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PROTOLYTIC REACTIONS AND THE FUNCTIONING OF F-ATPases

F-ATPases synthesize ATP driven by a protonmotive, in some cases by a sodiummotive force. Their construction is bipartite with a membrane integral channel portion and a peripheral catalytic portion, both consisting of more than 20 polypeptides. These are remarkably conserved in tertiary and quaternary but not in primary structure. Exchange of subunits between remote organisms as *E.coli*, *Synechocystis sp PCC 6803* and spinach by proteinchemical or genetical manipulations has led to partially or fully functional *chimeric enzymes*. It is conceivable that they function as electrochemical-to-mechanical-to-chemical, or proton-to-conformation-to-ATP transducers. The *extreme proton selectivity* of the channel portion of the chloroplast enzyme ($H^+/K^+ > 10^6$) sharply contrasts with the sodium dependence of *P. modestum*. *This observation also points to a non-chemical*, indirect action of protons [1-5].
We have attempted to resolve partial reactions of the proton by application of *flash spectrophotometry to the chloroplast enzyme in the thylakoid membrane*, using both pH and voltage probes. A clue came from studies on a proton slip in the absence of nucleotides. *F-ATPases contain a total of six* nucleotide binding sites that can be grouped into two subsets, catalytic and non-catalytic ones, according to their specificities and affinities. Increasing the concentration of ADP the enzyme is transformed between three states with different proton conductance: uncoupled ("proton slip") ⇒ closed ⇒ coupled (ATP-synthesis). Proton slip is inhibited by the binding of ADP (K=200nM, plus Pi) to a first binding site. Proton flow coupled to ATP-synthesis is initiated by the binding of another molecule of ADP (K=7µM, plus Pi) to at least another site. Already the first site belongs into the subset of potentially catalytic sites. We interprete the above state transition as a consequence of an alternating site mechanism of catalysis. A mechanical model is presented which links *proton flow to the extrusion of spontaneously synthesized* and bound ATP. This model explains ATP synthesis under multisite and unisite conditions as well as *ATP hydrolysis driven proton pumping*.
Under conditions of proton slip we have demonstrated for the first time that *the binding, the transmembrane transport, and the extrusion of protons* can be selectively affected by nucleotides and inhibitors of the enzyme. Evidence for a "proton well" construct (Mitchell) at the inlet and the *outlet side of the* enzyme is presented [2, 6, 7].

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Biol. Chem. Hoppe-Seyler 374: 741-742