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Electrical Behavior of Molecular Junctions Incorporating α-Helical Peptide

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We synthesized an α-helical peptide containing two terminal thiol groups and demonstrated the method of preparation of a self-assembled monolayer (SAM) on gold with uniform orientation of the molecules on the surface. The monolayers were employed as model systems for the investigations of mediated electron transfer. The measurements of electron transfer efficiency through the peptide were performed using scanning tunneling spectroscopy (STS). The molecules were trapped between the gold tip and the substrate using a Au−S linkage. The electron transfer behavior of the peptide was examined as a function of the tip−substrate distance at fixed bias voltage and as a function of bias voltage at a fixed distance between the tip and the substrate. The data obtained from these experiments indicated that the electron transfer through α-helical peptide is very efficient, and its conductivity is comparable to those observed for dodecanedithiol. There is also a directional dependence of electron transmission through the peptide, which is connected with the electric field generated by the molecular dipole of the helix.

The process of efficient and controllable electron transport is one of the primary regulation mechanisms in biology.[1] A number of factors limit and regulate the rate and efficiency of electron transfer in the complex biological system. One of the current issues in understanding this process is the importance of the chemical structure of peptides, which act as mediating bridges. In the past few years, numerous studies of long-range electron transfer through synthetic peptides have been reported.[2−11] Results of electron transfer studies through polypeptides indicate that the length and chemical nature of the peptide influence the rate of electron transport, but the detailed mechanism of the electron transfer through peptides is still a subject of discussion.[11] For example, Isied and co-workers reported that long-range electron transfer across peptide bridges may occur according to a superexchange or hopping mechanism, with the first one dominating for short bridges while the second one contributes to the overall process for longer peptides.[3] Apart from the mechanism, the rate of electron transfer through peptides usually decreases with increasing length of the bridge, and the ability of the peptide to promote the electron transfer can be described by the decay constant (β). The magnitude of β can be considered a measure of the electron transfer efficiency for a particular system. In the case of peptide-mediated electron transfer, the decay constant depends on the secondary structure of the bridge. For example, the values of β reported in the literature for α-helical systems vary from 0.66 to 1.17 $Å^{-1}$.[9−13] These results suggest that the helical peptides can be considered efficient mediators for electron transfer. Another interesting feature of electron transfer through peptides is its directional dependence, which means that the electron transfer may be strongly affected by the molecular dipole of the peptide. Such effect was observed mainly for helical peptides; however, recently Tao and co-workers reported the asymmetric current−voltage characteristics for very short peptides trapped within the junctions.[8] This suggests that the carefully designed peptide molecules may be considered potential rectifiers.[14] Here, we report the electron transfer behavior of an α-helical peptide trapped within the molecular junction, and the conductivity of the peptide was compared with dodecanedithiol. The junctions were formed using scanning tunneling microscope (STM). Several groups demonstrated that the application of probe microscopy (e.g., current-sensing atomic force microscopy or scanning tunneling microscopy) allows the measurement of the conductivity of single molecules or nanostructures.[8,15−19]

To obtain reproducible measurements of peptide conductivity, we designed the molecule with two terminal thiol groups, which can form covalent bonds with the gold substrate and the STM tip. We also developed the procedure of formation of the self-assembled monolayer, which leads to uniform orientation of the peptide molecules on the gold surface. We synthesized the helical peptide consisting of fourteen amino acid residues and a cysteamine linker. The sequence of the amino acids in the peptide chain was chosen as helix-favoring. The details of the amino acid sequence are shown in Scheme 1. The peptide contained the cysteine residue at the N-terminus of the peptide chain, and the sulfur atom of cysteine was protected with an acetamidomethyl group (Acm).[20] The synthesis of the peptide was performed manually by solid-phase techniques starting from cysteamine 4-methoxytrityl resin. Successively, *N*-fluorenylmethoxycarbonyl (Fmoc)-protected amino acids (with exception of cysteine, which was protected with *tert*-butoxycarbonyl) were coupled using *N*-hydroxybenzotriazole and diisopropylcarbodiimide as coupling reagents. The cleavage of the final product was performed by 95% trifluoroacetic acid in water containing 2.5% triisopropylsilane. Then, the peptide was purified by preparative RP-HPLC and analyzed by mass spectrometry [M + 3H]$^{3+}_{found}$ m/z = 488.2 ([M + 3H]$^{3+}_{calcd}$ m/z = 488). The

