Single-molecule high-resolution structure and electron conduction of Photosystem II from scanning tunneling microscopy and spectroscopy

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Abstract

Scanning tunneling microscopy (STM) and spectroscopy (STS) were used to obtain the first direct high resolution (~0.3 nm) images of single isolated Photosystem II (PS II) molecules, and to determine the supramolecular organization of oxygen-evolving PS II core complexes and PS II membrane fragments including the identification, assignment, location and dimensions of the polypeptide units. Our results predict a unique structural model which we then compare with alternative models. We show that the combination of quasi-constant-height mode STM operation, STS and suitable choice of sample-substrate preparations can be used to enable investigation of the structure and function of single PS II particles under normal thermodynamic and hydration conditions without the requirement and complications of ordered PS II arrays or crystals. STS was also used to characterize single-molecule electron conduction and tunneling mechanisms in PS II including the semiconduction and photoconduction behavior of the reaction center and photoexcitation effects in the light-harvesting complex LHC II. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Photosystem II is one of the two plant membrane-bound protein systems which catalyze the conversion of light energy from the sun into electrochemical energy on which plants, and hence life on earth, depend [1-3]. Over the last decade, there has been great interest in the two- (2D) and three-dimensional (3D) structure of PS II. Although the 3D X-ray structure of the reaction center of *Rhodopseudomonas viridis* was reported by Deisenhofer et al. [4] in 1985, the corresponding X-ray structure for PS II from higher plants has remained elusive partly because of difficulties in crystalizing PS II. Consequently, most of the models of PS II structure and function have been based on the structure of the bacterial reaction center together with the large body of spectroscopic information obtained for PS II. So far, detailed structural information on PS II is limited in general and completely lacking at high resolution (~0.5 nm) [5]. Electron microscopy (EM) studies of PS II particles and 2D arrays [6–14] have provided valuable information concerning the structure of PS II. Unfortunately, these EM studies often involve averaging over many images which can lead to a reduction in resolution (to typically 1–3 nm) and possible complica-
tions associated with image projection and registration. Recent electron crystallographic studies by Rhee et al. [14] have achieved resolutions of ~0.8 nm and have renewed interest in this area. Because of the expected difficulty of scanning probe studies of PS II, the only attempts of this kind so far have been on platinum-coated PS II replicates [15] for which internal structural detail is lost due to the effects of coating. Despite these advances, the long-standing controversies surrounding the detailed structure, protein arrangement and oligomerization state of PS II remain unresolved. At the same time, there has also been a lack of single-molecule spectroscopic information on PS II. This latter problem is important because, in the absence of single-molecule spectroscopic data, the relationship between true single-molecule properties and the large body of spectroscopic information on bulk PS II samples may be ambiguous.

Since its inception, STM [16] has proved a particularly powerful technique for atomic-resolution imaging of the surfaces of conductive materials and has spawned a variety of scanning probe microscopies which exploit different interactions between the tip and the sample. However, STM has only very rarely been applied successfully to biological specimens [17–19]. Most biological materials are considered to be insulators with low conductivity and hence low tunneling currents. Biological STM is extremely difficult because of the low conductance, ‘softness’ and ‘mobility’ of most biological specimens together with complications associated with the choice of substrate and optimization of the substrate–specimen binding properties. For these reasons, the standard constant-current and constant-height mode STM techniques have so far been unable to yield meaningful STM images of biological specimens. Here, we show that a quasi-constant-height mode operation, in which the feedback gain is slightly enabled, can allow direct STM images to be obtained while the significance and assignment of features in the resulting images can be determined by STS. A great attraction of the approach used here is the fact that the conductivity requirement for STM can be elegantly fulfilled by the unusual photoconductive and electronic properties of PS II without the need for a metallic coating. This then allows high-resolution imaging of the surface structure of single PS II molecules, the ability to spectroscopically distinguish PS II subunits and particles from other biological and substrate species present, and a powerful method for investigation of electronic processes in single PS II particles. Another important advantage of STM is the ability to study, under normal thermodynamic conditions (room temperature and pressure), samples that more closely approximate the in vivo case than those, such as microcrystals or arrays, which are studied under the atypical conditions of reduced hydration and high vacuum found in an electron microscope. We show that the extremely high resolution and sensitivity of STM/STS allows single-molecule imaging, spectroscopy and structure determination on typical PS II samples without the requirement of producing 2D or 3D crystals or ordered arrays.

