of **A** (imaging parameters; bias voltage = 799 mV, tunnel current = 30 pA). The STM images were low-pass filtered to remove high-frequency noise. The scale bar represents 10 Å.

Similar studies of adenine adsorbed to the surface of the synthetic crystal HOPG showed a different image by STM (Fig. 2). In these images the adsorbate had a striped appearance and the molecules were resolved inequivalently, but with submolecular detail in which the ring structures of individual molecules were clearly visible. This effect is believed to be a result of the electronic differences between the two substrates. Interaction between the molecular orbitals of the adsorbed molecules and those of the substrate atoms can affect the local density of states imaged by STM (Smith et al. 1992). The technique of penetrating the adsorbate with the STM probe to image the substrate and the adsorbate in a single-image frame allowed us to determine the exact location of the adsorbate molecules on the substrate by extrapolation. The rectangular lattice structure of the adsorbate unit mesh that is coincident with the underlying substrate is drawn onto the STM image and does not suggest enantiomorphism as seen in the adenine adsorbate on the molybdenite surface. Repeated investigation of the adenine adsorbate on HOPG showed that the observed adsorbate structure was invariant.

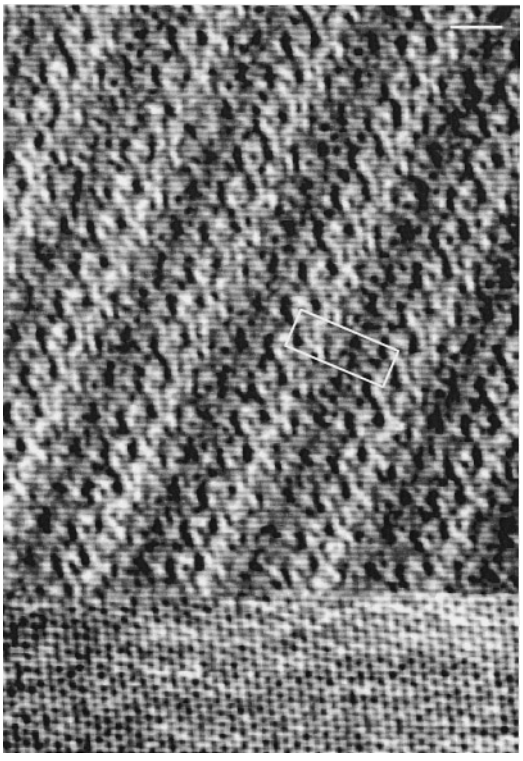

Fig. 2. STM image showing an ordered array of adenine molecules adsorbed to the HOPG surface (imaging parameters; bias voltage = 800 mV, tunnel current = 200 pA) and, in the same frame, the underlying substrate showing the uppermost carbon atoms of HOPG, 2.46 Å apart, obtained by reducing the bias voltage so that the STM tip pierced the adsorbate to image the substrate (imaging parameters; bias voltage = 20 mV, tunnel current = 200 pA). The image frame shows both adsorbate and substrate data simultaneously and allows the position of the adsorbate molecules on the substrate to be determined precisely. The *white rectangle* describes the adsorbate unit mesh that is coincident with the underlying substrate. The STM image was low-pass filtered to remove high-frequency noise. The *scale bar* represents 10 Å.

The models proposed to account for the formation of enantiomorphic adenine structures on molybdenite (Fig. 3A), and the symmetrical adsorbate structures on HOPG (Fig. 3B), are based on the experimentally determined dimensions of the adsorbates and the well-established intermolecular hydrogen-bonding capabilities of adenine.

