VIP Molecular Recognition

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Tracking the Chiral Recognition of Adsorbed Dipeptides at the Single-Molecule Level**

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Specific molecular recognition depends on the precisely defined arrangement of atoms in complementary structures interacting by short-range forces.[1,2] The mutual interactions between complementary molecules can result in their recognition even if their static molecular structure is not optimized for specific binding.[2–4] This mechanism of dynamic induced fit, originally introduced by Pauling in 1948[2] and developed by Koshland for enzymatic activity,[5] is today the generally accepted picture in biomolecular recognition processes.[6] Dynamic mutual conformational adjustments should also be important in chiral recognition, that is, in the discrimination of stereoisomeric molecules. However, no experimental studies have so far addressed the issue of tracking the conformational dynamics of interacting enantiomers on the single-molecule level, and chiral recognition is mainly discussed within the static three-point model[7] adapted from Fischer's lock-and-key picture.[1,8] Herein we report on the direct observation of chiral recognition events of adsorbed diphenylalanine by scanning tunneling microscopy (STM). The interaction among individual di-D-phenylalanine (D-Phe-D-Phe) molecules and the discrimination of D-Phe-D-Phe from its enantiomer L-Phe-L-Phe on Cu(110) is followed by STM and rationalized by using first principles and classical molecular dynamics techniques. We find that the stereoselective assembly of adsorbed di-phenylalanine enantiomers into molecule pairs and chains takes place through mutually induced conformational changes, thereby illustrating at the single-molecule level the more than half a century old prediction of Pauling.

The di-phenylalanine dipeptide contains two chiral carbon centers linked through a central amide bond (Figure 1a). It constitutes a key motif in molecular recognition for many biological processes,[9–11] and, as recently demonstrated by Reches and Gazit, it contains all the molecular information needed to mediate the self-assembly of peptide nanotubes.[10]

After co-depositing L-Phe-L-Phe and D-Phe-D-Phe on the Cu(110) surface (Figure 1b) at room temperature and low coverage, STM imaging reveals both single-molecule adsorption and supramolecular organization in the form of homochiral chains (Figure 1c). Each single molecule is characterized by two bright protrusions corresponding to the electron-rich phenyl rings and by a central dimmer part associated with the peptide backbone (Figure 1d).

The main axis through the center of the two phenyl rings of single L-Phe-L-Phe molecules is rotated 34° clockwise from the $[1\bar{1}0]$ substrate direction, while for D-Phe-D-Phe it is rotated 34° counterclockwise (Figure 1d). The identification of the rotation angle is done on enantiopure-covered surfaces, where just one rotation is observed. The two enantiomers are mirror images with respect to the plane perpendicular to the surface through the $[1\bar{1}0]$ axis and cannot be superimposed onto each other by rotation or translation. The *molecular chirality* of the adsorbed molecules evident in the STM topographies[12–18] (Figure 1d) indicates that their stereogenic centers are involved in the molecule–surface interaction.[19] The system shows also *supramolecular chirality*, which results from the stereoselective self-assembly of two or more dipeptides in homochiral chains (Figure 1c). The main molecular axis of the single molecules is rotated by a further

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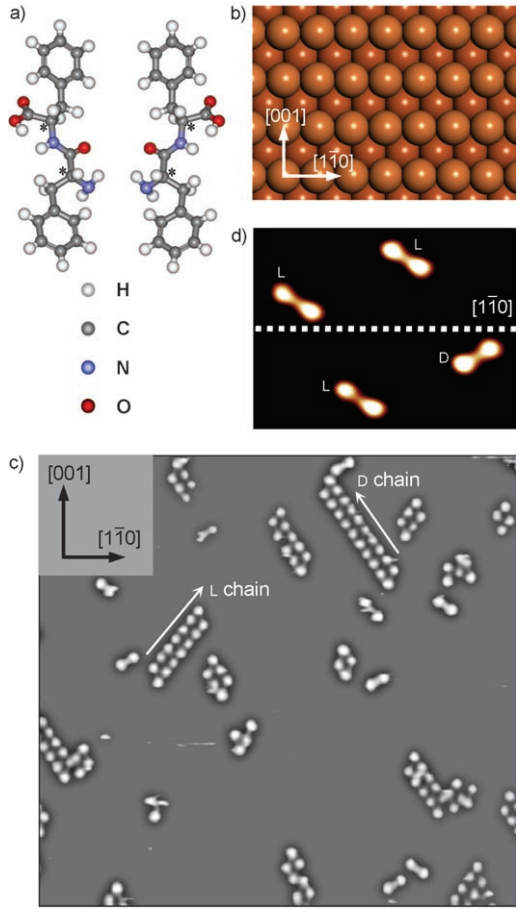