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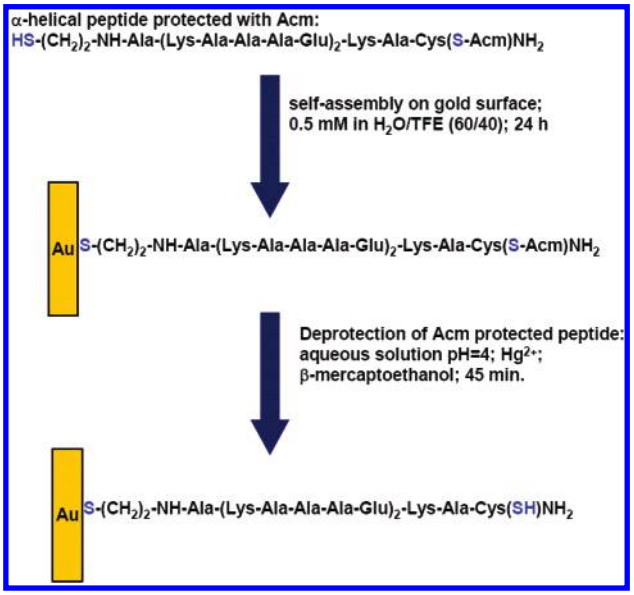
SCHEME 1: Preparation of Peptide SAM with Uniform Orientation of the Molecules on the Surface



helicity of the peptide was confirmed by FTIR measurement in KBr pellets. We found that the amide I and amide II bands appeared at 1660 and 1545 cm^{-1}, respectively, which corresponds to α-helical structure of the peptide. Monolayers on the gold surface were prepared according to Scheme 1. Freshly prepared Au(111) substrates were soaked in water/trifluoroethanol mixture containing 0.5 mM of peptide for 24 h. We used water/TFE mixture as a solvent, since it is well-known that such mixtures have the helix-enhancing propensity.[21] Thus, the secondary structure of the peptide is preserved during the self-assembly process. This was confirmed by FTIR-RAS measurements. We found that the amide I and amide II bands were located at 1659 and 1546 cm^{-1}, respectively, which corresponds to α-helical conformation of the peptide.[22,23] Using the absorbance intensities of the amide bands, we also calculated the tilt angle of the helix axis from the surface normal.[4,9,10] It was found that the tilt angle of the helix from the surface normal is 45 ± 2°. It should be noted that the molecules were adsorbed with uniform orientation, since the thiol group of cysteine was protected with Acm. After the deposition of monolayer, the modified substrates were subjected to the deprotection procedure in order to remove the Acm group from cysteine.[20] Finally, the samples with free external SH groups were used to form junctions. We also prepared the self-assembled monolayers of dodecanedithiol on gold, and this system was used as a reference in our experiments. Self-assembly was carried out from a 1 mM solution in ethanol, and the incubation time was 18 h. After deposition, the modified substrates were rinsed with ethanol and water and then used to form junctions.

The junctions were created by the method similar to those described by Haiss.[16,17] Briefly, the junctions were formed by placing the gold STM tip at a given location on the surface at a distance, which was determined by the current setting. Usually, the currents were between 0.05 and 0.5 nA, and the initial resistance of the tip−sample gap was in the range 5×10^8−5×10^9 Ω. We found that these settings were sufficient to bring the tip into contact with the molecules forming the monolayer, since the STM images taken at these conditions were very noisy and indicated that the tip penetrates the monolayer. On the other hand, the gap resistance applied to form the junction was always high enough to avoid crushing the tip. Our observations based on STM imaging of the samples indicated that the tip crushed