On molybdenite the adsorbate crystals are composed of adjacent parallel rows of molecules which point in the opposite direction with adjacent molecules linked by intermolecular hydrogen bonds to form a bimolecular row of symmetrically bonded adenine pairs. The center-to-center molecular distance between commensurate locations of adjacent bimolecular rows is 10.4 Å and extends along adsorbate lattice vector *a.* Along the bimolecular rows, head-to-tail adenine molecules are placed with an intrarow, center-to-center molecular distance of 8.4 Å, described by adsorbate lattice vector *b.* Each molecule within each single row is symmetrically hydrogen bonded to two other molecules which are both in the adjacent row and oriented by a 180° screw axis. The hydrogen-bonding configurations which extend along the direction of adsorbate lattice vector *a* are through the N9 ring nitrogen proton donors and the N3 ring nitrogen acceptors of adjacent molecules, and in the opposite direction along adsorbate unit cell vector *a,* through the N6 amino proton donors and the N7 ring nitrogen acceptors of adjacent molecules. This is consistent with the intermolecular hydrogen bonding seen in the three-dimensional crystal of adenine hydrochloride hemihydrate (Kistenmacher and Shigematsu 1974). Adjacent bimolecular rows are also connected by symmetrical hydrogen bonding through the N6 amino proton donors and the N1 ring nitrogen acceptors of adjacent molecules which extend along the direction of adsorbate lattice vector *a* and are also related by a 180° screw axis. The proposed hydrogen-bonding configurations implicate each molecule in hydrogen-bonding interactions with the three nearest neighbors. The members of each pair of molecules involved in intermolecular hydrogen bonds are all related by a 180° screw axis. Consequently all molecules in the adsorbate are of the same configuration, related to each other only by rotation and not by mirror reflection. Both of the observed adenine structures adsorbed on molybdenite can thus be accounted for by a reflection perpendicular to the plane of the substrate. On HOPG, however, the observed structure of the adenine adsorbate was rectangular. The proposed model differs from those previously published (Allen et al. 1991; Tao and Shi 1994) but supports measurements made on the adenine HOPG system using low-energy electron diffraction (LEED) (Freund et al. 1995).

Similar to the structure of the adsorbate on molybdenite, adjacent rows of commensurate head-to-tail adenine molecules on HOPG form bimolecular rows in which each molecule within each row is symmetrically hydrogen bonded to molecules of the adjacent row. The intrarow, center-to-center molecular distance of 8.5 Å is comparable to that for the equivalent measurement of the adsorbate on molybdenite and is also described by the adsorbate lattice vector *b.* However, along adsorbate vector *a,* commensurate locations occur at a distance of 22.1 Å, almost twice that for the adenine adsorbate on molybdenite. The identification of aromatic-like ring structures from the STM images suggested an intermolecular assembly of the adsorbate structure which correlated well with the LEED observations that agreed with the unit cell dimensions and showed the presence of two glide reflections. Although the symmetric hydrogen bonding proposed between the molecules of the bimolecular rows is identical to that of adenine on molybdenite, adjacent bimolecular rows are related by a glide reflection and are hydrogen bonded only through a single interaction between the N9 ring nitrogen proton donor and the N3 ring nitrogen acceptor of adjacent residues in the direction of adsorbate lattice vector *a.* The internal symmetry of the adsorbate unit mesh would thus exclude

