High-resolution Electron Crystallography of Light-harvesting Chlorophyll a/b-Protein Complex in Three Different Media

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Large two-dimensional crystals of the light-harvesting chlorophyll a/b-protein complex (LHC-II) from the photosynthetic membrane of pea chloroplasts were grown by a new method from detergent solution. The structure of these crystals was examined by electron crystallography, using three different media to preserve high-resolution detail: vitrified water, glucose and tannin. The crystals diffracted electrons to at least 3.2 Å resolution in all three media. R-factors between the three data sets of electron diffraction amplitudes ranged from 6.4% to 14.3%. Fourier difference maps were generated and compared to a projection map of the complex at 3.4 Å resolution. No significant differences were found, proving that all three media preserved the native structure of LHC-II at high resolution. The probability of recording high-quality electron diffraction patterns with tannin was 90%. With glucose and water this probability was lower by a factor of 10 to 20, suggesting that tannin may be preferable as a preserving medium for sensitive biological specimens.

1. Introduction

A prerequisite for determining the structure of biological macromolecules and their assemblies to atomic or near-atomic resolution by electron crystallography is the preservation of the specimen in its native, hydrated state when placed in the high vacuum of the electron microscope. This has been achieved in several different ways. Taylor & Glaeser (1974) demonstrated that frozen crystals of catalase diffract electrons to high resolution when examined at low temperature. Unwin & Henderson (1975) found that a thin film of dried glucose solution preserved two-dimensional (2D) crystals of bacteriorhodopsin and thin 3D crystals of catalase to high resolution, as judged by electron diffraction. Akey & Edelstein (1983) observed that a dilute solution of tannic acid can be used to preserve catalase crystals for high-resolution diffraction. In its neutralized form, tannin, this medium proved to be particularly suitable for preserving 2D crystals of the light-harvesting chlorophyll a/b-protein complex (LHC-II) (Kühnbrandt & Downing, 1989). To date, the best results in electron crystallography have been obtained with specimens that were prepared by one of these three methods and cooled in the electron microscope to a temperature in the range −115°C to −269°C (4 K) in order to reduce the effect of radiation damage caused by inelastic scattering. In this way, electron diffraction patterns of a variety of 2D and thin 3D protein crystals have been recorded at 3.4 Å resolution (1 Å = 0.1 nm) or better, including frozen-hydrated crotoxin crystals (Jeng & Chiu, 1983), purple membrane in glucose (Baldwin & Henderson, 1984), the bacterial porins PhoE (Jap, 1988) and OmpF (Sass et al., 1989), both in glucose, and LHC-II in tannin (Kühnbrandt & Downing, 1989). By processing high-resolution images of 2D crystals, the projected structures of bacteriorhodopsin (Henderson et al., 1986; Baldwin et al., 1988), LHC-II (Kühnbrandt & Downing, 1989) and PhoE (Jap et al., 1990) have been determined at resolutions of 2.8 Å, 3.7 Å and 3.5 Å, respectively. Recently, the 3D structure of bacteriorhodopsin has been solved at high resolution (Henderson et al., 1990) by this technique.

For future applications of biological electron crystallography it is important to establish how reliably these media preserve high-resolution detail. It is usually assumed that rapid freezing in a thin film of vitrified water preserves biological specimens perfectly. However, we observed that this treatment destroys the high-resolution detail of the large majority of 2D crystals of LHC-II. If water can have such a drastic effect, the influence of air-drying

Abbreviations used: 2D, two-dimensional; 3D, three-dimensional; LHC-II, light harvesting chlorophyll a/b-protein complex; NG, n-nonyl-β-D-glucopyranoside; OG, n-octyl-β-D-glucopyranoside.
in glucose or of treatment with tannin seems even less predictable. Glucose was not much better than water for preserving our crystals, whereas a 0.5% solution of tannin proved to be highly effective.

Tannin (also referred to as Chinese gallotannin) is a natural plant product. Chemically, it consists of a glucose molecule substituted with several, partly oligomerized, gallic acid residues. The ionic form, tannic acid, is widely used as a mordant prior to heavy-metal staining of tissues for ultramicrotomy (Simionescu & Simionescu, 1976) and has been shown to preserve the fine structure of complex assemblies such as insect flight muscle (Reedy & Reedy, 1985) in thin sections.

