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Introduction

Approximately 2.5 Gyr ago, an enzyme emerged which dramatically changed the chemical composition of our planet and set in motion an unprecedented explosion in biological activity. This enzyme used solar energy to power the thermodynamically and chemically demanding reaction of water splitting. In so doing, it provided biology with an unlimited supply of the 'hydrogen' (electrons and protons) needed to convert carbon dioxide into the organic molecules of life. Prior to this, biology had been dependent on hydrogen/electron donors such as H_2S, NH_3, organic acids and Fe^{2+}, which were in limited supply compared with the 'oceans' of water with which the planet Earth is blessed. The by-product of the water-splitting reaction is molecular oxygen. The release of this gas had dramatic consequences for biology: the world changed from anaerobic to aerobic and, as oxygen built up in the atmosphere, the ozone layer was formed. With oxygen available, the efficiency of metabolism increased dramatically since aerobic respiration provides in the region of 20 times more cellular energy than anaerobic respiration. This improved efficiency in energy conversion was probably a major factor responsible for the subsequent evolution of eukaryotic cells and multicellular organisms. The build-up of the ozone layer provided a shield against harmful UV radiation allowing organisms to explore new habitats and, in particular, to exploit the terrestrial environment.

Biological water splitting using sunlight had simply solved biology's energy problem allowing life to prosper and diversify on an enormous scale, as witnessed by the extent and variety of organisms in the fossil record and living on our planet today. The enzyme that gave rise to this 'big bang of evolution' is known as photosystem II (PSII) and is therefore an enzyme of global significance. It is a multiprotein complex contained within the thylakoid membranes of all types of plants, algae and cyanobacteria. In contrast to chemical and electrochemical water splitting, which are thermodynamically highly demanding, the PSII-catalysed biological water-splitting mechanism is truly remarkable since it proceeds with very little driving force and has only moderate activation energies; in the language of the electrochemist, it has a small over-potential.

These properties have led chemists to cast an envious eye on the water-oxidizing enzyme, looking for clues on how to construct efficient photochemical systems to mimic the natural reaction. The gain in efficiency from such catalysts could be very significant and could contribute greatly to alternative fuel production (electrochemical and photochemical water splitting) and usage (fuel cells).

Over the years, molecular enzymologists have slowly revealed the secrets of the photosynthetic water-splitting enzyme. The photochemical reaction centre was shown to be similar to the more banal and much better understood purple bacterial reaction centre. This was first demonstrated in the 1980s using spectroscopy, biochemistry and molecular biology, and confirmed more recently by crystallography. The catalytic site for water oxidation has, however, remained poorly defined. Certainly, there were vast amounts of information available. A precise kinetic model of the enzyme's redox intermediates has been established for decades. The active site was all but universally accepted as being made up of a cluster of four Mn ions close to a redox-active tyrosine group. A role for a nearby Ca^{2+} was also indicated. Good arguments for which amino acids were the probable ligands to the Mn had been painstakingly obtained, mainly by site-directed mutagenesis and spectroscopy. Moreover, a vast array of spectroscopic information reflecting the structural geometry of the cluster had been accumulated. But because Mn is one of the less cooperative of the transition metals, an unambiguous structure of the water-splitting site had not been defined and, consequently, mechanistic studies were held back. It sometimes seemed that the subject was bogged down in controversy fed by structural ambiguities.

The appearance of X-ray crystallographic models changed that perception. The first crude structural models verified the existing models for the positions and geometries of the cofactors of the photochemical reaction centre and the basic aspects of the protein structure: it was as expected, 'a purple bacterial reaction centre with Mn stuck on the bottom'. Some long-standing ambiguities were also resolved: the position of the Mn cluster and on which side of the reaction centre cytochrome b559 is located, for example. The state of knowledge at that time is reflected in a volume of this journal resulting from a discussion meeting held at the Royal Society in 2002 (see Barber, J. & Anderson, J. M. (eds) 2002 Photosystem II: molecular structure and function. *Phil. Trans. R. Soc. B* **357**, 1321–1512).

The first refined crystallographic model, including most of the amino acid side chains of the many subunits, first appeared in 2004. This provided not only many immediate new insights but also a rich resource of structural information for a wide range of current and future studies. While the crystal structures available today have yet to provide a clear, unambiguous model of the structure and geometry of the water-splitting site, they have provided a marked

One contribution of 20 to a Discussion Meeting Issue 'Revealing how nature uses sunlight to split water'.

improvement on previous models and at the same time they have removed large areas of ambiguity.

