COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Evidence for spatially-coherent trans-molecular electron tunnelling through two-dimensional arrays of Photosystem II core complexes

Philip B. Lukins* and Christopher S. Barton
School of Physics A28, University of Sydney, NSW, 2006 Australia

Received (in Cambridge, UK) 30th October 2002, Accepted 23rd January 2003
First published as an Advance Article on the web 3rd February 2003

Localised spatially-coherent electron tunnelling through single Photosystem II complexes and into atomically-flat graphite is observed.

Photosystem II (PS II) is responsible for light-to-electrochemical energy conversion and water oxidation in photosynthesis. Consequently, the structure and functional properties of PS II have been of interest for many decades. The three-dimensional structure of PS II has been determined by electron crystallography with 0.6 nm resolution[1] and, more recently, by X-ray crystallography with 0.38 nm resolution.[2] There have also been several earlier electron microscopy studies including those on two-dimensional crystals or arrays of PS II.[3–5] Single-molecule scanning tunnelling microscopy (STM) of PS II[6–8] has also been used to determine the supramolecular organization in the protein complex and characterize the electron conduction processes in the key molecular components. An attraction of the later technique is that subtle molecule processes such as tunnelling phenomena, conduction processes and surface vibronic effects can be studied. The combination of the unusual electronic properties of PS II, the feasibility of preparing highly-ordered two-dimensional crystals of PS II in which the PS II complexes are essentially non-interacting, and the ability of STM to probe conduction processes in biomolecules provides an opportunity to study trans-molecular electron conduction in well-defined single protein complexes. Here, we present evidence for localised and spatially-coherent trans-molecular electron tunnelling and conduction through PS II. These phenomena may have implications for conduction in PS II aggregates and artificial photosynthetic analogues.

Oxygen-evolving PS II dimeric core complexes were prepared by the procedure of van Leeuwen *et al.*[9] and deposited onto single-crystal graphite by a process similar to electrically-assisted Langmuir–Blodgett film deposition. We formed the dense film across the substrate by movement and contraction of a droplet of aqueous PS II solution exposed to a fixed 1 V electrical bias to achieve the desired film orientation in a fashion analogous to that used by Yasuda *et al.*[10] who employed a conventional LB trough. STM was carried out under ambient conditions using a Burleigh STM system and composite current–height scan techniques similar to those used previously for single-molecule PS II imaging.[6] The reproducibility of the results was limited by non-uniformity and amorphisation of the electrodeposited PS II 2D crystals. Of the several highly-ordered 2D crystals produced, all gave consistent STM images and tunnelling behaviour with the results in Fig. 1 being for images with minimised scan noise.

As seen from the STM image in Fig. 1a, PS II core complexes form highly regular arrays that closely resemble the 2D crystals observed by others using electron microscopy.[3–5] The edges of these arrays typically align to steps, edges and terraces in the underlying graphite substrate and extend over several microns across atomically-flat regions of the graphite.

A closer view (Fig. 1b) shows the dimeric structure with two resolved monomer units. The packing of the dimers in the arrays is approximately hexagonal with densities of $\sim 2.6 \times 10^{-3}$ dimers per nm^2 which are slightly lower than those usually found for PS II 2D crystals used in electron microscopy studies. Single PS II core dimer complexes have lateral dimensions of 18.3×9.7 nm which are consistent with previous crystallography[1–5] and STM[6] studies. The complexes are ~ 10 nm thick which is similar to the electron microscopy result of Haag *et*

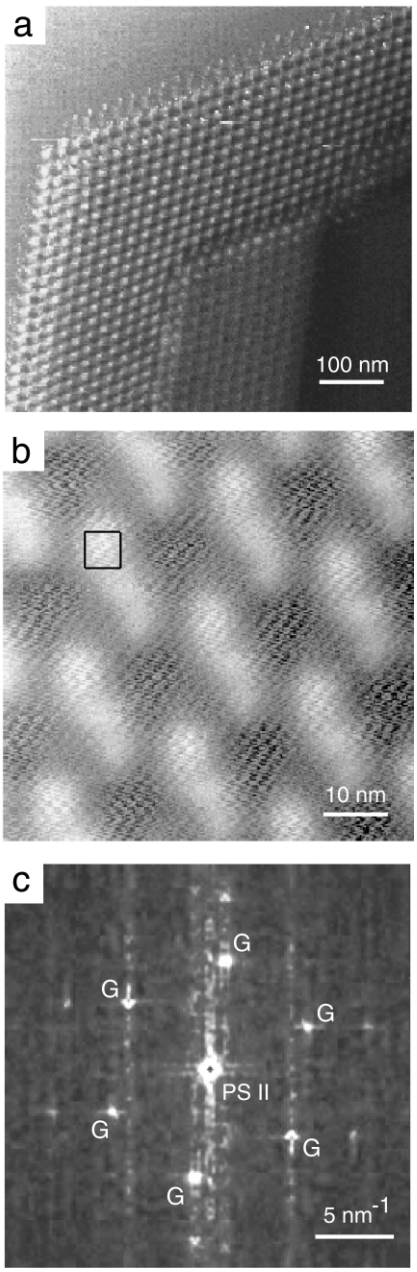

Fig. 1 STM images of two-dimensional arrays of PS II core dimers showing (a) an extended array on a graphite substrate, (b) regular packing of highly-aligned dimers and (c) the 2D-FFT of the region bounded by the box in (b) with the graphite (G) and PS II peaks labelled. The current setpoint and tip bias voltage are 1 nA and 20 mV respectively.

