Bioenergetics (1973), 4, 521-532

Studies on Stabilization of the Oxidative Phosphorylation System

III. The Effects of Substrates and ATP on Sensitivity of Various Energy-Linked Functions of Submitochondrial Particles to Phospholipase A from *Crotalus terrificus* Venom

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Received 9 January 1973

Summary

The following results have been obtained from the study of the action of phospholipase A from *Crotalus terrificus* venom on sonic submitochondrial particles:

(1) The rate of ATP-dependent NAD^+ reduction by succinate and the rates of ATP- and succinate-dependent transhydrogenase reactions (NADH → $NADP^+$) rapidly decreases on treatment of submitochondrial particles with phospholipase A. The rate of the ATP-P_i exchange reaction does not thereby significantly decrease. NADH: fumarate oxidoreductase and energy-independent transhydrogenase (NADH → $NADP^+$) activities of the particles do not display a high sensitivity to phospholipase A either.

(2) When the particles subjected to the action of phospholipase A carry out NADH oxidation or ATP hydrolysis, some energy-dependent functions are disturbed less readily. This refers to reversed electron transfer, the ATP-dependent transhydrogenase reaction and partially to the succinate-dependent transhydrogenase reaction. The protective effect is sensitive to uncouplers. The ability to catalyze the ATP-P_i exchange reaction is not preserved in these conditions.

(3) These results suggest that coupling sites I and IV of sonic ("inside out") submitochondrial particles are highly sensitive to phospholipase A from *Crotalus terrificus* venom.

Abbreviations: CCCP, carbonyl cyanide, m-chlorophenyl hydrazone; BSA, bovine serum albumin; NAD^+, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide, reduced form; $NADP^+$, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form, ADP, adenosine-5′-diphosphate; ATP, adenosine-5′-triphosphate; P_i, inorganic phosphate.

(4) The relation is discussed of the protective effects of NADH and ATP to energy transformation in submitochondrial particles. Some new data on the energized state of particles are reported.

Introduction

One of the puzzles about mitochondria is why these organelles, which are so labile *in vitro,* possess such a remarkable viability in the living cell. It is a widespread view now that ageing of mitochondria largely depends on the action of mitochondrial phospholipase A_2 [1-10]. The suggestion has also been put forward that lysomal phospholipase A_1 plays an active role in the degradation of mitochondria *in vivo* and *in vitro* [10, 11]. The following ways of decreasing the effects of phospholipases on mitochondria have been considered: (1) binding of free fatty acids and lysophosphatides (which can be uncouplers) by serum albumin [5, 6, 12-14]; (2) addition of phospholipids to compensate for those digested by phospholipases [12, 16] and (3) competitive inhibition of phospholipase A_2 by certain phosphoric esters and exogenous phospholipids [1, 17]. Finally, the possibility is discussed of an ATP-dependent reincorporation of free fatty acids in the phospholipid fraction, involving mitochondrial acyltransferase [8, 18, 19].

Unfortunately, nothing was known until recently about how the resistance of mitochondrial membranes to phospholipases depends on the functional state of the oxidative phosphorylation system. And this information is very valuable, as it is well known that generation and transformation of energy in mitochondria is accompanied with conformational changes in its inner membrane [20-25] and hence can result in changed sensitivity of the membrane to phospholipases. Some aspects of this problem have been studied in this laboratory. Among other things, electron transfer has been proved, to be very important for stabilizing the respiratory chain, which is one of the constituents of the inner mitochondrial membrane, to cobra venom phospholipase [26, 27]. The present investigation has been carried out for elucidating the conditions in which the inner membrane components responsible for energy transduction display a higher resistance to phospholipase A. The results obtained thereby show that when NADH is oxidized, suppression of the ATP-dependent NAD^+ reduction by succinate and the ATP-dependent transhydrogenase activity of the particles is retarded. In the presence of ATP, in addition to these functions, the succinate-dependent transhydrogenase activity is somewhat stabilized. The results obtained suggest that the effects of NADH and ATP are associated with energy transduction in the membrane.

Materials and Methods

Ultrasonic submitochondrial particles, $ETP_H(Mg^{2+}, Mn^{2+})$, were obtained from heavy beef heart mitochondria, according to Beyer [28].

