Journal of Bioenergetics (1976), **8**, 109–119

Coupling Factors ATPases from Photosynthetic Bacteria

B.A. Melandri and A. Baccarini Melandri

Institute of Botany, University of Bologna, Bologna, Italy

Received 3 June 1975

Introduction

Considerable information on the mechanism of ATP synthesis in energy transducing membranes has been obtained by studies concerning the detachment of the membrane bound ATPase and its reconstitution from purified membrane components. Although the first successful utilization of such an approach was described for the respiratory system of an aerobic bacterium [1], the most extensive efforts in this field have been subsequently spent on respiratory and photosynthetic organelles from eukaryotic cells (for recent reviews, see [2, 3]. Only recently bacterial ATPases [4–8] have again gained the interests of biochemists mainly because of their possible involvement in bacterial active transport.

Among prokaryotic photosynthetic organisms the resolution and reconstitution of the ATP synthesizing system has been achieved only for three genera of photosynthetic bacteria, namely, two members of the family Rhodospirillaceae (*Rhodopseudomonas capsulata* [9] and *Rhodospirillum rubrum* [10] and one of Chromatiaceae (*Chromatium vinosum*, strain D [11]). Only for the first two species, however, have methods for purification and a first characterization of the protein been reported [12, 13].

This review is concerned specifically with studies on the ATPase of these three organisms; comparative considerations have been included relative to other ATPases, mitochondrial, plastidial, and bacterial, for which the reader must refer to specific review articles (e.g., [2, 3, 5]). Emphasis has been placed on the modulation of ATPase activity in intact or reconstituted membranes since these newer aspects in the study of

energy transducing ATPases might be of general relevance for the mechanism of ATP synthesis.

Reversible Resolution of the Phosphorylating System

Different experimental procedures have been used successfully for solubilizing coupling factors from photosynthetic bacteria. Brief sonication in the presence of 1 mM EDTA decreases of about 90% light induced ATP synthesis and of 80% Mg^{2+}-dependent ATPase activity of membranes from *Rps. capsulata* [9]; the same technique can also be applied to *Rh. rubrum* membranes, but a more intense sonication is required in this latter instance [10]. Detachment of coupling factor from *Chromatium vinosum*, strain D can be achieved simply by washing the membranes with low ionic strength buffers [11]. Improvement in resolution has been obtained by treatment of phosphorylating membranes from *Rps. capsulata* and *Rh. rubrum* with 2 M LiCl solutions [14–16]. Low concentrations of Triton X-100 have been also used for detaching ATPase from membranes of *Rps. spheroides* [17].

The purest preparation of coupling factor protein from photosynthetic bacteria described in the literature appears to be that from *R. rubrum* [13]. The procedure used for purification is essentially similar to that originally reported for coupling factor from *Rps. capsulata* [12], but it includes further steps of purification. Recently Berzborn, Johansson, and M. Baltscheffsky (personal communication) have further improved this method and have succeeded in obtaining a protein electrophoretically and immunologically homogenous. The purified protein shows a molecular weight of 350.000 daltons, a sedimentation coefficient $s_{20,w} = 13.1 \times 10^{-13}$ *s* [13] and appears to be composed of five subunits, when analyzed by SDS acrylamide gel electrophoresis [18]. The subunit pattern is similar to that observed in coupling factors from other sources [2–7]; however, a big difficulty in further characterization of the physiological role of these subunits is represented by the very high instability and the very low yield of purified material, which does not allow, at the present time, the pursuing of further studies.

Difficulties have been met, at first, in the definition, in classical terms, of the coupling factor from photosynthetic bacteria, the light-dependent electron transport system of these organisms being cyclic in nature. This does not allow in fact a direct measurement of the rate of electron flow in untreated and treated membranes. However, it was observed that, in contrast to other energy transducing systems (e.g. [19, 20]), removal of coupling factor, while decreasing markedly photophosphorylation, ATPase, and ATP-^{32}Pi exchange activities, did not affect at all light induced proton uptake [21], indicating clearly that the resolution of ATPase from the membranes did not impair cyclic electron flow.