Figure 1. a) Schematic drawing of the L-Phe-L-Phe (right) and D-Phe-D-Phe (left) molecules; the asterisks show the stereocenters. b) Ball model of the Cu(110) surface. c) STM image (36×34 nm) of co-adsorbed L-Phe-L-Phe and D-Phe-D-Phe on Cu(110) at room temperature. The arrows indicate the growth direction of the homochiral chains. d) STM image (8.3×6.4 nm) of individual di-phenylalanine molecules showing that the two enantiomers are mutual mirror reflections with respect to the plane perpendicular to the surface through the [1$\bar{1}$0] axis. The color scales are adapted in each image to enhance the molecular contrast.

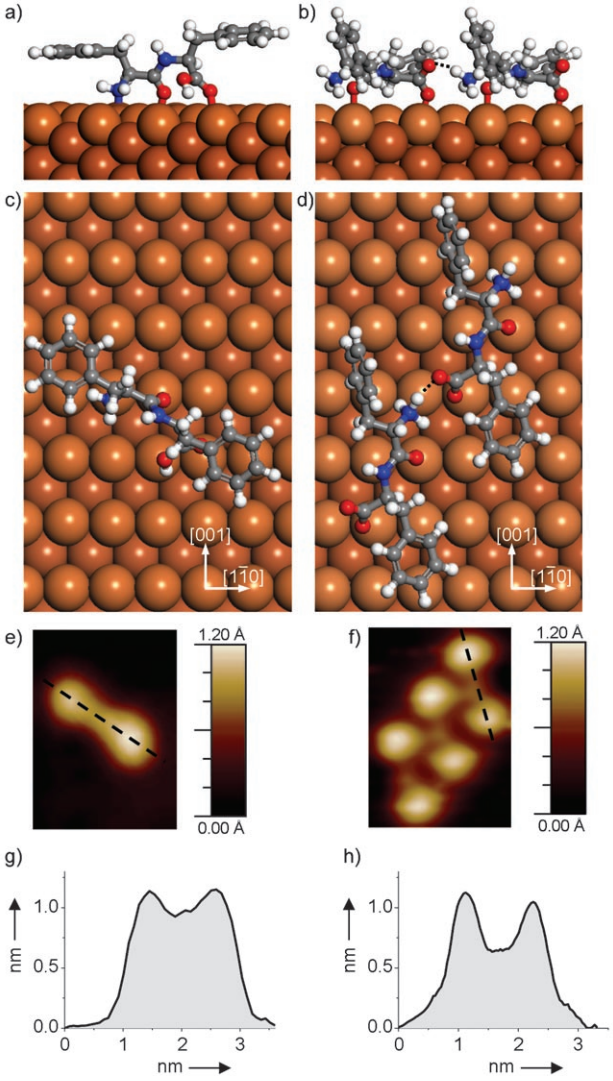

Figure 2. a), c) Minimum-energy structures of an isolated L-Phe-L-Phe dipeptide obtained in DFT simulations. The carboxylic and amino groups are located on the same side with respect to the molecular main axis (conformer C). b), d) Adsorption geometry of L-Phe-L-Phe dipeptides organized in homochiral chains. The carboxylic and amino groups are located on opposite sides with respect to the molecular axis (conformer S). e), f) Representative high-resolution STM images of an isolated L-Phe-L-Phe molecule and of L-Phe-L-Phe molecules in a chain, respectively. g), h) Corresponding height line scans through the main molecular axes (dashed lines in (e) and (f)). Note that the difference in height between the phenyl rings in the DFT equilibrium structure appears to be averaged out at room temperature, and is not visible in the STM images.

