JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Solid-State Electron Transport across Azurin: From a Temperature-Independent to a Temperature-Activated Mechanism

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ABSTRACT: The temperature dependence of current−voltage values of electron transport through proteins integrated into a solid-state junction has been investigated. These measurements were performed from 80 up to 400 K [above the denaturation temperature of azurin (Az)] using Si/Az/Au junctions that we have described previously. The current across the ∼3.5 nm thick Az junction was temperature-independent over the complete range. In marked contrast, for both Zn-substituted and apo-Az (i.e., Cu-depleted Az), thermally activated behavior was observed. These striking temperature-dependence differences are ascribed to the pivotal function of the Cu ion as a redox center in the solid-state electron transport process. Thus, while Cu enabled temperature-independent electron transport, upon its removal the polypeptide was capable only of supporting thermally activated transport.

Inter- and intramolecular electron transfer (ET) across organic molecules and proteins in solution is a widely investigated field of research, and the parameters determining k_{ET}, the rate constant for ET between a donor (D) and an acceptor (A), have been widely studied both theoretically and experimentally.[1−3] These parameters include $H_{DA}(l)$, the electronic coupling strength between D and A, which in turn depends exponentially on the D−A separation distance l; E_a, the activation energy of the process; and the temperature, T:[1]

$$k_{ET} \propto H_{DA}^2(l) \exp(-E_a/k_B T) \quad (1)$$

where k_B is Boltzmann's constant. In many experimental studies of the ET properties of organic and biological molecules, the distance "l" separating the D and (optically detected) A[4] is varied (in contrast to solid-state organic molecular electronics, where "l" is the distance between the electrodes, which can be varied by altering the molecular length). This approach requires elaborate modifications of the sample under examination.

Carrying out similar studies of electron transport across proteins (ETp) in a solid-state configuration is problematic because in the sandwich sample configuration that is often used, the relevant protein dimension sets the ETp distance, as is also the case for room-temperature, solid-state, single- or few-molecule ETp measurements on azurin (Az) and other proteins.[5] Another experimental way to study the mechanism of ETp through proteins is to measure current (I) and voltage (V) as a function of temperature, as done in the present study of Az.

In several studies,[6−8] the temperature dependence of ET has been investigated by freezing a protein solution in a glassy state. We are aware of one report of solid-state-like $I-V-T$ measurements on a dry-film (rather than a monolayer) protein sample.[9] More results have been reported for better-defined samples of organic molecules,[10] polymers,[11,12] and DNA in a solid-state configuration.[13] We recently reported results of ETp measurements on Az incorporated in a monomolecular layer of a solid-state electronic junction.[14] Measurements of $I-V$ characteristics as a function of temperature in this configuration are relatively straightforward. However, immobilization of the "dry" protein for these measurements limits the technique to proteins that do not denature under such conditions. Such is the case for Az, which under the solid-state conditions used here has shown optical absorption and fluorescence spectra indicating that the native conformation is maintained even when only tightly bound water is retained.[14] Such stability for varying times (from minutes to years) has been reported for several proteins.[15]

Junction formation was carried out as previously described.[14] In brief, p^{++}-Si covered with ∼1 nm silicon oxide (SiOx) provided a conducting substrate with a very smooth surface (rms roughness <0.2 nm) to which Az was coupled covalently via a monolayer of (3-mercaptopropyl)trimethoxysilane (MPTMS) that self-assembled on the SiOx. The use of MPTMS yielded an oriented assembly of Az on the surface via S−S bonds. The protein monolayers were characterized by atomic force microscopy (AFM) and ellipsometry. Ready-made Au pads were then deposited to complete the junction, without damaging the soft protein monolayer. The method used was lift-off, float-on (LOFO),[16] which eliminates the possibility of Au metal penetration through the monolayer or thermal damage to the protein, as is likely with direct metal evaporation. Use of LOFO produced reproducible junction $I-V$ characteristics and a ∼50% yield of such samples (over more than 100 different junctions). The "sandwiched" sample was placed in a variable-temperature probe station (Desert) evacuated to 0.1 mbar and connected to femtoampere $I-V$ measurement electronics (Keithley). The sample and probe temperature was varied between 80 and 400 K and controlled to within ±0.1 K. Measurements were carried out after thermal equilibration had been reached. Control experiments on SiOx/MPTMS/Au junctions without the protein yielded currents in the linear regime of the $I-V$ plot (+50 mV) that were constant over the whole temperature range (i.e., temperature-independent) (see the × data in Figure 1). Remarkably, as Figure 1 shows, for the immobilized Az-based junction we observed temperature-independent ETp (○) with