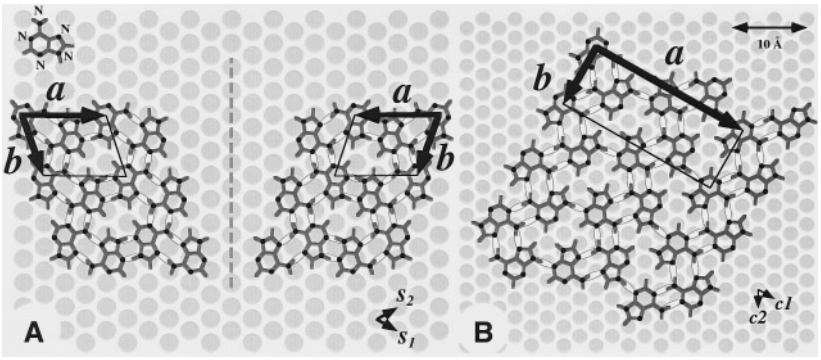

Fig. 3. Scale models of the adenine adsorbates. **A** Enantiomorphic adenine adsorbates on the basal plane surface of molybdenite. The adsorbate lattices that are coincident with the underlying substrate lattice (where *s1* and *s2* are both 3.16 Å) are described by lattice vectors, $a = 10.9$ Å and $b = 8.4$ Å, that are separated by an angle of 70°. Putative intermolecular hydrogen bonds of length 2.9 Å (N to N), are shown in *white.* The two models of the adenine adsorbate on molybdenite are related by a reflection perpendicular to the plane of the substrate, shown by the *dashed line.* **B** Adenine adsorbed to the basal plane surface of HOPG. The adsorbate lattice that is coincident with the underlying substrate lattice (where *c1* and *c2* are both 2.46 Å) is described by lattice vectors, $a = 22.1$ Å and $b = 8.5$ Å, that are separated by an angle of 90°.

the possibility of enantiomorphism for the adenine on HOPG.

Discussion

Our results show that the achiral purine base adenine can easily self-assemble into enantiomorphic two-dimensional arrays on the surface of the naturally occurring mineral molybdenite, whereas on the surface of the synthetic crystal HOPG, the adsorbate structures are symmetrical and consequently are not enantiomorphic. We propose that epitaxial registration requirements of the adsorbate with the underlying substrate together with the intermolecular hydrogen-bonding requirements of the adsorbate molecules are responsible for the observed differences between the structures of the two adenine adsorbates. Although adenine is achiral in solution, it loses a rotational degree of freedom upon immobilization on a surface and its planar structure allows for two configurations for adsorption. Real-time observation of spontaneous purine adsorbate formation on HOPG in saline solution shows anisotropic line tensions in nucleating monolayer clusters, suggesting that direct incorporation rather than diffusion is the predominant mechanism for adsorbate growth (Tao and Shi 1994). It is likely that in both cases the mechanisms of adsorbate growth are similar. Although the mechanism of primary nucleation is unclear, it would seem sensible that growth of adsorbate structures would be directed by the stereochemical requirements placed on the approaching adsorbate molecules by the growing crystal edge. The stereochemical requirements would thus be a function of both the registration requirements of the adsorbate molecules with the substrate and of restrictions imposed on the hydrogen-bonding orientations for incoming molecules by the growing crystal edge.

This model suggests that, in both cases of adsorbate formation, the predominant interaction is symmetrical intermolecular hydrogen bonding between molecules related by a 180° screw axis that results in bimolecular rows of adenine pairs, along the direction of adsorbate lattice vector *b* (Fig. 3A and B). In the case of adenine on molybdenite, adjacent bimolecular rows are each composed of molecules of the same orientation and every second molecule is commensurate with the substrate along adsorbate lattice vector *a.* Consequently, all of the molecules on the molybdenite are of the same configuration with respect to the plane of the substrate, and the planar structure of adenine gives rise to the possibility of two adsorbate structures. On HOPG, however, the substrate structure does not allow commensurability of every second adenine molecular along adsorbate lattice vector *a* unless every second molecule is flipped. This gives rise to the two glide reflections. As a consequence, on HOPG the mirror symmetries within the unit mesh exclude the possibility of enantiomorphism.

Comparison of the two STM images of adenine on molybdenite illustrated in Fig. 1 shows that the two possible lattices are oblique and nonsuperimposable in the real space images and consequently represent both enantiomorphs of the chiral adenine adsorbate on molybdenite. This is an analogous situation to recent findings that achiral calcium arachidate fatty acid molecules can spontaneously form chiral domains in a Langmuir-Blodgett film on a mica substrate (Viswanathan et al. 1994). Similarly, the loss of a rotational degree of freedom by the ordered immobilization in space of an achiral liquid crystal enables it to exhibit the chiral properties of optical activity and circular dichroism (Cherepkov and Kuznetsov 1991).

