significantly the kinetics of the thermal reactions that follow the primary photochemical event.

The discovery that the Rh_2^{2+} ion reacts spontaneously with 12 M HCl to form H_2 suggests a possible mechanism for photoreaction (2):

$$Rh_4Cl_2^{4+} \underset{\Delta}{\overset{h\nu}{\rightleftharpoons}} Rh_2^{2+} + Rh_2Cl_2^{2+} \quad (9)$$

$$Rh_2^{2+} + HCl \xrightarrow{\Delta} {}^1/_2Rh_4Cl_2^{4+} + {}^1/_2H_2 \quad (10)$$

At low concentrations of HCl, where net formation of H_2 does not occur upon irradiation of $Rh_4Cl_2^{4+}$,[15] presumably both the thermal back-reaction (eq 9) and reaction of Rh_2^{2+} with Rh_4^{6+} (from $(Rh_4Cl_2^{4+})$ to give Rh_6^{8+} compete effectively with the H_2-producing step (eq 10). We are currently examining the temperature dependences of the photoreactions of $Rh_4Cl_2^{4+}$ in HCl solutions,[22] in the hope that under suitable conditions we can observe the photoproduction of Rh_2^{2+} directly.

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Electronic Relaxation in Azurin: Picosecond Reverse Charge Transfer

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Abstract: Ultrafast relaxation of the 625-nm charge-transfer absorption band of a blue copper protein, azurin, is studied by the use of subpicosecond light pulses. The blue protein solution is transiently bleached, but the absorption reversibly reappears with a time constant of 1.6 ± 0.2 ps, due to reverse charge transfer. A fast transient (<0.5 ps) interpreted as excited-state vibrational relaxation/internal conversion is also observed. The results suggest that ligand rearrangement is not rate determining in the biochemical reactivity of azurin.

Azurin is a small (mol wt 14000) deep blue protein found in a number of bacteria, in particular *Pseudomonas*. The blue color is due to a single copper(II) ion which is related to the apparent role of the protein as an electron carrier. This blue copper center[2] is ubiquitous, occurring in such proteins as plastocyanins, an essential component in plant photosynthesis, in numerous oxidases, and even in the human blood as ceruloplasmin.

The intense absorption band (orders of magnitude stronger than that of $Cu^{2+}(aq)$ in the red) peaks at 625 nm. Spectroscopic evidence has been put forth[3,4] to show that this band is due to a charge-transfer transition of a σ electron from a cysteine sulfur atom to the singly occupied Cu 3d orbital. Preliminary X-ray structures of oxidized azurin[5] and plastocyanin[6] have shown that the Cu^{2+} is coordinated to the nitrogen atoms of two imidazole side chains, the sulfur atom of a methionine, and the negatively charged sulfur of a cysteine ion. The charge-transfer model for the optical transition is thus well supported.

Until now little has been known about the electronic relaxation rate associated with this blue color. Inasmuch as no fluorescence is observed, the lifetime of the upper state must be in the subnanosecond range. Accordingly, an experiment was done by using subpicosecond light pulses at 615 nm to measure this lifetime.

Experimental Section

The picosecond dynamic studies are performed by using a pump–probe technique in which one pulse excites the sample and a second pulse monitors the induced, transient absorption change.[7] Subpicosecond pulses from a cavity-dumped, passively mode-locked, CW dye laser[8,9] are divided into two beams. The more intense beam, the pump, traverses a variable length optical delay, and the weaker beam, the probe, traverses a fixed optical path. Both beams are focused to a common 10-μm diameter spot at the sample. The pump beam is chopped, and the modulation of transmission induced on the probe beam is observed by using a diode detector and a lock-in amplifier. The variable length delay is controlled by a digital stepping motor which also indexes a multichannel analyzer. As the path delay is repetitively scanned, the time profile of the induced transmission is displayed and averaged. The pulses used in the present experiments have a wavelength centered at 615 nm, duration of 0.5 ps, and 5-kW peak power. Seventy-five percent of the pulse energy is contained in the pump beam. The pulse repetition rate is 10^5 Hz for most experiments, but the results are not changed when a pulse repetition rate of 10^4 Hz is used.