Received: November 7, 2010
Published: February 4, 2011

dx.doi.org/10.1021/ja109989f | J. Am. Chem. Soc. 2011, 133, 2421–2423

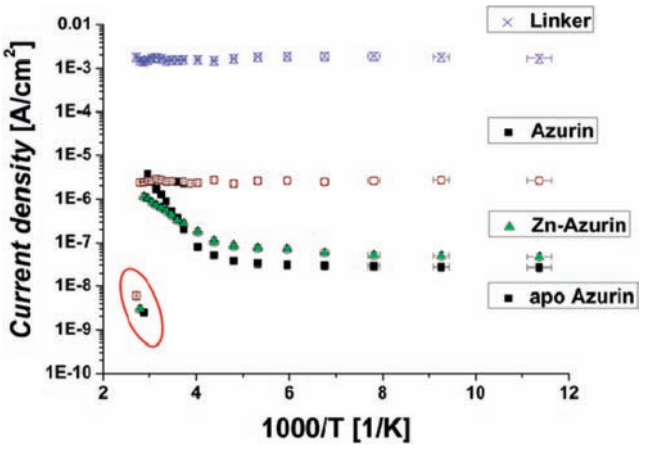

Figure 1. Current density at 50 mV (i.e., in the linear $I-V$ regime) plotted on logarithmic scale as a function of inverse temperature for holo-Az, apo-Az, and Zn-Az junctions. Also shown are the results of measurements on the junction without Az but with the MPTMS linker (×), which exhibited temperature-independent ETp. Similarly, the current across the holo-Az-based junctions was independent of temperature (○) until an irreversible sharp decrease occurred near the Az denaturation temperature. The currents across junctions with apo-Az (■) and Zn-Az (▲) were temperature-dependent down to 200 K; for $T < 200$ K, no significant temperature dependence was measured. In addition, the currents for apo-Az and Zn-Az decreased sharply and irreversibly near 350 K, corresponding to protein denaturation. The red circle shows the currents for the denatured proteins.

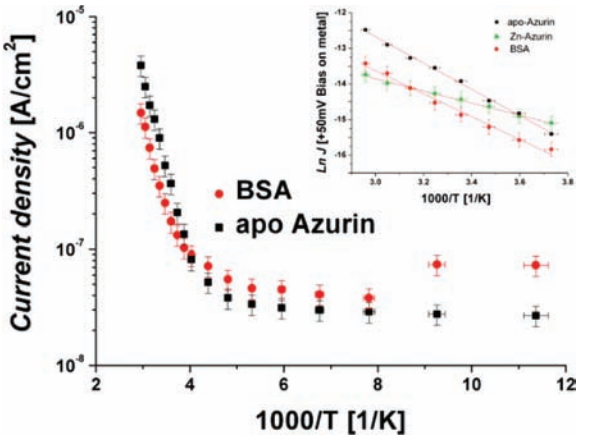

Figure 2. Current density at 50 mV (i.e., in the linear $I-V$ regime) plotted on logarithmic scale as function of inverse temperature for BSA and apo-Az junctions. The two junctions behaved similarly down to ~120 K. The activation energies (see the Arrhenius plots in the inset) for apo-Az, BSA, and Zn-Az are 320, 280, and 150 meV, respectively. To focus on the temperature-dependence effect, the very low currents after denaturation at high temperature are outside the scale and not shown.

currents attenuated by over 3 orders of magnitude relative to the control junction, as expected from the addition of an extra separating layer (i.e., the protein). Heating the sample above 360 K caused a sharp irreversible drop in the current, which corresponds well with the known Az thermal denaturation process that occurs at ~360 K. This result indicates that the observed ETp via Az requires its native state. Further heating of the sample up to 400 K did not significantly change the currents from their 360 K values (data not shown).

To assess the role of the Cu redox center of Az in ETp, its metal-free form (apo-Az) and Zn-substituted form (Zn-Az) were prepared using a published procedure[17] and $I-V-T$ measurements on apo-Az and Zn-Az junctions were carried out. Cu depletion or Zn substitution cause rather minor changes in the protein's three-dimensional structure, as determined by both crystallography and spectroscopy.[18]

The effect of Cu removal or its substitution by Zn on the $I-V$ behavior was dramatic in that the ETp changed from temperature-independent for the holo-Az junction to temperature-activated. Only below 200 K did apo-Az and Zn-Az junctions also show temperature-independent ETp (see the ■ and ▲ data in Figure 1). Cu removal or Zn substitution hardly affect the proteins' structure.[18] Based also on thermal stability studies[19] over the temperature range studied here (except after denaturation for $T \gtrsim 350$ K), we postulate that Cu removal or its substitution by Zn changes the ETp mechanism over the complete temperature range studied.

The temperature dependence of the current for the apo-Az and Zn-Az junctions is similar to that for bovine serum albumin (BSA). BSA, which is also a barrel-shaped protein, forms monolayers with its short axis perpendicular to the surface, so its barrel diameter, which is similar to the height of Az, determines the monolayer thickness. BSA has no known ET activity. We therefore hypothesize that the observed conductances via BSA, apo-Az, and Zn-Az may represent some type of general ETp ability of proteins (Figure 2).