2. Materials and methods

Photosystem II membrane fragments (BBY samples) were prepared by the method of Berthold et al. [20]. Thylakoid membranes from spinach were treated for 10 min with 5% Triton X-100. The solubilized PS II membrane fragments were then collected by centrifugation and washed twice in a standard MES buffer (20 mM MES, 400 mM sucrose, 15 mM NaCl, 5 mM CaCl₂, 5 mM MgCl₂; pH 6.0). Oxygen-evolving PS II core complexes were prepared using a variation of the procedure of van Leeuwen et al. [21]. PS II membrane fragments were treated with n-dodecyl-β-D-maltoside in BTS 400 buffer (20 mM Bis-Tris, 400 mM sucrose, 20 mM MgCl₂, 10 mM MgSO₄, 5 mM CaCl₂; pH 6.5). The incubation times were 10–30 min for mixtures at a final concentration of 1.7 mg chlorophyll (Chl)/ml and 1.25% (w/v) detergent. The core complexes were then purified using a Q-Sepharose anion exchange column, with the PS II eluted by a stepwise increase in MgSO₄ concentration to 75 mM. We adopted the approach of using shorter milder detergent treatments in order to produce core complexes that retain as much of the integral PS II core proteins as possible. The protein fragment distributions were characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and the activity and charge separation for these preparations were checked by oxygen evolution and P680⁺ reduction measurements using ap-
paratus described previously [22]. The oxygen evolution and P680 reduction measurements were obtained using MES and BTS400 buffers for the BBY samples and core complexes, respectively. In each case, K3Fe(CN)6 was used as electron acceptor and the measurements were carried out at 22°C.

Specimens for STM were prepared by dispersing a suitably prepared suspension of the above PS II particles onto a freshly cleaved crystal surface of highly oriented pyrolytic graphite. All STM and STS experiments were carried out under ambient conditions on a modified Park Scientific Instruments Autoprobe SPM system fitted with an STM head. Probe tips of either tungsten or platinum–iridium were made, inspected by optical microscopy and evaluated by STM of a freshly cleaved graphite surface. Scale calibration of the instrument was performed using the known inter-atomic separation of carbon atoms on graphite surfaces. The lateral resolution was ~0.02 nm for graphite and 0.2–0.5 nm for PS II core samples, while the vertical resolution was <0.01 nm in each case. The effective resolution for the PS II samples was obtained by evaluating the root mean square value for the resolution based on a consideration of the effects of scanning resolution, vertical and lateral instrument resolutions, scanning rate effects, the maximum gradient in the signal seen over an entire composite current–height image, repeatability and reproducibility, the pixel resolution and signal noise. We obtain resolutions of typically 0.2–0.5 nm for the range of several images and ~0.3 nm for the images presented. This procedure for determining the resolution was used in preference to the use of cross-correlation techniques since the latter (a) is unnecessary in view of the true single-molecule resolution of PS II achieved here, and (b) can be unreliable when applied to complex images such as the current–height images obtained here or images containing regions where the signal may not vary greatly on the local scale, as occurs for parts of some subunits or at the center of BBY particles. Scanning tunneling spectroscopy was performed by first obtaining an STM image of the sample, locating the tip above each region of interest at an appropriate height and then measuring the tunneling current I as the tip voltage V was ramped from ~−1 V to ~+1 V for a chosen tip bias voltage. The resulting current–voltage (I–V) and conductance–voltage ((dI/dV)–V) curves were corrected for tip bias and analyzed by fitting the curves with polynomials of the form \( I(V) = \sum A_n V^n \) using standard least squares fitting techniques. The final quoted particle dimensions were obtained after applying detergent layer corrections of 1.7 nm for Triton X-100 and 2.5 nm for n-dodecyl-β-D-maltoside. These corrections were evaluated by calculating the length of the detergent molecules and using a steric correction.

