Single-Molecule Conductance

Changes in the Conductance of Single Peptide Molecules upon Metal-Ion Binding**

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As the field of silicon-based microelectronics attempts, with difficulty, to head towards the nanoscale, the construction of electronic devices with individual molecules becomes an attractive alternative[1] and has stimulated a recent surge of interest in the study of the electronic properties of single molecules.[2,3] As well as displaying excellent electronic properties, single molecules can also recognize other molecules through specific binding interactions, which is something that current silicon-based technology is unable to offer. This capability of molecular recognition is used with astonishing accuracy and efficiency in biological systems and serves as an important design principle for chemical and biological sensors. Various molecular recognition processes have been studied and applied to sensor applications, but most methods to date measure an optical, electrochemical, or mechanical signal that arises from a large number of molecules.[4–8] Herein we demonstrate that the binding of a guest species onto a single host molecule can be studied electrically by wiring the host molecule to two electrodes. The measurement of electron-transport processes through a single molecule also allows the rectification properties of asymmetric host molecules and host–guest complexes to be studied.

Peptides were chosen as the host molecules because of the unlimited choice of different sequences that can be tuned to obtain optimal binding strength and specificity for a metal ion—our chosen guest.[7] Four peptides were studied, cysteamine-Cys, cysteamine-Gly-Cys, Cys-Gly-Cys, and cysteamine-Gly-Gly-Cys (Cys = cysteine, Gly = glycine), which each have two thiol termini that can form reproducible contact to Au electrodes for electrical measurement. These peptides were expected to bind transition-metal ions, such as Cu^{2+} and Ni^{2+}, specifically through deprotonated peptide bonds.[9] The binding configuration and the binding constant are sensitive to the pH of the peptide local environment. To form the most stable metal–peptide complexes and also to avoid the precipitation of metal hydroxides on the Au electrodes, the pH of the solution was maintained at 8 and 9 for Cu^{2+} and Ni^{2+}, respectively. Under the experimental conditions, the metal ions and the peptides were expected to form mainly 1:1 metal-to-ligand complexes. For cysteamine-Cys, cysteamine-Gly-Cys, and Cys-Gly-Cys, the peptide bonds are completely deprotonated so the number of deprotonated

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peptide bonds is 1, 2, and 3, respectively, whereas for cysteamine-Gly-Gly-Cys, the number of deprotonated bonds is 2 or 3.[10]

To reliably measure the conductance of a single molecule,[11–15] we used two complementary approaches. The first was a statistical approach, which has been described in detail elsewhere.[16,17] Briefly, individual molecular junctions were created by repeatedly moving an Au scanning tunneling microscope (STM) tip into and out of contact with an Au substrate in a solution that contained the sample molecules (1 mM, pH ≈ 8, Figure 1a). The process was controlled by a feedback loop that started by driving the electrode into contact with the substrate by using a piezoelectric transducer (PZT). Once the contact was established, the feedback loop activated the PZT to pull the electrode out of contact. After breaking the contact, a series of steps appeared in the conductance that signaled the formation of the molecular junctions (Figure 1b for cysteamine-Gly-Cys). The conductance steps correspond to the breakdown of the contact of individual molecules to the electrodes.[18] When the last molecule was broken, we then repeated the above process to quickly obtain a large number of conductance curves. The histogram of the conductance curves exhibit well-defined peaks that are located at integer multiples of a fundamental conductance value, which is identified as the conductance of a single molecule (Figure 1c). The first peak for cysteamine-Gly-Cys is located at $4.2\times10^{-6}\,G_0$ ($G_0 = 2e^2h^{-1} \approx 77\ \mu S$), which gives a conductance of ≈ 0.3 nS or a resistance of ≈ 3 GΩ. This statistical approach has allowed us to determine the single-molecule conductivity values for a variety of systems,[16,17,19] but important features associated with the individual molecular junctions may be lost. For example, the current–voltage (I–V) curve in the statistical approach is assembled from the positions of the peaks in the conductance histograms, which are obtained at different bias voltages and which smears out possible rectification behavior of asymmetric peptides. To overcome this difficulty, we used a second approach, which is similar to the break-junction method,[11] to measure the I–V characteristics of the peptides in this work. First, the Au tip was brought into contact with the substrate and then the tip was gently pulled out of contact by controlling the PZT whilst the conductance was measured continuously. Once the conductance dropped to the last step, which corresponds to the formation of a single molecule bridged between the electrodes, the position of the tip was fixed and I–V curves were measured.

