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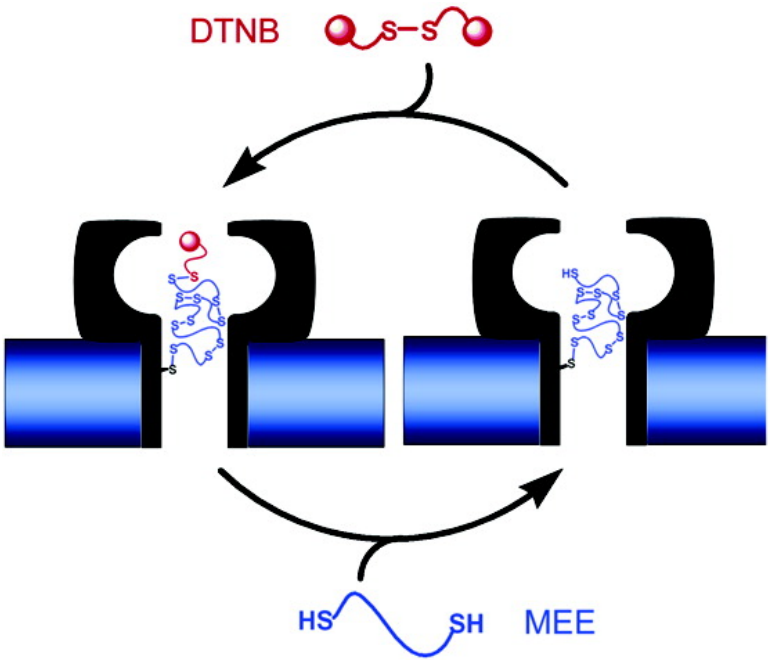

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Stepwise Growth of a Single Polymer Chain

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Single-molecule techniques are powerful tools for the detection of intermediates in dynamic systems that can be concealed in ensemble experiments.[1−4] Many single-molecule studies in solution have been dedicated to biological problems, such as the folding of macromolecules[2,4−6] and the turnover and conformational changes of enzymes[7,8] and ribozymes.[4] In general, these studies have involved optical or force measurements.

The chemistry of individual small molecules has received less attention.[9,10] Recently, we investigated bond making and bond breaking at the single-molecule level in aqueous solution.[11−13] In this work, the staphylococcal α-hemolysin (αHL) pore was adopted as a "nanoreactor". Chemical reactions occurring near the internal surface perturbed the current flow through a single pore located in a lipid bilayer, allowing the detection of reaction intermediates on the basis of their size, shape, and charge. In the present study, we visualize the growth of a single polymer chain. Previously, the enzymatic elongation and shortening of nucleic acids was examined by scanning probe microscopy[14] and with optical tweezers,[15,16] but single-base resolution has not yet been achieved.[16] Two dendronized polymers have been joined photochemically,[17] as detected by scanning probe microscopy, but step-by-step growth of an individual polymer has not been observed. A single polymer chain has been formed from fullerene oxides inside a nanoreactor, in this case a carbon nanotube, but the product was examined by electron microscopy only after its formation.[18]

In previous studies, a version of the αHL pore, P_{SH}, which contains a single thiol in the barrel was used as the nanoreactor, allowing the examination of chemistry involving spatially separated reactants.[13] For example, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and DL-dithiothreitol (DTT) were respectively placed in the cis and trans compartments of the recording apparatus, separated by the lipid bilayer (Figure 1a). In this case, DTNB first reacted with the internal thiol to yield a mixed disulfide. DTT, from the trans compartment, then cleaved the disulfide and cyclized to yield free P_{SH}, permitting another reaction with DTNB.[13] Numerous such cycles followed, each step of which could be observed at the single-molecule level.

In the present work, DTT was replaced with (mercaptoethyl)-ether (MEE) (Figure 1a). In this case, after P_{SH} had reacted with DTNB and MEE had cleaved the mixed disulfide, the adduct (b_1) failed to cyclize and instead reacted again with DTNB to form the extended activated adduct a_1. The adduct a_1 again reacted with MEE to form the tethered dimer b_2 (Figure 2a). Subsequently, the same chemistry repeated and a polymer formed (Figure 1b). The "downward" step (increased conductance) from a_n to b_{n+1} is most likely caused by replacement within the nanoreactor of the bulky, charged TNB group with MEE.

