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Scanning tunnelling spectroscopic imaging of Photosystem II

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Introduction

The current interest in Photosystem II (PS II) structure has led to several studies (Barber 1998; Boekema et al. 1995; Rhee et al. 1997, 1998; Zouni et al. 2001) which aim to determine the high-resolution structure, generally by either electron or Xray crystallography. However, an alternative approach, based on single-molecule imaging of PS II using scanning tunnelling microscopy (STM) and scanning tunnelling spectroscopy (STS), has also been shown (Lukins and Oates, 1998; Lukins, 1999, 2000) to yield structural information on this important biomolecule.

These two types of approaches have different advantages and disadvantages. Crystallography effectively gives atomic density maps which can be interpreted as structures by overlaying plausible molecular configurations or, if the resolution is sufficient, 3D structures directly. STM gives 2D or psuedo-3D projection images of the distribution of electronic density of states which again gives high-resolution structural information. Comparison of structures from these two approaches is normally not a straightforward matter because the contrast mechanisms are very different. Crystallography has the advantages of being a well-established technique that can yield 3D structures. However, there can be issues associated with the assignment of structures and the influence of solid-state interactions due to the crystalline nature of the specimen. STM has the advantages of extremely high resolution imaging of both single molecules and aggregated phases, and the ability to carry out STS with ~ 1 meV energy resolution enabling definitive assignment of certain component structures in the images. Although STM can achieve ~ 0.05 Å vertical resolution, it provides only limited depth resolution due to the confined tunnelling region. The interpretation of STM images, particularly those for biomolecules, can also be complicated. The best resolutions reported to date for PS II are 3.8 Å from Xray crystallography and 3 Å from STM.

Here, we present some selected results on STM/STS of PS II to demonstrate the usefulness of the technique and clarify some aspects of PS II structure.

Materials and methods

PS II membrane fragments and PS II core complexes were prepared using slight modifications of the procedures described by Berthold et al. (1981) and van Leeuwen et al. (1991). Two-dimensional arrays of PS II cores on graphite were prepared by electrodeposition. STM measurements were carried out on Nanosurf EasyScan, Park Autoprobe SPM and Burleigh ISTM systems.

Results and discussion

STM imaging clearly shows that in the isolated-molecule state, PS II membrane fragments and core complexes exist almost entirely in their respective dimer forms (Fig. 1a,b). A statistical analysis based on extensive STM of a wide variety of PS II specimens indicates that >90% of isolated PS II are dimers. The structures and protein assignments for these two PS II types are consistent with electron and Xray crystallographic data. There has been some debate surrounding the precise positioning of CP43 and CP47 but the locations of these proteins as shown in Fig. 1 is consistent with that expoused in most previous reports. As mentioned earlier, direct comparison of STM and crystallographic structures is difficult but there is broad consistency between the two approaches in terms of the salient structural features. The proposed protein organization is supported by similar STM images of CP43-depleted cores (Barton and Lukins, 2001) shown in Fig. 1c. Some differences between free-molecule structures from STM and bound-molecule structures from crystallography are to be expected.