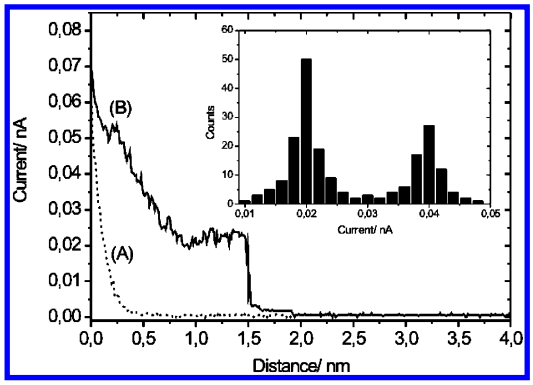


Figure 1. Current−distance curves for bare (A) and peptide-modified (B) gold substrate recorded in single experiments at −0.3 V bias. The inset presents a histogram for the current values obtained for Au−peptide−Au junctions at the bias of −0.3 V.

at the initial gap resistance lower than 2×10^8 Ω. When the proper distance between the tip and the sample was achieved, the molecules were expected to form chemical bonds through Au−S linkage with the tip. Further, the STM tip was lifted (at the rate of 4.96 nm/s) while keeping a constant $x-y$ position, and the current as a function of distance was recorded. To obtain reliable statistics, this procedure was repeated many times (at least 200) for each investigated value of the bias voltage. It should be noted that our method of measurement is obviously destructive for monolayer structure, since the initial distance between the tip and the substrate is smaller than the monolayer thickness. Thus, at the initial stage of the experiment, the molecule (or molecules) trapped within the junction is most probably forced to tilt, which is caused by vertical movement of the tip during the approach. Further, during the measurement, the molecule is lifted as long as the Au−S linkage breaks.

Figure 1 shows typical current−distance curves for bare (A) and peptide-modified (B) gold substrate recorded in single experiments at −0.3 V bias. Curve A represents a fast exponential decay of the current with distance, which is typical for tunneling between the tip and the bare gold substrate. Curve B recorded for the peptide-modified sample is significantly different. At the initial stage, the current decays with increasing distance between the tip and the substrate, and then, it is followed by a current plateau, which is attributed to conduction through a molecular bridge chemically bonded to the tip and the substrate.[15−17] As long as the molecule is bonded to the metallic contacts, the current remains nearly constant, but as soon as the chemical bond between the molecule and the tip or the substrate is broken, the current falls instantly, reflecting lower conductivity of the gap. The value of the current plateau corresponds to the conductivity of the molecule (or molecules) trapped within the junction.[15−17] The inset in Figure 1 shows a histogram of the current values constructed on the basis of the current−distance curves obtained for particular bias voltage. The apparent maxima are located at multiples of the fundamental value of the current. Thus, it can be concluded that consecutive peaks which can be seen on the histogram correspond to conductivity through one or two molecules trapped between the tip and the substrate.[16,17] Figure 2 shows analogous current−distance curves recorded for bare (A) and dodecanedithiol-modified (B) gold substrates at the bias voltage of 0.2 V. The interpretation of these curves is exactly the same as that given above.

The conductivity of the junctions was measured within the bias range from −0.4 V to +0.4 V. Figure 3 presents the dependence of the current as a function of the bias voltage for the junctions incorporating dodecanedithiol (triangles) and

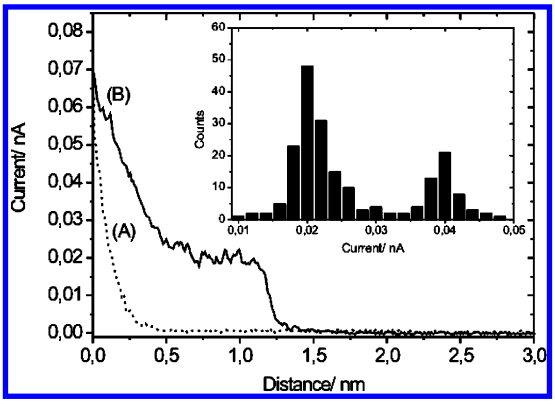


Figure 2. Current−distance curves for bare (A) and dodecanedithiol-modified (B) gold substrate recorded in single experiments at 0.2 V bias. The inset shows a histogram for the current values obtained for Au−dodecanedithiol−Au junctions at the bias of 0.2 V.

