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Properties and regulation of the H^+-ATP synthase of mitochondria

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A brief survey is made of the function of the H^+-ATP synthase of mitochondria with emphasis on how it is regulated. A main regulatory factor is a low molecular weight protein whose binding to the enzyme appears to be essential for optimal accumulation of ATP as driven by electron transport. The ATP synthase is also controlled by ADP that, by binding to a site in the enzyme, inhibits ATP hydrolysis. Data on the spontaneous synthesis of a tightly bound ATP are discussed. Apparently, this requires proper subunit interactions to yield a competent catalytic site.

1. Introduction

In the energy-transducing membranes of mitochondria, chloroplasts, photosynthetic bacteria, and the plasma membrane of bacteria, electron transport results in the formation of electrochemical H^+ gradients. These in turn are utilized by H^+-ATP synthases for driving synthesis of ATP from ADP and phosphate [1–3]. The enzyme may also function as an ATPase, i.e., it hydrolyzes ATP with formation of electrochemical H^+ gradients. The H^+-ATP synthases may be classified among the various ion-translocating ATPases. However, the structure of the former is more complex, at least with respect to the number of subunits which is significantly much larger in the H^+-ATP synthases [3,4].

In order to understand fully the mechanism through which the ATP synthases carry out the synthesis of ATP, there are essentially three points that must be clarified. The first is catalysis; in contrast to other ion-translocating ATPases, ATP synthases have three catalytic sites [5], and no phosphorylated enzyme intermediates have been detected during the operation of catalytic cycles. Thus, the precise molecular events that occur during synthesis and hydrolysis of ATP appear to be difficult to unveil. A second point is how the enzyme is regulated. In recent years, evidence has accumulated that indicates that the enzyme has complex regulatory mechanisms. These essentially involve the function of a low-molecular-mass protein known as the ATPase inhibitor protein [6], and of adenine nucleotides that may bind to the enzyme. With respect to adenine nucleotides, it has been shown that the enzyme possesses six binding sites for adenine nucleotides, three being catalytic whereas the other three are noncatalytic [5,7,8]. Finally, since the function of the enzyme is to produce ATP, the most important point is to ascertain the mechanism of how the enzyme transforms the energy of electrochemical H^+ gradients into that of the β–γ pyrophosphate bond of ATP. It is clear that all three processes are intimately related, and that in the background of the process is a complex molecular structure. Nevertheless, for the sake of clarity, these three points will be treated separately stressing the regulation of the enzyme and energy transduction, since these are two aspects of the enzyme which we have studied

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in more detail. The reader is referred to excellent recent reviews on various aspects of H^+-ATP synthases [1–4,9,10].

At this point, it is worthwhile to recall briefly some aspects of the structure of the enzyme that are relevant to the experimental data that will be discussed. The H^+-ATP synthases contain a membrane sector known at F_0 that is deeply embedded in the membrane. Its function is to mediate the transport of H^+ across the membrane to a component known as F_1 where synthesis and hydrolysis take place. F_0 is composed of at least three definite subunits (a–c) [11,12]. The most thoroughly studied component [13] is a proteolipid (subunit c) that binds dicyclohexylcarbodiimide (DCCD); its binding abolishes H^+ translocation. H^+-ATP synthases also contain a molecular entity known as F_1; this is where synthesis and hydrolysis of ATP are catalyzed. F_1 may be easily detached from the membrane and purified as a water-soluble protein [14,15]. In its soluble form, F_1 catalyzes the hydrolysis of ATP. The latter is the function of F_1 that has been most extensively studied, but recently it has been found that the enzyme may also catalyze the synthesis of enzyme-bound ATP [16–19]. F_1 is composed of five different subunits α_3, β_3, γ, δ, and ϵ in the indicated stoichiometry and in order of decreasing molecular mass. The molecular mass of the complex has been calculated to be 347 kDa [14].

In mitochondria from bovine heart, the proper attachment of F_1-F_0 for tight coupling requires additional proteins, oligomycin-sensitivity conferring protein (OSCP) [20], F_6 [21], and a protein that has been extensively studied by Sanadi and co-workers [22], viz., factor B. These proteins are devoid of catalytic properties, but it is very likely that they affect catalysis by F_1 and certainly H^+ conduction through F_0 to F_1. In some energy-transducing membranes such as those of *Escherichia coli*, OSCP does not exist as a separate molecular entity, but instead its structure is embedded in other subunits of the enzyme [23].

Finally, H^+ -ATPases have a low-molecular-mass protein that inhibits the hydrolytic activity [6]. It has been reported that it controls the kinetic properties of the enzyme [24]; it also affects H^+ conduction [25] through F_0 even though the protein binds to one of the β-subunits of F_1 [26].