Prebiotic Relevance

The observation that nucleic acid base molecules can form enantiomorphic surface structures suggests a

mechanism for localized symmetry breaking under plausibly prebiotic conditions. The formation of both enantiomorphs in the absence of any chemical or physical asymmetry should result in a statistically equal proportion of each enantiomorph. The structure of nucleic acid base arrays is constrained by the intermolecular hydrogen bonding and the registration of the molecules with the underlying substrate. The surface structure of molybdenite has been well characterized by STM (Weimer et al. 1988). The [001] surface to which the adsorbate molecules register is a sulfur lattice with perfect threefold symmetry and would not be expected to direct the chirality of the adenine adsorbate other than the registration requirements for incoming molecules at the growing crystal edges. The observed real-time growth of guanine adsorbate crystals along the grain boundaries of the HOPG substrate (Tao and Shi 1994) might suggest a mechanism for creating a localized bias of one enantiomorph over the other if a grain boundary on the molybdenite surface imparted some type of asymmetry on the system.

The functionalization of inorganic surfaces by the crystallization of prebiotically synthesized nucleic acid bases would be expected to provide a novel surface with potential hydrogen-bonding capabilities. The enantiomorphic topography resulting from the adsorption of a mixture of nucleic acid bases could lead to physiochemically different surface locations where different combinations of base molecules lie juxtaposed on the surface. The subsequent adsorption of other prebiotically synthesized molecules which might specifically bind to the nucleic acid base array through stereospecific hydrogen bonding could result in enantiospecific co-adsorption that favors one configuration over the other. This would result in a spatially localized enantiomeric excess of chiral stereoisomer.

The condensation of amino acids into peptides under plausibly prebiotic conditions can be achieved through a variety of processes, and the resulting peptides have been shown to exhibit some catalytic activity (Brack 1993). The primitive nature of sulfur-reducing hyperthermophilic organisms which inhabit hot springs and hydrothermal vents suggests that these vents are plausible locations for the origin of life. Their steep chemical and temperature gradients and abundant iron-sulfur chemistry suggest conditions conducive to the formation of organic molecules (Blöchl et al. 1992; Hennet et al. 1992; Shock 1992) and peptide bonds (Keller et al. 1994) and have been proposed to provide catalytic inorganic surfaces for organic molecule adsorption (Russell et al. 1993; Wächtershäuser 1988). These locations might also be considered as suitable environments for nucleic acid base synthesis and subsequent two-dimensional adsorbate formation.

The formation of peptide bonds between adjacent amino acids adsorbed to an array of nucleic acid bases, and their release by subsequent rehydration, could result in catalytically active peptides that were composed of enantiomerically pure amino acids. These might then be expected to act asymmetrically on their substrates, and the catalytic activity of such polymers could provide a mechanism for the abiotic synthesis of other more complex biomolecule precursors such as enantiomerically pure carbohydrates. The stereochemical interaction between immobilized base molecules and amino acids would thus transfer the chiral surface information from the nucleic acid base arrays into solution by way of polymerization of amino acid stereoisomers of the same handedness. Additionally, the physiochemical interactions between specific amino acids and different combinations of juxtaposed base molecules could conceivably provide a primitive coding mechanism for prebiotic peptide synthesis. Currently available surface science techniques provide practical methods for testing these hypotheses.

Acknowledgments. We thank M.P. Murphy, A.J. Bridgman, P.A. Stockwell, M. Reiter, T. Reiter, and P. Stranglmeier for helpful discussions. This research was supported by the New Zealand Lottery Grants Board and the Health Research Council of New Zealand, and the Deutsche Forschungsgemeinschaft (W.M.H.) S.J.S. thanks the Health Research Council of New Zealand for a postgraduate scholarship and the Deutscher Akademischer Austauschdienst for a fellowship.

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