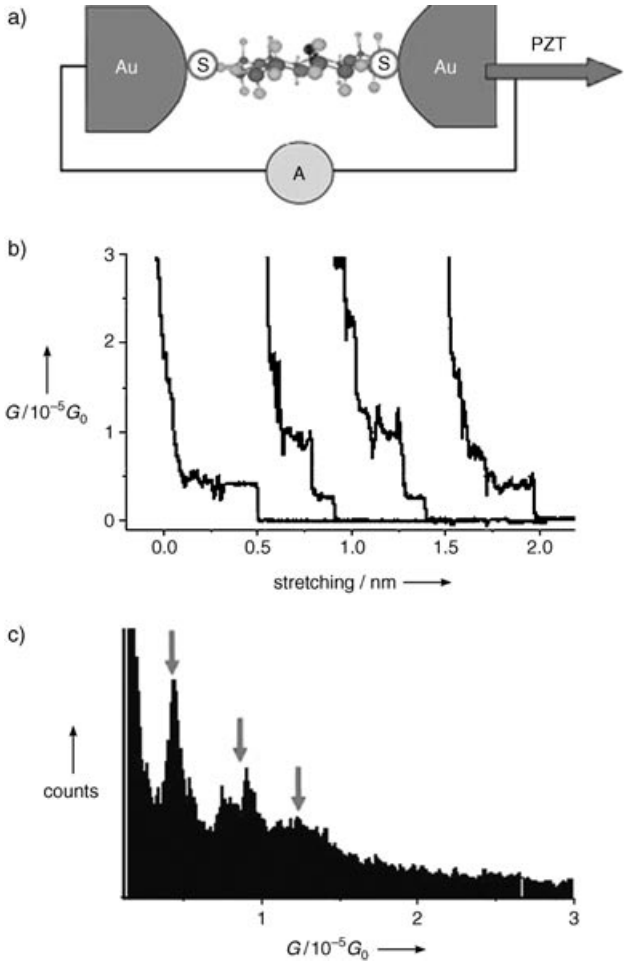

Figure 1. a) Schematic illustration of a molecular junction formed by the separation of two electrodes (PZT = piezoelectric transducer); b) several typical conductance curves of cysteamine-Gly-Cys during the stretching of the molecular junctions; c) conductance histogram constructed from over 500 individual conductance curves.

Figure 2, a–c, shows the I–V curves obtained for three of the peptide sequences. The slopes of the I–V curves near zero bias voltage give the conductance values of these peptides, which agree with the conductance values extracted from the conductance histograms. The most striking feature shared by all the peptides is asymmetry in the I–V curves. For clarity, only the I–V curves with the same polarity (i.e. the current at negative bias is greater than the current at positive bias) are shown in Figure 2. In reality, the polarity of the asymmetric I–V curves varies from one junction to another owing to the random orientation of the molecules in these individual molecular junctions. Rectification behavior in peptides is expected because of the asymmetry and the electric dipoles of the molecules. In a control experiment, I–V curves were measured for 1,8-octanedithiol, which gave rather symmetric curves (Figure 2d). Rectification is one of the most actively pursued goals in molecular electronics because of its potential application in molecular diodes.[1] Reichert et al. reported asymmetric I–V curves for asymmetric molecules by using a break-junction method.[11] Early observation of rectification behavior in a molecular system was observed on a Langmuir–Blodgett film of molecules that contained donor and acceptor groups.[20] More recently, Whitesides and co-workers demonstrated rectification behavior in a molecular junction, which involved two molecular layers sandwiched between silver and mercury electrodes.[21] In general, the observation of rectification requires asymmetric molecular junctions.[22–24]

The binding of Cu^{2+} to each of the peptides, which were self-assembled on the gold substrate, was studied by the introduction of Cu^{2+} (2 mM) into $NaClO_4$ (0.1M) and adjustment of the pH to 8 with NaOH at which value Cu^{2+} is expected to bind to the peptides.[10,25] Figure 3 shows the conductance curves of cysteamine-Gly-Gly-Cys during the formation of individual molecular junctions in the absence