DOI: 10.1039/b210596a

al.[11] Although the resolution of the STM images for these arrays (~1 nm) is reduced compared to that which we have seen previously[6] for single-molecule STM imaging (0.3 nm), the imaging under these tunnelling conditions show, in addition to the structural nature of the PS II arrays, unusual tunnelling phenomena not seen previously. An intriguing aspect of these STM images of PS II arrays is the fact that the images appear as a convolution of the images of the PS II complexes with those of the optimised imaging for the atomically-resolved (~0.02 nm resolution) single-crystal graphite substrate even though the PS II complexes on the substrate are ~10 nm thick. This effect, which we believe has not been previously reported, is noticeable because of the particular tunnelling conditions which preserved atomic resolution for the graphite but reduced resolution for the PS II which appears very diffuse and therefore offers greater contrast with the underlying graphite.

The 2D Fourier transform of the region defined by the square box in Fig. 1b is shown in Fig. 1c. The FFT contains low-frequency contributions due to the PS II complex together with six hexagonally-oriented peaks characteristic of the regular hexagonal graphite lattice. The vertical and horizontal lines passing through the peaks are partly due to tip–sample interactions. Although these later signals are not desirable, they do provide useful supporting evidence for the existence of the PS II and G peaks. Importantly, this square region lies solely within the boundaries of the PS II dimer complex. This confirms that the image for the bounded region is a convolution of the image of the PS II complex in this region with that of the image of the underlying graphite substrate in this same region. The image can only be interpreted as demonstrating tunnelling through the PS II complex into the graphite below. This is an interesting result since STM is generally considered to be a surface-sensitive technique which probes only the surface or first few sub-surface atomic layers due to the characteristic tunnelling interaction distance of ~0.1 nm. However, in the present case, we see tunnelling through the ~10 nm thick PS II complexes into the graphite surface below in such a way that the spatial distribution of the tunnelling is conserved. Our observation that this trans-molecular tunnelling does not lead to any significant diffuseness of the substrate image indicates that the trans-molecular tunnelling is highly spatially coherent.

We have previously shown[8] that the main electron conduction mechanisms in PS II involve semiconductivity, photovoltaic behaviour, ohmic and non-local conduction, and hopping conduction processes. For the 2D arrays, the PS II orient such that the plane of the dimer is parallel to the substrate surface and the trans-molecular electron conduction is normal to the plane of both the dimer and the substrate. Under the present conditions, the tunnelling current is not only preferentially in the direction normal to the surface, but the tunnelling behaviour has a high degree of spatial coherence or confinement while lateral conduction appears to be suppressed. The proposed mechanism leading to this coherence involves electron tunnelling between amino acids and along the peptide structure including along the trans-molecular α-helices which span the PS II complex. This mechanism is consistent with the structures of PS II from X-ray diffraction[2] and single-molecule STM.[6] There will also be a contribution from semiconduction associated with the primary electron transfer pathway Mn_4-Y_z-P680-pheo-Q_A-Q_B but this effect will be localised and only important in the vicinity of D1/D2. These effects should be observable in other thin self-oriented monolayer systems comprising molecules with uniaxial electron conduction normal to the surface of a substrate which has unique, identifiable and resolvable atomic structure.

We thank the Australian Research Council for financial support.

Notes and references

1 K.-H. Rhee, E. P. Morris, J. Barber and W. Kuhlbrandt, *Nature*, 1998, **396**, 283.
2 A. Zouni, H. Witt, J. Kern, P. Fromme, N. Krauss, W. Saenger and P. Orth, *Nature*, 2001, **409**, 739.
3 A. Holzenburg, M. C. Bewley, F. H. Wilson, W. V. Nicholson and R. C. Ford, *Nature*, 1993, **363**, 470.
4 M. K. Lyon, K. M. Marr and P. S. Furcinetti, *J. Struct. Biol*., 1993, **110**, 133.
5 E. J. Boekema, B. Hankamer, D. Bald, J. Kruip, J. Nield, A. F. Boonstra, J. Barber and M. Rogner, *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 175.
6 P. B. Lukins and T. Oates, *Biochim. Biophys. Acta*, 1998, **1409**, 1.
7 P. B. Lukins, *Biochem. Biophys. Res. Commun*., 1999, **256**, 288.
8 P. B. Lukins, *Chem. Phys. Lett*., 2000, **321**, 13.
9 P. J. van Leeuwen, M. C. Nieveen, E. J. van de Meent, J. P. Dekker and H. J. van Gorkom, *Photosynth. Res*., 1991, **28**, 149.
10 Y. Yasuda, H. Toyotama, M. Hara and J. Miyake, *Thin Solid Films*, 1998, **327**, 800.
11 E. Haag, K.-D. Irrgang, E. J. Boekema and G. Renger, *Eur. J. Biochem*., 1990, **189**, 47.