

BRIEF COMMUNICATION

Localisation of CP43 in single molecules of photosystem 2 using protein deletion and scanning tunnelling microscopy

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Abstract

Scanning tunnelling microscopy of intact D1/D2/CP47/CP43 photosystem 2 (PS2) core complexes and CP43-deleted D1/D2/CP47 core complexes shows definitively that the CP43 subunits reside at the ends of the dimeric core complex. The CP43-removal procedure produces CP43-deleted cores with minimal conformational distortion to the D1/D2/CP47 residual core complex. There was excellent agreement between the X-ray and STM structures for the intact core complex, and between the STM image for the CP43-deleted core complex and the X-ray model with the components assigned to CP43 omitted.

Additional keywords: CP43 deletion; model; STM; X-ray structure.

The current interest in photosystem 2 (PS2) supramolecular structure has led to several studies (Rhee *et al.* 1998, Hankamer *et al.* 1999, 2001, Zouni *et al.* 2001, Kamiya *et al.* 2003, Ferreira *et al.* 2004) which aim to determine the high-resolution structure, usually by either electron or X-ray crystallography. However, an alternative approach, based on single-molecule imaging of PS2 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. Both crystallography and STM have yielded structures with ~0.3 nm resolution. However, definitive and direct visualisation of the positioning of the CP43 subunits has not previously been achieved because the crystallographic and probe techniques have relied, to some extent, on biochemical evidence together with overlaying plausible supramolecular structures onto the density maps or images obtained. Specific protein deletion studies in conjunction with these structural techniques would have the advantage that the position and boundaries of the deleted fragment could be determined definitively. Apparently, the only previous CP43-deletion study of PS2 was that by Eijkelhoff *et al.* (1997) who obtained electron crystallography data for PS2 core dimer complexes with and without CP43 present. However, the resolution of ~2.5 nm and the small density changes

observed made precise location of the subunits difficult. The use of multiparticle averaging can also introduce uncertainties due to contributions from dissimilar particles unless sample purity is high. On the other hand, STM offers both high resolution (~0.3 nm) and true single-molecule imaging (as distinct from "single particle analysis" in crystallography which still involves diffraction from many molecules in the sample). For both these reasons, STM is a natural choice of imaging technique for use in protein-deletion studies such as this one. This communication presents the first direct evidence for the positioning of the CP43 subunits through direct single-molecule comparison of intact core complexes containing CP43 with the same core complexes where the CP43 has been removed. We also present the first comparison of STM and X-ray structures for PS2.

Comparison of structures from STM and crystallography is normally not a straightforward matter because the contrast mechanisms are very different. Although atomic force microscopy and crystallographic techniques have been compared previously (Heymann *et al.* 1999, McPherson *et al.* 2001) for crystalline samples, similar comparisons have not been made for the higher resolution technique of STM nor for single-molecule imaging. Indeed, to our knowledge, there has been no previous direct comparison of the crystallographic and STM structures for photosynthetic systems. This study provides a

Received 10 May 2005, accepted 24 October 2005.

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convenient opportunity to compare the relative protein imaging and structural analysis capabilities of these techniques.

PS2 core complexes were prepared using the procedure of van Leeuwen *et al.* (1991) and CP43-deletion was achieved using a 1 M potassium thiocyanate treatment as described by Yamaguchi *et al.* (1988). The protein fragment distributions were characterised by SDS-PAGE. STM measurements were carried out on *Nanosurf EasyScan* and *Park Autoprobe* systems. Samples for STM imaging were prepared using the methods described by Lukins and Oates (1998). Approximately 5 mm³ of PS2 solutions, diluted to various concentrations in milli-Q water, were deposited on freshly-cleaved HOPG and air-dried for 15–30 min. Intact and CP43-deleted PS2 core complexes were imaged with resolutions of 0.3 and 0.6 nm, respectively.

