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# Conductance Titration of Single-Peptide Molecules

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The ability to measure the conductance of a single molecule wired to two electrodes is a basic requirement toward electronic devices based on single molecules.[1−11] It also promises a fresh approach to study various chemical processes and reactions on a single-molecule basis by simply monitoring the conductance of the molecule. We report here a study of electron transport in single peptides covalently bonded to two electrodes. By monitoring the pH dependence of the peptide conductance, we provide the first conductance titration measurement of a single molecule. We chose peptides because they are the building blocks of proteins, in which electron transport is directly relevant to many biological functions.

We focus here on three simple peptides (Scheme 1), containing 1, 2 and 3 amino acids, respectively. To reproducibly measure the conductance of a single-peptide molecule, we introduced two thiol groups as terminal groups so that they can form covalent bonds with Au electrodes. Peptide 1 has an amino acid, cysteine, linked to a cysteamine via a peptide bond. The thiol groups in the cysteamine and cysteine provide two terminals that can bind to Au electrodes. Peptide 2 is similar to Peptide 1, except that it has an extra amino acid, glycine, inserted between the cysteamine and cysteine. Peptides 1 and 2, each containing an amine group, can be protonated by changing the solution pH. Peptide 3 has three amino acids, cysteine, glycine, and cysteine, sequentially linked via peptide bonds, where the two cysteine groups are natural terminals. Unlike Peptides 1 and 2, it contains an amine and a carboxyl group.

We created individual molecular junctions by repeatedly moving a Au electrode into and out of contact with a Au substrate in a buffer containing 1 mM of the sample molecules (Figure 1a).[9] The experiment was controlled by a feedback loop that started by driving the electrode into contact with the substrate using a piezoelectric transducer (PZT). Once the contact was fully established, the feedback loop activated the PZT to pull the electrode out of contact. After breaking the contact, the measured conductance did not simply decay exponentially with the pulling distance as we might expect for a tunneling process. Instead, a series of steps appeared in the conductance, signaling the formation of molecular junction. We have shown that the conductance steps are due to the breakdown of individual molecules as a result of contacting the electrodes.[12] As a further control experiment, we performed the measurement in the absence of sample molecules and observed only smooth exponential decay in the conductance. When the last molecule was broken, we then repeated the above process which allowed us to quickly perform a large number of measurements.

Figure 1b shows several typical conductance curves recorded during the formation of Peptide 1 junction in pH = 2 solution with a bias voltage of 0.1 V, where conductance steps are marked by arrows. The lowest-conductance step occurs around $2.5 \times 10^{-4}$ $G_0$, where $G_0 = 2e^2/h \approx 77$ $\mu$S. There is a significant run-to-run variation in the step position, reflecting the variation in the microscopic detail of the molecule−electrode contact.[12] Thus, a statistical analysis over many measurements is necessary to obtain

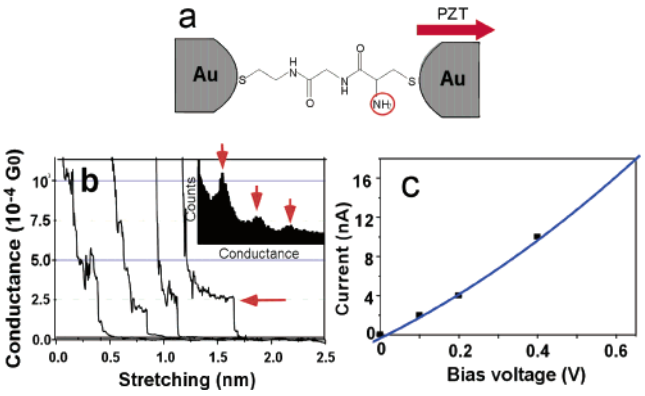
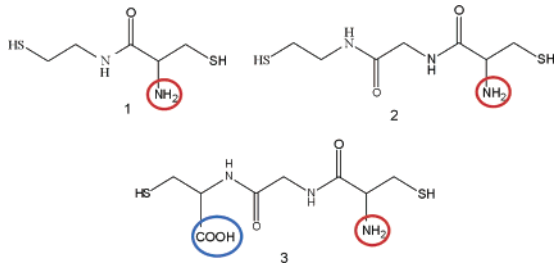