As the polymer chain grew, the conductance of the pore decreased steadily (Figure 2b,c), and spikes toward lower conductance appeared. For example, for levels "a", the frequency of occurrence of the spikes increased with n, with a plateau of ∼25 s^{-1} at $n = 6-9$ (300 Hz filter). The amplitude of the spikes also

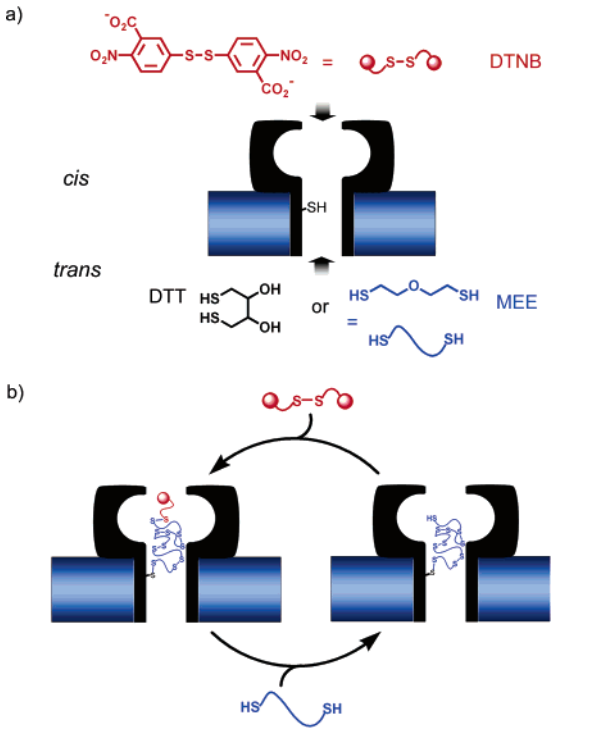

Figure 1. Chemistry in a nanoreactor with spatially separated reactants. (a) P_{SH} is a heptameric αHL pore, which acts as the nanoreactor. P_{SH} contains one mutated subunit with a cysteine residue at position 117 and six cysteine-free wild-type subunits. A single copy of P_{SH} was allowed to insert into a lipid bilayer within a recording apparatus.[13] DTNB was added to the cis chamber and DTT or MEE to the trans chamber. When DTT is present, a reaction cycle occurs.[13] (b) When MEE is present, a polymer grows on the wall of the nanoreactor.

increased with n, eventually reaching the ceiling of complete block for $n \geq 8$. The mean spike duration was ∼500 μs for $n \leq 8$, rising to 1.4 ms at $n = 10$. The spikes most likely arise from the movement of the polymer inside the pore. Similar events have been observed in related experiments, for example, when a single poly(ethylene glycol) (PEG)[19] or DNA[20,21] molecule is tethered within the αHL pore.

The mean lifetimes of a_n and b_n (τ, the time period before conversion to a_{n+1} and b_{n+1}, Table 1) could be converted to rate constants by using $k = 1/(\tau[A])$, where [A] is the concentration of MEE or DTNB.[13] The mean k value for the "a" steps, involving reaction with MEE, is 2.7×10^3 M^{-1} s^{-1}, and that for the "b" events, involving reaction with DTNB, is 1.6×10^4 M^{-1} s^{-1}. These values are somewhat lower (but less than 10-fold) than those for comparable reactions in the literature,[13] which is to be expected as MEE and DTNB must diffuse to the reaction site within the pore. The mean lifetimes of a_n and b_n became on the whole shorter as the chain grew in length (Table 1), although this effect is modest (see also Figure 2b), suggesting that there is little hindrance to growth or substantial change in the environment of the end of the

