Complementary base-pair-facilitated electron tunneling for electrically pinpointing complementary nucleobases

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Molecular tips in scanning tunneling microscopy can directly detect intermolecular electron tunneling between sample and tip molecules and reveal the tunneling facilitation through chemical interactions that provide overlap of respective electronic wave functions, that is, hydrogen-bond, metal-coordination-bond, and charge-transfer interactions. Nucleobase molecular tips were prepared by chemical modification of underlying metal tips with thiol derivatives of adenine, guanine, cytosine, and uracil and the outmost single nucleobase adsorbate probes intermolecular electron tunneling to or from a sample nucleobase molecule. We found that the electron tunneling between a sample nucleobase and its complementary nucleobase molecular tip was much facilitated compared with its noncomplementary counterpart. The complementary nucleobase tip was thereby capable of electrically pinpointing each nucleobase. Chemically selective imaging using molecular tips may be coined “intermolecular tunneling microscopy” as its principle goes and is of general significance for novel molecular imaging of chemical identities at the membrane and solid surfaces.

Results and Discussion

The nucleobase molecular tips were prepared by chemical modification of underlying metal tips with thiol derivatives of adenine, guanine, cytosine, and uracil (see Materials and Methods) (for their chemical structures, see Fig. 1b; for their preparations, see Supporting Text, which is published as supporting information on the PNAS web site), and the outmost single nucleobase adsorbate probes intermolecular electron tunneling to or from a sample nucleobase molecule. Importantly, the tunneling current increases when sample and tip molecules form a chemical interaction that provides overlap of electronic wave functions between them. The current increase is ascribed to the facilitated electron tunneling through the overlapped electronic wave functions. Electron tunneling observed here occurs without any net chemical oxidation/reduction of the involved bases. Fig. 2 a–c shows typical STM images of guanine SAMs observed with complementary cytosine tips, noncomplementary adenine, and unmodified tips, respectively (see Materials and Methods). Cross-sectional profiles of the images are shown in Fig. 2d, which represents the extent of electron tunneling between the tip and nucleobase. The complementary cytosine tip exhibited the most facilitated electron tunneling and therefore the brightest guanine images among the three tips. Similarly, for adenine, cytosine, and uracil, their complementary nucleobase tips gave the brightest images of their counterparts, the results of which are shown in Fig. 2e together with those using irrelevant tips for validation. We have differentiated the complementary nucleobases from the noncomplementary ones by the tip heights for the sample nucleobases in absolute terms. The height is a quantitative measure of the current, because the tunneling current $I$ is related to the tip height $h$ by the relation as $I \propto \exp(-2kh)$, where $k = h^{-1}(2\pi e)^{1/2}$ and $e$ is the work function of the sample (24). The tip height $h$ is usually recorded rather than the current $I$ for the instrumental convenience, keeping the current $I$ constant. For example, with the cytisine tips, the heights of the tips were found to be $197 \pm 23$ pm for the complementary guanines (Fig. 2e, black columns) and $102 \pm 5$ pm, $98 \pm 9$ pm, and $99 \pm 7$ pm for the noncomplementary adenines, uracils, and cytosines, respectively (Fig. 2e, yellow columns). These heights quantitatively represent the tunneling currents flowing within the base pairs. On the contrary, with unmodified tips, or with gold tips modified with 2-mercaptobenzimidazole (MB) and thiophenol (TP) (chemical structures, Fig. 1b), which have a pyrimidine- and pyridine-like structure, respectively, but no particular functional groups for hydrogen-bond formation with nucleobases, selective facilitation of electron tunneling was not detected for any nucleobases (Fig. 2e, yellow columns), as shown in an STM image.

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Abbreviations: STM, scanning tunneling microscopy; SAM, self-assembled monolayer; PNA, peptide nucleic acid; MB, 2-mercaptobenzimidazole; TP, thiophenol.

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nary bases with the nucleobase tips was not caused by the result indicates that the chemical differentiation of complemen-
panying the complementary nucleobases. (b) The chemical structures for thiol derivatives of adenine (1), guanine (2), cytosine (3), uracil (4), MB (5), and TP (6) are shown.

(Fig. 5, which is published as supporting information on the PNAS web site). Taken together, it is concluded that the complementary combinations of the tip and sample base pairs facilitated the largest electron tunneling compared with the noncomplementary combinations, and particular nucleobases were thus discriminated from other nucleobases in STM images by using the complementary nucleobase tips.