40° upon assembly, and is thus oriented at −74° from the [1$\bar{1}$0] direction for the L-Phe-L-Phe chains and at 74° for the D-Phe-D-Phe chains. Stable heterochiral chains are never observed.

Further insight into the molecular configurations and binding modes is obtained by first principles (FP) and classical molecular dynamics (MD) modeling. Classical MD simulations at room temperature in the gas phase indicate that the L-Phe-L-Phe molecular conformation with the carboxylic and the amino groups located on the same side with respect to the main molecular axis (conformer C) is more stable than the other possible conformation, in which the two functional groups point in opposite directions (conformer S).

In a density functional theory (DFT) simulation, the isolated dipeptide adsorbed on the Cu(110) surface in the C conformation binds to the surface through the nitrogen atom of the amino group, one oxygen atom of the carboxyl group, and the oxygen atom of the carbonyl group (Figure 2a,c). This three-point binding mode is frequently observed in analogous systems.[20,21] In the optimized molecular geometry the angle between the molecular axis and the [1$\bar{1}$0] direction is −30°, in good agreement with the measured value (Figure 2e) and the calculated molecule–surface interaction energy amounts to about 1.6 eV. By contrast, calculations on the adsorbed isolated molecule in the S conformation predict a 0.5 eV lower interaction energy at an orientation of about −14° (Figure S1 in the Supporting Information), which is never observed in the STM images.

While our results indicate that the isolated molecule adsorbs in the C conformation, we note that only the S adsorption geometry with the amino and carboxyl groups

pointing to opposite sides is compatible with the head-to-tail bonding necessary to form the observed supramolecular chains. We thus performed DFT simulations in which periodically repeated L-Phe-L-Phe S conformers are adsorbed at a distance compatible with the chain orientation and spacing observed in the STM images. In the minimum-energy structure the molecular axis is tilted by about −74° with respect to the [1$\bar{1}$0] direction (Figure 2d), matching the experimental value (Figure 2f). The main intermolecular interaction is a strong hydrogen bond between the carboxyl group of one molecule and the amino group of the adjacent one. In addition, spontaneous proton transfer between the two groups occurs during the simulations, further strengthening the bond and yielding a chain of zwitterionic molecules (Figure 2b,d and video V1 in the Supporting Information). Consistently, the X-ray photoemission N 1s spectrum for an adsorbed di-phenylalanine monolayer shows a large peak at 401.2 eV, associated with the charged ammonium group (NH_3^+) and a second peak around 400.3 eV, assigned to the amide (NH) and the neutral amino (NH_2) groups. Furthermore, the O 1s peak at 531.9 eV and the C 1s peak at around 288.6 eV are consistent with an amide carbonyl group and a deprotonated carboxyl group.[19]

A detailed analysis of the structural models also reveals that the rotation of the molecular axis observed by STM after assembly is only the most evident consequence of a more complex conformational rearrangement. Namely, to form supramolecular chains the monomers need to switch from the C to the S conformation while the oxygen atoms of the adsorbed functional groups change their relative positions along the [1$\bar{1}$0] direction (compare Figure 2c,d). At the same time, the process involves a rotation of the inherently planar peptide backbone, so that the initially upright NH group (Figure 2a) flattens down towards the substrate. A direct experimental evidence of this conformational change is given by high-resolution STM images which show that the peptide backbone of isolated molecules appears almost twice as high (height = 96 ± 8 pm, Figure 2e,g) as the backbone of molecules arranged in chains (height = 55 ± 6 pm, Figure 2f,h).