Here we report the results of an electron diffraction study of LHC-II in the three media, vitrified water, glucose and tannin. Structure factor amplitudes up to 3.2 Å resolution and Fourier difference maps between the projected structure in these media are compared.

2. Materials and Methods

(a) Crystallization

The 2D crystals of LHC-II were grown of LHC-II isolated from pea chloroplasts (Kühlbrandt et al., 1983). For 2D crystallization, the complex was first precipitated from stock solution (2 to 4 mg chlorophyll/ml) in 0.3 to 0.4% (w/v) Triton X-100 purified for membrane biochemistry (Boehringer-Mannheim, Germany) by diluting a portion 10-fold with distilled water and adding KOH to a final concentration of 300 mM. The 2D crystals were then obtained by 1 of 2 methods, referred to as method A and B. For method A, the precipitate was dissolved in Triton X-100 and glycerol at final concentrations of 23% and 40%, respectively. The final chlorophyll concentration was 0.7 mg/ml. 2D crystals formed during incubation at 38°C for 2 h.

(b) Specimen preparation

Thin carbon films were prepared by evaporating carbon rod (Agar Aids) on to freshly cleaved mica in an Edwards 306 evaporator at 5 × 10⁻⁶ torr. For vitreous water embedding, a small (about 4 mm × 4 mm) piece of carbon film was floated off on a clean water surface and picked up with the shiny side of a 400 mesh copper grid. A small volume (1 to 3 μl) of crystal suspension was injected with a Gilson pipette into the small lens of liquid that formed. The grid was then blotted shiny side up for 10 to 20 s on Whatman no. 4 filter paper and immersed immediately in liquid nitrogen before mounting in liquid nitrogen.

(c) Electron diffraction and cryo-electron microscopy

Low-dose electron diffraction patterns were recorded by the method of Unwin & Henderson (1975), adapted to a JEOL electron microscope (Kühlbrandt, 1988a) on to Kodak SO 163 electron emulsion film at an acceleration voltage of 100 kV and a nominal camera length of 1200 mm in a JEOL 2000EX electron microscope equipped with a cold fork anticontaminator. A 40 μm condenser aperture was used. Exposure times ranged from 4 to 10 s to record low resolution reflections to 32 s for high-resolution data. The stage temperature was kept at approximately −120°C to −130°C for tannin-embedded specimens, and at about −135°C for glucose- and vitreous-water-embedded specimens to minimize the risk of ice contamination that occurred at stage temperatures below −140°C. A Gatan television image intensifier was used to identify crystals at very low electron dose rates, estimated using reference curves for electron speed supplied by Kodak. Crystals larger than 3 μm (method A) or 7 μm (method B) in diameter were selected. Films were developed in full strength D10 developer for 12 min. High-resolution electron micrographs of tannin-embedded crystals were recorded with a JEOL 100C electron microscope equipped with a field emission gun at 100 kV and a temperature of −115°C at the Lawrence Berkeley Laboratory (Berkeley, CA) as described (Kühlbrandt & Downing, 1989).

(d) Data processing and calculation of difference maps

Electron diffraction patterns showing sharp diffraction spots beyond 3.5 Å resolution and distinct 6nm Lute symmetry were selected for processing. Areas of 2048 steps × 2048 steps were digitized on a Perkin-Elmer 1010-M microdensitometer with a step size of 15 μm and 15 μm × 15 μm square aperture. Diffraction patterns were processed according to the method of Baldwin & Henderson (1984). Corrections for radial background, drift of the densitometer light source and for the curvature of the Ewald sphere were applied. Background-corrected electron diffraction intensities were extracted and merged, and temperature factors were refined, with programs written by Henderson and coworkers (Baldwin & Henderson, 1984; Céska & Henderson, 1990). Several different R-factors were calculated. First, for each data set the internal R-factors for Friedel pairs, and the merging R-factor for combining data from different films, were generated from expressions given by Baldwin & Henderson (1984):

\[ R_{sym} = \frac{\sum |I_{h,k} - I_{-h,-k}|}{\sum (I_{h,k} + I_{-h,-k})} \]

and:

\[ R_m = \frac{\sum |I_{h,k} - I_{h,k-1}|}{\sum (I_{h,k} + I_{h,k-1})} \]