The gels (Fig. 1) show that the CP43 has been strongly depleted from the PS2 as predicted by Yamaguchi *et al.* (1988). Some CP26/CP29 is present in the intact core complexes and these subunits are also removed along with the CP43. There is also a reduction in the 30 and 32 kDa components on treatment with 1 M potassium thiocyanate. An advantage of single-molecule STM imaging is that those particles which one wishes to image can be identified and selected even in the presence of impurities or unwanted protein fragments. Therefore, a high degree of sample purity is not essential for STM whereas high purity levels are necessary for crystallography. In this case, we select for imaging only the intact PS2 core complexes and the CP43-deleted core complexes. Within the resolution of the STM images, we did not observe any significant differences between the D1/D2/CP47 region of the intact core complex and the CP43-deleted core complex. This indicates that the CP43-deletion method used here caused negligible, if any, structural or conformational changes to the D1/D2/CP47 PS2 core complex.

STM clearly showed that in the isolated-molecule state, PS2 core complexes and CP43-deleted core complexes exist almost solely in their respective dimeric

forms (Fig. 2). The dimensions (uncorrected for detergent monolayer) of the complexes and the resolutions obtained are: intact PS2 core complex, 23.9×14.4 nm, 0.3 nm; CP43-deleted PS2 core complex, 17.0×15.0 nm, 0.6 nm. A comparison of the STM images in Fig. 2A,B clearly shows that the CP43s reside at the ends of the core complex. These structures and protein locations are consistent with electron and X-ray crystallographic data, previous STM images, and biochemical data.

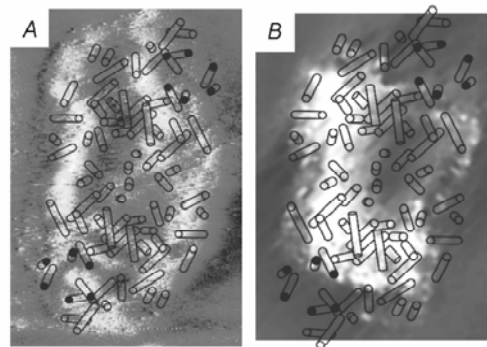


Fig. 2. Overlay of the X-ray crystal structure of photosystem 2 (PS2) onto the STM single-molecule images of (A) the intact PS2 core complex (image size 17×25 nm), and (B) the CP43-removed PS2 core complex (image size 17×19 nm). The α -helices with the filled-in ends at the extremities of the complexes correspond to those assigned to CP43 in the X-ray structure of Zouni *et al.* (2001).

A direct comparison between the single-molecule STM images and the atomic model of PS2 obtained using X-ray crystallography can be made simply by overlaying the X-ray model for the PS2 core complex (Zouni *et al.* 2001) onto the STM images in Fig. 2. For clarity, only the α -helices are shown but this is perfectly adequate for the purposes of this comparison. Those α -helices assigned to CP43 by Zouni *et al.* (2001) are shown with filled-in ends. There is excellent agreement between the X-ray and STM structures for the intact core complex. Similarly, there is excellent agreement between the STM image for the CP43-deleted core complex and the X-ray model with the components assigned to CP43 omitted. This comparison again confirms that the CP43 subunits are located at the ends of the core complex.

In conclusion, we show definitively that the CP43 subunits are located at the ends of the D1/D2/CP47/CP43 core dimer complex and that CP43 and CP47 reside on opposite sides of D1/D2 in the monomer complex. These results also indicate that the CP43-removal procedure used produces CP43-deleted cores with minimal conformational changes to the D1/D2/CP47 residual core complex. Analogous experiments using antibodies are also possible. Finally, single-molecule STM images and X-ray crystal structures of photosynthetic proteins are broadly consistent and the two techniques provide complementary information on PS2 structure.

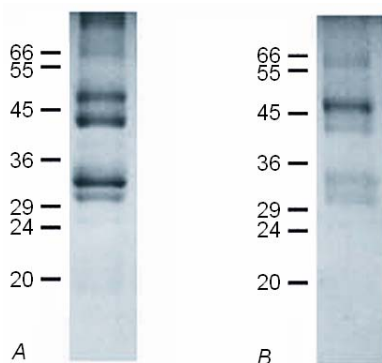


Fig. 1. SDS-PAGE gels of (A) intact D1/D2/CP47/CP43 core complexes, and (B) CP43-deleted D1/D2/CP47 core complexes.

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