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Introduction

Proton-transfer reactions in bioenergetics

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The term 'bioenergetics' generally refers to the discipline which confronts problems associated with the understanding of the mechanisms by which energy provided by light or by oxidation-reduction (redox) reactions is utilised by living organisms, for example for the synthesis of ATP. As outlined by Peter Mitchell in the *chemiosmotic theory*, an intermediate in the energy conversion in biological systems is an electrochemical potential difference ('proton gradient') across a membrane. Consequently, transmembrane proton-transfer reactions play a central role in Bioenergetics, which motivates this special issue of *Biochim. Biophys. Acta* on 'Proton-Transfer Reactions in Bioenergetics'.

The build-up and utilisation of the proton gradient are performed by membrane-bound protein complexes. Only during the last 10 years have the detailed three-dimensional structures of some of these complexes been revealed (see Fig. 1). These major breakthroughs have led to an enormous advancement in the field of Bioenergetics and experiments aimed at targeting specific mechanisms can now be designed and evaluated within the framework of the known protein structures. The advancement of the field is also illustrated by the fact that since 1988 two Nobel Prizes in Chemistry have been awarded for major discoveries in the field of Bioenergetics.

Proton diffusion in water is special as the proton is part of the solvent in which the reaction takes place, which is manifested by the much larger diffusion coefficient of protons than of any other ion. The transfer of a proton from one place to another in water does not necessarily require that the proton is transferred bodily. Instead, the charge of the proton may be propagated through a chain of hydrogen-bonded water molecules by alternating the exchange of hydrogen and covalent bonds between neighbouring water molecules (see [1]). This mechanism is most likely also utilised for proton transfer involving chains of water molecules through proteins. One extensively studied and well-characterised such system is the transmembrane gramicidin channel. The conductance of protons through this channel is about 15 times larger than that of potassium ions (see [2]), which is striking because the size of H_3O^+ and K^+ are about the same (see [3]). Along the same line of thinking, Nagle and Morowitz [4] introduced the concept of a 'proton wire' to illustrate the mechanism by which protons may move inside proteins along pathways provided by a network of hydrogen-bonded amino-acid side chains. So far, the structure–function studies of proton transfer in many proteins indicate that the transfer involves a combination of both chains of water molecules and protonatable amino-acid residues.

Even if the structural information is extremely important for the understanding of the functional properties of a biological energy transducer, it may not reveal dynamical features, such as e.g. transient configurations essential for efficient proton transfer. Consequently, an important challenge in future investigations will be the determination of high-resolution structures of transient intermediate states as has already been pioneered by the determination of the

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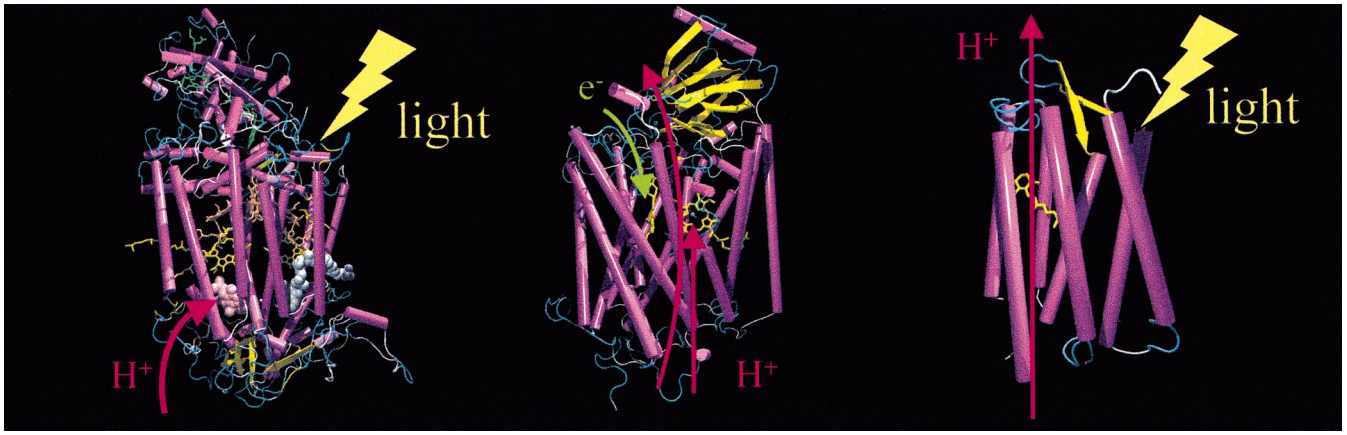

Fig. 1. Illustration of the structure and function of three of the membrane-bound protein complexes that are discussed in this Special Issue (the membrane is not shown): (from left) bacterial photosynthetic reaction centres (1PRC, [35]), cytochrome *c* oxidase (1AR1, [36]) and bacteriorhodopsin (1C3W, [37]). The figure was prepared using the Visual Molecular Dynamics software [38].

structure of a photosynthetic reaction centre in the charge-separated state [5] and more recently by the determination of the structure of intermediate states in the reaction cycle of bacteriorhodopsin [6,7].