\[ I_{h,k-1} \] is the averaged intensity of reflection (h, k) after merging of data from all films. \( R_{sym} \) and \( R_m \) were calculated separately for the total number of reflections, for the subsets of strong and weak reflections, and in a resolution bands from 30 Å and 32 Å, with roughly equal numbers.
of reflections per band. Second, 3 other R-factors were calculated between the merged, symmetry-averaged data sets obtained from crystals in the 3 different media to quantify structural differences between them. These R-factors were $R_m$ (tannin versus vitreous water), $R_g$ (glucose versus vitreous water), and $R_t$ (glucose versus tannin). Fourier difference maps (see Blundell & Johnson, 1976) were calculated from difference structure factors $F' - F$, using the phases obtained by high-resolution image processing of tannin-preserved specimens (Kühlbrandt & Downing, 1989).

3. Results
(a) Crystallization

The 2D crystals of LHC-II (layer group $p21$, $a = b = 127$ Å) prepared by method A typically measured 2 to 4 μm in diameter. Larger crystals measuring up to 10 μm across were rarely observed. Crystals grown by this method were more robust than those obtained by method B. In the crystallizing solution, they could be kept at 4°C for two months or longer without detectable deterioration, as assessed by electron diffraction. Due to their greater stability they were used preferentially for the preparation of frozen-hydrated specimens. On average, however, they were too small for routine data collection, in contrast to purple membranes of this size, which are adequate for recording high-quality electron diffraction patterns (Baldwin & Henderson, 1984; Ceska & Henderson, 1990). We found that 2D crystals of LHC-II need to measure at least 5 to 6 μm across in order to yield data of comparable quality. This is due to the large unit cell and low packing density of the protein (Kühlbrandt, 1988a), which means that diffraction spot intensities are estimated to be, on average, 7 times weaker than those of purple membrane.

Fortunately, 2D crystals measuring between 7 and 10 μm in diameter can be grown routinely by method B, in a detergent mixture of Triton X-100 and NG. Crystals prepared by this method seemed to grow in three stages. During the first stage, small 1 to 3 μm crystals formed within 12 hours at 25°C. These fused into larger polycrystalline arrays measuring 5 to 7 μm in diameter after another 36 hours at the same temperature. Electron diffraction patterns of these arrays showed sharp continuous rings at spacings corresponding to strong electron diffraction spots, indicating that they were mosaics of small crystalline arrays. In the final stage, during incubation at 40°C for two hours, these crystalline arrays merged into an extensive single lattice. At this stage the size of 2D crystals increased further to an average diameter of 7 to 10 μm. The crystal form was identical with that grown by method A, but the crystals appeared to be less stable. Kept at 4°C, at least 90% of them diffracted to 3.3 Å resolution or better in the first two weeks. The crystals then deteriorated gradually so that after five weeks fewer than 20% diffracted to high resolution. They also seemed to be more sensitive to disruption by treatment with water or glucose solution than were crystals grown by method A.

(b) Electron diffraction patterns

For electron diffraction, crystals were located in the defocused diffraction mode with decreased filament current, using the television image intensifier. We estimated the electron dose rate under these conditions to be less than $4 \times 10^{-4}$ electrons/Å² per second. Thus, the pre-irradiation of the specimen was negligible, and virtually all electrons hitting the crystal were recorded on film. The total dose for diffraction patterns recorded with 32 second exposure time was below 0.3 electrons/Å², less than the critical dose for purple membrane at room temperature (Unwin & Henderson, 1975). Figure 1 shows an electron diffraction pattern taken at a temperature of -125°C of a LHC-II crystal preserved with tannin. The pattern has almost perfect 6mm symmetry, indicating that the crystal was virtually untented. On the best negatives, diffraction spots were visible by eye to 24 Å resolution. Reflections were processed to 3.2 Å resolution. The region near the unscattered, central beam was completely blackened due to electrons scattered by non-periodic objects in the beam path, mainly the carbon support film, and by inelastic scattering from the specimen. To reduce this effect as much as possible, we used carbon film of roughly 100 Å thickness. This was thin enough to enable us to record weak diffraction spots while providing the necessary mechanical stability. By reducing the exposure time we were able to record most of the low-frequency data. However, structure factor amplitudes of reflections at resolutions below 30 Å could not be measured reliably by this technique, due to the high, rapidly varying background in this region. Amplitudes of these reflections were therefore taken from the scaled and merged set of amplitudes and phases obtained by image analysis of tannin-preserved, untented 2D crystals (Kühlbrandt & Downing, 1989). Processed electron diffraction patterns yielded a total of about 2140 Friedel pairs of reflections per film. As expected, the average intensity per diffraction spot was much lower than for purple membrane.