More direct demonstration of the specific role of this protein has come from studies comparing light and ATP induced quenching of fluorescent amines [22, 16]. While the ATP induced energization of membranes from *Rps. capsulata* was completely abolished by the detachment of coupling factor and was reconstituted by its reinsertion into the membrane [22], light induced energization was completely unaffected. Similarly ATP (or pyrophosphate) induced reduction of cytochrome *b* and the transient ADP-dependent oxidation of cytochrome *b* were reconstituted by addition of crude coupling factor in *Rh. rubrum* chromatophores [23]. This indicates that the protein is functioning only in the last step of ATP synthesis and hydrolysis. Other light-dependent reactions, such as carotenoid band shift and enhancement of ANS fluorescence, are only partially affected by the decoupling procedures ([16, 23], and B.C. Jackson, personal communication).

Facultative photosynthetic bacteria, like *R. rubrum* and *Rps. capsulata,* can grow easily in a complete heterotrophic condition; in this case energy is supplied by substrate oxidation. The photosynthetic and respiratory electron transport chains, which are localized in the cytoplasmic membrane and its invaginations [24], share most probably common electron transport components [25–27]. Support to the idea of a possible interaction between the two ATP synthesizing systems was provided by experiments showing that coupling factor purified from photosynthetically grown cells of *Rps. capsulata* could restore oxidative phosphorylation in decoupled membranes prepared from heterotrophically grown cells and vice versa [28]. Coupling factor restored the homologous or the heterologous function with the same effectiveness [29, 30]; moreover, a specific antibody prepared against photosynthetic coupling factor was able to inhibit oxidative phosphorylation [28]. Studies on the chemical properties of the purified protein, together with the observed lack of large structural effects of the bacterial coupling factor, indicate strongly a complete identity of the two proteins [28–31]. These conclusions have been recently confirmed also in *Rh. rubrum* chromatophores by immunological techniques (B.C. Johansson, personal communication).

Properties of Membrane Bound and Solubilized ATPase

Chromatophores from *R. rubrum* and *Rps. capsulata* exhibit a rather active Mg^{2+}-dependent ATPase activity that is sensitive to uncouplers and energy transfer inhibitors [32, 33]. Other metal cations such as Mn^{2+}, Ca^{2+}, and Ni^{2+} can substitute for Mg^{2+}, although with less efficiency [23, 31, 32]. On the contrary, membranes from *Chromatium* show a very low Ca^{2+} and Mg^{2+}-dependent ATPase activity [34], which can,

however, be enhanced from five- to eight-fold by tryptic digestion [35]. In this organism Mg–ATP or Ca–ATP complexes were shown to act as substrates for ATP hydrolysis with a Km of 1 mM for both Mg^{2+} and Ca^{2+} complexes. Free cations or free ATP inhibit competitively both activities [34]. A light-dependent enhancement of ATPase activity in *Rps. capsulata* chromatophores has been reported [36]; conformational changes of the enzyme, induced by cyclic electron flow, are likely to be involved in this phenomenon (see below).

Solubilization of the enzyme from the membranes of *Rh. rubrum* results in changes in the catalitic properties of the protein, especially in regard to cation requirements [13]. This phenomenon, which has been reported also for other phosphorylating systems [2], appears to be related to the procedure used for the detachment of the coupling factor. A protein purified from an acetone powder of *Rh. rubrum* membranes shows a very high Ca^{2+}-dependent ATPase activity that is purified in parallel with the recoupling activity [13]; Mg–ATP complex is not hydrolyzed and acts as a competitive inhibitor of Ca–ATP in this reaction. On the other hand, supernatants obtained by EDTA sonication treatment, in the presence of sulfhydryl reagents, of membranes from the same organism have been reported to exhibit a Mg^{2+}-dependent activity [15].

In *Chromatium* both Ca^{2+} and Mg^{2+} activities are present in the crude soluble enzyme; both activities are increased by tryptic digestion [35].

A weak ATPase activity, in the presence of Mg^{2+}, has been observed in purified coupling factor from *Rps. capsulata* [33].