The availability of detailed structural descriptions of proton-transfer pathways makes it possible to apply theoretical methods to understand such processes on a molecular level, interpret experimental data and design new experiments. The article by Krishtalik (p. 6–27) is a detailed summary of various means of thinking about proton transfer from a theoretical perspective. One important such aspect is the origin of the kinetic isotope effects. The article of Scheiner (p. 28–42) focuses on the origin of these effects and it contains a very informative integration of the contribution of proton tunnelling and the role of the hydrogen bond. Showen et al. (p. 43–62) present an interesting summary of various experimental data related to the problem of proton transfer and discuss these data within the context of theoretical models. They, as well as Krishtalik, also discuss the 'proton-inventory technique', which has been used to understand the details of specific proton-transfer reactions in biological systems.

The Marcus theory (for review see [8]) was originally developed to understand the principles of the kinetics of electron transfer and it has been applied extensively to studies of biological systems (see [9–13]). In recent years, the theory has also found applications in the study of biological proton transfer (Silverman, p. 88–103). However, this development has not been as rapid as in the case of electron transfer, primarily because it is much more difficult to study proton-transfer reactions than electron-transfer reaction since, in contrast to redox reactions, protonation reactions are generally not associated with spectral changes. In addition, it is often difficult to define the properties (e.g. pK_a values) of the donor–acceptor pair. One important exception is carbonic anhydrase, a well-characterised enzyme in which the proton donors and acceptors are well defined and can be manipulated in a controlled way. In his article, Silverman discusses the Marcus theory for proton transfer, using as a starting point the studies of this enzyme.

Since proton transfer in proteins takes place in a medium which on average has a low dielectric constant, electrostatic interactions are of fundamental importance. In fact, such interactions are most likely utilised by redox-driven proton pumps to regulate the proton-transfer rates, which is necessary for an efficient function of a proton pump (see below). The theoretical approach towards an understanding of such interactions in proteins is very difficult, as all charged species interact with each other and the whole of the protein must always be considered. The article by Gunner and Alexov (p. 63–87) summarises theoretical methods used for quantitative investigations of electrostatic interactions in proteins. In addition, it describes a new method which also takes into account protein structural changes as a result of changes in the charge state. This new ap-

proach has proven to be extremely valuable in the investigations of the bacterial photosynthetic reaction centres [14] and it is likely that it will also contribute towards the understanding of more complicated systems, such as proton pumps.

A limiting factor in many studies of proton-transfer reactions is the time resolution imposed by the mixing time of liquid solutions. While this time resolution is difficult to extend beyond the millisecond time scale, many proton-transfer reactions occur on much shorter time scales. In a few systems, such as e.g. bacterial (see Okamura, p. 148–163 and [15]) and plant photosynthetic complexes (see Tommos and Babcock, p. 199–219 and [16]), and bacteriorhodopsin (Heberle, p. 135–147, and [17]), the inherent light-sensitivity of the biological system of interest has been used to monitor light-induced proton-transfer reactions using e.g. pH-indicator dyes. In other cases, such as e.g. cytochrome *c* oxidase (see Mills et al., p. 180–187; Zaslavsky and Gennis, p. 164–169; and Wikström, p. 188–198 and [18,19]) artificial, light-sensitive states can be prepared so that reactions can be initiated by means of a short laser flash. However, most biological proton-transfer reactions are not initiated by light. Therefore, the development of the laser-induced proton-pulse technique by Gutman and Nachliel (see Brandsburg-Zabary et al., p. 120–134) has provided an important tool for the investigation of proton-transfer reactions in a large number of biological systems. The paper by Brandsburg-Zabary et al. describes results from experiments performed on a wide range of different biological systems and discuss fundamental chemical-physical mechanisms for proton transfer.

One basic feature of a redox or light-driven bioenergetic device, which has to transfer protons against a gradient, is alternating access of the proton to the two sides of the membrane, i.e. regulation of the intraprotein proton-transfer rates[1]. Thus, an important aspect of proton transfer is the mechanisms by which rates of proton transfer can be regulated by a protein. This aspect of proton transfer is discussed by DeCoursey and Cherny (p. 104–119) in their article, which mainly focuses on proton conduction through selective proton-transfer pathways. They also discuss the properties of a very interesting voltage-gated proton channel, which may have important implications for the understanding of mechanisms controlling proton transfer in Bioenergetics.