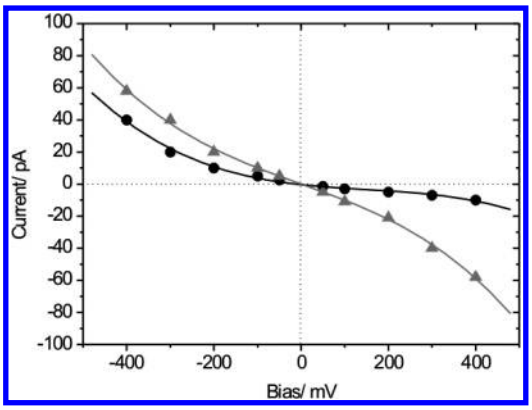


Figure 3. Current vs bias voltage curves for the junctions incorporating dodecanedithiol (triangles) and α-helical peptide (circles). The curves were constructed on basis of current−distance curves recorded at different bias voltages.

peptide (circles). The current−voltage curves were constructed using the current value corresponding to the first peak on the histogram at a given bias voltage. The characteristic obtained for dodecanedithiol is symmetric, and the resistance corresponding to a single molecule measured at low bias (−50 mV) is 10^{10} Ω. This curve was used as a reference in order to compare the results obtained for the peptide-bridged junction. The current−voltage characteristic for peptide is asymmetric, but the values of the current are not very different from those obtained for dodecanedithiol. This indicates that the conductivity of the peptide is comparable to that of the C_{12} alkyl chain, which is rather surprising, since the peptide molecules are significantly longer than dodecanedithiol. In the case of the peptide, the length of the molecules calculated as a distance between the terminal sulfur atoms is about 25.0 Å, while for dodecanedithiol, the length of the molecule is about 16.9 Å.[24] Assuming a superexchange model, such a difference should lead to at least several orders of magnitude higher resistance for the peptide. The value determined from the current−voltage curve for a single peptide at low bias voltage is about 2×10^{10} Ω, which is only two times higher than for dodecanedithiol. In our opinion, this result may support the theoretical model of electron transfer through peptides described by Petrov and May.[25] They postulated that the peptide-mediated electron transport obeys biexponential distance dependence, because it occurs according to the superexchange mechanism for short bridges, while for the long bridges, the electron transfer is governed by the hopping mechanism. In the latter case, the distance dependence of electron transfer is much weaker, and as a result, the process occurs very efficiently over relatively long distances. Biexponential distance dependence for electron transfer across peptides was also obtained experimentally by Isied and co-workers.[3] The relatively high conductivity of the helical peptide found from our experiments suggest that the distance dependence for such a system is rather weak, supporting the thesis that the hopping mechanism may significantly contribute to the overall electron transfer process. This is in agreement with the results of electron transfer measurements reported by Kimura and co-workers.[4] The authors investigated long-range electron transfer through helical peptides using electrochemical methods, and they found that the rate constants are higher than expected on the basis of superexchange model. Finally, they concluded that the process is governed by the hopping mechanism. In our recent work, we also observed relatively fast electron transfer for α-helical polyalanine bridges.[10]

