Antennas and Reaction Centers of Photosynthetic Bacteria

Structure, Interactions, and Dynamics

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Contents

Crystallization of the B800–850-complex from *Rhodopseudomonas acidophila* Strain 7750

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Recently, methods have become available for producing good quality three-dimensional crystals of integral membrane proteins, in the presence of detergents [1,2]. We were especially encouraged in this respect by the successful determination of the structure of the reaction centre from Rhodopseudomonas viridis [3], which has been so well described at this meeting.

We adopted a 'shotgun' approach to try and get crystals from a bacterial antenna complex. A range of antenna complexes were prepared and tested to see whether they would form crystals, using the vapour diffusion method [2]. Crystals were obtained in two cases, the B800-850-complexes from Rhodopseudomonas sphaeroides and Rhodopseudomonas acidophila. The B800-850-complex from Rps. acidophila forms crystals much more readily, and so we have concentrated our efforts upon this antenna complex. The B800-859-complex from Rps. acidophila contains bacteriochlorophyll a and carotenoids non-covalently bound to three low molecular-weight polypeptides [4].

We have experimented with the conditions for crystal formation, varying the detergent (lauryl-dimethylamine-N-oxide (LDAO), N-octyl-rac-2,3 dioxypropyl-sulphoxide or β-ocytl-glucoside), the precipitant (ammonium sulphate, potassium phosphate or polyethylene glycol), the pH (pH7.0 to 10.0), the temperature (4°-20°C) and the type of small amphiphile present (1,2,3 heptane-triol, piperidine-2-carbonic acid and benzamidine hydrochloride).

So far, the best results have been obtained with phosphate as the precipitant and at pH values above pH9.0. The crystal form seems to be very dependent upon the type of small amphiphile present. This is illustrated in Figs. 1-4.

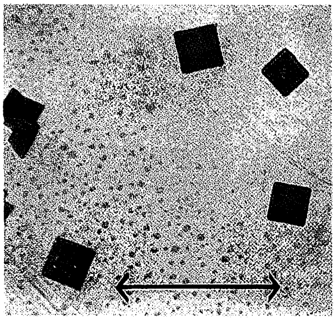
Crystallisation in the presence of heptane-triol/piperidine-2-carbonic acid mixtures gives rise to long needles or large, flat, plate-like crystals, while use of piperidine-2-carbonic acid alone yields rather square crystals. So far our largest crystals have been obtained in the presence of benzamidine hydrochloride, and in this case the crystals are usually wedge-shaped or rhomboid.

The degree of order of the pigment molecules within the crystals has been investigated optically, by looking for dichroism. Figure 5 shows the result of photographing some crystals with polaroid light. The colour of the crystals in the visible region is dominated by the absorption bands of the carotenoids. A comparison of the two pictures in Fig. 4 shows quite clearly that the carotenoids, within these crystals, exhibit a high degree of dichroism, and are therefore well-ordered within the crystals.

Fig. 1 Crystals grown in the presence of heptane-triol and piperidine-2-carboxylic acid

Fig. 2 Crystals grown in the presence of heptane-triol and piperidine-2-carboxylic acid

In each case the bar represents 100 μ



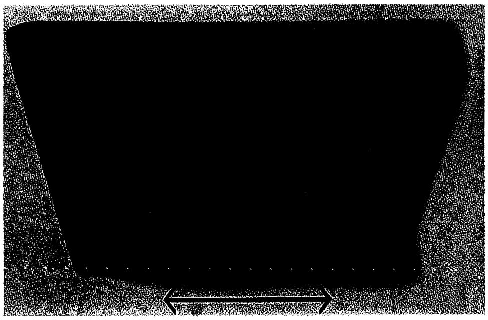
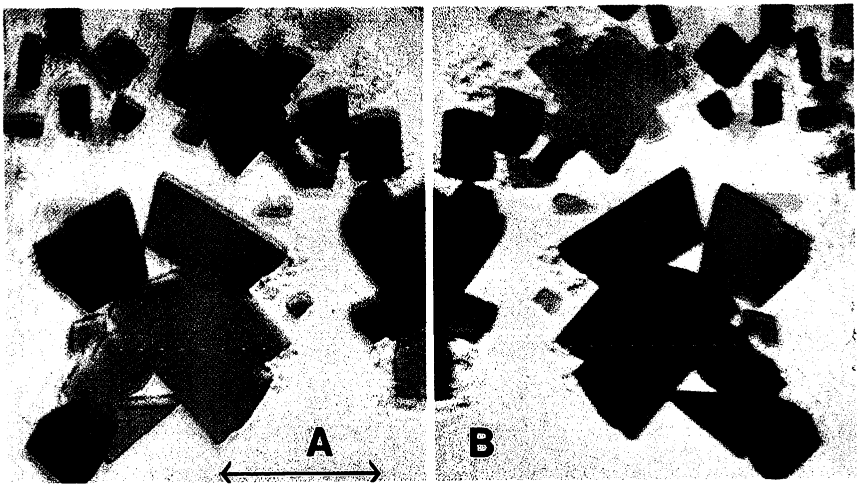


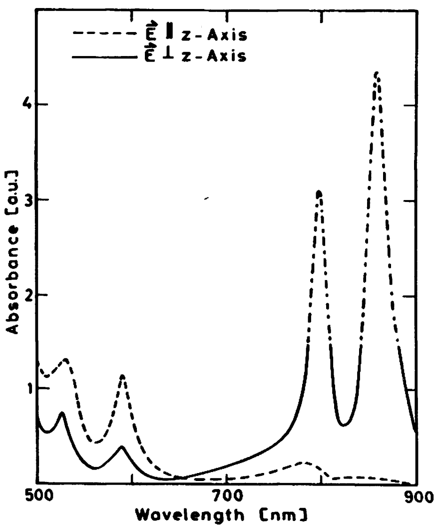
Fig. 3 Crystals grown in the presence of piperidine-2-carboxylic acid

Fig. 4 Crystal grown in the presence of benzamidine hydrochloride

In some preliminary studies we have looked at this phenomenon in more detail,by determining the linear dichroism spectra of some thin needle-shaped crystals (Fig. 6). Even though the crystals were, optically, rather too dense in the region of the 800 and 850 nm absorption bands, the linear dichroism spectra do indicate a remarkable degree of order in these bacteriochlorophyll *a* *Qy* absorption bands. In these bands,the dichroic ratio is clearly ⩾20x. In the *Qx* band at ~590 nm the sign of the dichroism is reversed,and the dichroic ratio is about 3:1. These initial results are very encouraging and indicate well-ordered crystals.

We are now continuing with this project,trying to get larger crystals that will be suitable for X-ray diffraction studies.





Figs. 5 A/B Crystals photographed under polarised light. The polarised light was 90° out of phase in the two photographs. The bar represents 100μ.

Fig. 6 The linear dichroism spectrum of some long, thin, crystals.

Where the line goes above 1.5 absorbance units the spectrum has been computed by reference to the absorption spectrum of the antenna complex in solution.

Acknowledgements

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