The sample is contained in a 0.1-mm path length cell which is translated continuously during the experiments, at a rate of 0.3 cm/s. At the 100-kHz pulse rate, the exposed sample volume is thus changed every 300 pulses, in order to avoid excessive heating and possible damage. The sample shows no spectral changes or other evidence of damage, even after several hours of irradiation. The optical density at 630 nm of the azurin solution in the 0.1-mm cell is 0.2, which corresponds to a concentration of 5×10^{-3} M.[3] The preparation of the azurin from the bacteria *Psuedomonas aeruginosa* has been previously described.[10]

Results

A typical experiment utilized about 2 h of signal averaging and resulted in a curve such as A in Figure 1. The sample response is a transient bleaching which fully recovers on a picosecond time scale. Curve A was obtained for the case of parallel pump and probe pulse polarization and corresponds to a maximum modulation (at $t = 0$) of 0.7% in the probe pulse transmission. For such small modulations, transmission changes are linearly proportional

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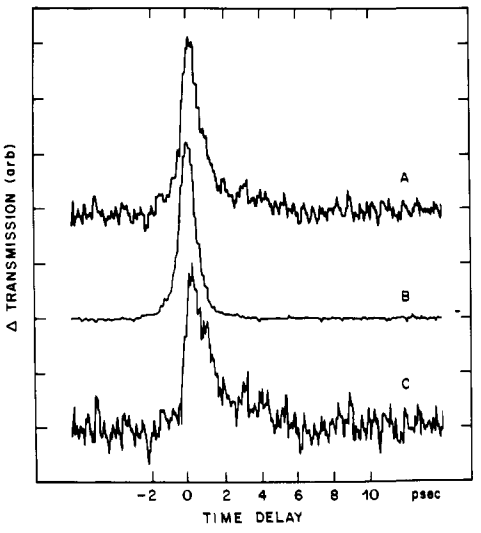

Figure 1. Time resolved data: curve A, probe pulse transmission as a function of time delay; curve B, pulse autocorrelation function; curve C, molecular response. See text for details.

to absorbance changes. The decay curves obtained were the same for both parallel and perpendicular polarizations of the probe pulse relative to the pump pulse, except that the entire signal was 3 times weaker in the case of perpendicular polarizations. This result implies that all electronic transitions interrogated in this experiment have parallel polarizations.

In these picosecond transient absorption experiments the measured modulation of the probe beam is the sum of two terms: the molecular response and a coherent coupling contribution.[11] The coherent coupling contribution, which peaks at zero time delay, has a temporal width which is determined by the coherence of the optical pulses and is thus independent of the dynamical response of the sample. The shape of the coherent coupling contribution in our experiments is essentially identical with that of the pulse autocorrelation function, and its amplitude at $t = 0$ is rigorously equal to the amplitude of the molecular dynamic response at $t = 0$.[11] Proper analysis of the data requires consideration of the coherent coupling contribution. The probe transmission as a function of time delay is curve A in Figure 1, and the pulse autocorrelation function, measured by noncolinear second harmonic generation,[12] is curve B. For removal of the coherent coupling contribution, the magnitude of curve B at $t = 0$ is made equal to half the magnitude of curve A at $t = 0$ and curve B is subtracted from curve A. The result, curve C in Figure 1, is the molecular response. The amplitudes of the curves in Figure 1 are normalized to the same peak value.

Figure 2 shows a semilogarithmic plot of the data of curve C. The data clearly show that the bleaching recovers in two steps. The initial rapid decay has a shape that is identical with the pulse autocorrelation function. This is evidence that the initial, partial recovery occurs in a time that is short compared to the 0.5-ps pulse width. The slower decay, evident at longer times, corresponds to complete recovery with an exponential time constant of 1.6 ± 0.2 ps. The error limit is the standard deviation of seven different measurements of the transient bleaching. The fast decay may be due to relaxation to an intermediate state which has a different cross section for probe pulse absorption than the initially prepared state. If the absorption cross sections of the initially prepared and the intermediate relaxed states were known, the rate of the fast decay process could be calculated from the amplitude of the rapid decay contribution. Since the cross sections are not known, however, only an upper limit of 0.5 ps may be determined for the rapid decay process. The intermediate state relaxes and the system recovers completely with a 1.6-ps time constant.