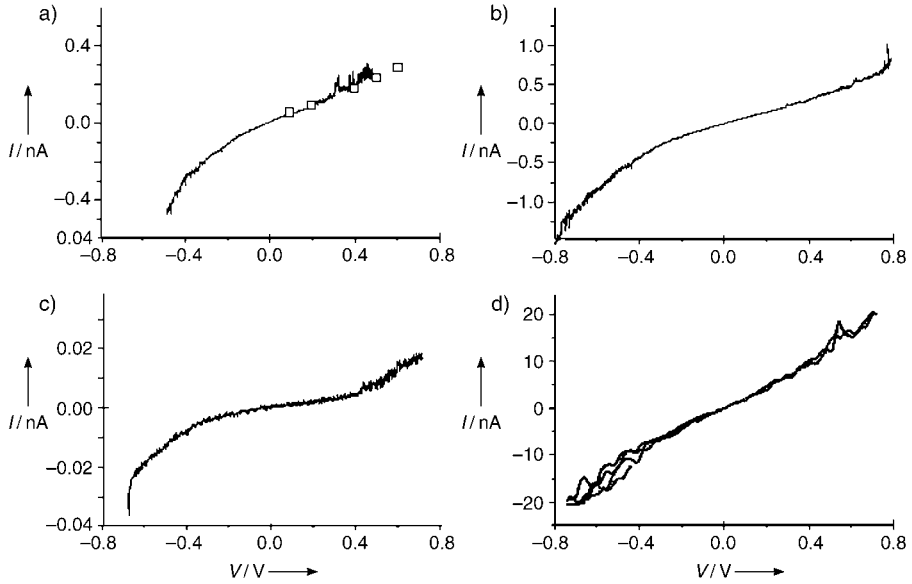

Figure 2. Asymmetric *I–V* curves of a) cysteamine-Gly-Cys, b) Cys-Gly-Cys, and c) cysteamine-Gly-Gly-Cys; *I–V* data which were determined from conductance histograms for cysteamine-Gly-Cys are also shown (□); d) symmetric *I–V* curves of symmetric 1,8-octanedithiol.

(Figure 3a) and presence (Figure 3b) of Cu^{2+}. The last conductance steps in these curves correspond to the formation of single molecule junctions. The conductance steps of the peptide occur at values that are two orders of magnitude higher in the presence of Cu^{2+} ion than those in the absence of Cu^{2+} ion which shows that the Cu^{2+} binding event drastically changes the conductance of the peptide. From the corresponding conductance histograms (Figure 3, a and b, insets), conductance values of the peptide and the peptide–Cu^{2+} complex are $5x10^{-7}$ and $1.6x10^{-4}$ G_0, respectively. A typical *I–V* curve of the peptide complex is shown in Figure 3d which is also asymmetric, but its slope (conductance) near zero bias voltage is much greater than that of the peptide itself (Figure 3c).

Cyclic voltammetry measurements were also made on an Au substrate, which was coated with monolayers of the peptides, before and after exposure of the substrate to Cu^{2+}. Pronounced redox peaks after exposure to Cu^{2+} were observed. As shown in Figure 4, the quasi-reversible peaks around 160 mV (half of the sum of the potentials of the anodic and cathodic peaks) correspond to Cu^{2+}/Cu^{+} redox processes, which is in agreement with a similar Cu–peptide complex,[26] and confirms the formation of the Cu–peptide (deprotonated) complex. Previous cyclic voltammetry studies also reported such redox process for Cu^{2+}–diglycine and Cu^{2+}–triglycine complexes, but at potentials below −200 mV.[27,28] The positive shift of the redox potential may arise from surface adsorption.[26,29] No other redox features were observed which indicates the absence of Cu^{2+} ions that are simply coordinated with carboxyl or amine groups.[30,31] This is reasonable because all of the peptides studied here are flexible so they can fully use their binding sites. As a result, the complexes have high binding constants at pH 8. In a further control experiment, after conductance measurements of solutions of the free peptide and Cu^{2+}–peptide complex, respectively, we acidified the solutions with $HClO_4$ (10 mM) to pH ≈ 2 and again measured the conductance values of the substrates. As expected, the conductance of the single