As mentioned above, the current−voltage characteristic for the junction incorporating the peptide is asymmetric with lower currents recorded at positive bias voltage. Positive bias induces negative tunneling currents, which means that the electrons flow from the tip to the substrate. At negative bias, the electrons flow in the opposite direction, i.e., from the substrate to the tip. The asymmetry of the $I-V$ curve indicates that the electron transfer from the substrate to the tip is more efficient in comparison to the opposite direction. The currents recorded for negative bias are up to four times larger than those recorded for the positive bias. Such rectification behavior of the junction can be explained by considering that the α-helical peptide trapped within the junction has a large dipole moment oriented along the molecular axis of the helix. This dipole produces an effective positive charge at the N-terminus and an effective negative charge at the C-terminus of the peptide. As a result, the electron transfer through an α-helical peptide can be strongly influenced by the electric field generated by the dipole of the helix. Namely, the electrons should be more efficiently transferred along the molecular dipole of the helix in comparison to the opposite direction. In our experiment, the peptide molecules are uniformly oriented with the C-terminus located near the substrate and the N-terminus located near the tip. Thus, the larger currents recorded at negative bias correspond to electron transfer along the molecular dipole, i.e., from C-terminus to N-terminus. It should be noted that the observation of the rectification was possible only at the conditions where the peptide molecules were uniformly oriented on the gold surface. In the case of random arrangement of the molecules, the current−voltage curve was symmetric, and the spread of the current values measured for each bias was much broader.

It is rather obvious that the method of the conductivity measurement used in this work can result in distortion of the secondary structure of the helix. The peptide molecules can be squeezed or stretched by the tip vertical movement. To verify how this distortion affects the ratio of asymmetry, we investigated the electrical properties of the junction as a function of bias voltage at fixed distance between the tip and the substrate; thus, the vertical tip movement was eliminated in this case. The tip was located at the distance, which was controlled by the tip−substrate gap resistance. Its value was set to 10^{10} Ω for the dodecanedithiol monolayer and 2×10^{10} Ω for the peptide monolayer at a bias voltage of −0.6 V. At these conditions, the tip is expected to be located outside the monolayer. After positioning the tip, current−voltage characteristics were recorded in a single scan from −0.6 to +0.6 V. Figure 4 shows the representative examples of the $I-V$ curves obtained for dodecanedithiol (A) and peptide (B). As expected, the shape of curve

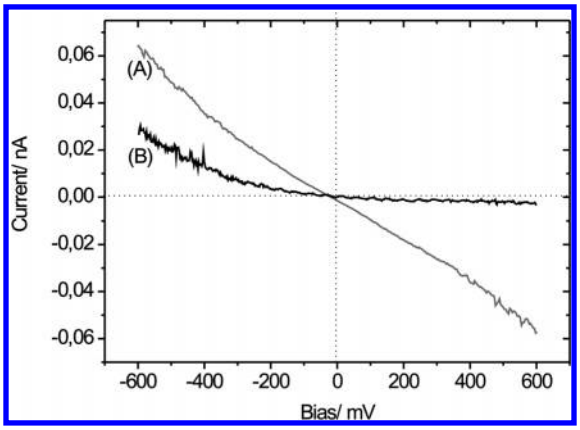


Figure 4. Current vs bias voltage curves obtained at fixed tip−sample distance for the junctions incorporating dodecanedithiol (A) and α-helical peptide (B). Representative spectra are averaged from 50 individual measurements.

A is symmetric, while curve B displays significant asymmetry, which confirms the results obtained from current−distance measurements. However, the positive current is up to 10 times higher than the negative current measured for a given absolute value of the bias voltage. This means that the current ratio is higher than measured previously, and it proves that the less destructive conditions lead to better rectification behavior.

In conclusion, we demonstrated the method of preparation of helical peptide SAM with uniform orientation of the molecules, and we found that said molecules could be considered relatively efficient mediators for electron transfer. Moreover, the helical peptide seems to be a good candidate for an electrical rectifier. It should be noted that the degree of rectification is significant, although the method of measurement can result in distortion of the secondary structure of the helix due to the squeezing and stretching of the molecules. It can be expected that the application of a less destructive method would allow achievement of an even higher degree of rectification as indicated by the STS $I-V$ measurements. We are now investigating the other helical peptides having different lengths and dipole moments.