Seventeen electron diffraction patterns of untented LHC-II crystals stabilized with tannin were processed, including three recorded with short exposure times (Table 1). In total there were 28,881 Friedel pairs of independent measurements. We determined the intensities of all 375 reflections between 3.2 and 30.0 Å resolution in the asymmetric unit. Roughly 40% of the observed spots had intensities of at least two times their average standard deviation. Together, these strong reflections accounted for more than 75% of the total diffracted intensity to 3.2 Å. The measurements for these spots were highly accurate and they were therefore the most significant for determining the crystal structure. The low Friedel R-factor $R_{sym}$ of 13.0% provided additional proof of the high quality of these intensities. The strong reflections accounted for roughly 80% of all measurements in the resolution range 30 to 36 Å and were evenly distributed.
amongst four resolution bands (Table 1). In the highest resolution range (3.6 to 3.2 Å), about 10% of the reflections fell into this category. The remaining (weak) reflections, which together accounted for less than 25% of the diffracted intensity, had an averaged $R_{\text{sym}}$ of 54.8%. The overall Friedel $R$-factor for all spots was 22.1%. The best electron diffraction pattern had an overall $R_{\text{sym}}$ of 14.7%. The averaged $R_{\text{sym}}$ of the three diffraction patterns recorded with a lower electron dose was 36.6% because of the less favourable signal/noise ratio.

During data merging, scale factors and temperature factors were determined for each individual pattern by a conventional least-squares procedure, based on Wilson plots (Baldwin & Henderson, 1984). The merging $R$-factors $R_m$, averaged over all 17 films, were 26.2%, 55.9% and 34.7% for strong, weak and total reflections, respectively. Initially, $R$-factors were calculated assuming that all crystals were untilted. In reality, however, each crystal was tilted by some small amount. After refinement against a merged set of diffraction data recorded from highly tilted crystals (results not shown), the actual tilt angles of the 17 patterns were determined to range from 0.2° to 3.6°. When these small tilts were taken into consideration, the merging $R$-factor for all reflections improved by 8% to 26.7%. This overall $R_m$ was only slightly higher than that found for purple membrane (Ceska & Henderson, 1990).

When using tannin solution as embedding medium, about 90% of the LHC-II crystals measuring more than 4 μm in diameter diffracted to 3.5 Å or better, whereas only 15% to 20% of all crystals diffracted to this resolution when 10% glucose solution was used instead. With vitreous

**Table 1**

<table>
<thead>
<tr>
<th>Resolution range (Å)</th>
<th>Number of reflections in asymmetric unit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
</tr>
<tr>
<td>30.0-7.2</td>
<td>75</td>
</tr>
<tr>
<td>7.2-5.1</td>
<td>78</td>
</tr>
<tr>
<td>5.1-4.1</td>
<td>74</td>
</tr>
<tr>
<td>4.1-3.6</td>
<td>75</td>
</tr>
<tr>
<td>3.6-3.2</td>
<td>73</td>
</tr>
</tbody>
</table>

The zone ranging from 30 Å to 3.2 Å resolution was divided into 5 resolution bands, such that each band contained roughly equal numbers of reflections. Strong reflections with intensities of more than twice the standard deviation are distributed evenly to about 3.6 Å.
Friedel and merging R-factors of the LHC-II electron diffraction data

<table>
<thead>
<tr>
<th>Medium</th>
<th>Patterns processed</th>
<th>Friedel pairs</th>
<th>Resolution range (Å)</th>
<th>Friedel R-factor (%)</th>
<th>Merging R-factor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Strong</td>
<td>Weak</td>
</tr>
<tr>
<td>Tannin</td>
<td>17</td>
<td>26,881</td>
<td>3.2-30.0</td>
<td>13.0</td>
<td>54.8</td>
</tr>
<tr>
<td>Water</td>
<td>4</td>
<td>6470</td>
<td>3.2-9.1</td>
<td>12.4</td>
<td>54.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>9</td>
<td>15,849</td>
<td>3.2-15.5</td>
<td>15.7</td>
<td>57.5</td>
</tr>
</tbody>
</table>

Friedel R-factors and merging R-factors of the electron diffraction patterns recorded from LHC-II crystals preserved in 1 of the 3 media, vitreous water, tannin or glucose. Merging R-factors were calculated assuming that all crystals were untilted. Small deviations from zero tilt cause an increase in R_m. When the tilt angles of individual crystals were refined against a 3D data set, the merging R-factor for tannin-embedded crystals improved to 26.7%. Similar improvements would be expected for R_m in the other 2 media.