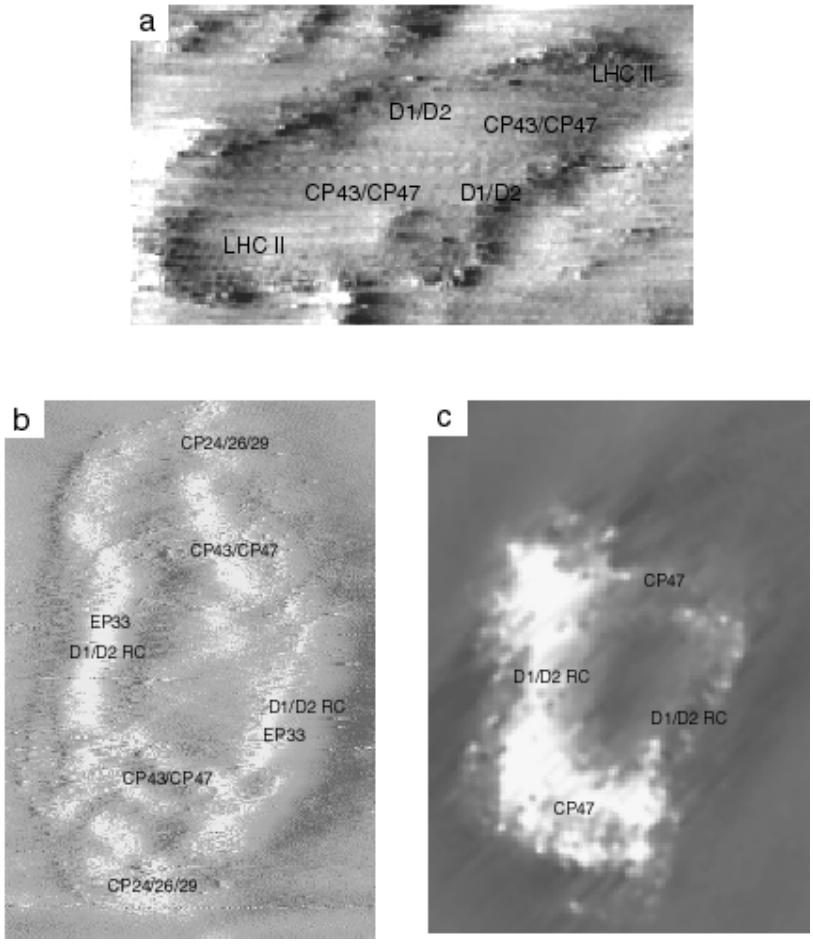

Fig. 1. STM images of single PS II particles and proposed models of the supra-molecular organization. (a) PS II membrane fragments, (b) PS II core complex and (c) CP43-deleted PS II core complex. D1/D2, reaction centre; CPmn, chlorophyll protein of mass mn kDa. Image sizes are (a) 50 x 35 nm, (b) 18 x 25 nm, (c) 18 x 25 nm.

The location of D1/D2 is definitively determined by STS since the spectra for D1/D2 are unique and clearly distinct from all other proteins in the complex. These spectra show the expected semiconductive, photoconductive and photovoltaic behaviour. On the other hand, removal of selected proteins such as LHCII and CP43 has allowed identification of these species. LHCII resides at both ends of the membrane fragment particle while CP43 resides at the ends of the core complex. Other accessory proteins are also seen in the vicinity of CP43 and LHCII. In fact, STM provides a particularly convenient means of observing these accessory proteins.

Similar STM experiments (Lukins and Barton, 2001) on 2D arrays of PS II core complexes gave results (Fig. 2) consistent with electron microscopy data. The structure of individual core particles in these films were also consistent with the isolated molecule structures (Fig. 1b). There were some differences in the packing and film density but we attribute this to the electrodeposition technique used for their preparation.

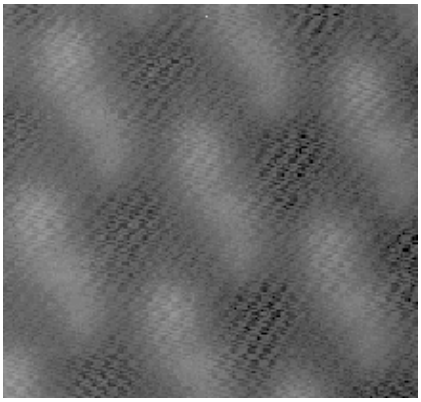

Fig. 2. STM image of a 2D array of PS II core complexes (image size, 60 x 60 nm).

STS provides a powerful tool for single-molecule electronic spectroscopy as well as STM image interpretation. Figure 3 shows the differential conductance, which is related to the electronic density of states, as a function of voltage for a single PS II reaction centre in the dark and exposed to light. The corresponding I(V) spectra are very well described by a simple conduction model which includes a semiconduction term describing the primary electron transfer pathway in PS II together with ohmic, delocalized and hopping conduction terms. This can be expressed by the relation (Lukins, 2000)

$$I = I_0 [\exp (e(V - IR_s) / qkT) - 1] + (V - IR_s) / R_{sh} + AV^2 (1 + BV) .$$

The fitted parameters (dark, excited) are reverse saturation current (1 nA, 2.5 nA), diode quality factor (5, 4), parallel resistance (30 MΩ, 15 MΩ) and series resistance (10 MΩ). These values are similar to those achieved by artificial hybrid organic photovoltaics. This highlights the importance of PS II as a model in biomimetic applications including the development of organic photovoltaic and artificial photosynthetic materials and devices.

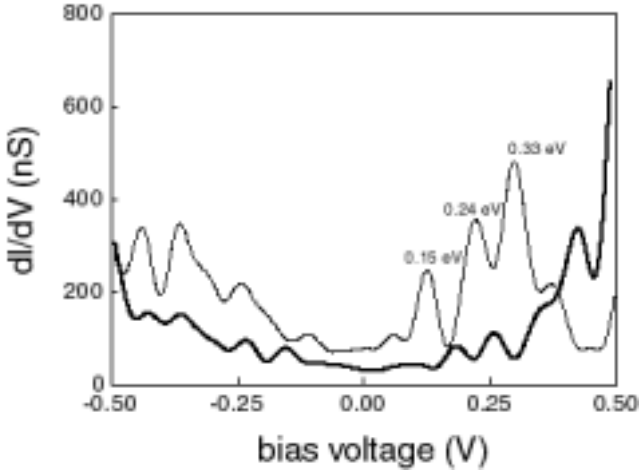

Fig. 3. Differential conductance spectra of a single PS II reaction centre in the dark (thick line) and on illumination with visible light (thin line).

At present, the highly delocalized electronic structure of PS II appears to place a limit of 2 – 4 Å on the attainable resolution using STM. We have recently carried out a thorough evaluation of the sample and instrumental factors influencing performance of STM on a wide variety of specimens including diamond (Lukins et al., 2001) and DNA (Zareie and Lukins, 2001). These systems can now be imaged with 0.2 Å and 0.5 Å resolution respectively. We are therefore confident that further resolution improvements on PS II are possible using our more recent STM/STS techniques.

Acknowledgements

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