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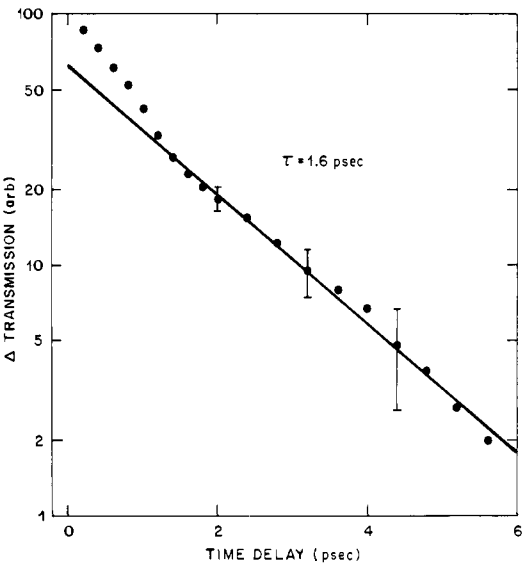

Figure 2. Semilogarithmic plot of the molecular response (curve C of Figure 1). The initial rapid decay is in shape identical with the pulse autocorrelation function; the slower component is attributed to the reverse charge-transfer process.

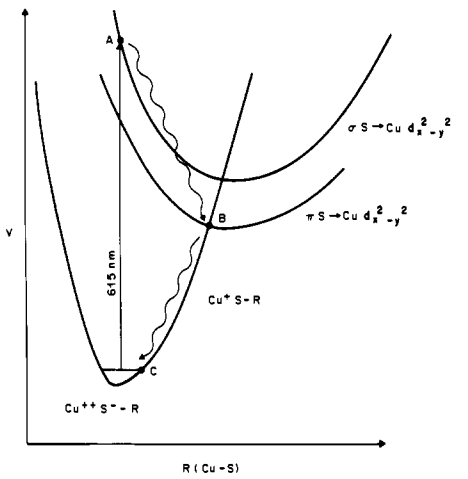

Figure 3. A schematic of a model of the pathways of a charge-transfer absorption followed by a reverse charge transfer at point B and deactivation. The process A → B indicates internal conversion/vibrational relaxation.

Discussion

The charge transfer accompanying the absorption can be described by eq 1 and the radiationless relaxation process by eq 2.

$$R\text{–}S^- Cu^{2+} + h\nu \rightarrow R\text{–}S\cdot Cu^+ \quad (1)$$

$$R\text{–}S\cdot Cu^+ \rightarrow R\text{–}S^- Cu^{2+} + \text{phonons} \quad (2)$$

Thus, the relaxation is characterized by a reverse charge-transfer transition. The process is shown schematically in Figure 3. The electronic level reached via the red absorption band is the second excited charge-transfer state of azurin.[3,4] As indicated, the Cu–S bond distance is expected to be elongated in the excited charge-transfer states.[3] The overall relaxation process, following excitation to A, will require vibrational relaxation and perhaps internal conversion to the lowest charge-transfer state, followed by reverse charge transfer by crossing to the ground state (shown schematically at B), and vibrational relaxation within the ground state (B → C). Vibrational relaxation and internal conversion between excited states in large molecules can occur on a subpicosecond time scale.[13,14] For azurin, the similar electron distributions of

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the lowest two charge-transfer states and an expected small energy gap between them[15] supports the interpretation of the fast decay component as relaxation to the lower, $\pi S \rightarrow Cu\ d_{x^2-y^2}$, charge-transfer state. The crossing to the ground electronic state (eq 2) involves a larger rearrangement of ligands due to the change in electrostatic interaction between Cu and S and probably a larger energy gap and so is expected to be slower and rate determining. The observed 1.6 ± 0.2-ps decay is thus interpreted as the reverse charge transfer, eq 2. The reverse charge-transfer process could also involve excited ligand field states of Cu^{2+} which lie lower in energy than the $\pi S \rightarrow Cu\ d_{x^2-y^2}$ charge-transfer state.[3]