In analogy with other energy transducing systems [2, 3, 5, 37] loss of sensitivity to uncouplers and energy transfer inhibitors accompanies the solubilization of the enzyme from the membranes of photosynthetic bacteria [33, 13, 35]. That sensitivity to oligomycin requires a direct interaction of the coupling factor with specific components, which are still present in the decoupled membranes, has been suggested by the observed stimulation of light induced proton uptake [21] by this antibiotic in ATPase stripped membranes (EDTA sonicated [21] or LiCl treated vesicles from *Rps. capsulata* [14]). LiCl treated particles washed with NH_4OH at pH 9.2 failed to restore oligomycin sensitive ATPase on addition of soluble coupling factor [14]; a crude factor, obtained from the ammonia supernatant by $(NH_4)_2SO_4$ precipitation, can partially restore oligomycin sensitivity in ammonia extracted membranes [14]. These preliminary data resemble closely the situation observed in mitochondria, in which a protein required for the reconstitution of oligomycin sensitive ATPase (OSCP = oligomycin sensitivity conferring protein) is also solubilized by ammonia washes (reviewed in [3]). However, support from further studies is needed in order to elucidate the molecular arrangement of the different components of the energy transducing bacterial ATPase.

Modulation of Membrane Bound ATPase Activity

Coupling factor ATPase from some photosynthetic bacteria, when bound to the membrane, can be affected markedly by various experimental conditions, since its activity depends by the interaction of the protein with substrates and with the electron transport chain. The factors affecting ATPase include light, phosphorylation substrates (such as ADP and phosphate) uncouplers, and energy transfer inhibitors. This particular aspect will be covered in some detail in this review since evidence is accumulating that these phenomena are common, although not identical, in many energy transducing systems of respiration and photosynthesis and might therefore be related, in the authors' opinion, to a general mechanism of the reaction present in all phosphorylating membranes.

The first phenomenon of this kind, discovered in *Rps. capsulata,* was a considerable increase of the ATPase activity following illumination of the membrane [36]. The activation of the enzyme, about two to three times as great as the dark activity, requires a few minutes of preillumination and is promptly reversed in the dark. This activation appears to depend upon the interaction of the enzyme with cyclic electron flow since it can be completely prevented by addition of Antimycin A [36]. The presence in the assay of an enzymatic trap for ADP (phosphoenolpyruvate and pyruvate kinase) allows an immediate activation of the enzyme without any need for preillumination. This suggested an antagonistic action of ADP against the photoactivation; indeed, the addition of micromolar concentrations of ADP before preillumination blocks completely the increase of activity by light: the concentration of ADP, reducing photoactivation by 50%, is very low (of the order of 1 μM). The relevance for energy transduction of the activity elicited by light is testified by its complete sensitivity to the energy transfer inhibitors oligomycin and aurovertin [38]. Illumination of the membranes in the absence of ADP brings about also structural modification of the coupling factor protein, which can be demonstrated by the response of the activity to the thiol reagent, *N*-ethylmaleimide (NEM) [38]. Incubation of the membranes with NEM in the dark does not cause any effect on ATPase activity; however, preincubation in the light produces an irreversible inactivation of the enzyme that can be observed both in phosphorylation and in ATPase activities. ADP, if added alone or, better, in association with phosphate, protects the enzyme against NEM photoinactivation. It has been suggested, on this basis, that light induces a conformation change of the enzyme that makes some thiol groups susceptible to the attack by NEM and that ADP and Pi can counteract the effect of light [38]. Moreover, it was also suggested that this phenomenon might be related or coincident with the

photoactivation of ATPase and its inhibition by ADP [38]. Phosphate, which partially protects the coupling factor against NEM, has also an activating effect on ATPase activity [39]. The activation by Pi in the dark (about two- to three-fold), which corresponds to an increase in the apparent V_{max} for ATP and is completely sensitive to aurovertin and oligomycin, is always observed in freshly prepared, well washed chromatophores. The stimulatory effect of phosphate is simulated by arsenate; when this phosphate analog is used, an activation of ATPase can be also observed in the light (the presence of an interfering light-dependent ATP–Pi exchange precludes the addition of Pi during the measurements of ATPase in the light). The stimulation of ATPase activity by Pi corresponds to an accelerated generation of the high energy state dependent on the hydrolysis of ATP in the dark; this observation together with the sensitivity of the Pi induced activity to energy transfer inhibitors confirms that the whole energy transducing ATPase complex is involved in these regulatory phenomena [39].