The aforementioned need for regulation of proton-transfer rates is crucial for an efficient function of proton pumps, such as bacteriorhodopsin and many respiratory oxidases. Bacteriorhodopsin is a light-driven proton pump. It has been characterised extensively using a wide range of biophysical methods, which in combination with detailed structural information makes it one of the best-characterised proton pumps. In his article, Heberle (p. 135–147) summarises experimental results obtained by many research groups and gives a detailed overview of the current understanding of the structure–function relations in this proton pump.

Cytochrome *c* oxidase couples the one-electron oxidation of four cytochrome *c* molecules to the four-electron reduction of dioxygen to water, creating an electrochemical proton gradient. In addition, many terminal oxidases conserve part of the free energy released in this reaction by translocating (pump) protons across the membrane. Even though the recent determination of the three-dimensional structures of cytochrome *c* oxidase, in combination with the use of site-directed mutagenesis and biophysical techniques has led to major developments in the oxidase research during the last couple of years (for review see [24,25]), the detailed mechanism of proton pumping remains unknown. Zaslavsky and Gennis (p. 164–179) and Mills et al. (p. 180–187) summarise the structural and functional information known to date. Zaslavsky and Gennis also comment on the ongoing discussions [26,27] that relate to the reaction steps involved in proton pumping. In addition, they propose a hypothetical mechanism by which the enzyme may control the proton-pumping activity. Mills et al. discuss the roles of the proton-transfer pathways in cytochrome *c* oxidase and focus on the proton-exit pathways of the enzyme, which is a very important issue that is poorly understood. In his article, Wikström (p. 188–198) proposes a mechanism of proton pumping. This mechanism is based on the histidine cycle, previously proposed by Wikström's

[1] The concept of energy-linked conformational changes in proteins has been discussed in general terms by Lumry [20] (see also [21]). General rules for the operation of energetically coupled vectorial systems have been discussed by Jencks [22] and Tanford [23].

research group [28] and incorporates the most recent experimental data. The model will undoubtedly stimulate new experimental investigations, especially relating to electron-proton control mechanisms [29].

The first three-dimensional structure of a membrane protein involved in energy transduction to be modelled at atomic resolution was the bacterial photosynthetic reaction centre ([30], see also [31]). The reaction centres catalyse the light-driven one-electron oxidation of cytochrome *c* and the two-electron reduction of the secondary acceptor quinone to quinol. The intermediate one-electron reduction of the primary and secondary acceptor quinones, respectively, are associated with fractional proton uptake. In their article, Okamura et al. (p. 148–163) summarise results from studies of proton uptake that follows the light-induced reduction of the quinones on the acceptor side in photosynthetic bacterial reaction centres. Factors controlling the driving force and rates of electron transfer between the primary and secondary acceptor quinones, as well as stabilisation of the charge-separated state, are discussed. The roles of individual amino acid residues, electrostatic interactions between these residues and the role of protein structural changes are described in detail.

A more elaborate relative of the bacterial reaction centre is photosystem II from plants. Instead of cytochrome *c* as an electron donor it uses water, which is oxidised as a result of the light-induced charge separation. The abstraction of four electrons and protons from two water molecules and formation of dioxygen takes place in the manganese-containing water-splitting complex and it is a complicated process which involves electron and proton/hydrogen transfer, and the transient formation of a tyrosyl radical. In particular, the coupling of electron and proton transfer has been studied extensively [16,32–34]. Babcock and Tommos (p. 199–219) present a summary of the experimental data pertaining to the mechanism of water-splitting in photosystem II and present an insightful model, based on both experimental data and theoretical calculations, which illustrates the mechanism by which the light-induced charge separation may be coupled to the oxidation of water.

As seen from the above description, this Special Issue contains a collection of articles summarising various aspects of biological proton transfer, including theoretical approaches, experimental techniques, biological model systems, membrane-bound energy transducers and complex molecular machines. I would like to thank all the authors for having contributed to making this Special Issue a collection of fascinating articles summarising the recent developments, controversies and ideas. In addition, many new, creative and original ideas are presented which hopefully will stimulate new developments and ways of thinking. I hope that this Special Issue will serve both as a reference and inspiration for future research within the field of Bioenergetics. I would also like to thank Andromachi Katsonouri for providing Fig. 1 in this summary and Martin Karpefors for providing the cover illustration. Finally, I would like to thank Wolfgang Junge and Mårten Wikström for suggesting to publish this Special Issue.

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