Incubation Conditions

The particles (1.0-2.0 mg protein per ml) were incubated at 30°C in a medium contained 20 mM Tris-HCl (pH 7.5), 100 mM sucrose, and 4.0 mM $MgSO_4$. Concentration of phospholipase A* was 12-40 μg per mg particle protein. When necessary, the incubation medium also contained a NADH-generating system: (1) 0.1 mM NAD^+, 30 mM ethanol, 0.15 mg/ml alcohol dehydrogenase and 8 mM semicarbazide or (2)0.4 mM $NADP^+$, 0.6 mM NAD^+, 10 mM glucose-6-phosphate, and 0.038 mg/ml glucose-6-phosphate dehydrogenase. Aeration was effected by continuously shaking the suspension in Warburg vessels. Whenever necessary, other components indicated in the figure captions were added to the medium.

Assays

(1) *Measurements of the rate of ATP-dependent NAD^+ reduction by succinate* were carried out according to Löw and Vallin [29]. 0.3 ml aliquots were taken from the incubation medium and placed into a spectrophotometer cuvette, containing: 50 mM Tris-HCl (pH 7.8), 3 mM $MgSO_4$, 250 mM sucrose, 10 mM succinate, 0.7 mM NAD^+, 1 mM KCN and 2 mg of BSA per ml (final volume 3.0 ml). The reaction was initiated by adding ATP (final concentration 2.6 mM). An increase in the optical density of the solution at 340 nm was recorded. The assays were carried out at 30°. The action of phospholipase A slowed down greatly when the incubation mixture was diluted by the assay medium. In the case when the incubation medium contained a NADH-generating system, NADH (0.05 mM) and semicarbazide (4.0 mM) were added to the assay medium to suppress alcohol dehydrogenase. In some cases it was sufficient to make corrections for NADH production by the alcohol dehydrogenase system. The rates of ATP-dependent NAD^+ reduction by succinate were 150-180 nmoles of NAD^+ reduced per min per mg protein at 30°.

* Phospholipase A from *Crotalus terrificus* venom was purchased from Calbiochem, Inc.

The action of the uncoupler (CCCP or oleate), some amount of which had been added to the assay medium together with the incubation medium, was interrupted completely by addition of BSA.

(2) *The ATP-dependent transhydrogenase activity* was measured as described by Danielson and Ernster [30]. The assay medium contained 250 mM sucrose, 50 mM Tris-HCl (pH 7.8), 3 mM $MgSO_4$, 1 mM KCN, 0.4-0.6 mM $NADP^+$, 0.1 mM NADH, 50-100 μg of alcohol dehydrogenase per ml, 30 mM ethanol, 4 mM semicarbazide, 1.33 mM ATP, 1.33 mg of BSA and 0.15 mg of particle protein per ml. The reaction was initiated by addition of ATP.

The medium for measuring succinate-dependent transhydrogenase activity contained 250 mM sucrose, 50 mM Tris-HCl (pH 7.8), 3 mM $MgSO_4$, 0.5 μg of rotenone per ml, 0.4 mM $NADP^+$, 0.1 mM NADH, 14 μg of oligomycin per ml, 100 μg of alcohol dehydrogenase per ml, 30 mM ethanol, 10 mM succinate, 4 mM semicarbazide, 1.33 mg of BSA and 0.15 mg of particle protein per ml. The reaction was initiated by addition of succinate.

The rate of the energy-independent transhydrogenase reaction ($NADH \rightarrow NADP^+$) was measured in the same conditions as those of the ATP-dependent reaction, but for the fact that the assay medium contained no ATP. The concentration of the particle protein was 0.3 mg/ml.

All the measurements were carried out at 32°. The specific activities of ATP-dependent, succinate-dependent and energy-independent transhydrogenases were 130-140, 130-160 and 10 nmoles of $NADP^+$ reduced per min per mg particle protein, respectively.

(3) *The NADH: fumarate oxidoreductase activity* was measured as described by Sanadi and Fluharty [31]. The assay medium contained 80 mM potassium phosphate (pH 7.5), 1 mM EDTA, 0.2 mM NADH, 3 mM KCN and 1.33 mg of BSA per ml. Aliquots of the particle suspension (final concentration 0.15 mg particle protein per ml) were added to the medium and KCN-insensitive NADH oxidation was recorded, after which 10 mM fumarate was added. NADH: fumarate oxidoreductase activity was estimated, taking into account the KCN-insensitive NADH oxidation by oxygen. This correction did not exceed 20% of the value to be determined. The specific activity was 40-50 nmoles of NADH oxidized per min per mg protein at 30°.