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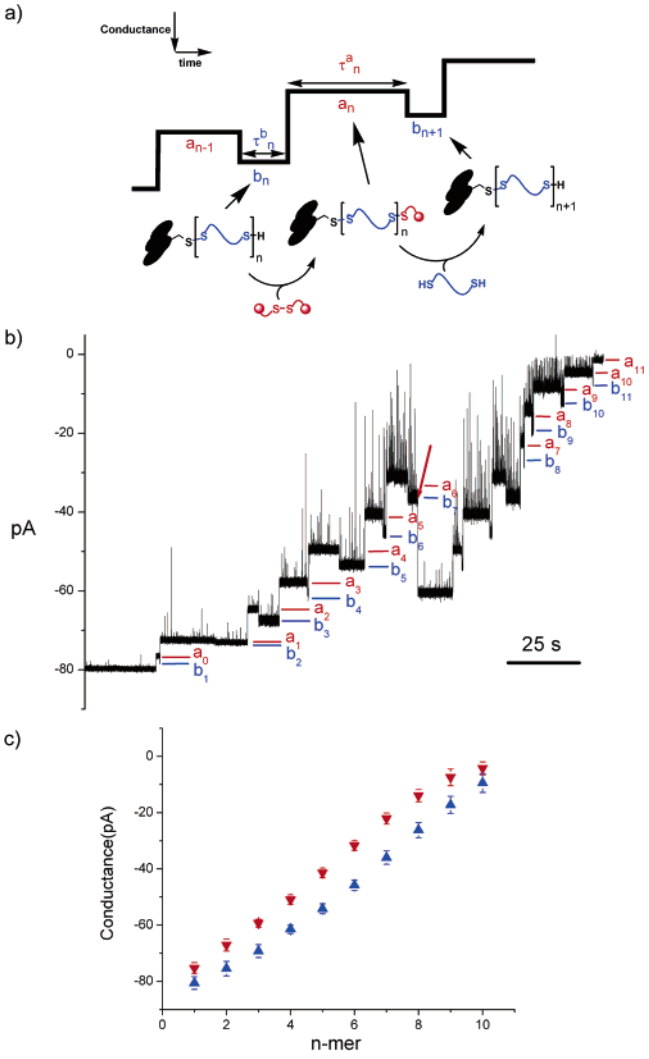

Figure 2. Stepwise growth of a polymer chain. (a) Schematic showing the lifetimes (τ) and conductance levels of the intermediates. Two series of intermediates are depicted. One series, a_n, has an activated disulfide bond at the end of the polymer chain, and the other series, b_n, has a free thiol group (n is the number of (mercaptoethyl)ether units in the chain). The sequence of chain propagation is a_0, b_1, a_1, b_2, a_2, ..., b_n, a_n. $\tau^a{}_n$ and $\tau^b{}_n$ are the lifetimes of a_n and b_n. (b) Representative single-channel recording. The current passed by an individual P_{SH} pore was recorded with 2 M KCl, 30 mM 3-(4-morpholinyl)-1-propanesulfonic acid (MOPS), pH 8.5, and 100 μM ethylenediaminetetraacetate (EDTA) in both chambers. The cis chamber was at ground and the trans chamber held at an applied potential of −50 mV. DTNB (50 μM)[23] was added to the cis chamber and MEE (42 μM)[23] to the trans chamber. The signal was low-pass Bessel filtered at 1 kHz and sampled at 5 kHz. The current trace was digitally filtered at 300 Hz for display. During propagation, the chain was occasionally broken by either an intrachain cyclization or an internal disulfide bond cleavage by MEE (red arrow). In the example shown, a disulfide in b_7 was cleaved to yield b_4, and propagation then resumed. (c) Mean conductance values with standard deviations for intermediates observed during chain propagation (▼, red, a_n; ▲, blue, b_n). The conditions were as in Figure 2b. In this case, DTNB (100 μM) was added to the cis chamber and MEE (42 μM) to the trans chamber. Around 10 determinations were made for each point (as in Table 1).

chain during the 10 extension steps that can be observed. Since the extension reaction occurs at the free terminus of the growing chain, the initial steps might be hindered by the internal surface of the pore, as suggested by the higher values for $\tau^b{}_1$ and $\tau^a{}_1$.

Table 1. Mean Lifetimes of Polymer Intermediates in Seconds[a]

n-mer	1	2	3	4	5	6	7	8	9	10
a_n	44	16	15	18	8.8	7.3	3.3	5.5	12	9.0
	(11)	(10)	(11)	(10)	(11)	(10)	(9)	(10)	(8)	(6)
b_n	3.3	1.9	2.0	1.3	1.3	0.51	0.98	0.46	0.33	0.21
	(11)	(9)	(11)	(10)	(9)	(11)	(10)	(10)	(9)	(6)

[a] The number of measurements is in parentheses. For conditions, see Figure 2c (legend).

In summary, we have shown that the individual covalent reaction steps involved in the growth of a single polymer chain can be monitored by using a protein nanoreactor. Because the mean lifetimes of each intermediate can be obtained from a collection of current traces, the approach may prove useful for studying the detailed kinetics of polymer growth. In addition, the current spikes associated with the elongating chain can provide information about polymer movement under confinement, especially since a series of polymers with pertinent lengths can be generated transiently in a single experiment. Finally, protein pores with ligands attached through individual polymer chains are useful for stochastic sensing of macromolecules.[22] The new findings suggest means to synthesize the required "molecular fishing lines" in situ, by capping the chain with a ligand at a desired length by using a thiol-directed reagent.

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