Some indications on the interaction of the coupling enzyme with the high energy state of the membrane have been obtained examining the response of ATPase to uncouplers. This response is quite different when the activity is measured in the light or in the dark and has to be described in some detail. Any uncoupler produces a marked stimulation of the activity when added in the dark at suboptimal concentrations, i.e., concentrations that only partially inhibit photophosphorylation; at higher concentration, however, a decrease of the activity is always observed, and a nearly complete inhibition of all the oligomycin sensitive activity is obtained at saturation [38]. In the light, on the other hand, only the inhibition at high concentrations of uncoupler is observed, the activity being completely unaffected at lower levels.

A straightforward interpretation of this phenomenon is not easy. Following the classical concept of uncoupler action it must be concluded that ATPase activity is controlled by energy dissipation in the dark (an energized state of the membrane following ATP hydrolysis in the dark can indeed be demonstrated), but this activity is not energy dissipation-limited in the light. The inhibition at high concentrations of uncouplers, which affects ATPase in the dark and in the light, in the absence or in the presence of Pi might indicate that the active state of the enzyme requires an energized state of the membrane above a minimal threshold level.

A possible correlation of these last phenomena with energy induced structural changes of the enzyme is suggested by the observation that FCCP can prevent the inhibition by NEM in the light; the protective effect of FCCP parallels exactly the inhibition of ATPase activity [38]. In summary, the activity of ATPase (in particular its V_{max}) in photosynthetic membranes of *Rps. capsulata* is controlled by the energy level of the membrane and by the presence of ADP and Pi, which exert

opposite effects; these modulations of the activity appear to be associated with some structural change of the enzyme, as indicated by the photo inhibition by NEM.

Preliminary experiments in *Rh. rubrum* (B.C. Jackson, personal communication) have indicated that some of the phenomena described above can be observed also in this organism under special experimental conditions. Activation of ATPase by light and inhibition by ADP could be observed when a low concentration of uncoupler was present, indicating that in this preparation ATPase activity was always under control of energy dissipation. Also in this system, however, high levels of uncoupler inhibited markedly ATPase activity.

The properties described above are not unique to ATPase from photosynthetic bacteria. A comparable behavior has been described also for the coupling enzyme from spinach chloroplasts, whose activity is triggered by light [40] and maintained by the energized state of the membrane, generated in the dark by ATP hydrolysis [41]. In this system the return of the enzyme to the latent state is accelerated by uncouplers [42] and by ADP [43], whereas phosphate stabilizes the active state [43]. Analogously to the bacterial ATPase, chloroplast photophosphorylation and ATPase are inhibited by NEM only following preincubation in the light in absence of substrates [44]: More specifically, it has been shown that NEM binds to the γ subunit of CF_1 in a 1:1 molar ratio [45]. These phenomena are most probably related to a conformational change of this protein, first discovered by Ryrie and Jagendorf [46, 47], who demonstrated the incorporation into the chloroplast coupling factor of nonexchangeable ^3H, during energization of the membrane. Phosphate was shown to accelerate the rate of ATP hydrolysis also in submitochondrial particles from rat liver [48]. An activation of the uncoupler stimulated ATPase, dependent on electron flow and/or on a low ATP/ADP ratio was demonstrated in beef heart mitochondria [49]; this last phenomenon was attributed to the release of a specific peptide inhibitor [50] present in the native ATPase complex.

This behavior of ATPase of photosynthetic bacteria finds analogies, therefore, in many other systems, photosynthetic or respiratory, prokaryotic or eukaryotic; it is therefore likely that all these phenomena reflect specific properties common to all energy transducing ATPases.

In many coupling enzymes several indications for multiple binding sites for adenine nucleotides have been described [51–54]. The presence in membrane from *Rh. rubrum* and presumably on the coupling factor of tightly bound, acid soluble ATP, has been directly established [55]. Indirect evidence for multiple binding sites for ADP can be drawn from the studies on the ATPase of *Rps. capsulata*. ADP, in fact, is able to inhibit light activation of ATPase with an apparent Ki around 1 μM [36], a value comparable with that of the Km for ADP in

photophosphorylation (about 10 μM); these figures are much lower than the apparent Ki for ADP in the competitive inhibition of ATPase, which was determined to be 0.26 mM at pH 8.0 [36].