Although constant-height mode has been found to produce better resolution under ambient conditions than constant-current mode, neither method was satisfactory for this work. Furthermore, since the conductance of a biological sample varies, height information obtained from constant-current STM images is not reliable for these specimens. We therefore used quasi-constant-height mode operation to enable images to be obtained while achieving the best compromise between image quality and minimal tip–sample interactions. For quasi-constant-height mode, a small amount of feedback is used so that the tip height varies slightly in response to the average tunneling current leading to a current–height image. A consequence is that when the tip encounters an increase in topographic elevation, the increased current causes the tip to slowly retract from the sample. When the topographic height decreases, the conductance values are decreased due to the increased tunneling gap. This leads to a shadowing effect on the trailing edge of the scan over a topographic feature together with a region of slightly higher tunneling current on the leading edge where the topographic feature begins. We have found this effect very useful in imaging PS II specimens particularly for contrast improvement and edge detection. This effect arises from the modified gain and integrating time response of the STM feedback network. Final images were obtained by Hilbert transform 2D deconvolution of the instrument response function from the raw image.

3. Results and discussion

3.1. PS II sample characterization

SDS-PAGE of the PS II core complexes (Fig. 1A) gave well-resolved protein distributions that are very similar to those reported by van Leeuwen et al. [21].
However, in our modified preparation using a shorter and milder detergent treatment with \(n\)-dodecyl-\(\beta\)-\(D\)-maltoside the bands in the range 23–29 kDa and near 55 and 62 kDa are slightly more intense. These results indicate that the core complexes contained D1, D2, cytochrome (cyt) \(b\)-559, CP43, CP47, CP24, CP26, CP29 and the donor side extrinsic proteins including the 33 kDa protein. This preparation appears to lead to core complexes that contain more of the PS II core integral proteins, such as CP24/CP26/CP29, than the preparations used in previous EM studies.

The oxygen evolution capacities for the BBY and core complexes were checked at 22°C using a Clark electrode. Rates \(\sim 500\) and \(\sim 1100\) \(\mu\)mol \(O_2/\text{mg Chl/h}\) were observed for the BBY samples and core complexes, respectively. The nanosecond decay kinetics for P680\(^+\) reduction (Fig. 1B) were similar for the BBY and core preparations except that the nanosecond decay is faster and the microsecond amplitude greater in the case of the core complexes. The relative amplitudes and time constants for the BBY nanosecond decay kinetics were 0.27 at 28 ns and 0.54 at 115 ns for a two-exponential fit and 0.21 at 24 ns, 0.48 at 85 ns and 0.15 at 250 ns for a three-exponential fit (84% due to nanosecond components). The corresponding values for the core particle nanosecond decays were 0.31 at 18 ns and 0.41 at 105 ns for a two-exponential fit and 0.19 at 6 ns, 0.40 at 55 ns and 0.18 at 280 ns for a three-exponential fit (77% due to nanosecond components). These parameters and trends are very similar to those found previously for BBY samples [22,23] and oxygen-evolving core complexes [22]. The well-defined gels, good oxygen-evolution rates and good P680\(^+\) reduction kinetics indicate that the PS II samples have intact structures with high activity. This conclusion is further reinforced by the apparently high proportion of structurally intact PS II particles seen during STM scans.

