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COMMUNICATIONS

Published on Web 09/06/2006

Photoisomerization of an Individual Azobenzene Molecule in Water: An On−Off Switch Triggered by Light at a Fixed Wavelength

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The α-hemolysin (αHL) protein pore has been used as a "nanoreactor" for the observation of small-molecule chemistry in water at the single-molecule level. By monitoring the ionic current passing through a pore, changes in the size, shape, and polarity of a reactant tethered to the internal wall can be monitored. By comparison with other single-molecule techniques, such as fluorescence measurements and force spectroscopy, the approach offers a minimum of interference with the reaction under observation. Reaction conditions, such as reagent concentration, pH, and temperature, can be varied in a straightforward manner. This powerful approach has been used to monitor the three-step photoinitiated breakdown of a 2-nitrobenzyl-protected carbamate,[1] the reversible adduct formation between a thiol and an organoarsenic(III) compound,[2] the formation of a mixed disulfide and its cleavage by dithiothreitol,[3] and the step-by-step growth of a polymer chain.[4] In the present work, we have observed the photoinduced trans−cis isomerization of an azobenzene. The reaction constitutes a digital on−off switch that can be operated by light at a fixed wavelength.

Azobenzenes are photochromic dyes,[5] which are converted from the trans to the cis form by light of short wavelength (e.g., 380 nm) and from the cis to the trans state by light of longer wavelength (e.g., 450 nm) or by thermal relaxation. Because the trans and cis states have overlapping absorption bands, complete conversion to the cis form cannot be achieved in bulk solution. The mechanism of isomerization (inversion, rotation, or hula-twist[6,7]) remains unclear despite extensive experimental and computational work.[7−9]

We constructed an αHL pore in which only one of the seven subunits was modified with an azobenzene derivative (Figure 1). A mutant αHL monomer, αHL-T117C−D8, was derivatized at the single cysteine residue at position 117 with the water-soluble azobenzene derivative **1** (Figure 1a). The modified subunit was assembled together with wild-type (WT) subunits, and the heteroheptamer WT_6(T117C-**1**-D8)$_1$ (P_{AZO}, Figure 1b) with only one modified subunit was purified by SDS-polyacrylamide gel electrophoresis, relying upon the electrophoretic shift caused by the C-terminal octa-aspartate (D8) tail[1,10] on the modified subunit (see Supporting Information for details). Compound **1** carries a sulfonate group for aqueous solubility and for maximizing the effect of isomerization on the ionic current.

A single P_{AZO} was inserted into a planar lipid bilayer, and the current carried by aqueous ions passing through the pore was measured at a constant applied potential (Figure 2). The bilayer was illuminated with light from a 300 W Xe lamp brought to a sapphire window in the bilayer chamber with a fiber optic bundle.[1] At the start of the recording shown, the current was −89 pA. This level is taken to represent the thermally stable trans state as it was the current passed when P_{AZO} first inserted into the bilayer. When the bilayer was irradiated at 330 nm ($+h\nu$), transitions between the initial level and a second level at −92 pA were apparent. The second level is taken to represent the cis state, as substantiated by its characteristics, described below. The light source was then shuttered ($-h\nu$) with P_{AZO} in the cis state, and no further transitions were seen until irradiation was continued ($+h\nu$). Finally, the light source was shuttered ($-h\nu$) with P_{AZO} in the trans state, and again no further transitions were seen. Over 40 light-dark cycles have been recorded in a single experiment, with P_{AZO} remaining in the trans or cis state during the dark periods, depending on which state was present when the light source was shuttered.

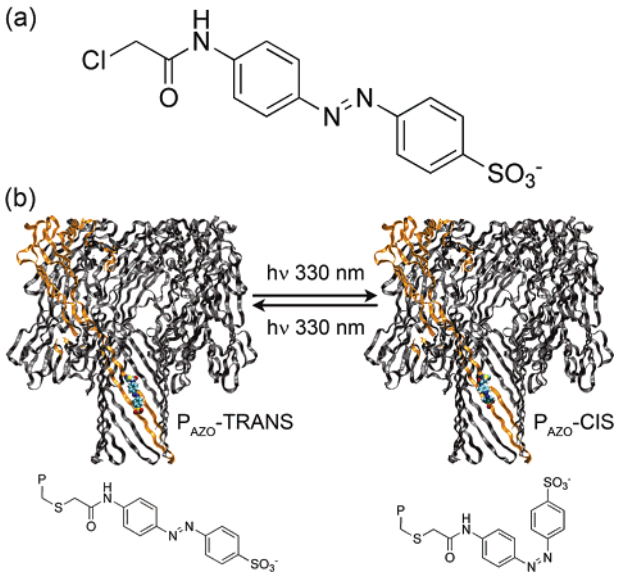

Figure 1. P_{AZO}, an α-hemolysin protein pore containing an azobenzene group in the lumen. (a) 4-((4-(2-Chloroethanoamido)phenyl)diazenyl)-benzenesulfonate, **1**, the alkylating agent used to prepare P_{AZO}. (b) Representations of P_{AZO} in the trans and cis forms.