Mechanisms for ATP synthesis in oxidative and photosynthetic phosphorylation, based on conformation changes of the ATPase and on the consequent changes in affinity constant for adenine nucleotides, which have been found to be bound to coupling factors in several instances, have been recently proposed [55–57]. It is conceivable that the activation of ATPase activity by light, its inhibition by ADP, and all the other phenomena described above, which were previously interpreted mainly as regulatory events, might represent experimental conditions in which the conformational equilibrium of the enzyme is shifted in one direction and, as a consequence, a specific catalytic function of the enzyme related to this conformation, enhanced.

Open problems

The problems still open in the study of photosynthetic bacterial ATPases are multiple; some of them are specific for these particular systems, others are of general interest for all coupling enzymes.

The first difficulty to be overcome is the setting up of a purification procedure that allows large yields and a reasonable stability of the protein. This will enable one to study in detail the molecular organization of the enzyme, its subunit structure, the presence of regulatory subunits (peptide ATPase inhibitor), number of binding sites for substrates, and their localization on the subunits themselves.

Like all other coupling factors, however, the intimate study of the EDTA detachable enzyme cannot solve completely the problem of energy transduction, since other hydrophobic components present in the membrane are essential for the complete mechanism of ATP synthesis coupled to electron transport or of energy transducing ATP hydrolysis [3]. In this respect our attempt to isolate from *Rps. capsulata* [14] a factor analogous to the oligomycin sensitivity conferring protein of McLennan and Tzagoloff [58] is only at a very preliminary stage.

According to the concepts of chemiosmotic coupling the whole ATPase complex should act as an electrogenic primary active transport system for protons linked to ATP hydrolysis [59]. Experiments supporting this view have been obtained for mitochondrial ATPase [60–62] using reconstituted lipoprotein vesicles; although the unavailability of an oligomycin-sensitive ATPase preparation precludes performing analogous experiments in photosynthetic bacteria, some indications along this line have been obtained with native membranes [22, 62, 63]. A clear-cut proof that proton translocation is an intrinsic property of all energy coupling ATPase will come, however, only when

proton translocation will be demonstrated in a reconstituted system with protein components purified to homogeneity, a condition that has not been met in any system studied so far.

The physiological meaning of the regulatory phenomena observed in isolated membranes remains unknown. In fact, the presence of multiple sites of different affinity for the binding of ADP in photophosphorylation, for "allosteric" interactions, and for competitive inhibition of the ATPase, suggests the possibility that two different and rather unrelated conformational states of the enzyme could exist, endowed with different catalytic properties in ATP synthesis and hydrolysis.

Acknowledgments

The authors would like to thank Drs R. Berzborn, A. Binder, C. Carmeli, B. Jackson, and B.C. Johansson for making available to us preprints of their manuscripts. Thanks are also due to Consiglio Nazionale delle Ricerche (Italy) for general support.