(c) Comparison of data sets and difference maps

For a detailed comparison of the three data sets for generating difference maps, amplitudes of structure factors were calculated directly from the merged intensities in each case. R-factors between the structure factor amplitudes of crystals preserved in vitreous water, tannin or glucose are shown in Table 4. Their values ranged from 64% to 143%, The lowest of these R-factors, R_sym, was that found between glucose and vitreous water, indicating the highest degree of structural similarity. Note, however, that all three values are considerably lower than typical R-factors between X-ray diffraction data and a protein structure solved by X-ray crystallography. Table 5 indicates that the R-factors factors between the three data sets were roughly the same in all resolution ranges.

Difference structure factor amplitudes were calculated between the three data sets after absolute scaling (Blundell & Johnson, 1976). Fourier difference maps were calculated using projection phases obtained by processing high resolution images of three nominally untilted 2D crystals in tannin (results not shown). The phase data extended to a resolution of 3.4 Å, which was somewhat higher than was previously reported (Kühlbrandt & Downing, 1989). Difference maps are shown in Figure 2(a), (b) and (c). The first contour is drawn at two times standard deviation of the map. The step size is equal to the standard deviation in each case. A 3.4 Å projection map of the complex in tannin is shown in Figure 2(d). Comparing this projection with the difference maps it is clear that difference peaks are more or less at the noise level of the projection map. The high-resolution electron diffraction patterns demonstrated that the three media are capable of preserving the crystals equally well, to at least 3.2 Å. From the absence of significant peaks in the difference maps and form the low R-factors between data sets we conclude further that all three media preserve the native structure of the protein. We are aware that our method is less sensitive to differences that may exist at low resolution (below about 15 Å), arising from the different electron-scattering densities of vitreous water, ice and glucose (Kühlbrandt, 1982). Differences of this...
kind can be detected by comparing the phases of structure factors at low resolution, obtained from Fourier transforms of images. However, such low-resolution differences have no bearing on the state of preservation of the protein at high resolution.

4. Discussion

(a) Formation of two-dimensional crystals

Precipitation of LHC-II with salt previous to 2D crystallization was essential for reproducible crystallization conditions. It seems that some of the microcrystalline aggregates that formed upon precipitation did not quite dissolve when detergent was added to the precipitate and thus acted as microseeds for the 2D crystals. Crystallization conditions were quite different when we used unprecipitated stock solution instead, and crystal formation tended to be erratic. On average, the crystals were much smaller. According to a previously reported method, 2D crystals of LHC-II formed upon dialysis against 200 mM-KCl (Kühnbrandt, 1984). KCl does induce crystallization but also causes 2D crystals to stack. We found that 40% glycerol was even more effective in promoting 2D crystallization. Stacking did

Table 4

R-factors (%) between the three data sets

<table>
<thead>
<tr>
<th>Difference</th>
<th>Amplitudes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strong</td>
</tr>
<tr>
<td>t/w</td>
<td>96</td>
</tr>
<tr>
<td>g/w</td>
<td>3.6</td>
</tr>
<tr>
<td>g/t</td>
<td>100</td>
</tr>
</tbody>
</table>

R-factors between the 3 sets of structure factor amplitudes collected from LHC-II crystals in vitreous water (w), tannin (t) or glucose (g). Strong and weak reflections are as defined in Table 2.

Table 5

R-factors between different data sets in resolutions ranges

<table>
<thead>
<tr>
<th>Resolution range (Å)</th>
<th>$R_w$</th>
<th>$R_{ew}$</th>
<th>$R_\mu$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.0-7.2</td>
<td>10.4</td>
<td>6.2</td>
<td>17.8</td>
</tr>
<tr>
<td>7.2-3.1</td>
<td>12.5</td>
<td>6.6</td>
<td>13.4</td>
</tr>
<tr>
<td>5.1-4.1</td>
<td>10.1</td>
<td>4.8</td>
<td>11.6</td>
</tr>
<tr>
<td>4.1-3.6</td>
<td>14.3</td>
<td>5.9</td>
<td>14.5</td>
</tr>
<tr>
<td>3.6-3.2</td>
<td>14.2</td>
<td>10.1</td>
<td>15.5</td>
</tr>
</tbody>
</table>

For definitions of subscripts, see Table 4.
not seem to occur under these conditions and large 2D crystals were obtained more reproducibly than with the KCl procedure.