The switch between molecular conformations described above implies that the process of chain formation does not result from a mere juxtaposition of molecules, as expected within the static "lock-and-key"[7] picture of the three-point model.[15] To attain a more detailed dynamic description of the chiral-recognition phenomenon we have tracked with STM the conformation and orientation changes that two D-Phe-D-Phe molecules undergo while they approach and attempt to assemble (video V2 in the Supporting Information). Eight snapshots corresponding to the observed formation path of a homochiral pair are displayed in Figure 3.1a–h. A quantitative representation of the relative molecular motion is obtained by plotting the distance between the uppermost phenyl rings of each molecule as a function of the elapsed time (graph in Figure 3.1). The STM video V2 (Supporting Information) starts with two isolated molecules of D chirality (Figure 3.1a). After meeting, they experience a first change in the adsorption geometry, forming an initial pair (Figure 3.1b). This complex is not in the optimal configuration, and undergoes several further rearrangements (Figure 3.1b–g)

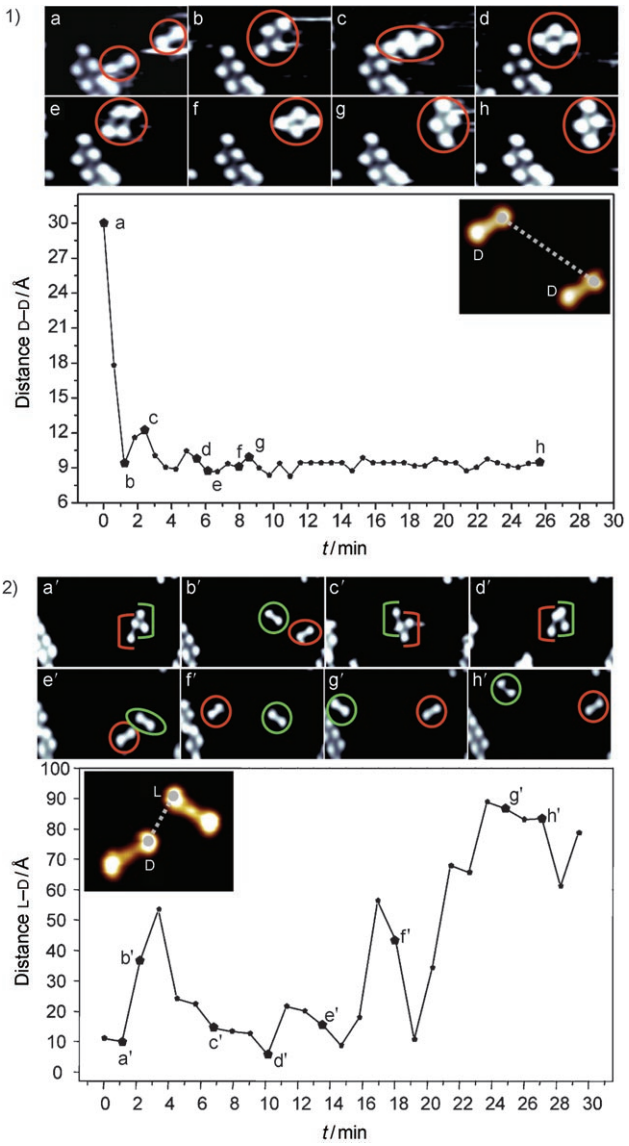

Figure 3. 1) Selected frames (a–h) of the STM video V2 (see the Supporting Information) showing the formation path of a homochiral pair of D molecules (in red circles). The final pair stays stable without further breaking or reorganization (from frame g to h). 2) Selected frames (a′–h′) of the STM video V3 (see the Supporting Information) showing the evolution of the interacting heterochiral L–D pair (L in green circles, D in red circles). The full color scale corresponds to 1.33 Å. The corresponding graphs illustrate the relative molecular motion of the full videos.

until the final state corresponding to the stable pair is reached (Figure 3.1g,h). The molecular arrangement within this structure is identical to that observed in longer homochiral chains (Figure 1c).

