Enantiomeric interactions between nucleic acid bases and amino acids on solid surfaces

Q. CHEN* AND N. V. RICHARDSON
School of Chemistry, North Haugh, University of St Andrews, St Andrews, Fife KY16 9ST, UK
*e-mail: qc@st-and.ac.uk

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Molecular interaction between nucleic acid bases and amino acids is a fundamental process in biology. The adsorption of the molecules on surfaces provides the opportunity to study such interactions in great detail by exploiting the high-resolution imaging capabilities of scanning tunnelling microscopy (STM). The chemisorption of prochiral molecules, such as adenine, on a metal surface causes the adsorbed species to become chiral[1]. Subsequent interactions with inherently chiral molecules may then lead to the formation of diastereoisomers, if the enantiomeric interaction process is sufficiently strong. In the case of adenine adsorption on Cu{110}, the chiral adsorbates form homochiral chains. Here, we show that the adenine chain direction is fully correlated with the chirality, and that the α-amino acid, phenylglycine, shows a strong chiral preference in its interaction with these chains. STM images clearly demonstrate that S-phenylglycine (R-phenylglycine) binds only to chains rotated 19.5° (anti-) clockwise from the [001] direction. Closer examination reveals that the enantiomeric interaction involves double rows of phenylglycine molecules and the adenine chains. This is the first observation at the molecular level of diastereoisomeric interaction, and demonstrates that STM is a powerful method for studying the details of these interactions.

STM studies have shown that adsorption of a single enantiomer of a chiral compound on metal[2–5] and graphite[6,7] surfaces can lead to the formation of two-dimensional chiral arrays. Furthermore, a racemic mixture of enantiomers may separate into domains consisting of only a single enantiomer[2,8–12] showing chiral self-interactions in two-dimensional crystal formation. More directly, and recognisable by their STM signature when adsorbed on a Au{110} surface, cysteine molecules are capable of chiral self-interaction, because dimers formed at low coverage are exclusively homochiral[13]. The origin of this recognition lies in the 'three-point contact' of each molecule, encompassing a modified surface and its partner molecule. As yet, direct observation of diastereoisomeric interactions arising from enantiomeric interactions between dissimilar molecules has not been reported.

Molecules of appropriately low symmetry, which are achiral as isolated gas-phase or solution-phase species, are described as prochiral if they become chiral when structurally constrained, for example by chemisorption on a surface. The nucleic acid base adenine is prochiral,

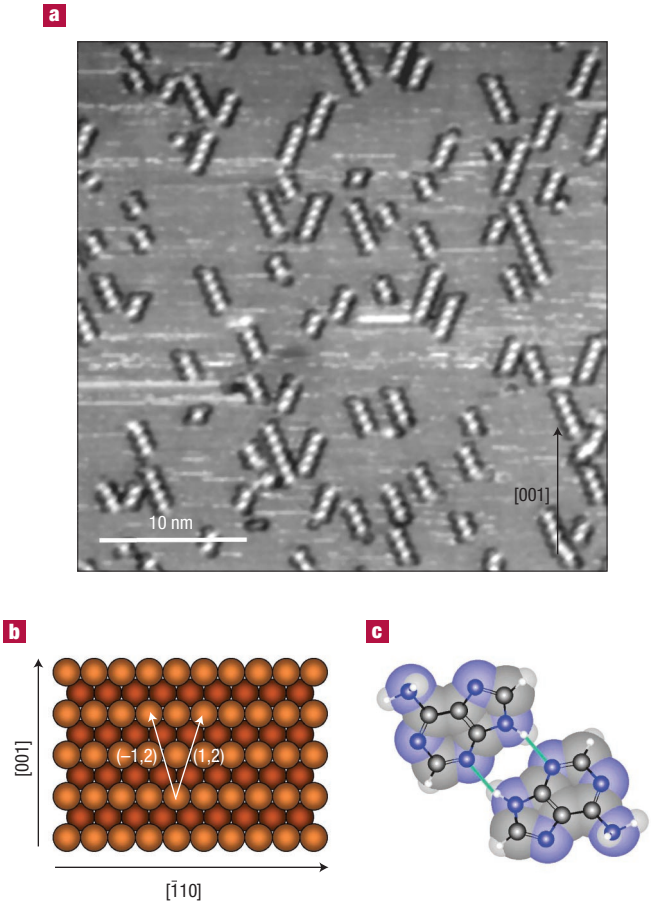

Figure 1 Adenine molecules form chains aligned along (±1,2) directions of a Cu{110} surface. a, STM image of Cu{110} surface with sub-monolayer coverage of adenine obtained in ultra-high vacuum at room temperature using a tungsten tip in constant current (1.24 nA) mode and sample bias of –1.07 V. **b**, Model of {110} surface defining the (±1,2) directions. The translation vectors, **i** and **j**, form a right-handed coordinate system directed along [$\bar{1}$10] and [001] respectively, in units of the substrate lattice unit cell. The orientation is the same as that of **a**. **c**, Homochiral dimer of adenine molecules.