Incubation temperature and time proved to be equally important. Incubation at 40°C for two hours apparently provided the mosaic patches with the necessary free energy to rearrange into large single 2D crystals. Significantly longer incubation periods or higher temperatures yielded aggregated, partly degraded crystals. Although the crystallization of membrane proteins is still far from routine, the choice of detergent and the protein/detergent ratio have been found to be key factors in 3D crystallization (Michel, 1983; Kühlbrandt, 1988b). Our results suggest that the correct ratio of detergent to protein is also critical for the formation of 2D crystals.

A decisive improvement of crystal size occurred when we introduced a second detergent, NG. In our opinion, this changed the size and surface properties of the mixed detergent/protein micelles in a way that favoured crystallization. Even if crystal contacts are made largely by the hydrophobic domains, as seems likely for 2D crystals of LHC-II and other membrane proteins, the properties of the detergent micelle surrounding the hydrophobic parts of the protein are clearly important for crystal formation. This is suggested by the detergent structure within 3D crystals of the reaction centre of the photosynthetic bacterium *Rhodopseudomonas viridis*, which forms an extended network that is spatially complementary to the structure of the protein (Roth et al., 1989). From this observation it seems clear that detergent micelles merge during crystallization of membrane proteins. The temperature at which micelles in solution coalesce into a separate phase is characteristic of each detergent. If two detergents are present, this temperature is a function of the molar ratio of both. It is known that the attractive forces between micelles of Triton X-100 become stronger with increasing temperature, whereas micelles of n-octyl-β-D-glycopyranoside (OG), and, presumably, also those of its nonyl analogue, interact more strongly at the temperature decrease (for a review, see Kühlbrandt, 1988b). We therefore believe that other 2D crystals of biological macromolecules may be equally sensitive to changes in environment. This may be especially true of 2D crystals of membrane proteins that grow in the presence of a particular detergent, or combination of detergents, and that therefore may be stable only in equilibrium with the crystallizing solution. We hope that the tannin procedure described here will prove useful in such instances.

In this case, the crystallizing solution contained 40% glycerol. It was therefore too viscous to form a thin film upon blotting so that it was necessary to dilute it or to wash the crystals on the support film. We found that washes with water, 1% glucose or 1% glycerol tended to disrupt high-resolution detail within seconds. However, we had some measure of success with these media after adopting the lens technique of specimen preparation (see Materials and Methods). By this technique, a small volume of crystallizing solution was mixed with glucose or water on the grid before blotting. We estimated that, after mixing, the glycerol concentration was between 10% and 20%, depending on the amount of liquid on the support grid. Since, under otherwise identical conditions, the success rate with glucose appeared to be considerably higher than with water, we believe that this medium does help to preserve the order of biological specimens.

Vitrification of the specimen was achieved routinely without plunge-freezing in liquid ethane (see Dubochet et al., 1988), presumably due to the presence of glycerol and other solutes that seem to inhibit the formation of crystalline ice. Devitrification was not observed at temperatures up to −135°C with glucose and water, and up to −110°C with tannin. Cooling was, of course, essential because electron diffraction patterns of 2D crystals of LHC-II could not be recorded at room temperature due to radiation damage (Kühnbrandt & Downing, 1989).
We observed that tannin, even at a concentration of 0.1%, caused immediate precipitation of LHC-II to 3 by careful control of conditions during crystal formation. Critical parameters are, in order of importance: detergent concentration, detergent composition, temperature and time.

Tannin is more effective than glucose or vitreous water in preserving the high-resolution detail of 2D crystals of light-harvesting complex, presumably due to its favourable interaction with the protein and detergent molecules in the crystals. The technique of specimen preparation is critical for recording high-resolution electron diffraction data.

High-resolution electron diffraction patterns of light-harvesting complex crystals indicate that the structure of the complex is the same in all three media. This structure has been determined at 3.4 Å resolution in projection by electron crystallography of 2D crystals in tannin.

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References


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