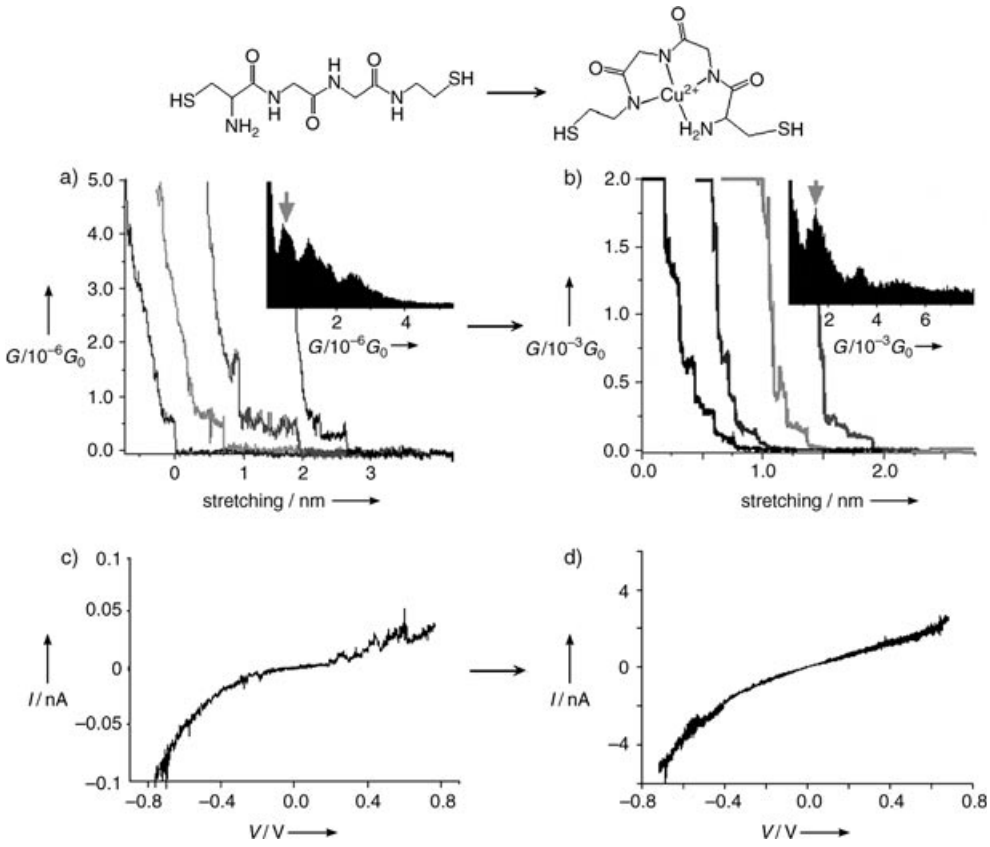

Figure 3. Individual conductance curves of cysteamine-Gly-Gly-Cys a) before and b) after binding to Cu^{2+}; the insets show the conductance histograms. *I–V* curves of cysteamine-Gly-Gly-Cys c) before and d) after binding to Cu^{2+}.

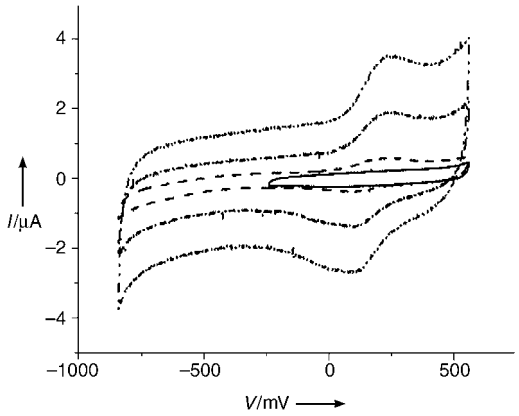

Figure 4. Cyclic voltammogram (vs. Ag/AgCl) of a (Cys-Gly-Cys)-modified electrode before (——) and after (----- and •••••) exposure to copper(II) solution recorded in Cu^{2+}-free buffer solution (pH ≈ 8). Cu^{2+} was accumulated at the (Cys-Gly-Cys)-modified electrode at open circuit for 10 min and then removed. Sweep rates: —— and -----: 100 $mV s^{-1}$; •••••: 500 and 1000 $mV s^{-1}$.

molecule complex returned to the value found for the free peptide, an indication of the dissociation of Cu^{2+}–peptide complex in acidic solution.

