Excitation of the ligand-to-metal charge transfer band induces electron tunnelling in azurin

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Optical excitation of azurin blue copper protein immobilized on indium-tin oxide, in resonance with its ligand-to-metal charge transfer absorption band, resulted in a light-induced current tunnelling within the protein milieu. The related electron transport rate is estimated to be about $10^5\,s^{-1}$. A model based on resonant tunnelling through an azurin excited molecular state is proposed. The capability of controlling electron transfer processes through light pulses opens interesting perspectives for implementation of azurin in bio-nano-opto-electronic devices.
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Nanostructured materials with specific optical properties have been widely investigated in the last years, and many efforts have been devoted to develop optically controlled devices, to be applied, for instance, in controlled drug delivery, optical therapeutics, and biosensing.[1] Within this context, nanosized proteins, which are endowed with optical and electron transfer (ET) properties, are highly promising, since they may combine peculiar optoelectronic capabilities with biocompatibility.

Among these, Blue Copper Proteins (BCPs) are particularly interesting, since they are ET proteins characterized by unique spectroscopic properties,[2,3] showing remarkable biorecognition abilities[4,5] and efficient electron conjugation within hybrid nanostructures.[6–8] Moreover, both the ET mechanism and the optical characteristics of BCPs are tightly connected with the vibrational modes involving their metal-containing active site. Indeed, BCPs contain a copper ion that is able to switch between two different oxidation states (Cu^{++}/Cu^{+}) and that shows an "entatic" state arising from the peculiar distorted tetrahedric symmetry of the metal-ligand field. Therefore, the corresponding phonon modes are able to modulate the metal ion coordination geometry, resulting in a subtle tuning of the protein redox potential, and in the consequent thermal, physiological ET.[9,10] Moreover, BCPs are endowed with an intense Ligand-to-Metal Charge Transfer (LMCT) absorption band in the visible, whose excitation activates the peculiar phonon modes of the active site and, to a minor extent, of the molecular milieu, as shown by Resonant Raman spectroscopy.[11–13] It has been thus hypothesized that the optical excitation of the LMCT band with visible light could induce, in turn, the activation of phonon-assisted ET processes, mimicking the physiological one.[13,14]

Within this context, we have explored the effect of the LMCT band excitation on the electron tunnelling through Azurin (AZ; Sigma-Aldrich Co.), a widely investigated BCP characterized by an intrinsic robustness,[15,16] rectification capabilities,[17] and specific molecular pathways underlying its intramolecular charge transfer.[9,18,19] AZ has been assembled (50 μl of 100 μM solution in acetate buffer, pH 4.6) on a surface of indium-tin-oxide (ITO), which is a semiconductive material able to fully preserve the protein optical properties and activity.[20] The experiments have been carried on with the home-assembled apparatus schematically sketched in Fig. 1(a). The current tunnelling through the AZ proteins has been measured by Scanning Tunnelling Microscopy (STM). The proteins have been illuminated by a pulsed laser source in resonance with the LMCT transition (635 nm wavelength), and the STM signal has been recorded at the laser pulsation frequency by a lock-in amplifier (LIA), to select the tunnelling current component synchronous with the light.

Tunnelling currents, as measured by STM in the presence of illumination, may be crucially affected by light-induced thermal effects on the tip elongation, which significantly depend on the incident light power, the pulsation frequency, and on the tip absorption coefficient.[21,22] Accordingly, we have carried out some detailed preliminarily tests to evaluate the incidence of these experimental conditions on the photo-induced current. We found that a light power increase above 0.15 mW resulted in a tip thermal expansion, exceeding the STM sensitivity in the z direction (10^{-10} m). This would have made difficult to decouple thermal from protein-mediated effects on the photocurrent. On the other hand, setting the light power below 0.10 mW led to a compromised photocurrent signal-to-noise ratio. We decided then to work at 0.10 mW and a pulsation frequency above kHz (see Fig. 1(b)). Under these conditions and by using Au tips, which show low absorption coefficient, the local temperature increase at the molecular site under the STM tip (as sketched in Fig. 1(c)) was estimated[22] to be negligible (around 10^{-3} K), thus not implying any protein damage.