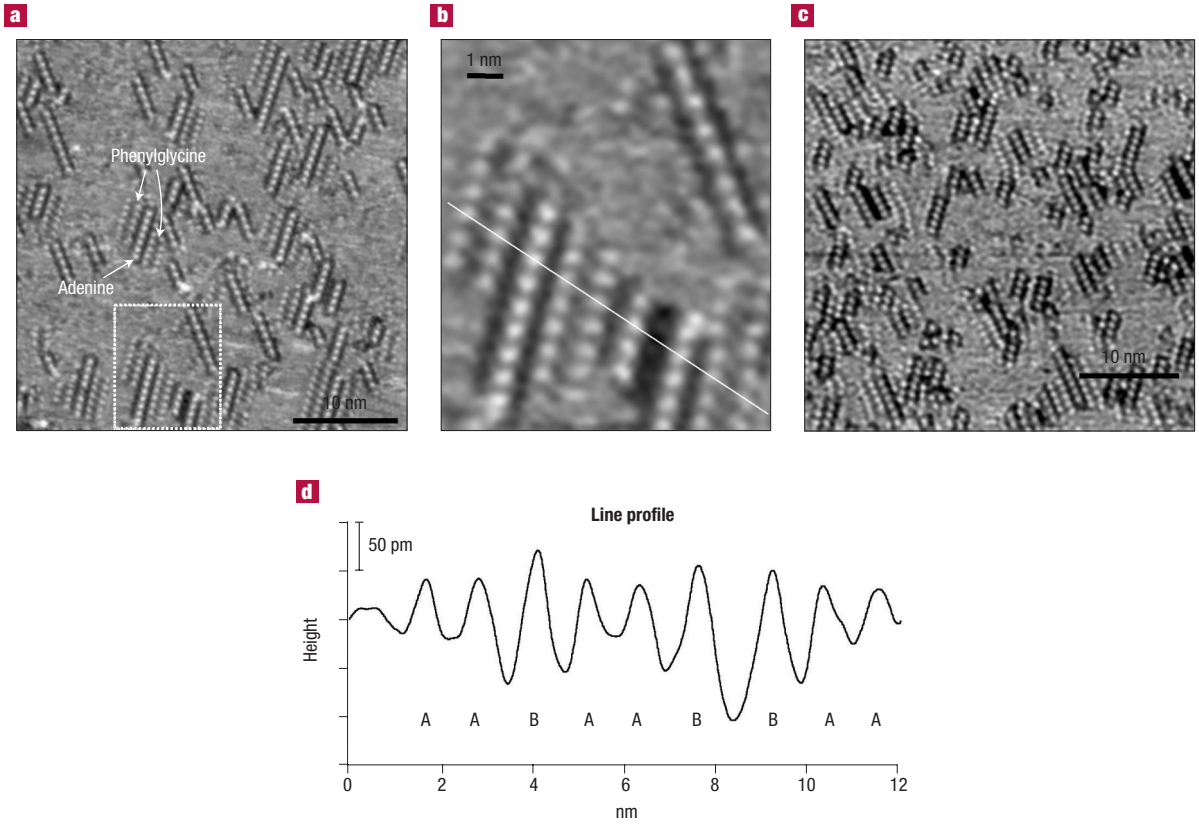

Figure 2 Phenylglycine shows a strong chiral preference in its interaction with chemisorbed adenine. a, STM image (Bias 0.02 V, 0.27 nA) of Cu{110} surface with a low coverage of pre-adsorbed adenine, subsequently exposed to S-phenylglycine, showing decoration of adenine chains in the (1,2) direction by S-phenylglycine molecule. **b**, Magnification of the region outlined by the dashed white line in **a**. **c**, STM image of Cu{110} surface pre-covered with a low coverage of adenine and subsequently exposed to R-phenylglycine, now showing decoration of adenine chains in the (–1,2) direction by R-phenylglycine molecules. **d**, Profile along the white line marked in **b**, Features marked A correspond to S-phenylglycine molecules and those marked B to adenine dimers. The adenine molecules result in somewhat brighter features than do the phenylglycine molecules. They are also flanked by deeper valleys, particularly so when adenine rows are close together.