3.2. STM of protein and substrate features

Fig. 2A, which shows an STM image of a small protein fragment, demonstrates the ability of the quasi-constant-height mode method to image biological samples and illustrates how an image can be calibrated using the atomic separation of carbon atoms in the graphite substrate which appears in the background of the image. Biological material exhibits lower resolution than the atomically resolved substrate because of the lower conductance of the protein compared with that of graphite and the greater topographic irregularity of the biological material. The band-like structures in Fig. 2B,C were observed for BBY samples with each band typically \(\sim 40\) nm in size. In these images, the PS II membrane fragments are viewed from side on or along the plane of the membrane and the tendency for planar stacking and preferred orientation is evident.
Fig. 2C also shows internal structures within each band which we attribute to PS II integral membrane proteins embedded in the bilayer structure. Deposition of these BBY particles at low densities on graphite substrates containing steps and terraces leads to preferential binding of the PS II particle to the edge region (Fig. 2D). Protein molecules were often observed at such sites because of the additional intermolecular forces that exist due to the graphite dislocation and the magnitude and anisotropy in the binding potential for PS II attachment to graphite.

3.3. STM and STS of PS II membrane fragments

In Fig. 3A we see two overlapping elliptical structures representing PS II complexes with dimensions of \(33.5 \times 15.9\) nm (\(30.1 \times 12.5\) nm with detergent correction). The long axis dimensions of these particles corresponds well with the length of the band structures observed in Fig. 2B,C. A dimeric anti-parallel twofold symmetric structure for the PS II complex is seen in Fig. 3A and represented in the topographic...
diagram in Fig. 3B. Note that the interior of the particles is not well resolved because the BBY particle appears relatively flat and the presence of lipid leads to a reduction of both height and conductance resolution. The dotted regions represent the boundaries of the observed topographic features seen on these subunits but the actual full size of some of these subunits will be greater than the feature dimensions indicated in the figure legend because of the above-mentioned effects.

The $I-V$ curves for the region 1 in Fig. 3B were qualitatively different from the $I-V$ curves for the regions 2-4 which were all similar. However, the curves for region 1 were similar to those obtained for the reaction center region of the core complex (Fig. 5A) which will be discussed in Section 3.4. Therefore, we assign region 1 as the reaction center complex D1/D2/cyt b-559. Unfortunately, the presence of lipid and other material tended to obscure features of interest in the $I-V$ curves in a similar way to the reduction in both height and conductance resolution observed in the STM images. The regions 3a,b appear as circular fragments at each end of the BBY dimer complex. The overall structure of these BBY particles, which are well known to contain LHC II, can be compared with the overall structure of the core complexes (see Section 3.4) which do not contain LHC II. This comparison indicates that region 3 corresponds to LHC II, a conclusion that is in agreement with previous EM studies [3,9,10,13,14]. The shape and size of the LHC II (circular complex of 7.5 nm apparent diameter or 4.1 nm diameter after detergent correction) are consistent with the electron crystallographic data of Kuhlbrandt et al. [24]. Region 2 is tentatively assigned as elevated features on either CP43 or CP47, or both. Unfortunately, the actual boundaries of these fragments are not clear because of the reduced conductance resolution for these specimens and the presence of absorbates. Finally, region 4 is tentatively assigned as link-
er or chlorophyll-binding proteins. So far, we have not yet been able to clarify the identity of these proteins for BBY particles from STS measurements. However, EM studies show that the corresponding positions in similar PS II particles also have enhanced densities but the identity of these features remains unclear. There is excellent agreement between these overall structures and those proposed by Rogner et al. [3] and Boekema et al. [10]. For example, the overall dimensions of the BBY complex obtained by Boekema et al. [10] were 26.8 × 12.3 nm. We emphasize here that our structures are based on single BBY fragments.