(4) *The rate of the ATP-P_i exchange reaction* was determined according to Christiansen *et al* [32]. The assay medium contained 125 mM sucrose, 25 mM Tris-HCl (pH 7.5), 4 mM $MgSO_4$, 10 mM $^{32}PO_4$ with a specific activity of $0.5 \times 10^5 - 1.5 \times 10^5$ counts per min per μmole, 5 mM ATP, 0.5 mM EDTA and 1.0 mg of BSA per ml. The reaction was initiated by adding the particle suspension (final concentration 0.2 mg of particle protein per ml) and interrupted in

10 min by adding 0.1 ml 50% trichloroacetic acid. The content of the labelled ATP was determined as described earlier [33]. Specific activity was 100-130 nmoles of ATP labelled per min per mg particle protein at 30°.

Results

Suppression of Some Energy-Dependent Functions of Submitochondrial Particles by Phospholipase A

Figure 1 demonstrates that the levels of certain activities in submitochondrial particles incubated with phospholipase A decrease. As is clearly seen, it is the ATP-dependent NAD^+ reduction by succinate and the succinate-dependent transhydrogenase that become suppressed most rapidly (Fig. 1, curves 1 and 2). The ATP-dependent transhydrogenase inactivates somewhat slower in the same conditions (Fig. 1, curve 3). As to the NADH: fumarate oxidoreductase and energy-independent transhydrogenase activities of the particles and their ability to catalyze the ATP-P_i exchange reaction (Fig. 1, curves 4, 5, 6), these functions become suppressed by phospholipase A comparatively

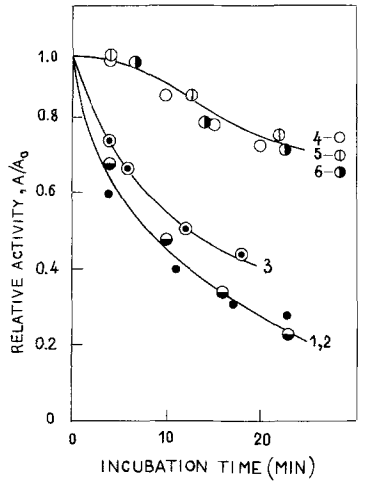

Figure 1. Changes in some functions of the submitochondrial particles induced by treatment with phospholipase A. 1, ATP-dependent NAD^+ reduction by succinate, 2, succinate-dependent transhydrogenase, NADH $\rightarrow$ $NADP^+$ (succinate); 3, ATP-dependent transhydrogenase, NADH $\rightarrow$ $NADP^+$ (ATP); 4, exchange reaction ATP-P_i ; 5, NADH: fumarate oxidoreductase; 6, transhydrogenase, NADH $\rightarrow$ $NADP^+$.

The incubation medium contained 1-2 mg particle protein per ml and 15-20 μg of phospholipase A per mg particle protein. A/A_0-relative specific activity. In all experiments represented on this and other figures the initial activities of the particles (A_0) were measured immediately after introducing phospholipase A.

slowly. It should be emphasized that the above effects are not associated with the uncoupling action of the products of degradation of phospholipids as all the measurements were carried out in the presence of a large amount of BSA (see "Material and Methods"). Hence these effects are to be looked upon as resulting from damage to the structure of the membranes of submitochondrial particles.

The Effects of ATP and Substrates on the Maintenance of Reversed Electron Transfer in Submitochondrial Particles

The phospholipase A-induced suppression of the ATP-dependent NAD^+ reduction by succinate was retarded when a NADH-generating system was added to the incubation medium together with phospholipase A (Fig. 2A). As the protective effect did not depend on the composition of the NADH-generating system (see "Materials and Methods") and appeared only in aerobic conditions (Fig. 2A), it may be attributed to electron transfer. The sensitivity of this effect to uncouplers is its important peculiarity. In fact, the protective effect of NADH does not take place in the presence of CCCP (0.6 nmoles per mg protein) or potassium oleate (80 nmoles per mg particle protein, see