Fig. 2(a) shows that illumination of the AZ/ITO samples results in a current of 18 ± 2 fA tunnelling through the proteins, which decreases to 4 ± 2 fA when the light is off. The latter value is comparable with the background signal registered, at the same conditions, between the STM tip and the bare ITO surface independently from the illumination conditions, as shown for comparison in Fig. 2(b). Thus, the optical excitation of AZ molecules, in resonance with their LMCT band, enables the proteins to sustain a light-induced electron tunnelling current, over the STM set point current.

The intensity of this light-induced current has been mapped over the sample surface, in connection with its

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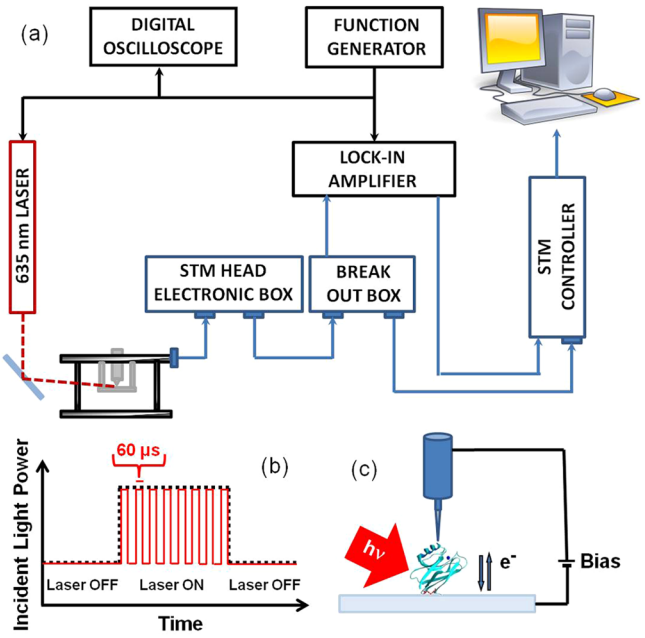

FIG. 1. (a) Schematic representation of the experimental apparatus: the current measured by the STM (PicoLE 5100, Agilent, USA) is extracted by a break out box and compared by the lock-in amplifier (SR530, Stanford Research, USA) at the light modulation frequency of a pulsed laser (635 nm, 1 mW, model Premier-PWM, Global Laser, UK), which is driven by a square wave provided by the function generator. (b) The laser light is mechanically switched (black, dotted line); when the laser is on, the sample is illuminated by light flashes, separated by equivalently long dark periods (red, continuous line). In order to reduce thermal expansion tip effects, the light has been pulsed at 8 kHz, resulting in 60 μs light flashes. (c) Detail of an AZ molecule assembled on the ITO surface under light irradiation, with the STM tip on top.