***Figure 1.*** (a). Schematic illustration of a molecular junction formed by separating two electrodes. (b). Several typical conductance curves of Peptide 1 during the formation of molecular junctions. The discrete changes in the conductance are due to the breakdown of individual molecules. The inset in (b) is a conductance histogram constructed from over 500 individual conductance curves, showing peaks near integer multiples of $2.5 \times 10^{-4}$ $G_0$. (c) $I-V$ characteristic of Peptide 1 determined from conductance histograms at different bias voltages.

***Scheme 1***




a complete picture. As we will discuss below, the statistical analysis also provides us with an identification of single-molecule conductance. A typical conductance histogram is shown as an inset in Figure 1b. It reveals well-defined peaks near 1×, 2×, and 3× a fundamental conductance value, $\sim 2.5 \times 10^{-4}$ $G_0$. We assign these peaks to 1, 2, 3,... Peptide 1 molecules and the fundamental value to the conductance of Peptide 1 at pH = 2. This is similar to the Milikan oil-drop experiment that determined the charge of a single electron 90 years ago.[7] The peaks are pronounced, but have finite widths, which reflect the run-to-run variation in the molecule−electrode contacts. We have performed the measurement at various bias voltages. From the peak positions of the corresponding conductance histograms, we have determined the current−voltage ($I-V$) characteristic curve for Peptide 1 at pH = 2 (Figure 1c). We note that only positive bias data are shown here because the $I-V$ curve was obtained from the conductance histogram that averaged out asymmetrically in the $I-V$ curves. Below 0.5 V, the $I-V$ curve is rather linear.

We have obtained similar conductance histograms for the three peptides at various pHs. The results for Peptide 1 at several pH values are shown in Figure 2a−c. At low pH, the conductance peaks



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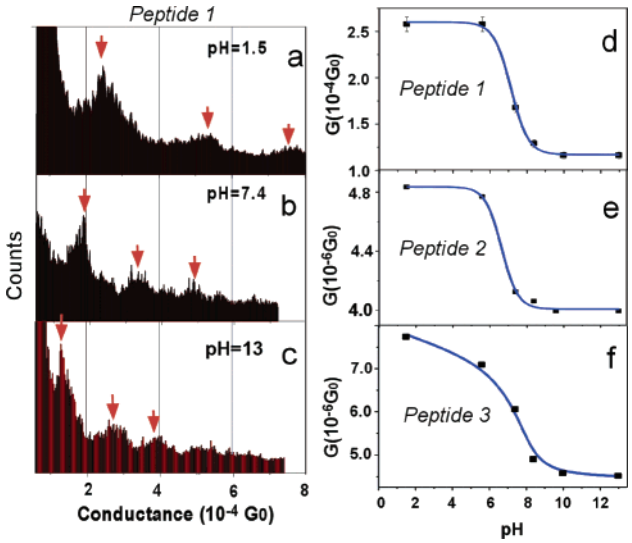


***Figure 2.*** (a−c) Conductance histograms of Peptide 1 obtained at various solution pH. Conductance vs pH for Peptides 1 (d), 2 (e), and 3 (f). The solid lines are guide for the eye.