While the temperature independence of ETp over a range of physiological temperatures can be rationalized by evolution-selected stabilization of ET against temperature fluctuations, it is more difficult to rationalize the observations much below physiological temperatures. The observed temperature independence of ETp across Az differs from results of the earlier solution ET studies[7,8,20−22] in that in the present experiments, ETp occurs across the whole protein length in a "dry" configuration rather than via part of it. In the present experiments, transport occurs over a distance (~35 Å) that is at the very limit of the range over which single-step tunneling can be invoked in nonconjugated systems. We can surmise that ETp via Az presents an electron-relay system with a sequence of exquisitely fine-tuned, near-activationless transport steps involving Cu and its ligands, as long as no major structural changes in the protein occur. Once this structure is modified, the transport becomes "normal" for such a thick barrier, i.e., thermally activated. The thermally activated ETp behavior of apo-Az and Zn-Az can be understood as a result of hopping, probably affected by thermal fluctuations in the protein.

The similar ETp observed for the latter and BSA junctions suggests that proteins without a redox-active site exhibit a temperature-dependent yet markedly lower ETp. For these systems, we found that from 200 to 350 K, the current at 50 mV obeys an Arrhenius relation with activation energies of 320 meV (31 kJ/mol) for apo-Az, 150 meV (14.8 kJ/mol) for Zn-Az, and 280 meV (27 kJ/mol) for BSA (Figure 2 inset). These proteins showed temperature-independent ETp below 200 K, a result that is in line with a recent theoretical simulation[23] suggesting a general structural transition in the proteins below 200 K to a glasslike state, which may affect the ETp. Moreover, ETp across BSA showed a reproducible "jump" in the current magnitude at ~120 K. Because our measurements were not only sensitive to current magnitude but also to structural changes (denaturation), we can speculate that this change in current occurred because of intrinsic modifications in this protein. We note

dx.doi.org/10.1021/ja109989f | J. Am. Chem. Soc. 2011, 133, 2421–2423

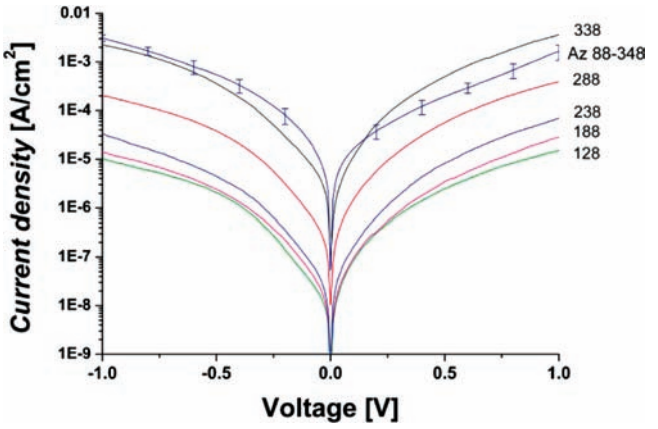

Figure 3. Current density plotted on a logarithmic scale vs voltage for apo-Az at different temperatures. The temperature dependence at the high temperatures can be seen even at biases higher than twice the activation energy of the protein. For comparison, the $I-V$ plot for holo-Az, with its standard deviation, is also shown for all temperatures measured.

that the ETp of apo-Az and Zn-Az at low temperatures had values more than 2 orders of magnitude smaller than those of Az, although all were characterized by similar conformation and thickness. Since it is reasonable to assume that a tunneling mechanism is dominant at low temperatures, it is concluded that the Cu redox center also significantly affects the tunneling mechanism.[24]

The temperature dependences of the current remained unchanged under applied voltages of up to ± 1 V, i.e., well above twice the calculated activation energies. Therefore, the barriers between the protein and the electrode contacts cannot be the cause of the temperature dependence (Figure 3). Figure 3 also shows that the ETp for holo-Az remained temperature-independent up to an applied bias of ± 1 V. We note that all of our results were obtained on large-area samples (0.2 mm^2) containing 10^9-10^{10} protein molecules.

In summary, we have observed temperature-independent electron transport across holo-Az in a solid-state junction over a wide temperature range. Upon removal of the Cu ion or its substitution by Zn, temperature-activated Arrhenius behavior was observed. These observations extend the known function of Cu in this metalloprotein from ET to ETp. It is possible that this temperature-independent ETp reflects the robust functional nature of Az as an ET protein. The thermal activation energies of ETp across apo-Az, Zn-Az and BSA are comparable, which may suggest that these ETp properties are intrinsic to an organized structure of an amino acid polymer devoid of a redox-active site. This possibility is of fundamental interest in our efforts to understand how and why proteins conduct so well. Thus, experiments on additional proteins and synthetic polypeptides aiming to answer these questions are in progress.

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ACKNOWLEDGMENT

We thank Dr. Y. Selzer for fruitful discussions and the Nancy and Stephen Grand Centre for Sensors and Security, the Schmidt Minerva Centre for Supramolecular Architecture, the Israel Science Foundation (D.C.), and the Kimmelman Center for Biomolecular Structure and Assembly (M.S.) for partial support. M.S. holds the Katzir—Makineni Chair in Chemistry and D.C. the Sylvia and Rowland Schaefer Chair in Energy Research.

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