A careful analysis of the STM videos reveals that the central core becomes dimmer whenever the molecules adopt the + 74° orientation. This result suggests that the rotation of the main molecular axis is always associated with a C-to-S conformational switch of the adsorbed monomers, as previously described. A further analysis of the full video sequence confirms that these conformational changes are induced by the mutual intermolecular interaction since they

occur only when two molecules are close to each other. The occurrence of *metastable* pairs of molecules in the S conformation (Figure 3.1 b, e) implies that the C-to-S conformational change is necessary, but not sufficient for the formation of a stable pair. Our DFT simulations suggest that this happens only if the mutual position of the amino and carboxylic groups in adjacent molecules allows the creation of a strong, polar hydrogen bond (compare Figure 2 d and video V1 in the Supporting Information).

To investigate the stereoselectivity of the recognition process, the interaction between two molecules with opposite chirality (L-Phe-L-Phe and D-Phe-D-Phe) has been recorded in video V3 in the Supporting Information and is shown in Figure 3.2. The distance between the two enantiomers is, on average, much larger than in the case of homochiral molecules and undergoes more pronounced oscillations (graph in Figure 3.2). Moreover, no stable pair structure is reached, but only *metastable* heterochiral pairs form temporarily (Figure 3.2 a′, c′, d′). In these structures the two molecules assume the $-74°$ and $+74°$ orientations (for L-Phe-L-Phe and D-Phe-D-Phe, respectively) and the dimmer appearance of the peptide backbone indicates once again a C-to-S conformational switch as the result of their mutual intermolecular interactions. However, the different chirality of the two stereoisomers prevents their functional groups from reaching the optimal position for forming a stable electrostatic bond (Figure S2 in the Supporting Information).

In conclusion, our results show at the single-molecule level that chiral recognition is a dynamic process that involves mutually induced conformational adjustments. A general theory of biological specificity based on the conformational flexibility of interacting molecules for the expression of molecular complementarity was formulated by Pauling in the 1930s and 1940s.[4] Koshland later coined the term "induced fit"[5] to describe the dynamic recognition process in enzymatic reactions. Chiral recognition, on the other hand, is usually discussed in the context of the static three-point model.[7,22] Only recently attempts were made to implement a dynamic picture of enantioselective molecular interactions to account for possible mutual conformational adjustments.[7,23] Our experiments constitute the first single-molecule confirmation of this dynamic recognition picture, which follows the steps proposed by Booth, Wahnon, and Wainer:[7] 1) formation of the selectand–selector complex (tethering, Figure 3.1 b, 3.2 a′); 2) positioning of the selectand–selector complex to optimize interactions (conformational adjustments, Figure 3.1 c–f, 3.2 c′, d′); 3) formation of secondary interactions and expression of the molecular fit (chiral recognition, Figure 3.1 h).

Experimental Section

The samples were prepared and characterized in an ultrahigh vacuum (UHV) system (base pressure $\approx 2\times10^{-10}$ mbar) equipped with a variable-temperature UHV-STM. The Cu(110) surface was cleaned by repeated cycles of Ar^+ sputtering and subsequent annealing. Preparation and characterization of the sample were performed at room temperature. Commercially available di-phenylalanine (Bachem AG) in powder form was deposited by organic molecular beam epitaxy (OMBE) from a Knudsen cell constantly held at 400 K during evaporation. STM measurements were performed in the constant-current mode. Typical tunneling current and sample bias voltages were $I = 0.9$ nA and $V = -0.9$ V for molecular imaging and $I = 0.7$ nA and $V = -0.3$ V for measuring the Cu surface with atomic resolution. The STM images were processed by average filtering to eliminate high frequency noise.

DFT-based first principles molecular dynamics simulations[24] were performed with the parallel code LAUTREC[25] by using the PW91 GGA exchange and correlation functional and norm-conserving pseudopotentials. The calculations were performed by using periodic boundary conditions and a plane-wave basis set for the valence electrons up to a kinetic energy cut-off of 70 Ry. The Cu(110) surface was modeled within the scheme of reference [26] by a periodically repeated four-layer slab, whose two bottom layers were kept fixed during the simulations. A time step of 5 au was used throughout the FPMD simulations, which cover a total of about 15 ps (in particular, video V1 in the Supporting Information covers a simulated time of 4.2 ps). Classical MD simulations of dipeptide molecules and pairs in the gas phase were performed by using the AMBER 8 suite of programs.[27]

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