SubBioen Su-AM-1

PROTON TRANSFER IN ENZYMATIC REACTIONS

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Proton transfers that are important for the catalytic cycle occur between sites on the enzyme and/or between the enzyme and a reaction intermediate in the formation of product.

Transfers that are internal are difficult to demonstrate but may be inferred from D_2O effects in some cases. Transfers between enzyme and intermediate may be demonstrated by use of T to label the enzyme using pulse/chase methods or using a T donor in a specific reaction.

Pulse/chase experiments that follow the formation of T product with time can show the presence of hydrogen relays and explain the basis for sticky protons- those that exchange slowly in transfer between donor and acceptor substrates. To detect donor protons in a reaction in which T would not be chemically stable in the product a pulse/chase method on a D_2O rate effect (solvent shift) can be used. These methods will be described in studies with fumarase.

SubBioen Su-AM-2

PROTON TRANSFER IN THE CYTOPLASMIC REGION OF BACTERIORHODOPSIN IS FACILITATED BY A CLUSTER OF INTERACTING RESIDUES.

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We have examined the roles of the residues D96, T46 and R227 in the proton transfer reactions of the photocycle near the cytoplasmic surface of bacteriorhodopsin, and in general the relationship between the reprotonation of the Schiff base and the subsequent proton uptake from the cytoplasmic side. The phenotypes of single and double mutants suggest close functional interaction among D96, T46, R227, and bound water. The inhibitory and stimulatory influences of T46 and R227, respectively, on D96 as a proton donor compensate one another and ensure the effective reprotonation of the Schiff base. T46 and D96 mediate, in turn, proton uptake at the cytoplasmic surface. Although ultimately this will reprotonate D96, the observation of proton uptake from the bulk in R82Q without reprotonation of the aspartate residue suggests that the direct proton acceptor is not D96. It appears that, as proposed earlier for the extracellular region, proton exchange with the cytoplasmic bulk medium is facilitated by bound water, liganded in this case to T46 and D96. We suggest that hydrogen-bonded networks of protein residues and bound water facilitate the passage of the transported protons through both extracellular and cytoplasmic domains.

SubBioen Su-AM-3

PROTON TRANSFER IN BACTERIAL REACTION CENTERS FROM *RB. SPHAEROIDES*.* M.L. Paddock, S.H. Rongey, P.H. McPherson, A.C. Juth, G. Feher and M.Y. Okamura; UCSD, Physics Dept. 0319, 9500 Gilman Dr., La Jolla, CA., 92093-0319, USA.

The bacterial reaction center (RC) is a light driven proton pump that converts light into chemical energy through a two electron and two proton reduction of a bound quinone molecule as shown in Eqn. 1:

$$Q_AQ_B^- \xrightarrow{H^+(1)} Q_AQ_BH^- \xrightarrow{H^+(2)} Q_AQ_BH_2 \qquad (1)$$

where Q_A and Q_B are the primary and secondary quinone molecules. Q_B is buried inside the protein matrix out of contact with the aqueous exterior (1). Characterization of site-directed mutants (2-5) has shown that several amino acid residues are important for proton transport from the exterior to the buried Q_B and that two sequential proton transfers along separate pathways are important for the formation of the quinol. The first proton transfer ($H^+(1)$) is coupled to electron transfer and involves Ser-L223 and Asp-L213 (Eqn. 1). The second proton transfer ($H^+(2)$) follows electron transfer and involves Glu-L212. The coupling of the electron and proton in the first step of Eqn. 1 is an area of active investigation. In addition, other work suggests that other proton transfer pathways can be activated (6,7).

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(1) Allen *et al.* (1988) Proc. Natl. Acad. Sci. USA **85**, 8487-8491. (2) Paddock *et al.* (1989) Proc. Natl. Acad. Sci. USA **86**, 6602-6606. (3) Paddock *et al.* (1990) Proc. Natl. Acad. Sci. USA **87**, 6803-6807. (4) Takahashi & Wraight (1990) Biochim. Biophys. Acta **1020**, 107-111. (4) McPherson *et al.* (1991) Biophys. J. **59**, 142a. (5) Rongey *et al.* (1991) Biophys. J. **59**, 142a. (6) Hansen *et al.* (1993) Proc. Natl. Acad. Sci/ USA **90**, 8929-8933. (7) Rongey *et al.* (1993) Proc. Natl. Acad. Sci. USA **90**, 1325-1329.

SubBioen Su-AM-4

PROTON TRANSFER IN BACTERIAL REACTION CENTERS STUDIED BY TIME-RESOLVED INFRARED SPECTROSCOPY

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We have analyzed the coupling of H^+ transfer to e^- transfer in photosynthetic RC from *Rb. sphaeroides* by transient infrared (IR) spectroscopy in the sub-μsec to sec time domain using tunable diode lasers. In the 1760 to 1700 cm^{-1} range characteristic for ASP/GLU COOH vibrational modes, a number of IR transient signals could be characterized which correspond to protonation or deprotonation of ASP/GLU residues, or to a change of environment of a protonated ASP/GLU side chain. The major signal at 1725 cm^{-1} disappears in ^2H_2O and in GLU L212 ⇒ GLN mutant RC and can thus be attributed to H^+ uptake by GLU L212 near Q_B. H^+ uptake shows little variation between pH 5 and 9, and and increases above, indicating a complex titration behavious typical for electrostatically interacting residues. The signals can be fitted with two time constants, a fast one (100 μsec at 5°C) characteristic for $Q_A^-Q_B \Rightarrow Q_AQ_B^-$ electron transfer, and a slow one (1 msec at 5°C). The fast component is proposed to arise from instantaneous rearrangements and protonation changes at the Q_B site upon electron transfer, while the slow component corresponds to H^+ uptake. A model for *a dynamic electrostatic reaction field* around Q_B, which serves to accomodate and to stabilize different states (Q, Q^-) of this cofactor, is proposed from these data.

SubBioen Su-AM-5

PROTON UPTAKE IN REACTION CENTERS, COMPUTATION AND EXPERIMENT. ((M.R.Gunner)) Physics Dept. C.C.N.Y N.Y., NY 10031

The coupling of proton uptake or release to electron transfer is what makes chemiosmosis work. Proton uptake following charge separation has been measured in photosynthetic reaction centers from Rps. sphaeroides (Maroti, P. and Wraight, C. A. (1988). Biochim. Biophys. Acta **934**: 329-347;McPherson, P. H., Okamura, M. Y. and Feher, G. (1988). Biochim. Biophys. Acta **934**: 348-368). Analysis of the protein's structure using continuum electrostatics provides insight into the experimental results. A group of acidic amino acids is found near the Q_B binding site. The charges in this cluster are stabilized by as much as 15 kcal by the protein backbone. However, with 5 acidic and only 1 basic residue in this region, some of the acids are neutral. The cluster therefore serves an internal buffer. This structure can reduce the pH dependence of proton uptake, reduce the sensitivity to mutation, and diminish the difference between the electrochemical midpoints for the first and second electron transfers to Q_B. Supported by GM48726-01.