In the mixed nucleobase SAMs (see Materials and Methods), nucleobase tips were capable of pinpointing complementary nucleobase images in the presence of other nucleobases. Fig. 3a shows a typical STM image of an adenine/guanine-mixed SAM observed with a cytosine tip. As shown in Figs. 3a Inset and 2f, the cytosine tip gave a high- and low-contrast image for the complementary guanine and noncomplementary adenine, respectively. The number of the high-contrast guanine images increased in proportion to the molar ratio of [guanine] to [guanine + adenine] in the sample-mixed solution for the SAM (compare Figs. 3a–d), the results of which are shown in Fig. 3e. Similarly, uracil tips were capable of selectively pinpointing the complementary adenine images in the presence of noncomplementary guanines in the SAM (Fig. 2g), and the number of the high-contrast adenine images also increased in proportion to molar ratio of [adenine] to [guanine + adenine] in the sample solutions as shown in Fig. 3e. These results indicate that in the mixed nucleobase SAMs the large electron tunneling through the complementary base-pairing allows us to pinpoint a particular nucleobase in the presence of other nucleobases. On the other hand, with unmodified tips, the four bases were observed as identical images even in the mixed SAM (Fig. 6, which is published as supporting information on the PNAS web site). This result indicates that the chemical differentiation of complementary bases with the nucleobase tips was not caused by the difference in heights of the four bases. Also, MB and TP tips did not show selective large facilitation of electron tunneling (Fig. 2e, yellow column), confirming that the observed difference is caused solely by the hydrogen bonds of complementary base pairs between bases on a tip and substrate.

We have earlier reported on the use of hydrogen bond-based molecular tips for selective STM imaging of hydrogen-bond acceptor or donor molecules and functional groups and on the use of other chemical interaction-based molecular tips, metal coordination bond-based molecular tips for selective STM imaging of metal species in metalloporphyrins (20), and charge-transfer interaction-based molecular tips for that of electron-rich porphyrin rings (23). Upon tailor-making the molecular tips with differing extents of hydrogen-bond acidity or basicity, we have succeeded in selectively pinpointing particular functional groups in sample molecules, including hydroxyl, carboxy, carboxylate, ether oxygens and their orientations, and a free-base porphyrin center (16–22). We herein added another example of hydrogen-bond-facilitated electron tunneling, i.e., complementary base-pair-facilitated electron tunneling (Fig. 1a). For example, before the cytosine tip was placed on a guanine base, the guanine base did not possess any greater electron density compared with other bases, but instead a greater electron density was induced along the hydrogen-bonding plane upon placing the cytosine tip on the guanine base. This induced increase in electron density translates into a greater electronic coupling between the two bases and thus an increase in the tunneling current between them. As a result, nucleobase tips gave large extents of electron tunneling currents only for its complementary bases. The direction of electron flow between bases on a tip and a substrate did not affect the extent of electron tunneling through the same combination of the material (Fig. 2e): for instance, cytosine and guanine tips gave the same extent of electron tunneling to their counterpart complementary bases. Therefore, the extent of overlap of electron wave functions of the base pairs solely plays the requisite role.

The formations of the specific hydrogen bonds through complementary base pairs require that coplanar configurations of the bases be achieved on the tip and surface. Although the plane of bases may likely be oriented randomly in mixed monolayers (Fig. 6) and orderly in pure monolayers (Fig. 2e), the nucleobase tips gave the selective large facilitation of electron tunneling for its complementary bases in both the pure and mixed monolayers. (Figs. 2f and 3a–d), indicating that the base–base coplanar orientation was in fact achieved, and thus the specific hydrogen bonds between the complementary base pairs were formed. The base–base coplanarity probably is attained by the rotation of a carbon–sulfur bond in the thio base on a tip, which is well known even in the close-packed structure of thiolate SAMs (25). Therefore, the complementary bases in the SAM were exclusively differentiated. Although other hydrogen bonds, such as the Hoogsteen and G:U Wobble base pairs, could be formed between bases on the tip and sample substrate, they were found to give only a small tunneling current similar to those of noncomplementary base pairs (Fig. 2e–g), and did not thereby interfere with the chemical differentiation based on complementary base pairs. A branch of supramolecular chemistry to use the paradigm of individual nucleobases exists. The thermodynamic stabilities and related characteristics of these nucleobases have been extensively studied, and many researchers have reported Watson-Crick-type specific interactions between mononu-
meric nucleobases (26). These reports substantiate the profound specificity observed in the present study, including strong preference for Watson-Crick binding and rejection of Hoogsteen and Wobble base-pairing.

Hole and electron transfer in a DNA strand occur via two pathways, along the DNA strand (intrastrand pathway) and through the base pairs (interstrand pathway). In the interstrand pathway, electron transfer occurs preferentially through the
hydrogen bonds of complementary base pairs (14). Kelly and Barton (14) constructed the DNA double strands that were linked to a donor and acceptor located on the different strand and found that larger electron transfer occurred through the interstrand connection of the complementary double strands relative to the other double strands containing mispairs. On the other hand, in the intrastrand pathway, electron hopping is known to occur through a pi-pi stacking interaction of base pairs (8–13). Of the two pathways, only the interstrand pathway is to be compared with the present results.

The molecular tips directly detected intermolecular electron tunneling between sample and tip molecules and revealed the tunneling facilitation through chemical interactions that provide overlap of respective electronic wave functions, that is, hydrogen-bond interactions (16–19, 21, 22), metal-coordination-bond interactions (20), and charge-transfer interactions (23). We have extensively studied chemical selectivity toward various functional groups based on hydrogen-bond interactions. The chemical selectivity can be tailored by controlling the extent of the hydrogen bond acidity or basicity of the molecular tips (22).
Larger facilitation of electron tunneling was observed at ether oxygens in a favorable orientation than those in unfavorable orientations, allowing us to discriminate between these differently oriented functional groups. These results substantiate the facilitated electron tunneling through hydrogen-bond interactions, which resulted in pinpointing complementary nucleobases in the present study. Hydrogen-bond-mediated electron-transfer process has been of great interest and studied by several groups using photo-induced electron transfer with acceptor/donor markers (15) because of its fundamental importance in chemical...
reaction processes and crucial roles in biological electron-transfer processes.