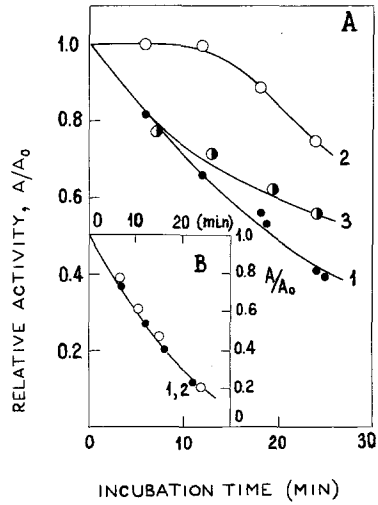

Figure 2. The influence of substrates on the sensitivity of the submitochondrial particles to phospholipase A (the ATP-dependent NAD^+ reduction by succinate). A, Protective effect of NADH. 1, 15 μg of phospholipase A per mg particle protein; 2, phospholipase A *plus* NADH-generating system (aerobic conditions); 3, phospholipase A *plus* NADH-generating system (anaerobic conditions). B, The effect of succinate. 1, 15 μg of phospholipase A per mg particle protein, 2, phospholipase A *plus* succinate (aerobic conditions).

Fig. 3A). Both uncouplers taken in these quantities almost completely suppressed the reversed electron transfer without inhibiting the NADH oxidase system of the particles or increasing their sensitivity to phospholipase A.

ATP* also retarded the suppression of the reversed electron transfer in submitochondrial particles by phospholipase A; this effect, like that of NADH, was not observed in the presence of CCCP or potassium oleate (Fig. 3B).

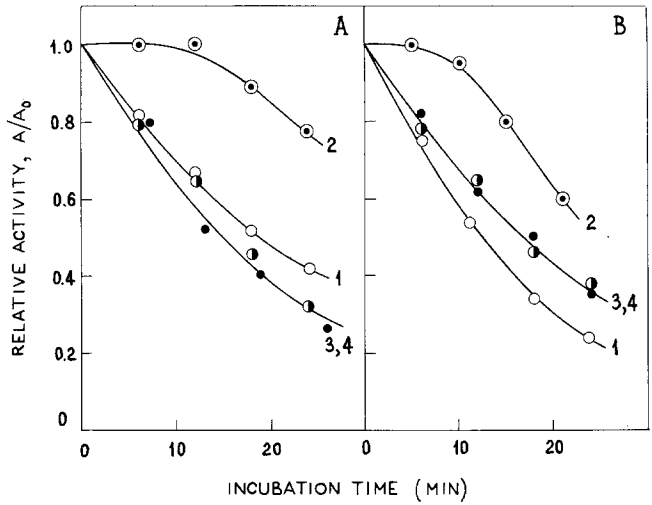

Figure 3. The effects of uncouplers on the protective action of NADH and ATP. (ATP-dependent NAD^+ reduction by succinate). A, The effect of potassium oleate on the protective action of NADH. 1, 25 μg of phospholipase A per mg particle protein; 2, phospholipase A *plus* NADH-generating system; 3, phospholipase A *plus* NADH-generating system *plus* 80 nmoles of oleate per mg protein; 4, phospholipase A *plus* 80 nmoles of oleate per mg protein. B, The effect of CCCP on the protective action of ATP. 1, 25 μg of phospholipase A per mg particle protein, 2, phospholipase A *plus* 6 mM ATP; 3, phospholipase A *plus* 6 mM ATP *plus* 0.6 nmoles of CCCP per mg protein; 4, phospholipase A *plus* 0.6 nmoles of CCCP per mg protein.

These results suggest that the above effects of NADH and ATP are in some way associated with energy transduction in submitochondrial particles.

In view of the above-said it seems surprising that succinate in aerobic conditions did not affect the reversed electron transfer suppression by phospholipase A (Fig. 2B).

* ADP taken at the same concentration as ATP did not prevent the submitochondrial particles from being activated.

The Effects of ATP and Substrates on the Suppression of the Energy-Linked Transhydrogenase Activity and the ATP-P_i Exchange Reaction by Phospholipase A

Figure 4A demonstrates the protective effects of NADH and ATP in the case of inactivation of the ATP-dependent transhydrogenase with phospholipase A. Unlike these agents, succinate did not markedly affect the rate of inactivation. The results shown in Fig. 4A coincide with those described above for the ATP-dependent NAD^+ reduction by succinate (see Figs. 2 and 3).