3.4. STM and STS of oxygen-evolving PS II core complexes

Although the STM/STS of BBY particles enabled the location of the reaction center complex and LHC II in PS II membrane fragments, the detailed protein arrangement was obscured by the reduction in height and conductance resolution due to the topology and the presence of lipid and other unwanted biological material. Therefore, the more highly purified core complexes were studied in order to better resolve the supramolecular organization of PS II. The STM image (Fig. 4A) of an oxygen-evolving PS II core complex, at ~0.3 nm resolution, shows a well-resolved dimeric anti-parallel twofold symmetric structure but, as expected, does not contain LHC II. The overall dimensions of the core complex are 23.9 × 14.4 nm (18.9 × 9.4 nm with detergent correction) along the orthogonal long and short symmetry axes, respectively. The angle between the long symmetry axis and the axis defined by the centers of the two regions labeled region 1 (which we later assign as D1/D2) is (75 ± 5)°. Fig. 4B shows a topographic representation of the complex, outlining the structure and both the shapes and dimensions of all six observed internal subunits. Identification of these subunits was made primarily on the basis of STS results for the tip positioned above the relevant subunit, together with symmetry considerations and the known constituents from gel electrophoresis. Slight differences in the observed sizes of fragments with corresponding labeling arise because of differential adhesion of small molecular absorbates. The overall shape and dimensions of the PS II core dimer complex are very similar to those reported previously using EM [10,13,14]. For example, Boekema et al. [10] find dimensions of 17.2 × 9.7 nm (present values 18.9 × 9.4 nm).

Region 1 is definitively assigned as the D1/D2 reaction center complex on the basis of the STS spectra (Fig. 5A) which show the expected semiconduction behavior whereas all other subunits show qualitatively different STS spectra with predominantly quadratic I–V dependencies. It is worth noting that this assignment is based on clear spectroscopic evidence whereas previous EM studies have hypothesized the location of the reaction center from plausible structural and biochemical arguments. The existence and size of the partial cavity in the center of the dimer suggests that the two reaction centers are largely decoupled.

Region 2 represents a well-defined elevated region lying above D1/D2 and is attributed to the 33 kDa extrinsic protein. We observe that the 33 kDa protein is highly compact and has a large extension (~3 nm) in the vertical (out of the page) direction. In the intact membrane-bound system, the 33 kDa protein protrudes from the membrane by ~3–5 nm [9,10,15].

Region 3, which is both structurally and spectroscopically distinct from region 1, is most probably cyt b-559. This assignment is consistent with the known proximity of cyt b-559 to D1/D2. The size of region 3 (4.0 × 3.7 nm) is larger than expected for the relatively small peptide cyt b-559 which has masses of ~4 kDa and ~9 kDa for the α and β subunits, respectively. However, this could be explained by the proposition that the cyt b-559 may be at least partly covered by the larger extrinsic proteins which would then be primarily responsible for the tunneling behavior seen in the STM images and for the size of region 3. This interpretation is supported by the fact that the I–V curves differ from those of region 1, which we have assigned as the reaction center, and that it is highly unlikely that the chlorophyll-binding proteins would be so large as to include region 3. Furthermore, we observe that region 3 is similar in size and shape between different particles in the same specimen. The composite current–height signal above region 3 is comparable to that above region 1 suggesting that the surfaces of these two regions are at a similar height. These
results are significant since apparently none of the earlier studies have addressed the location of cyt b-559. Our assignment is also consistent with the results of Nanba and Satoh [25], who isolated the minimal components for PS II photochemical activity, and the results of Marr et al. [12], who found that cyt b-559 and D1 are in close proximity since they both contribute to a high density peak in the EM map.

Regions 4 and 5 are assigned as CP47 and CP43, respectively, since their STS spectra and sizes are similar while EM evidence [3,10,13] indicates that CP43 is most probably at the extremities of the core dimer complex. Enami et al. [26] have found that there is an interaction between CP47 and the 33 kDa protein. It has also been suggested that CP47 should be in close proximity to both cyt b-559 and D1/D2 [12,27]. These points are consistent with our assignment in which CP47 and CP43 are adjacent to one another. The structure in Fig. 4B shows that CP47 is essential for the existence of the dimeric form of PS II and that, although CP29 and CP43 are present in these complexes, core preparations lacking CP29 or CP43 should also retain a dimeric structure.

From earlier EM work [3,10,13,14], PS II particles lacking 24/26/29 kDa fragments do not appear to show significant densities in locations corresponding to our region 6. The appearance of features at region 6 in our work on preparations containing these proteins therefore suggests that region 6 corresponds to CP24/CP26/CP29 or related linker proteins. There is evidence that these proteins interact with LHC II [26,28] and, in fact, are probably connected to LHC II [29]. The assignment of region 6 as these proteins is consistent with the above evidence since this region adjoins CP43 in the core complex and CP43 and LHC II in BBY particles. Indeed, we believe that the specific function of CP24/CP26/CP29 may be to bind LHC II to the PS II core.