An example of the detection of particular nucleobases was demonstrated here with the method in an 18-mer strand of a peptide nucleic acid (PNA), an analogue of DNA (27). A typical STM image with an unmodified tip of a PNA strand is shown in Fig. 4a, showing that bases in the strand were observed as rows of bright spots and the components of the strand, guanines and thymines, were not discriminated. On the contrary, cystine tips pinpointed the complementary guanines among the noncomplementary thymines in the strands (Figs. 4 b–d and Insets). The extent of electron tunneling along the strands shows that a single- and double-base substitution in the strands was distinguished with the cystine tip (Fig. 4e).

In conclusion, we found that hydrogen-bond-mediated electron tunneling occurs with the complementarity between the tip nucleobase and sample nucleobase. The electron tunneling increase is capable of electrically pinpointing each nucleobase. STM observations of nucleobases (28–30) and DNA oligomers (31–33) had been reported, but those studies failed to identify the chemical species of nucleobases because of the poor chemical selectivity of the STM images. The present approach made it possible to pinpoint particular nucleobases. Enhancement of electron tunneling occurred at specific functional groups and chemical species on the basis of hydrogen-bond, metal-coordination-bond, and charge-transfer interactions, and, as a result, allowed us to identify the location of the specific chemical species and functional groups. This technique may be coined “intermolecular tunneling microscopy” as its principle goes and is of general significance for novel molecular imaging of chemical identities at the membrane and solid surfaces.

Materials and Methods
Preparation of SAMs of Neat/Mixed Nucleobases. For preparing the sample SAMs of nucleobases (i.e., thiol derivatives of adenine, guanine, cytosine, and uracil), gold substrates were soaked into 10 mM sample ethanolic solutions (HPLC-grade ethanol, Wako Pure Chemical, Osaka) for 30 min, 45 min, or 1 h. After being taken out of the solution, the gold substrates were rinsed with ethanol to remove excess sample nucleobases physisorbed on the SAMs and dried in vacuum. The sample adenine/guanine-mixed SAMs were prepared from the aqueous 10 mM mixed solutions (sample negative) and a tunneling current of 1,200 pA. This work was supported by the Core Research for Evolutional Science and Technology of the Japan Science and Technology Agency and the Japan Society for the Promotion of Science.

Preparation of Nucleobase-Modified Tip (Nucleobase Tip). STM metal tips were prepared from a gold wire (0.25 mm diameter; Nilaco, Tokyo) by electrochemical etching in 3 M NaCl with ac 10 V and then washed in an ultrasonic bath or cleaned in piranha solution. For constructing nucleobase molecular tips, the gold tips were cleaned in piranha solution, and then immersed for 3 h in 10 mM ethanolic solution of thiol derivatives of nucleobases. The tips were then rinsed with ethanol and dried in a stream of argon or nitrogen.

STM Measurements of SAMs of Neat/Mixed Nucleobases. STM measurements were carried out on a Nanoscope E (Digital Instruments, Santa Barbara, CA) at room temperature in a constant current mode. A drop (5 μl) of 1,2,4-trichlorobenzene deposited on sample thio-base SAMs on Au(111) before the measurements. STM measurements were performed at the solution/gold interface under ambient condition at a bias voltage of ~500 mV (sample negative), and a tunneling current of 1,200 pA. It was confirmed that no polarity dependence was observed by applying the reversed potential. In the STM observation, ~45% of >30 nucleobase tips exhibited the facilitated electron tunneling in each combination of nucleobases on tip and substrate, and the others exhibited the same STM images as those observed with unmodified gold tips. The lack of the facilitation is most probably caused by the absence of a nucleobase molecule at the very apex of the underlying gold tip at the atomic level.

Preparation and STM Measurements of Single-Stranded PNA Oligomers. Three kinds of single-stranded, 18-mer PNA purified by HPLC, (i) H2N-TTTTTTTTTTTTTTTTTTCONH2 (containing 1 guanine and 17 thymines), (ii) H2N-TTTTTTTGTTTTTTTTTTTTCONH2 (containing 2 guanines and 16 thymines), and (iii) H2N-TTTTTTTTTTTTTTTTTTTTTCONH2 (containing 18 thymines), were purchased (Fasmac, Kanagawa, Japan) and used for STM measurements without further purification. Sample substrates were prepared by depositing a drop (5 μl) of a 1,2,4-trichlorobenzene solution containing PNAS (concentration, 0.5–1.0 mM) onto a Au(111). STM measurements were performed at the solution/gold interface by immersion, under ambient condition at a bias voltage of ~500 mV (sample negative) and a tunneling current of 1,200 pA.

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