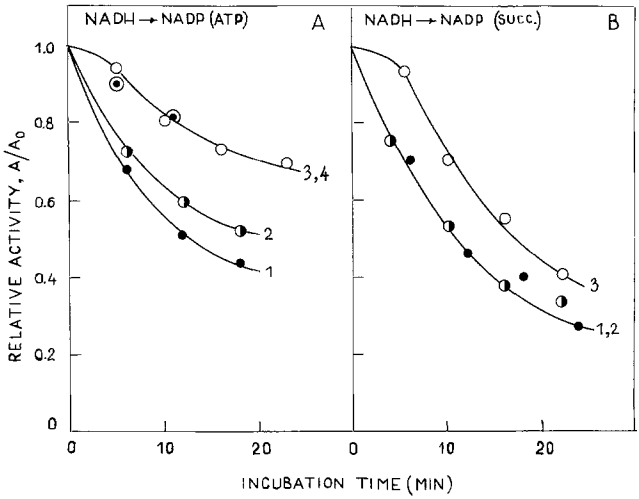

Figure 4. The effects of ATP and oxidizable substrates on the resistance of submitochondrial particles to phospholipase A (ATP-dependent and succinate-dependent transhydrogenases). A, ATP-dependent transhydrogenase. 1, 20 μg of phospholipase A per mg particle protein; 2, phospholipase A *plus* 10 mM succinate; 3, phospholipase A *plus* NADH-generating system; 4, phospholipase A *plus* 6 mM ATP. B, Succinate-dependent transhydrogenase. 1, 12 μg of phospholipase A per mg particle protein; 2, phospholipase A *plus* NADH-generating system; 3, phospholipase A *plus* 6 mM ATP.

Different results were obtained for the succinate-dependent transhydrogenase activity of submitochondrial particles. In this case the protective effect of NADH was not observed at all and that of ATP was relatively small (Fig. 4B).

Figure 5 shows suppression of the ATP-P_i exchange reaction during incubation of submitochondrial particles with phospholipase A in various conditions. It follows from this figure that neither ATP, nor NADH prevent the inactivating effect of phospholipase A from taking place.

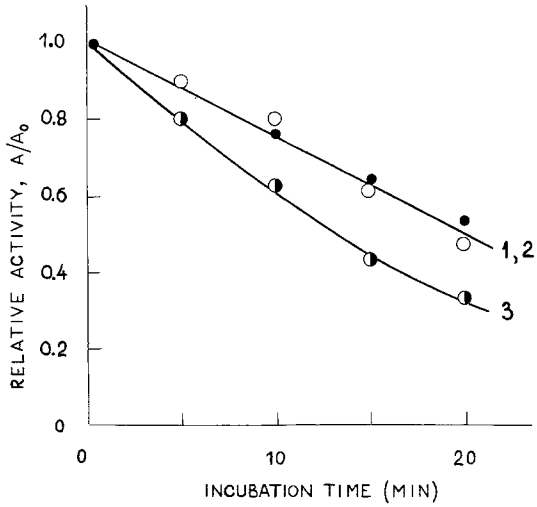

Figure 5. The decrease in the rate of the ATP-P_i exchange reaction induced by treatment of submitochondrial particles with phospholipase A under various conditions. 1, 40 μg of phospholipase A per mg particle protein; 2, phospholipase A *plus* 6 mM ATP; 3, phospholipase A *plus* NADH-generating system.

Discussion

The results of this investigation pertain to two main questions: (1) what pathways of energy transduction in submitochondrial particles are most sensitive to phospholipase A and (2) how sensitivity to phospholipase A changes with the functional state of the particles.

It has been shown above that the treatment of submitochondrial particiles with phospholipase A causes a rapid decrease in the rate of the ATP-dependent NAD^+ reduction by succinate. This effect may be due to the following kinds of damage: (1) in the respective fragment of the respiratory chain; (2) in coupling site I and (3) in the ATP-synthesizing system. The first of these reasons does not seem very consistent because of the comparatively low rate of inactivation of NADH: fumarate oxidoreductase. As the ATP-P_i exchange reaction is not strongly suppressed by phospholipase A, the changes in the ATP-synthesizing system can hardly account for the reversed electron transfer suppression. Thus, it is most likely that phospholipase A primarily attacks coupling site I. An alternative point of view is that the decrease in the rate of reversed electron transfer is due to changes induced by phospholipase A in the ATP-synthesizing system and these changes are not critical for the ATP-P_i exchange reaction. But there are no experimental facts to

support this suggestion, which is therefore just a speculation. Besides, if this were the case it would be difficult to explain the fact that ATP and NADH hinder "attenuation" of the reversed electron transfer during treatment of particles with phospholiphase A but display no protective effect in the ATP-P_i exchange reaction.