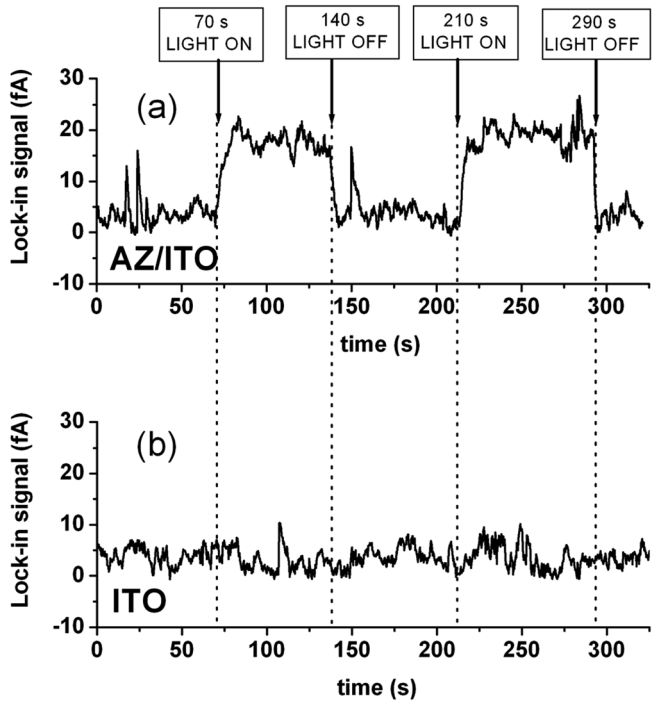

FIG. 2. Lock-in amplifier output signal recorded as a function of time and at different illumination conditions, corresponding to the STM current tunnelling from the tip to the ITO surface through the AZ molecules (a) and from the tip to bare ITO (b). STM parameters: $V_{bias} = 1.0$ V, $I_{setpoint} = 60$ pA. Lock-in parameters: light frequency $\nu = 8$ kHz, integration time constant $\tau = 3$ s, sensitivity $\sigma = 5\,\mu$V.

topography.[23] Fig. 3 shows the STM topography images of an AZ/ITO sample acquired at light-on (Fig. 3(a)) and light-off (Fig. 3(b)): both appear rough, with a number of circular structures whose dimensions almost correspond to the nominal protein size,[4] and no significant differences are visible as a function of the illumination. Moreover, the current tunnelling pattern appears more dense at specific sample locations when the light is on (Fig. 3(c)), with respect to what is observed when the light is off (Fig. 3(d)). If the corresponding current and topography maps are superimposed, the current spots observed at light-on essentially match with the circular features appearing in the corresponding topography image (see the coloured circles in Fig. 3(e)). Such an analysis, already successfully applied to resolve blurred scanning probe microscopy images,[7] has allowed us to detect single AZ molecules onto the rough ITO surface.

Let us now make some quantitative considerations on the light-induced electron tunnelling. By positioning the STM tip at sample points where current spots corresponding to single AZ proteins (Fig. 3) appear, a mean current of 18 ± 2 fA has been measured (see Fig. 2(a)). This value corresponds to an electron tunnelling rate of $(1.1 \pm 0.1) \times 10^5\,s^{-1}$, as obtained by dividing it by the net charge of a single electron (1.6×10^{-19} C). We remark that the optical excitation of AZ is somewhat responsible for the measured light-induced electron tunnelling within the protein milieu. If we consider that the protein is illuminated around 8×10^3 times per second by light flashes of 60 μs each (see Fig. 1(c)), the above estimated tunneling rate value could be

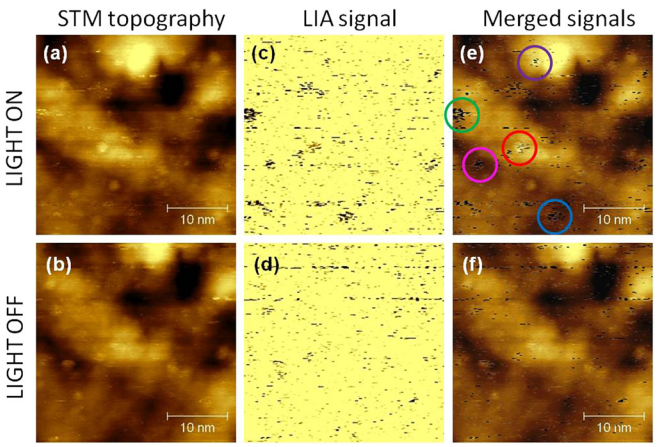

FIG. 3. AZ/ITO STM topography images (a) and (b), LIA output signal maps [(c) and (d): the darker the pixel, the higher the current] and their superimposition [(e) and (f)], from the same sample area, acquired with [(a), (c), and (e)] and without [(b), (d), and (f)] 635 nm laser illumination. STM parameters: $V_{bias} = 1.5$ V, $I_{setpoint} = 60$ pA, scan rate = 2.0 Hz. Lock-in parameters: $\nu = 8$ kHz, $\tau = 1$ ms, $\sigma = 200\,\mu$V.

