General discussion summary

This general discussion was chaired by A. W. Rutherford (*Service de Bioénergétique, Saclay, France*) and revolved around two major topics:

- (i) the implications of X-ray crystallography on the relationships between structure and function;
- (ii) the molecular mechanisms of the water-splitting process.

With regard to the X-ray structures the following series of questions were asked of P. Fromme (*Max-Volmer Laboratorium für Chemie und Biochemie, Technische Universität Berlin, Berlin, Germany and Department of Chemistry and Biochemistry, Arizona State University, Tempe, USA*).

T. A. Moore (*Department of Chemistry and Biochemistry and Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe, AZ, USA*) raised the point that given how important Ca^{2+} and Cl^- were in the mechanistic scheme presented by G. W. Brudvig (*Department of Chemistry, Yale University, New Haven, CT, USA*), at what stage of the crystallography will it be possible to see these cofactors? He extended the question to enquire about the possibility of identifying the positioning of the carotenoids in PSII. The latter question was endorsed by A. W. Rutherford, who reminded those present that the photooxidation of carotenoids in photosynthesis was unique to PSII.

P. Fromme explained that the quality of the X-ray data at present, especially the phase information, is simply not good enough to answer these questions and will require a structure closer to 2.5 Å.

J. De Las Rivas (*CSIC, Salamanca, Spain*) asked whether there had been an improvement of assignment of density to the extrinsic PsbO Mn stabilizing protein. P. Fromme said that they have now traced a considerable number of loops in addition to the β -sheet region. In reply to a question by A. W. Rutherford, she explained that none of these loops appeared to come within 10 Å of the Mn cluster suggesting that it was unlikely that any amino acids in this protein formed ligands with Mn. J. Barber (*Department of Biological Sciences, Imperial College of Science, Technology and Medicine, London, UK*) made the point that deletion of the *psbO* gene in cyanobacteria does not prevent the assembly of the Mn cluster or inhibit water oxidation, which is also the case for the deletion of the *psbV* gene. G. W. Brudvig (*Department of Chemistry, Yale University, New Haven, CT, USA*) raised the question of the stoichiometry of the PsbO, reminding the audience that some believe that there are two copies per PSII reaction centre. P. Fromme said that the X-ray structure clearly shows that there is only one copy of the PsbO and PsbV protein per reaction centre. A. W. Rutherford asked whether the β -sheet tubular nature of PsbO could act as a channel to the Mn cluster, either for H_2O or Mn delivery or for O_2 removal? P. Fromme explained that as long as the side chains within the tube are not identified, no final decision can be made whether there is a channel in the PsbO protein. Regarding PsbV, P. Fromme suggested that perhaps this cytochrome (cyt *c550*) has a protective role in mopping-up reactive oxygen species and therefore protecting PSII against photodamage. A. W. Rutherford thought this unlikely since A. Boussac at Saclay has found that the iron in cyt *c550* remains 6-coordinate in all conditions studied so far, notably over a wide range of pH. Thus there is no indication for an open coordination site on the metal that could act as a binding site for reactive oxygen species. Could this protein–protein interaction be some part of the redox control process, she asked?

I. Vass (*Department of Biology, Biological Research Centre, Szeged, Hungary*) asked whether the X-ray map indicated any close interactions of the Mn cluster with the D2 protein, while a similar question posed by S. Styring (*Department of Biochemistry, Lund University, Lund, Sweden*) enquired about the relationship of the AB lumenal loop of the D1 protein with the Mn cluster. P. Fromme said that there was no indication of any residues of the D2 protein coming closer than 10 Å to the Mn cluster, but in the case of the D1–AB loop it was possible that there could be some interaction. She went on to emphasize that the C/D loop and C-terminus of the D1 protein showed no homology with the corresponding region of the PsaA and PsaB proteins of photosystem I and that this may be because the D1 protein is involved in Mn ligation.

T. A. Moore (*Department of Chemistry and Biochemistry and Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe, AZ, USA*) asked which 'S state' the X-ray structure had been determined for? P. Fromme explained that the data collection has been performed in the dark, so most of the PSII complexes might have been in the S₁ state. However, we would not be able to detect small conformational changes of the Mn cluster upon oxidation at the present resolution. In fact, she made the point that not until a resolution is obtained that can resolve water molecules (2.5 Å or better) will it be relevant to address the mechanism of water oxidation and S-state cycling by X-ray crystallography. A. W. Rutherford concluded this discussion by saying that many of the ambiguities that had existed in structural–functional relationships in PSII have now been removed by the X-ray crystallography study. Clearly, however, a higher-resolution model is required to address many of the unanswered questions.

Discussions that focused on the mechanism of water oxidation raised more questions than answers. Picking up on the importance of Ca²⁺ in the water oxidation mechanism proposed by G. W. Brudvig, V. K. Yachandra (*Melvin Calvin Laboratory, Berkeley, CA, USA*) presented some EXAFS data that suggested that Ca²⁺ interacts closely with the Mn cluster, indicating that the interaction distance is *ca.* 3.4 Å. A. W. Rutherford wondered if V. K. Yachandra had conducted similar EXAFS experiments on the S₃ state where a change in the coordination of Ca²⁺ is predicted to occur. A. W. Rutherford also asked if the changes in the Mn–Mn distance seen when going from S₂ to S₃ could be explained by something other than the oxidation of the oxo-bridge. V. K. Yachandra felt that his data were consistent with the idea of a radical formation as an di-μ-oxo-bridge since it is difficult to explain the increase in Mn–Mn distance in any other way.

The discussion of water oxidation chemistry occurring on the S₃ state is inconsistent with a commonly held view that no such chemistry occurs until S₄, as emphasized by M. W. C. Evans (*Department of Biology, University College, London, UK*). C. Dismukes (*Department of Chemistry, Princeton University, Princeton, NJ, USA*) also thought it unlikely that the Mn⁵⁺ state existed as proposed in the Brudvig mechanism. He thought that the XANES data were more consistent with lower valency states. Indeed, his photoactivation studies favoured a maximum oxidation state of (Mn³⁺)₂(Mn⁴⁺)₂, although G. W. Brudvig doubted this conclusion. V. K. Yachandra disagreed with C. Dismukes's interpretation of the XANES and reiterated that the more recent X-ray absorption edge data favour the higher valence option, and stated that new data supports this conclusion. The prevailing discussions prompted a question as to whether G. W. Brudvig had achieved a Mn⁵⁺ state in his artificial Mn system? But apparently, the Mn⁴⁺ state has been the highest oxidative state characterized thus far.

GLOSSARY

EXAFS: extended X-ray absorption fine structure spectroscopy

PsbO: extrinsic 33 kDa protein

PsbV: extrinsic cyt *b*550 protein

PSII: photosystem II

XANES: X-ray absorption edge spectroscopy