Similar reasoning leads to the conclusion that inactivation of the ATP-dependent transhydrogenase is due to the action of phospholipase A on coupling site IV (Skulachev's term [34]) or zero (Mitchell's term [35]). As to the succinate-dependent transhydrogenase, it inactivates more rapidly, which may mean that, in addition to coupling site IV, phospholipase A attacks coupling sites II and/or III. However, for this suggestion to be realistic it should be better grounded.

The following results have been obtained when studying the effects of oxidizable substrates and ATP on suppression of the above-mentioned energy-linked functions of submitochondrial particles: (1) during oxidation of NADH coupling sites I and IV acquire some resistance to phospholipase A; (2) in the presence of ATP degradation of I and IV coupling sites is also retarded; (3) succinate displays no protective effect. That the effects of NADH and ATP are sensitive to uncouplers should be interpreted as meaning that they are associated with energy transduction in the membrane. The inability of succinate to protect the above energy-linked functions of submitochondrial particles from phospholipase A attack could be ascribed to the membranes being insufficiently energized. This, however, was disproved by the following data. In our experiments the rate of NADH oxidation was 40-200 nmoles per mg protein per min with the P/O ratio being 2.0-2.1. The succinate oxidation rate was 150-200 nmoles per mg protein per min, P/O ratio being 1.0-1.2. In other words, the two substrates should possess similar energizing activities.

It should be borne in mind that the methods by which the energized state of mitochondrial membranes is usually detected (electron microscopy [20, 21], the fluorescent probe [23, 24, 36, 37] and penetrating ions [38, 39] techniques) furnish but general information. The details, which depend on the specificity of interaction of succinate, NADH and ATP with the fixed components of the membrane, such as local changes in the membrane structure or in the charge on its surface [36, 37, 40], remain obscure. The results reported above may be regarded as elucidation of some of these traits. For example, it may be suggested that the protective effects of NADH and ATP is the result of local conformational changes involving coupling sites I and IV and accompanying the events which are usually regarded as being indicative of the energized state of the membrane. If this is the case the lack of protective effect with succinate (which interacts with different membrane components than ATP and NADH) is understandable. It should be

noted that the effects of NADH and ATP are not identical, ATP stabilizes succinate-dependent transhydrogenase and NADH does not.

The above said is supported by the data obtained previously when studying the action of trypsin on the respiratory chain [26, 27] and the phosphorylating system [33] of submitochondrial particles. The gist of the evidence was that electron transfer in the absence of ADP is accompanied by local conformational changes in the respiratory chain which do not extend to the trypsin-sensitive components of the phosphorylating system. On the contrary, the conformational changes associated with ATP synthesis or hydrolysis involves these components.

Acknowledgements

We wish to thank Professor I. V. Berezin for advice and encouragement and Professor V. P. Skulachev for valuable criticism. Grateful acknowledgements are offered to Miss T. I. Kheifets for translating the paper into English.

References

1. C. R. Rossi, L. Sartorelli, L. Tato and N. Siliprandi, *Arch. Biochem. Biophys.*, 107 (1964) 170.
2. C. R. Rossi, L. Sartorelli, L. Tato, L. Baretta and N. Siliprandi, *Biochim. Biophys. Acta*, 98 (1965) 207.
3. G. L. Scherphof and L. L. M. Van Deenen, *Biochim. Biophys. Acta*, 98 (1965) 204.
4. W. Chefurka, *Biochemistry*, 5 (1966) 3887.
5. I. Honjo, K. Ozawa, O. Kitamura, A. Sakai and T. Ohsawa, *J. Biochem.*, 63 (1968) 311.
6. K. Ozawa, O. Kitamura, T. Ohsawa, T. Murata and I. Honjo, *J. Biochem.*, 66 (1969) 361.
7. J. Nachbaur and P. M. Vignais, *Biochem. Biophys. Res. Commun.*, 33 (1968) 315.
8. J. Nachbaur, A. Colbeau and P. M. Vignais, *FEBS Letters*, 3 (1969) 121.
9. M. Waite, *Biochemistry*, 8 (1969) 2536.
10. I. Boime, E. E. Smith and F. E. Hunter, Jr., *Arch. Biochem. Biophys.*, 139 (1970) 425.
11. A. Mellors, A. L. Tappel, P. L. Sawant and I. D. Desai, *Biochim. Biophys. Acta*, 143 (1967) 299.
12. E. Petrushka, J. H. Quastel and P. G. Scholefield, *Can. J. Biochem. Physiol.*, 37 (1959) 889.
13. C. Burstein, A. Loyter and E. Racker, *J. Biol. Chem.*, 246 (1971) 4075.
14. C. Burstein, A. Kandrach and E. Racker, *J. Biol. Chem.*, 246 (1971) 4083.
15. R. Berezney, Y. C. Awasthi and F. L. Crane, *J. Bioenergetics*, 1 (1970) 445.
16. R. Berezney, Y. C. Awasthi and F. L. Crane, *J. Bioenergetics*, 1 (1970) 457.
17. C. R. Rossi, C. S. Rossi, L. Sartorelli, D. Siliprandi and N. Siliprandi, *Arch. Biochem. Biophys.*, 99 (1962) 214.
18. L. Wojtczak and A. L. Lehninger, *Biochim. Biophys. Acta*, 51 (1961) 442.