are located near integer multiple of $2.5 \times 10^{-4}$ $G_0$ (Figure 2a). Increasing the solution pH to 7.4, the peaks shift to integer multiples of $2.0 \times 10^{-4}$ $G_0$ (Figure 2b). Further increasing pH to 13, the conductance peaks shift to integer multiples of $1.2 \times 10^{-4}$ $G_0$. The conductance of peptide 1 vs pH is plotted in Figure 2d, which shows a sigmoid-like titration curve. The pH value at which the conductance reaches one-half of the total change is about 7. The p$K_a$ of the amine group in cysteine measured in solution phase is about 8. The deviation in the p$K_a$ can be attributed to the modification of hydronium ion concentration by the diffuse layers of the probing electrodes. Shifts in the p$K_a$ of surface-bound molecules have been reported using various techniques, including contact angle,[13] force,[14−15] differential interfacial capacitance,[16] and surface-enhanced Raman titration.[17] We have attempted but failed to observe such pH effects on the conductance of alkanedithiols; therefore, the observed pH dependence of the peptide conductance is due to the amine and carboxyl groups, rather than to changes in the S−Au bonds.

Peptide 2 exhibits a similar sigmoid-like pH-dependent conductance, which is expected because both Peptides 1 and 2 have an amine side group (Figure 2e). Peptide 3 is, however, quite different (Figure 2f). Instead of a sharp change around pH = 7 as found for Peptides 1 and 2, the conductance of Peptide 3 changes more smoothly with pH. This difference may be traced to the fact that Peptide 3 has an amine and a carboxyl group. Both are sensitive to pH with p$K_a \approx 8$ and 3, respectively, in bulk solution, but the p$K_a$ values are expected to shift due to the surface effect of the electrodes. The observed pH dependence of the conductance of Peptide 3 reflects a resultant effect of protonation/deprotonation of the amine and carboxyl groups.

Before discussing the origin of the pH-dependent conductance, it is necessary to determine whether the electron transport through the peptides is due to a coherent tunneling process (or superexchange), as found for alkanedithiols, or a sequentially hoping process. An important signature of tunneling or hopping is the length dependence of the conductance. Peptides 2 and 3 have similar lengths, and thus we have measured the conductance of a longer peptide, Cysteamine-Gly-Gly-Cys. The conductance ($G$) of the peptides vs length ($L$) can be described by $G = A \exp(-\beta L)$ with best fitting parameters, $A = 0.15G_0$ and $\beta = 1.1 \pm 0.1$ per carbon or nitrogen atom, or $0.87 \pm 0.7$ Å$^{-1}$ (Figure 3). As a comparison, we have also plotted the data for alkanedithiols[9] in the same

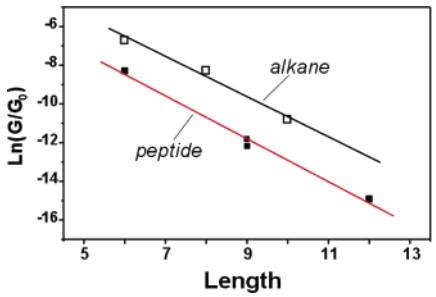


***Figure 3.*** Natural logarithm of conductance vs peptide length (number of carbon or nitrogen atoms in the peptides). The solid lines are linear fits that yield $\beta_N$. For comparison, the data for alkanedithiols are also plotted.

diagram, where $A = 0.65G_0$ and $\beta = 1.0 \pm 0.01$ per carbon atom. These findings are consistent with a coherent tunneling process.

Based on a simple tunneling model, the presence of charge in the tunneling barrier changes the tunneling barrier and thus the conductance. In the cases of Peptides 1 and 2, the amine group becomes positively charged at low pH, which lowers the tunneling barrier for electrons, leading to an increase in the conductance. The pH-induced conductance change of Peptide 1 is much greater than that of Peptide 2. This may be expected because Peptide 1 is shorter and its amine group controls a larger fraction of the tunneling barrier than Peptide 2. In the case of Peptide 3, the carboxyl group changes from negative at high pH to neutral at low pH, while the amine group changes from neutral to positive. The corresponding tunneling barrier decreases with the pH, which can explain the continuous increase in the conductance of Peptide 3 as pH decreases.

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**Supporting Information Available:** Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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