achieved if the protein can be excited more than 14 times during every light flash. Such a value could be reasonable if we consider that the characteristic timescale of the AZ optical excitation/relaxation processes has been found to be much faster than the light flash duration (the decay time constant of the AZ excited state has been measured to be about 270 fs (Ref. 13)).

We therefore propose the occurrence of a single-electron tunnelling process in resonance with an optically excited molecular state (AZ*) of AZ, as it is schematically depicted in Fig. 4. The AZ molecule, bearing the copper ion

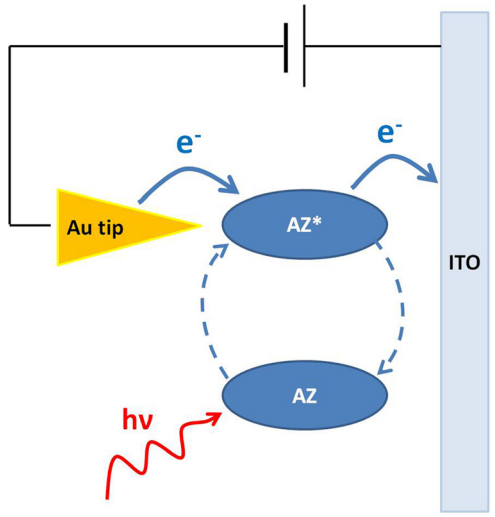

FIG. 4. Schematic representation of the light-induced tunnelling mechanism proposed in the AZ/ITO system.

in its native oxidation state (Cu^{++}), is assembled on the ITO surface and, upon absorption of the 635 nm radiation, switches to the AZ* state. Such a state may favour the electron tunnelling from the Au tip to the ITO substrate, consistently with the positive bias applied, and then the protein molecule decays, recovering the ground state. Indeed, a more favourable energy level alignment within the tip-AZ*-ITO system, with respect to that obtained with the ground state AZ, could be invoked as being responsible for the increased tunnelling probability through the excited state, as previously observed for other systems.[24]

On the other hand, if we take into account that the thermal ET in AZ involves the active site phonon modes,[9,10] and that the optical absorption in resonance with the AZ LMCT band is able to excite the relevant AZ phonon modes,[12,13] we could also hypothesize that the optical excitation might result in a phonon-assisted electron tunnelling within the protein milieu. This could lead to the opening of specific, efficient, intramolecular electron pathways, probably involving the copper-containing active site, or even to the mimicking of the thermal AZ ET process, as previously observed for photosynthetic proteins in analogous experiments.[25] In this connection, it is interesting to note that the electron tunnelling rate that we observed under light excitation of AZ is significantly higher that the thermal ET rates previously measured by electrochemistry for bulk AZ molecules assembled on electrode surfaces that range from $(8 \pm 4)\ s^{-1}$ to $(6 \pm 3) \times 10^3\ s^{-1}$.[26–29]

In conclusion, we have observed a light-induced electron tunnelling through single AZ molecules, when they are illuminated in resonance with their LMCT absorption band. The corresponding measured current is consistent with an electron tunnelling rate of $(1.1 \pm 0.1) \times 10^5\ s^{-1}$. The resonant tunnelling model proposed invokes a more favourable energy level alignment of the excited AZ molecule within the tip-AZ-ITO system, with respect to the ground state protein. We could also speculate about the possible opening of additional phonon-assisted electron pathways or even the activation of a phonon-assisted ET process mimicking the thermal one. While these latter aspects require further investigation, we believe that the control of the tunnelling current in ET proteins by using visible light could be a crucial step for developing new bio-optoelectronic devices of biomedical interest.

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