and adsorbs on Cu{110} in a flat-lying orientation through the delocalized π-orbitals of the ring system, and the lone pair on the nitrogen atom of the amino group[1]. This adsorption geometry destroys the mirror plane of the isolated species[14], so the adsorbed molecules exist in right- and left-handed forms. Of course, the energetic equivalence of the two enantiomers adsorbed on an achiral substrate, such as Cu{110}, leads to a racemic mixture. However, as for a racemic mixture of S- and R-phenylglycine on Cu{110}, diffusion and appropriate intermolecular interactions can lead to the separation of enantiomers on the surface[2]. In the case of adenine adsorbed on Cu{110} at room temperature, dimers form and then assemble into short chains[1] of average length 6.5 nm. Furthermore, these chains adopt only two directions on the single crystal substrate (Fig. 1a). The directions (±1,2) are not high-symmetry directions but are related by reflection across the [001] direction (Fig. 1b). This leads directly to the proposal that each chain consists of adsorbed molecules of a single chirality, and that this chirality is fully correlated with the direction adopted by a particular chain, that is, all molecules forming the chains in the (1,2) direction, rotated clockwise 19.5° from [001] have the same chirality, while those in the (–1,2) direction, rotated 19.5° anticlockwise from [001], have the opposite chirality. The correspondence between chirality and chain direction is driven by hydrogen bonding in the formation of dimers and then chains, and, crucially, by the preference of the nitrogen atoms of the amino group in adenine to adsorb directly above a copper atom[1]. The arrangement of copper atoms along the (1,2) direction constitutes a chiral template, which suits the interaction only with adenine chains of the correct chirality.

A Cu{110} surface with a 15–20% coverage of adenine chains was subsequently exposed to S-phenylglycine by vacuum deposition at room temperature, and the result studied using STM. Adenine and phenylglycine are readily distinguished in STM images because adenine molecules are brighter, equivalent to 26 pm height difference, and flanked by darker regions than are phenylglycine molecules. Note, this is not interpreted as adenine molecules projecting further from the surface than the phenylglycine, but rather as arising from the lower resistance to tunnelling through the π-system of adenine. Figure 2a,b clearly indicates that the amino acid molecules decorate adenine chains but, significantly, only those running in one direction. This supports the suggested homochirality of the chains and the correlation between chirality and chain direction. Most importantly, it is also direct evidence for enantiomeric interactions between the two molecules and a clear preference for the interaction between S-phenylglycine and chains oriented along (1,2) rather than (–1,2). Confirming the chiral origin of this preference, the separate adsorption of R-phenylglycine on an adenine-treated surface shows amino acid molecules now decorating chains aligned along (–1,2) (Fig. 2c).

The achievement of this enantiomeric interaction and bonding preference benefits both from the immobility and structural