19. M. G. Sarzala, L. M. G. Van Golde, B. de Kruyff and L. L. M. Van Deenen, *Eur. J. Biochem.*, 27 (1972) 401.
20. C. R. Hackenbrock, *J. Cell Biol.*, 37 (1968) 345.
21. R. A. Harris, M. A. Asbell, J. Asai, W. W. Jolly and D. E. Green, *Arch. Biochem. Biophys.*, 132 (1969) 545.
22. R. A. Harris, M. A. Asbell and D. E. Green, *Arch. Biochem. Biophys.*, 131 (1969) 316.
23. A. Datta and H. S. Penefsky, *J. Biol. Chem.*, 245 (1970) 1537.
24. A. Azzi, B. Chance, G. K. Radda and C. P. Lee, *Proc. Natl. Acad. Sci. US*, 62 (1969) 612.
25. J. M. Wrigglesworth and L. Packer, *Arch. Biochem. Biophys.*, 128 (1968) 790.
26. V. N. Luzikov, M. M. Rakhimov, V. A. Saks and I. V. Berezin, *Biokhimiya*, 32 (1967) 1234.
27. V. N. Luzikov and L. V. Romashina, *Biochim. Biophys. Acta*, 267 (1972) 37.
28. R. E. Beyer, in: *Methods in Enzymology* (R. Estabrook and M. Pullman,Eds), vol. X, Academic Press, New York, 1967, p. 186.
29. H. Löw and I. Vallin, *Biochim. Biophys. Acta*, 69 (1963) 361.
30. L. Danielson and L. Ernster, *Biochem. Z.*, 338 (1963) 188.
31. D. R. Sanadi and A. L. Fluharty, *Biochemistry*, 2 (1963) 523.
32. R. O. Christiansen, A. Loyter, H. Steensland, J. Saltzgaber, and E. Racker, *J. Biol. Chem.*, 244 (1969) 16.
33. V. N. Luzikov, V. A. Saks and V. V. Kupriyanov, *Biochim. Biophys. Acta*, 253 (1971) 46.
34. V. P. Skulachev, in: *Current Topics in Bioenergetics*, vol. 4, Academic Press, New York-London, 1971, p. 127.
35. P. Mitchell, *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation*, Glynn Research, Bodmin, 1966.
36. A. Azzi, P. Gherardini, M. Santato and G. Fabbro, *Fed. Proc.*, 30 (1971) 1016.
37. A. Azzi, P. Gherardini and M. Santato, *J. Biol. Chem.*, 246 (1971) 2035.
38. L. L. Grinius, A. A. Jasaitis, Yu. P. Kadziauskas, E. A. Liberman, V. P. Skulachev, V. P. Topali, L. M. Tsofina and M. A. Vladimirova, *Biochim. Biophys. Acta*, 216 (1970) 1.
39. L. E. Bakeeva, L. L. Grinius, A. A. Jasaitis, V. V. Kuliene, D. O. Levitsky, E. A. Liberman, I. I. Severina and V. P. Skulachev, *Biochim. Biophys. Acta*, 216 (1970) 13.
40. P. Dell 'Antone, R. Colonna and G. F. Azzone, *Eur. J. Biochem.* 24 (1972) 566.