References

1. G. Pinchot, *J. Biol. Chem.*, **205** (1953) 65.
2. A.T. Jagendorf, in: *Bioenergetics of Photosynthesis*, Govindjee ed., Academic Press, New York (1975) p. 413.
3. P.L. Pedersen, *Bioenergetics*, **6** (1975) 243.
4. R. Adolfsen and E.N. Moudrianakis, *Biochemistry*, **10** (1971) 2247.
5. A. Abrams and J.B. Smith, in: *The Enzymes*, P.D. Boyer, ed., Vol. X, Academic Press, New York (1974) p. 395.
6. E. Mũnoz, M.R.J. Salton, M.H. Ng, and M.T. Shor, *Eur. J. Biochem.*, 7 (1969) 490.
7. M. Futai, M.C. Sternweis, L.A. Heppel, *Proc. Natl. Acad. Sci. USA*, 71 (1974) 2725.
8. F.M. Harold, *Bacteriol. Rev.*, **36** (1972) 172.
9. A. Baccarini Melandri, H. Gest, and A. San Pietro, *J. Biol. Chem.*, **245** (1970) 1224.
10. B.C. Johansson, *FEBS Lett.*, **20** (1972) 339.
11. A. Hochman and C. Carmeli, *FEBS Lett.*, 13 (1971) 36.
12. A. Baccarini Melandri and B.A. Melandri, in: *Methods in Enzymology*, A. San Pietro, ed., Vol. XXIII, Academic Press, New York (1971) p. 556.
13. B.C. Johansson, M. Baltscheffsky, H. Baltscheffsky, A. Baccarini-Melandri, and B.A. Melandri, *Eur. J. Biochem.*, **40** (1973) 109.
14. B.A. Melandri, E. Fabbri, E. Firstater, and A. Baccarini Melandri, in: *Membrane Proteins in Transport and Phosphorylation*, G.F. Azzone, M.E. Klingenberg, E. Quagliariello, and N. Siliprandi, eds, Elsevier, North Holland (1974), p. 55.
15 A.W.T. Konings and R.J. Guillory, *J. Biol. Chem.*, **248** (1973) 1045.
16. Z. Gromet-Elhanan, *J. Biol. Chem.*, 249 (1974) 2522.
17. D.W. Reed and D. Raveed, *Biochim. Biophys. Acta*, **283** (1972) 79.
18. B.C. Johansson and M. Baltscheffsky, *FEBS Lett.*, 53 (1975) 221.

19. R.E. McCarty and E. Racker, in: *Energy Conservation by the Photosynthetic Apparatus,* Brookhaven Natl. Lab., Upton, New York (1967) p. 208.
20. P.C. Hinkle and L.L. Horstman, *J. Biol. Chem.,* **246** (1971) 6024.
21. B.A. Melandri, A. Baccarini Melandri, A. San Pietro, and H. Gest, *Proc. Natl. Acad. Sci. USA,* 67 (1970) 477.
22. B.A. Melandri, A. Baccarini Melandri, A.R. Crofts, and R. Cogdell, *FEBS Lett.,* 24 (1972) 141.
23. B.C. Johansson, M. Baltscheffsky, and H. Baltscheffsky, in: *Proceedings of IInd International Congress on Photosynthesis,* G. Forti, M. Avron, and B.A. Melandri, eds, Dr W.N.V. Junk, Publishers, The Hague, Netherlands (1971) p. 1203.
24. J. Oelze and G. Drews, *Biochim. Biophys. Acta,* 265 (1972) 209.
25. A. Thore, D.L. Keister, and A. San Pietro, *Arch. Mikrobiol.,* 67 (1969) 378.
26. J. Yamashita, S. Yoshimura, Y. Matuo, and T. Horio, *Biochim. Biophys. Acta,* 143 (1967) 154.
27. J.L. Connelly, O.T.G. Jones, V.A. Saunders, and D.W. Yates, *Biochim. Biophys. Acta,* 292 (1973) 644.
28. B.A. Melandri, A. Baccarini Melandri, A. San Pietro, and H. Gest, *Science,* 174 (1971) 514.
29. A. Baccarini Melandri and B.A. Melandri, *FEBS Lett.,* 21 (1972) 131.
30. S. Lien and H. Gest, *Arch. Biochem. Biophys.,* 159 (1973) 830.
31. A. Baccarini Melandri and B.A. Melandri, in: *Proceedings of the IInd International Congress on Photosynthesis,* G. Forti, M. Avron, and B.A. Melandri, eds, Dr W.N.V. Junk, Publishers, The Hague (1971) p. 1185.
32. T. Horio, K. Nishikowa, and Y. Horiuti, in: *Methods in Enzymology,* A. San Pietro, ed., Vol. XXIII, Academic Press, New York (1971) p. 650.
33. B.A. Melandri and A. Baccarini Melandri, in: *Proceedings of the IInd International Congress of Photosynthesis,* G. Forti, M. Avron, and B.A. Melandri, eds, Dr W.N.V. Junk, Publishers, The Hague (1971) p. 1169.
34. A. Gepstein, Y. Hochman, and C. Carmeli, in: *Proceedings of the IIIrd International Congress on Photosynthesis,* M. Avron, ed., Elsevier, North Holland, Amsterdam (1974), p. xxx.
35. A. Gepstein and C. Carmeli, *Eur. J. Biochem.,* 44 (1974) 593.
36. B.A. Melandri, A. Baccarini Melandri, and E. Fabbri, *Biochim. Biophys. Acta,* 275 (1972) 383.
37. M.P. Roisin and A. Kepes, *Biochim. Biophys. Acta,* 305 (1973) 249.
38. A. Baccarini Melandri, E. Fabbri, E. Fistater, and B.A. Melandri, *Biochim. Biophys. Acta,* 376 (1975) 72.
39. A. Baccarini Melandri, E. Fabbri, and B.A. Melandri, *Biochim. Biophys. Acta,* 376 (1975) 82.
40. B. Petrack and F. Lipmann, in: *Light and Life,* W.D. Mac Elroy and H.B. Glass, eds, Johns Hopkins, Baltimore, Maryland (1961) p. 621.
41. H. Hock and I. Martin, *Biochem. Biophys. Res. Commun.,* 12 (1963) 223.
42. C. Carmeli, *Biochim. Biophys. Acta,* 189 (1969) 256.
43. C. Carmeli and Y. Lifshitz, *Biochim. Biophys. Acta,* 267 (1972) 86.
44. R.E. McCarty, P.R. Pittman, and Y. Tsuchiya, *J. Biol. Chem.,* 247 (1972) 3048.
45. R.E. McCarty and J. Fagan, *Biochemistry,* 12 (1973) 1503.
46. I.J. Ryrie and A.T. Jagendorf, *J. Biol. Chem.,* 246 (1971) 3771.
47. I.J. Ryrie and A.T. Jagendorf, *J. Biol. Chem.,* 247 (1972) 4453.
48. P. Mitchell and J. Moyle, *FEBS Lett.,* 9 (1970) 305.
49. R.J. Van de Stadt, R.J. De Boer, and K. Van Dam, *Biochim. Biophys. Acta,* 292 (1973) 338.
50. M.E. Pullman and G.C. Monroy, *J. Biol. Chem.,* 238 (1963) 3762.
51. H. Roy and E.N. Moudrianakis, *Proc. Natl. Acad. Sci. USA,* 68 (1971) 464.