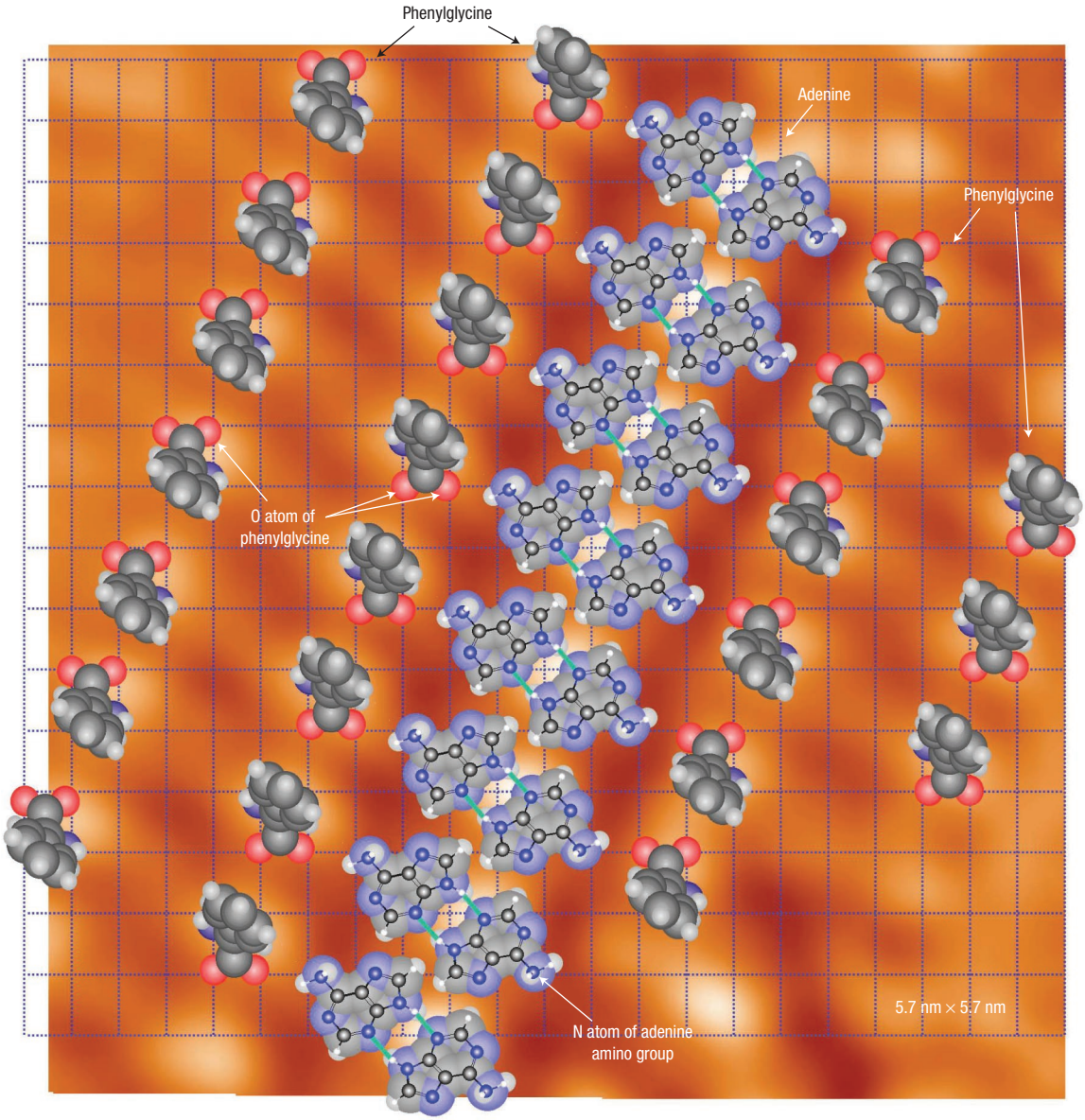

Figure 3 Model of the interaction between dimer rows of s-phenylglycine and adenine chains along (1,2) of a Cu{110} surface, superimposed on the STM image and showing the probable registry with the substrate lattice.

stability of the adenine chains at room temperature, and the fact that single phenylglycine molecules can readily diffuse, allowing them to locate their minimum energy sites in the vicinity of the appropriately oriented adenine chains. Further analysis of the assembly of the phenylglycine molecules with the adenine indicates that double rather than single rows (Fig.2b) of the amino acid lie parallel to the adenine chains, sometimes extending beyond the chains. The periodicity along these dimer rows matches precisely that of the adenine dimers along the chain at 765 pm, the length of the (1,2) vector. The separation between the adenine chain and the nearest phenylglycine row is about 1.15 nm measured along the <110> direction, (Fig. 2d), which is rather closer than the closest approach of adenine rows themselves in the full monolayer[1], which is six lattice spacings along <110> at 1.53 nm. This suggests that the phenylglycine rows are positioned 4.5 lattice spacings from the adenine row along <110>. The second phenylglycine row is then a further five lattice spacings away along <110>.

Adenine is considered to adsorb as the neutral species, although definitive spectroscopic evidence has not yet confirmed this[1]. Nevertheless, the species does lie flat on the surface, and bonding of the amino nitrogen atom directly to a copper atom is likely on the basis of the structure of other adsorbed amines[15–17], and the opportunity this provides to rationalize the link between chirality and chain direction. Hydrogen bonding is responsible for the formation of adenine dimers and the incorporation of these dimers into chains.

The adsorption of α-amino acids on copper surfaces is interpreted in terms of anionic species, $NH_2CH(R)CO_2^-$, interacting with the substrate such that both the carboxylate oxygen atoms and the amino nitrogen atom are directly above metal atoms[5,18–23]. Although the adsorbed species are anionic rather than zwitterions, which are