Why is this rate of back charge transfer so fast? One might well ask why it is so slow. The Cu-S bond distance is about 2 Å,[16] and a valence electron of typical energy requires about 10^{-16} s to travel from one atom to another. However, by charge transfer one means a transition from a state largely localized on one atom to a state largely localized on another. The rate of reverse charge transfer can be understood in the following way. The Cu-S vibration frequency is $1.2 \times 10^{13}\ s^{-1}$,[3] and the rate of charge transfer is $(1.6 \times 10^{-12})^{-1} = 6.3 \times 10^{11}\ s^{-1}$. The probability of charge transfer during a vibrational period is the ratio of these two rates (i.e., ~0.05); this is about what one obtains by inserting typical parameters into the Landau-Zener formula for the probability of an allowed crossing from one electronic state to another during a collision. Alternatively, the reverse charge-transfer rate can be explained by using multiphonon models for electron transfer[17,18] and a range of parameters typical for such models. Measurement of the reverse charge-transfer rate at other temperatures would be helpful in ascertaining the validity of these models and in fixing the parameters contained in them. The only previous measurement[19] of a back charge-transfer rate was in an aqueous solution of a diruthenium complex ion in which the electron was transferred between two ruthenium ions. The time measured was 2 orders of magnitude longer than that in azurin. The reason may be that solvent reorganization, absent in azurin, may cause a small increase in the potential barrier for electron transfer within the ruthenium complex.

The very fast spatial relaxation of the copper center implied by its very fast electronic relaxation has biochemical implications. In vivo, azurins and other proteins of its family exchange electrons with other metalloproteins, in particular, cytochromes. Rate constants found for this exchange[20] are in the range 5×10^5–$4 \times 10^7\ M^{-1}\ s^{-1}$. These rates are among the fastest known for protein electron-exchange reactions but are still one or more orders of magnitude slower than the rate of diffusion. The redox process involves a translational diffusion, a rotational diffusion so the proteins will fit in the best possible way, and a structural fluctuation which allows the two metal atoms to approach more closely than usual, followed by the electron jump. The change in electrostatic interaction between the Cu center and the cysteine S is larger upon excitation of the charge-transfer transition than when an external electron is captured by the Cu^{2+} and so the ligand rearrangement may be greater in the case of charge-transfer excitation (eq 3).

$$Cu^{2+}\cdot S^- \xrightarrow{h\nu} Cu^{+}\cdot S \qquad Cu^{2+}\cdot S^- \xrightarrow{e^-} Cu^{+}\cdot S^- \tag{3}$$

The picosecond experiment implies that the rearrangement of ligands around the Cu when its oxidation state changes is facile and is not rate determining for the electron transfer.

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Quenching of Pyrene Fluorescence by Single and Multivalent Metal Ions in Micellar Solutions

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Abstract: The temporal characteristics of the quenching of pyrene fluorescence in micellar solution, by several metal ions, are shown to depend on the charge and type of metal ion. The quenching by Tl^+ and Ag^+ is pseudo-first-order and dependent on the micelle concentration. For bivalent metal ions the fluorescence quenching curves show a two-component decay behavior. The biexponential decay behavior is dependent on the micelle concentration, the ionic strength of the solution, and the bivalent ion used. It is shown that some of these effects can be accounted for by assuming that micelle-micelle collisions allow the transfer of a bound metal ion from one micelle to the other. Intramicellar quenching constants were obtained for the quenching of excited pyrene by Cu^{2+} and Eu^{3+} ions in sodium dodecyl sulfate and sodium dodecyltrioxyethylene sulfate micelles and found to be dependent on both the metal ion and the micelle. From the experimental results it is also shown that bound metal ions are distributed among the micelles according to Poisson statistics.

Introduction

Ionic micelles, like charged colloids and ion-exchange resins, have the property of being able to bind oppositely charged ions. This aspect of micelles has been used in conjection with their ability to solubilize hydrophobic molecules to study a variety of reactions. For example, it has been shown that adsorbed metal ions on anionic micelles can act as efficient electron acceptors from excited micelle solubilized species,[1] can promote the rate of phosphorescence in some aromatic hydrocarbons,[2] and can "catalyse" metal/ligand

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