52. N. Nelson, H. Nelson, and E. Racker, *J. Biol. Chem.*, 247 (1972) 6506.
53. W.A. Catterall and P.L. Pedersen, *J. Biol. Chem.*, 247 (1972) 7969.
54. D.A. Harris, J. Rosing, R.J. van de Stadt, and E.C. Slater, *Biochim. Biophys. Acta*, 314 (1973) 149.
55. H.U. Lutz, J.S. Dahl, and R. Bachofen, *Biochim. Biophys. Acta*, 347 (1974) 359.
56. P.D. Boyer, R.L. Cross, and W. Momsen, *Proc. Natl. Acad. Sci. USA*, 70 (1973) 2837.
57. E.C. Slater, J. Rosing, D.A. Harris, R.J. van de Stadt, and A. Kemp, in: *Membrane Proteins in Transport and Phosphorylation*, G.F. Azzone, M.E. Klingenberg, E. Quagliariello, and N. Siliprandi, eds, Elsevier, North Holland, Amsterdam (1974) p. 137.
58. D.H. McLennan and A. Tzagoloff, *Biochemistry*, 7 (1968) 1603.
59. P. Mitchell (1968), "Chemiosmotic coupling and energy transduction", Glynn Res. Ltd., Bodmin.
60. Y. Kagawa and E. Racker, *J. Biol. Chem.*, 246 (1971) 5477.
61. E. Racker and A. Kandrach, *J. Biol. Chem.*, 246 (1971) 7069.
62. A.A. Jasaitis, I.B. Nemeck, I.I. Severena, V.P. Skulachev, and S.M. Smirnova, *Biochim. Biophys. Acta*, 275 (1972) 485.
63. P.I. Isaev, E.A. Liberman, V.D. Samuilov, V.P. Skulachev, and L.M. Tsofina, *Biochim. Biophys. Acta*, 216 (1970) 22.