The binding of different metal ions, such as Na^+, K^+, Cu^{2+}, Ni^{2+}, and Zn^{2+} to the different peptides in similar ways were also investigated. For Na^+ and K^+ ions, no changes in the conductance of the peptide were observed, which is expected because these ions do not bind to peptides. In the case of Zn^{2+}, a small increase in the conductance of the longest peptide was observed, which may be due to the weak Zn–peptide binding. However, the peaks in the conductance histograms for the Zn^{2+}–peptide complex are not well-defined and prevented further more-quantitative studies. The changes in the conductance upon the binding of Cu^{2+} and Ni^{2+} ions are rather dramatic, and the results are summarized in Table 1. The binding of the metal ions to the peptides increases the conductance, but the magnitude of the increase depends on the length and the sequence of the peptides as well as on the nature of the metal ions. For example, the change in the conductance is only ≈10% for the shortest peptide, cysteamine-Cys, and ≈300 times for cysteamine-Gly-Gly-Cys. The change in the metal-ion-binding-induced conductance also depends on the sequence of the peptides. Cysteamine-Gly-Cys and Cys-Gly-Cys have the same length, but the binding of Cu^{2+} changes the conductance of the two peptides by 2 and 4 times, respectively. This dependence on the sequence may be attributed to the difference in the number of Cu^{2+} ion binding sites in the two peptides (2 in cysteamine-Gly-Cys and 3 in Cys-Gly-Cys). The binding of Ni^{2+} ions to the peptides also increases the conductance of the peptide but by a smaller amount than Cu^{2+} ions. This difference between the binding of Cu^{2+} and Ni^{2+} ions shows that the measurement of conductance can be used to distinguish different metal ions with similar binding configurations to a host molecule.

Table 1: Effects of the binding of metal ions (Cu^{2+} and Ni^{2+}) on the conductance of peptides of various lengths and sequences.

Peptide	No. of binding sites	Conductance of peptide [G_0]	Conductance of peptide–ion complex [G_0]	Conductance ratio
Cysteamine-Cys	2	1.8×10^{-4}	1.9×10^{-4} (Cu^{2+})	≈1
Cysteamine-Gly-Cys	3	4.2×10^{-6}	9.1×10^{-6} (Cu^{2+})	2.2
			6.5×10^{-6} (Ni^{2+})	1.5
Cys-Gly-Cys	4	5.3×10^{-6}	2.3×10^{-5} (Cu^{2+})	4.3
Cysteamine-Gly-Gly-Cys	4	5.0×10^{-7}	1.6×10^{-4} (Cu^{2+})	320
			6.0×10^{-5} (Ni^{2+})	120

The binding of a metal ion to a peptide changes the conductance of a peptide in several ways. First, it affects the charge distribution of the peptide owing to the presence of the metal ion and the associated deprotonation of the peptide bonds. We recently observed that a change in the charge distribution of peptides can indeed change the conductance through a change in the tunneling barrier for the electrons, but the effect alone is usually small.[19] Furthermore, the effect of charge distribution cannot easily explain the sensitive dependence of the conductance of the metal-ion–peptide complex on the length of the peptide. Second, the presence of metal ions introduces new energy levels along the electron-transport pathway which may dramatically enhance the electron transport through a resonant tunneling effect.[32–36] Cyclic voltammograms indicate that the redox levels of Cu^{2+}–peptide complexes are closer to the Fermi energy levels of the electrode than those of corresponding Ni^{2+}–peptide complexes. This seems to explain the different changes induced by Cu^{2+} and Ni^{2+} in the conductance of the peptides. However, like the charge distribution model, this metal-ion-induced resonant tunneling mechanism alone cannot easily explain the sensitive dependence of the conductance of the metal-ion–peptide complex on the length of the peptide. Finally, the binding of a metal ion to a peptide can significantly change the conformation of the peptide. As we discussed in the previous sections, metal ions, under the experimental conditions, have a tendency to coordinate to all of the peptide binding sites which thus forces the peptide to adapt a new conformation (illustrated in Figure 3). This has been observed by X-ray crystallographic measurements and supported by other experimental data.[37,38] As the binding process does not significantly change the bond lengths and angles of the peptide bonds, one may argue that the conformational change should not change the conductance if electron transport through the peptide backbone is considered as the dominant conduction pathway.[39] However, the electrons may be transported through the chelate bonds, mediated by the metal ion, and thus provide a new pathway for electron transport. As the amount of conformational change increases with the peptide length, the metal-ion-mediated transport pathway is more efficient than the peptide backbone pathway for long peptides. On the basis of these considerations, we believe that the dominant mechanism for the observed changes in the conductance arises from conformational change together with

metal-ion-mediated tunneling, although effects such as changes in charge distribution may also play a role.

In conclusion, the conductance and *I–V* characteristics of single peptide molecules covalently bound to two Au electrodes have been measured. The *I–V* curves are highly asymmetric, which reflects the asymmetric structures and electric dipoles of the peptides. Upon binding of metal ions, the conductance of the peptides increases by an amount that depends on the sequence and length of the peptides. This work demonstrates a method to study molecular recognition on a single-molecule level.

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