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References and Notes

(1) Wasielewski, M. R. *Chem. Rev.* **1992**, *92*, 435.
(2) Fox, M. A.; Galoppini, E. *J. Am. Chem. Soc.* **1997**, *119*, 5277.
(3) Malak, R. A.; Gao, Z.; Wishart, J. F.; Isied, S. S. *J. Am. Chem. Soc.* **2004**, *126*, 13888.
(4) Morita, T.; Kimura, S. *J. Am. Chem. Soc.* **2003**, *125*, 8732.
(5) Sek, S.; Sepiol, A.; Tolak, A.; Misicka, A.; Bilewicz, R. *J. Phys. Chem. B* **2004**, *108*, 8102.
(6) Polo, F.; Antonello, S.; Formaggio, F.; Toniolo, C.; Maran, F. *J. Am. Chem. Soc.* **2005**, *127*, 492.
(7) Xiao, X.; Xu, B.; Tao, N. *J. Am. Chem. Soc.* **2004**, *126*, 5370.
(8) Xiao, X.; Xu, B.; Tao, N. *Angew. Chem., Int. Ed.* **2004**, *43*, 6148.
(9) Kitagawa, K.; Morita, T.; Kimura, S. *J. Phys. Chem. B* **2005**, *109*, 13906.
(10) Sek, S.; Tolak, A.; Misicka, A.; Palys, B.; Bilewicz, R. *J. Phys. Chem. B* **2005**, *109*, 18433.
(11) Long, Y.-T.; Abu-Irhayem, E.; Kraatz, H. B. *Chem.—Eur. J.* **2005**, *11*, 5186.
(12) Sisido, M.; Hoshino, S.; Kusano, H.; Kuragaki, M.; Makino, M.; Sasaki, H.; Smith, T. A.; Ghiggino, K. P. *J. Phys. Chem. B* **2001**, *105*, 10407.
(13) Shin, Y.-G. K.; Newton, M. D.; Isied, S. S. *J. Am. Chem. Soc.* **2003**, *125*, 3722.
(14) Metzger, R. M. *Chem. Rev.* **2003**, *103*, 3803.
(15) Xu, B.; Tao, N. J. *Science* **2003**, *301*, 1221.
(16) Haiss, W.; van Zalinge, H.; Higgins, S. J.; Bethell, D.; Höbenreich, H.; Schiffrin, D. J.; Nichols, R. J. *J. Am. Chem. Soc.* **2003**, *125*, 15294.
(17) Haiss, W.; Nichols, R. J.; van Zalinge, H.; Higgins, S. J.; Bethell, D.; Schiffrin, D. J. *Phys. Chem. Chem. Phys.* **2004**, *6*, 4330.
(18) Cui, X. D.; Primak, A.; Zarate, X.; Tomfohr, J.; Sankey, O. F.; Moore, A. L.; Moore, T. A.; Gust, D.; Harris, G.; Lindsay, S. M. *Science* **2001**, *294*, 571.
(19) Wold, D. J.; Frisbie, C. D. *J. Am. Chem. Soc.* **2000**, *122*, 2970.
(20) Veber, D. F.; Milkowski, J. D.; Varga, S. L.; Denkewalter, R. G.; Hirschmann, R. *J. Am. Chem. Soc.* **1972**, *94*, 5456.
(21) Kumaran, S.; Roy, R. P. *J. Peptide Res.* **1999**, *53*, 284.
(22) Miura, Y.; Kimura, S.; Imanishi, Y.; Umemura, J. *Langmuir* **1998**, *14*, 6935.
(23) Kennedy, D. F.; Chrisma, M.; Chapman, T. D. *Biochemistry* **1991**, *30*, 6541.
(24) The distance between the terminal sulfur atoms was calculated using HyperChem 6.0. It was assumed that the structure of the peptide is helical with dihedral angles: $\omega = 180°$, $\phi = -58°$, $\psi = -47°$. In the case of dodecanedithiol, we assumed all-trans conformation of alkyl chains.
(25) Petrov, E. G.; May, V. *J. Phys. Chem. A* **2001**, *105*, 10176.