The mean lifetimes of the trans and cis states at a given wavelength should be dependent on the incident light intensity, according to $\tau \approx 0.3/\phi\epsilon I_0$, where τ = mean lifetime; ϕ = quantum yield; ϵ = extinction coefficient of the absorbing species; I_0 = light intensity (see Supporting Information). By using neutral density filters to modulate the intensity of the incident light, I_0, we showed that τ indeed has a linear relationship with the reciprocal of the transmittance of the filter for both the trans and cis states at 330 nm (see Supporting Information).

The lifetimes in the trans and cis states should also exhibit a wavelength dependence, where $\tau_t/\tau_c = \phi_c\epsilon_c/\phi_t\epsilon_t$, for each wavelength examined. The validity of this relationship was tested by using a series of band-pass and interference filters (see Supporting Information). Values for ϵ_t and ϵ_c were taken from the absorption spectra of the trans and cis isomers of compound **1** (which should be closely similar to those of P_{AZO}), and values of ϕ_t and ϕ_c were from the literature. There is considerable disagreement over the latter, and

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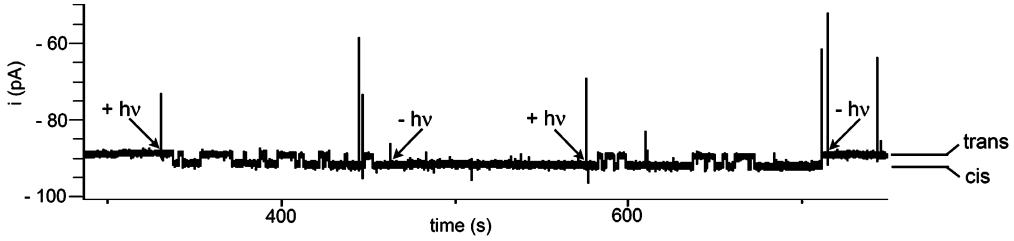

Figure 2. Trans−cis isomerization in a single P_{AZO} pore as demonstrated by current recording in a planar lipid bilayer. The buffer was 10 mM Tris-HCl, pH 8.5, 2 M KCl, 100 μM EDTA on both sides of the bilayer, and the applied potential was −50 mV. Irradiation was at 330 nm and 25 °C.

this difficulty as well as the unusual environment of the azobenzene within the lumen of the pore might explain the weak agreement, but correct trend, when the experimental and theoretical values of τ_t/τ_c are compared. For example, the experimental values were $\tau_t/\tau_c = 0.82$ at 330 nm and $\tau_t/\tau_c = 3.8$ at 465 nm, while the calculated values were $\tau_t/\tau_c = 0.18$ at 330 nm and $\tau_t/\tau_c = 1.5$ at 465 nm.

Interestingly, P_{AZO} could be switched to the trans or the cis state with light of a fixed wavelength (strictly a fixed band of wavelengths), simply by turning the light off after the desired transition (Figure 2). When P_{AZO} was in the cis state, no thermal relaxation was observed in the dark in individual experiments lasting up to 125 min (see Supporting Information). In total, P_{AZO} was observed for more than 8 h in the cis form with no transitions to the trans state. In bulk solution, containing 10 mM Tris.HCl, pH 8.5, 2 M KCl, 100 μM EDTA, the half-time for thermal relaxation at 25 °C was 10.3 ± 0.2 min for compound **1** and 44 ± 2 min for the product of its reaction with gluthathione, compound **2**. Hence the cis isomer is stabilized after the formation of **2**, and further stabilized within the αHL pore leading to the establishment of a simple switch. In general, the kinetics of single-molecule chemistry observed when reactants are tethered to the wall of the αHL pore are similar to those in bulk solution,[3] and further careful study will be required to determine the basis of the stabilization of the cis-azobenzene seen here.

The work described here is the first observation of the reversible photoisomerization of individual azobenzene molecules in an aqueous environment. While the thermal isomerization of a carbamate attached to the gramicidin channel has been observed,[11,12] when this approach was applied to the photoisomerization of an azobenzene derivative[13] or an hemithioindigo derivative,[14] individual isomerization steps were not directly detected. Light-sensitive ion channels incorporating azobenzenes have also been made but not with a view to the examination of individual molecules.[15,16] The isomerization of individual azobenzene derivatives has been reported in studies by scanning tunneling microscopy (STM).[17,18] However, the potential energy surface for the reaction is drastically altered under the conditions required for STM.

At the single-molecule level, a photostationary ratio of trans and cis forms cannot exist. Rather, as demonstrated here, the azobenzene can be flipped from one state to another by a fixed wavelength of light. As predicted from ensemble measurements,[8,19] no intermediates were seen on the millisecond time scale in the individual trans−cis transitions. Therefore, it might be possible to make fast digital nanoscale switches based on our findings.

Acknowledgment. This work was supported by the MRC and the ONR. H.B. is the holder of a Royal Society-Wolfson Research Merit Award.

Supporting Information Available: Materials and methods, derivation of the equations, tables, and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0642818