Santini et al. [8] found that removal of the oxygen-evolving complex extrinsic proteins and CP43 did not affect the extent of dimer formation whereas removal of CP47 converted all dimers into monomers. This indicates that CP47 is necessary for the formation of the dimer complex, a conclusion similar to that which we have drawn from the structure in Fig. 4. Furthermore, it has been suggested that CP43 is the last chlorophyll-binding protein to be incorporated during PS II core assembly and that lack of CP43 does not prevent assembly of a PS II complex in vivo [30,31]. The suggested location of CP24/26/29 is consistent with the proposed role of these proteins in the efficient transfer of excitation energy from LHC II to the reaction center core, possibly via CP47/CP43, even though CP24/26/29 contain only ~10% or so of the chlorophyll in the PS II system [32].

Eijckelhoff et al. [13] have proposed an alternative model for the PS II dimer structure in which the proteins have an approximately symmetric arrangement except that D1 and D2 are adjacent to one another but that CP43 and CP47 are on opposite sides of the D1/D2 complex in the monomer unit. Although their PS II particles did not contain CP43, Rhee et al. [14] also suggest a similar model where CP43 and CP47 reside on opposite sides of D1/D2 based on an analogy with PS I. In our assignments, which are based substantially on STS, we believe that the locations of the D1/D2 complexes and the 33 kDa proteins are definitively obtained. Our resulting structure of the PS II core dimer complex implies that the two D1/D2 complexes are in close proximity with no evidence for chlorophyll-binding proteins in the space between them. On this basis, we favor the model shown in Fig. 4 in which CP43 and CP47 reside on the same side of D1/D2. The alternative model [13] involves CP47 being partially interposed between the D1/D2 pairs, a possibility not so consistent with our STM/STS data. Our structure is similar to that suggested by Rogner et al. [3] and Boekema et al. [10], and is consistent with antibody labeling results [12] and other biochemical evidence [32].

3.5. The oligomerization state of PS II

The long-standing controversy [5,6,10] regarding the oligomerization state of PS II can now be addressed because STM can be used to determine the number of PS II centers per complex for the range of complexes present in a given region of the sample. This is an important capability since although previous EM on PS II arrays have also indicated dimeric structures, it has not been possible to show whether or not these dimeric forms are intrinsic to the PS II structure or due to intermolecular forces.
associated with the ordered 2D array. We find that, for the present PS II core particle preparation method, (90 ± 7)% of PS II exists in the dimer form and that the remaining <10% comprises monomers with the possibility of some tetramers but these were not observed. The dimer form is therefore most probably the predominant in vivo PS II structure.

Holzenburg et al. [6] have previously suggested that PS II normally exists as a monomer with dimensions of V18 U10 nm. These dimensions are similar to those obtained in the present work (18.9 × 9.4 nm) which shows clearly a dimeric core complex with twofold symmetry. Furthermore, several workers [3,10,13,14] have argued that PS II exists in the dimeric form, at least in the 2D crystalline state for which EM was carried out. This highlights the importance of obtaining high-resolution structures, and the need to exercise care in ascribing PS II particles as being monomeric or part of a polymeric complex within an ordered 2D array.