the basis of the bulk structure, bonding of the nitrogen to copper through the lone-pair electrons will create a positive charge on the nitrogen atoms. The two-dimensional arrangements of amino acids on metal surfaces such as copper are then a complex balance of dipole–dipole interactions, as in the bulk crystal but modified by the screening response of the metal, intermolecular hydrogen bonding, such as N-H---O, and the strong site preference of the carboxylate and amino groups. In the case of glycine adsorption on Cu{110}, adsorbate chains occur along the (±3,2) directions, although, at least in one domain, alternate molecules along these directions have opposite chirality[23]. Assuming O and N atoms are in on-top sites, the shortest N-H---O distance is the Cu-Cu nearest neighbour separation of 256 pm. This probably indicates significant hydrogen-bonding contributions, although 256 pm is a rather short hydrogen-bonding distance, and the true separation is probably somewhat greater because of displacements from the exact on-top sites and re-adjustment of the copper atoms in the outermost layer. The structure formed by a single enantiomer of phenylglycine on Cu{110} consists of adsorbate rows aligned 9° from the [001] direction with a spacing of 410 pm and a shortest N-H---O distance, in the idealized structure, again of around 250 pm (ref. 2). In marked contrast, the separation between phenylglycine molecules in a row adjacent to an adenine chain is much larger at 768 pm, essentially determined by the periodicity along the adenine chain. The shortest N-H---O distance is then about 450 pm, which is rather too large to support hydrogen bonding. The phenylglycine should therefore be considered as isolated molecules interacting with the adenine chain with weaker dipole–dipole interactions along the phenylglycine row. We have assumed that the phenylglycine molecules adopt the same geometry with respect to the substrate in the co-adsorbed system as when adsorbed without adenine. This is the stable geometry found for all simple α-amino acids bonded to copper surfaces, and optimizes the nitrogen and carboxylate interactions.

The separation of the centres of the adenine and phenylglycine rows at around 1 nm is fully consistent with a model (Fig. 3) in which the nitrogen atom of the adenine amino group is almost above a copper atom adjacent to one carrying an oxygen atom from a carboxylate group, at a separation of 256 pm, analogous to the separation found in glycine/Cu{110}. Hydrogen bonding is therefore likely to make a significant contribution to the bonding between the adenine chains and the phenylglycine, although attractive electrostatic interactions between the negatively charged oxygen atoms of the carboxylate group and the positively charged nitrogen atom of the adenine amino group will also contribute strongly.

What then is the bonding of second row of phenylglycine molecules, which appear to exactly match the first row in length, spacing and direction? STM features have the same intensities in the two rows, strongly indicating similar molecular orientations. Again, interactions along the row must be rather weak, because the intermolecular spacing is about 768 pm. However, the inter-row spacing is also very large (about 1.28 nm) precluding hydrogen bonding and suggesting metal-mediated, dipole–dipole interactions constrained by adsorbate preference for a specific substrate site. Care should be exercised here because the tunnelling mechanism in this molecule is not understood sufficiently well to be able to identify the bright region with a specific part of the molecule. The phenylglycine associated with the adenine limits at two rows, suggesting that these rows are related by a C_2 rotation rather than translation. Similarly, the rows immediately adjacent to the adenine are related by the C_2 operation, because this is also the symmetry within an adenine row. Given this relationship between molecules in adjacent phenylglycine rows, the molecular centres could be closer than 1.28 nm but probably not less than 800 pm. This large inter-row spacing remains something of a puzzle because the stability of the double-row system seems to suggest that the interaction is relatively strong, and comparable to that of the phenylglycine–adenine interaction. It is reminiscent of the large inter-row spacing (2.8 nm) observed for pentacene adsorption on Cu{110}, which was ascribed to the influence of adsorbate induced charge-density waves[24].

It follows that the pinning of the orientation of phenylglycine molecules, which ensures enantiomeric interactions of the fixed adenine chains, arises primarily from the simultaneous optimization of the interactions between the carboxylate group and the amino group of adenine, constrained by the site preferences of the main, metal-interacting functionalities on each molecule while avoiding short-range steric repulsions. Interactions between neighbouring phenylglycine molecules in the same row are most likely rather weak, and the formation of double phenylglycine rows is probably a second enantiomeric interaction step, templated by the first row. The structure is potentially over constrained in that only three points of contact are required to define enantiomeric interactions, including that to the target molecule. However, the other two points are defined by the adsorption site of the phenylglycine on the metal whereas the one-dimensional periodicity along the phenylglycine rows is incidental, arising from largely independent interactions of the phenylglycine molecules with the adenine rows.

The chiral preference in the interaction of phenylglycine with chemisorbed adenine points to key structural and bonding aspects determining the recognition process between an amino acid and a nucleic acid base, albeit significantly modified by interaction with the copper substrate. Considering the phenylglycine as a chiral probe molecule, this study confirms the hypothesis that the adenine chains are homochiral with a chirality correlated with chain direction. This first observation of diastereoisomer formation on a surface demonstrates the importance of STM for the investigation of chiral interactions between dissimilar molecules and points the way to studying even more complex two-dimensional interactions at the molecular level.

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Correspondence and requests for materials should be addressed to Q.C.

Competing financial interests

The authors declare that they have no competing financial interests.