The current volume contains several contributions which deal with the structure and environment of the Mn_4Ca^{2+} cluster. Barber & Murray (2008) provide a comparative overview of the refined crystallographic models of the cluster and its environment, and they put forward new models taking into account the most recent geometries from X-ray absorption (polarized extended X-ray absorption fine-structure, EXAFS) studies. The article from Yano *et al.* (2008) deals with the X-ray absorption work itself and, in particular, recent studies conducted on crystals. In addition, they raise interesting questions concerning the delocalization of the valence orbitals over the Mn cluster. This has particular importance, not only for understanding the chemistry, but also for interpreting other spectroscopic studies. Dau *et al.* (2008) also report extended X-ray absorption measurements but interpret their data as favouring a somewhat different geometry for the complex which they place within a ligand sphere taken from the crystal structure.

The appearance of the crystal structure opened the door to the application of other structural methods that could be used to confront and refine the model. The articles of Yano *et al.* and Dau *et al.* are examples of this; however, several other articles in this volume fall into this category. Yeagle *et al.* (2008) present a progress report on the use of advanced electron paramagnetic resonance (EPR) and its application to the study of hyperfine couplings from ligands to the Mn cluster. The article of Strickler *et al.* (2008) gives a synopsis of the ongoing elegant work from Debus and his colleagues in which vibrational (Fourier-transform infra-red, FTIR) spectroscopy together with site-directed mutagenesis is being used to map out the effects on the (potential) ligands to the metal cluster upon each step in the redox accumulation (S-state) cycle. The experiments presented here focus on the Glu ligand from the CP43 protein, the environment of which is found to be sensitive to oxidation of the cluster on the S_1 to S_2 step. The interpretation of these results is potentially influenced by the valence delocalization discussed in the paper of Yano *et al.* In his paper, Siegbahn (2008) also discusses how the redox state of the cluster might be expected to affect the ligands as detected by FTIR.

In the new era of a crystal structure of the enzyme, complex mechanistic models for the water-splitting reaction have become intrinsically less speculative. At the discussion meeting, many speakers presented specific schemes built on a range of structural frameworks that had their roots in the crystal structural models or their derivatives. Some of these schemes appear in the articles published here. Good structural models allow computational methods to be used and, since the appearance of the crystal structures, such approaches have become a feature of the field. In this volume, Siegbahn extends his efforts in this area and provides quantum chemical arguments for a specific water-oxidation mechanism involving a radical-like oxygen intermediate. Similarly, Zein *et al.* (2008) report density-functional theory (DFT) calculations based on the geometries suggested from recent polarized EXAFS measurements. This provides some weighting for which of the geometries are the most reasonable and again forms the basis for mechanistic thinking. Sproviero *et al.* (2008) have used minimized versions of the crystal structures and have applied quantum mechanical methods to investigate substrate binding in different charge accumulation states. This work seems to give chemical insights to explain substrate binding effects reported in the literature. At present, these computational approaches provide limits on and feasibility tests of the kinds of chemistry that may occur. They will benefit greatly from future improvements in the resolution of the experimentally determined structural models.

Experimental data on the subject of substrate (water) binding are presented by Singh *et al.* (2008), in which their time-resolved mass spectroscopy method was applied to the enzyme modified by mutagenesis. Brudvig (2008) also addressed the question of substrate binding by providing much needed reference data on water exchange in Mn model systems. Substrate binding was also studied by Noguchi (2008), who presented experimental data using FTIR in a range appropriate for detecting weakly H-bonded water molecules in the enzyme. This was achieved for each of the charge accumulation states and as a function of pH and hydration. This allowed him to propose a mechanistic model for substrate binding, proton release and the formation of the O–O bond.