3.6. STS of the PS II reaction center and the LHC II complex

Having resolved and assigned the internal structure of the PS II core complex, we are now in a position to use STS to investigate electron tunneling processes and light-induced conduction in a single PS II complex. Fig. 5 shows a comparison of the I–V curves for both the reaction center and LHC II before and after illumination with white light. The spectrum for graphite obtained under similar tunneling conditions is overlaid for comparison. Graphite shows a quadratic unipolar I–V dependence where the linear term represents an ohmic contribution and the term in V^2 arises from tunneling to the extended delocalized states of the planar aromatic network. Both the reaction center and LHC II components exhibit greatly increased tunneling current and conductivity in the presence of light. For the reaction center spectra (Fig. 5A) taken in the absence of light, there is a component that is asymmetric about V = 0 indicating a weak but significant ‘diode-like’ semiconductor behavior with an effective band gap ~0.4 eV. On illumination, this asymmetric component becomes more pronounced (Fig. 5A) indicating reaction center photoconduction arising from the normal linear electron transfer sequence [Mn]^4→Yz→P680→pheo→QA→QB. However, the major contribution to the I–V curves is a bidirectional conduction mechanism which we believe is ‘hopping’ conduction along the polypeptide chains which, in turn, is most likely due to an electron tunneling mechanism between amino acid residues. Delocalization and light-induced excitation of electrons in these protein systems may act as a source of ‘free’ electrons for hopping conduction. This interpretation also resembles that used to explain the unusual electronic properties and conduction processes seen for some amorphous carbons prepared under highly energetic ion bombardment conditions [33]. In the range ~300 mV < V < 300 mV, we see a linear ohmic effect arising from tip-sample interactions in the tun-
neling region together with a contribution in $V^2$ which, itself, contains contributions from both space charge limited current (SCLC) behavior [33] and tunneling into the delocalized vibronic molecular states near the surface of the reaction center. The current-limiting effect, which occurs for $V < -500$ mV and $V > +300$ mV, is due to saturated tunneling and gives a measure of both the maximum local density of states near the surface involved in the tunneling process and the maximum number of electrons available for excitation and conduction within the molecular framework near the tunneling region. The limiting tunneling current ($\sim \pm 100$ nA) is unusually high for a biological material and indicates an extremely high density of states near the PS II surface. On exposure to light, the tunneling current and conductance ($dI/dV$) increase dramatically (Fig. 5A) providing the first demonstration of photoconduction in a single PS II reaction center. As expected from the above discussion, the $I-V$ curves at higher tip voltages converge to similar limiting tunneling currents for both illuminated and unilluminated reaction centers.

The LHC II spectra (Fig. 5B) are symmetric but also show SCLC behaviour and a very marked increase in conductivity with illumination. We interpret these effects as being due primarily to tunneling involving the delocalized electronic states of the chlorophyll network comprising the LHC II. On excitation by light, the tunneling current increases due to the increased conductance of the LHC II surface structures in the excited state and the enhanced exciton and electron delocalization in the bulk LHC II structure. Further work is currently under way on highly oriented PS II structures to further investigate PS II photoconduction.

4. Conclusions

Previous structural studies of PS II involved the use of electron microscopy with its advantages of robust imaging of these particles in ordered arrays and crystals using well-established techniques. Unfortunately, these EM methods have some disadvantages which include (a) moderate resolution $\sim 1-2$ nm, (b) the need to use ordered arrays or crystals of low hydration under the conditions of high vacuum and high energy electron bombardment, and (c) the fact that no spectroscopic tool was used to characterize the physicochemical properties of the samples which may assist in the assignment of the protein components. Here, we have used STM and STS in a novel scanning mode to obtain new information on the structure and electron conduction in PS II with the highest resolution reported so far ($\sim 0.3$ nm). We describe the first direct STM of PS II, the first STS of PS II and some of the very few STM/STS results for any biological system. These techniques have allowed us to obtain, under normal ambient thermodynamic conditions, true single-molecule structures and single-molecule electronic spectra not possible previously using other techniques and without the need to use ordered arrays or crystals. In this way, it was possible to determine the polypeptide arrangement and sizes for two types of PS II particles and the typical oligomerization state. We also used STS to directly observe the semiconductive properties of single PS II reaction centers as well as the photoconductivity of single PS II reaction centers and LHC II complexes. It is now possible to achieve surface structure determination of single PS II molecules at high resolution simultaneously with single-molecule electronic spectroscopy. We believe that this type of single-molecule spectroscopic imaging of PS II could stimulate new areas of study of the structure and energetics of PS II, and have applications to other biomolecules.

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References