The search for intermediates in water chemistry was represented at the Royal Society discussion meeting by three different approaches. Junge talked about the elegant experiments by which he and his colleagues increased the concentration of the product (i.e. high O_2 pressure) to 'jam up' the crucial water-oxidizing steps and reveal the existence of intermediates in the reaction pathway. As mentioned in their paper, Dau and colleagues developed time-resolved X-ray absorption studies, a daunting task in itself, and have recently detected a short-lived intermediate which they ascribed to a deprotonation step in the S_3 state in the presence of the tyrosyl radical, $Tyr_Z^{\cdot}$. This intermediate seems to be different from those reported earlier using time-resolved optical methods. The paper by Boussac *et al.* (2008) describes the current state of procedures using low temperature and EPR spectroscopy to trap and study radical-metal states, an approach that is being used by several laboratories. In this case, new data are provided using a mutant that lacks the stable tyrosyl radical, which normally gives a signal that overlaps the spectral region of interest.

The crystal structure showed unambiguously that the Ca^{2+} makes up part of the catalytic metal cluster. Mechanistic studies have paid a good deal of attention to the role of the Ca^{2+} (see Brudvig's paper for example). It has been known for many years that the assembly of the Mn_4 cluster requires the presence of Ca^{2+} and, indeed, the article of Bartlett *et al.* (2008) reports a more detailed study of this phenomenon. This assembly process has been suggested to be facilitated by the presence of bicarbonate that lowers the redox potential of the Mn^{2+}/Mn^{3+} couple. The potential is so low that it seems that it can be oxidized by purple

bacterial reaction centres from a number of species, as reported by Khorobrykh *et al.* (2008). This finding is consistent with the idea that Mn^{2+}/bicarbonate may have served as an electron donor to a common ancestor related to the reaction centres of PSII and purple bacteria. The potential role of bicarbonate in the water-oxidizing enzyme remains controversial and the evidence is reviewed in the introduction to the paper by Khorobrykh *et al.*

There is still much to be understood concerning the details of the photochemical reaction centre that drives this enzyme, as emphasized at the discussion meeting at the Novartis Foundation immediately following the Royal Society discussion meeting. In this volume, there is one article on this subject. Schlodder *et al.* (2008) combine optical spectroscopy and site-directed mutagenesis to neatly remove the last ambiguity on the location of the reaction-centre chlorophyll triplet: it is indeed located on the chlorophyll now known as Chl_{D1}, as was first proposed more than two decades ago. This species has provided many structural insights and is considered to be responsible for much of the photodamage that limits the lifetime of the enzyme. As such, it remains the focus of research.

The appearance of crystal structures marked a change in the psychology of the subject. With a specific (albeit fuzzy) target structure, the synthesis of bio-inspired artificial water-splitting catalysts became an altogether more legitimate enterprise. Several chemistry laboratories have long been active in this area; indeed, the discovery of a multinuclear mixed valence Mn complex in the water-oxidizing enzyme in 1980 spawned a whole new field in inorganic chemistry. Recently, several more groups have joined the fray. The article by Armstrong (2008) provides an interesting overview of Mn chemistry and why this transition metal plays the unique role as a catalyst for water oxidation in biology. Brudvig's paper includes a discussion of the chemistry thought to be occurring in his now well-known dinuclear Mn complex that shows some water-oxidizing activity. Meelich *et al.* (2008) focus on some specific questions associated with enzyme activity that can be elucidated by the use of Mn models and the understanding of Mn chemistry. Meanwhile, Nocera in his paper, Betley *et al.* (2008), presents the two main mechanistic options for the O–O bond formation and describes specific molecules designed to perform these two distinct kinds of reactions. Hammarström & Styring's (2008) contribution represents the photocatalytic approach where the target is to produce an artificial charge-separating device that is hard-wired to the catalytic site, thereby mimicking another aspect of the biological system. The use of designer protein-based maquettes as a framework for constructing these types of hard-wired systems was presented by Dutton in his lecture at the Royal Society discussion meeting. Although thermodynamically efficient water oxidation has yet to be achieved with an artificial catalyst, there is a flurry of activity in this area, only a small fraction of which is represented here. It is surely just a matter of time and effort (not to mention money) before catalytic molecules see the light of day.

This volume serves as a snapshot of the main themes of research that are currently the centre of attention in this subject. The subject has entered a new era in which the structural questions are being resolved and the mechanistic questions are coming to the fore. This change is bringing stronger and stronger interest from chemists who wish to use the new information to make new catalysts that could contribute significantly to technological advances in the energy sector. The enzyme that solved the energy problem for biology 2.5 Gyr ago and thereby changed the planet may also contribute to solving what is considered to be humanity's greatest problem: the need for a sustainable energy supply that does not contribute to global warming.

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