2. Catalysis

Synthesis and hydrolysis of ATP are catalyzed by the F_1 component of the ATP synthase. As mentioned above, it is composed of five different subunits, α_3, β_3, γ, δ and ϵ. It is generally accepted that the catalytic site lies within the β-subunit; as there are three β-subunits per enzyme, there are three catalytic sites per molecular entity. Thus, any mechanism that is proposed for catalysis must account for the existence of three catalytic sites. In principle, the three could function independently (or two may be independent of the third one), or alternatively the three sites may function through a concerted mechanism. Based on the findings of Boyer and co-workers [27–30], the latter possibility has received considerably atttention. In the mechanism proposed by this group, it is visualized that release of product ATP in the synthetic direction (or ADP and P_i in the hydrolytic direction) is facilitated by the binding of substrate in an alternate catalytic site. Of relevance to this mechanism are the elegant experiments of Grubmeyer and Penefsky [31,32]; they were able to measure hydrolysis by a single catalytic site of F_1 by using concentrations of substrate lower than that of the enzyme. Under these conditions, it was found that the enzyme had very high affinity for substrate, and that hydrolysis took place at very low rates. When the enzyme was switched from single-site to two-site catalysis by raising the substrate concentration, the hydrolytic activity increased 10^6-fold. Moreover, the authors showed that the increase in activity was due to 10^6-fold increase in the rate of product release from one site upon binding of substrate to another.

Nevertheless, the proposed mechanism is not generally accepted [33,34]; indeed, there are a number of experiments that at least for the moment are difficult to explain unequivocally through a 'binding change' mechanism. Of relevance to the mechanism of catalysis by F_1 is that there exist data on the structure of F_1 at 9 Å resolution [35], and on its amino acid sequence [36] as well as cross-linking [37] studies indicating that the enzyme possesses an asymmetric β-subunit or an asymmetric α-β domain. The reason for this asymmetry is not well understood, but a priori it may be assumed that asymmetry has implications for the possible function of the three catalytic sites of F_1.

3. Regulation

The ATP synthase can express its catalytic properties either through synthesis or hydrolysis of ATP. Nevertheless, the data listed in table 1 show that through various treatments it is possible to prepare particles exhibiting a greater than 20-fold difference in hydrolytic activity; notwithstanding these differences, the various types of particles synthesize ATP at nearly equal rates. It is pertinent to add that as shown in previous works [24,38], the different behavior of the various types of particles is not due to a different degree of coupling or respiratory rates. Rather, the data reflect the fact that rates of hydrolysis do not necessarily correlate with rates of synthesis. That is, the enzyme possesses mechanisms of control that may be expressed to a different extent in either its hydrolytic or synthetic direction.

A well-known factor of control of the ATP synthase is a low-molecular-mass protein first isolated by Pullman and Monroy [6] from bovine heart mitochondria and described as being very effective inhibitor of ATP hydrolysis. Proteins with similar characteristics have been isolated from many types of energy-transducing membranes [39–43]. It has been documented that the different hydrolytic activity of the various types of particles as given in table 1 is due to their different content of inhibitor protein or to a change in the control of the enzyme by the protein [24,38,44]. Under the conditions of the experiment described in table 1, the data may suggest that the inhibitor protein does not affect ATP synthesis. However, this is not entirely correct, since studies of ATP synthesis as driven by electron transport in a time range of seconds showed that particles rich in inhibitor protein presented a 'lag' of 1–3 s before steady-state ATP synthesis was attained [45–47]. This was ascribed to the time required for relief of the inhibitory action of the protein in the process of ATP synthesis. Thus, the inhibitor protein does affect the enzyme not only in the hydrolytic, but also in the synthetic direction.

Although the data in table 1 indicate that equal rates of ATP synthesis may occur in particles which are rich or depleted of inhibitor protein, it should be pointed out that the experiments were carried out in the presence of an ATP trap, a condition in which the ATP formed was not allowed to accumulate in the media. In the absence of an ATP trap, it was found that much less ATP accumulated in the media when the enzyme contained particles devoid of the protein [38]. This was explained by means of a mechanism in which the inhibitor protein inhibited hydrolysis of ATP that was formed through oxidative phosphorylation. The action of the protein serves to affect the kinetics of the enzyme; its effect is to increase the time of release of ADP from the catalytic site when the enzyme works in the hydrolytic direction [24]. The overall data indicate that the protein is essential for maximal net formation of ATP in systems such as mitochondria which intrinsically contain high concentrations of ATP [49,50].

In addition to the inhibitor protein, the catalytic activity of F_1 is also controlled by other factors. An important one is ADP. There are numerous reports indicating that the binding of one ADP to F_1 promotes inhibition of hydrolysis [50–53]. Interestingly, the inhibitory ADP may

Table 1

Synthesis and hydrolysis of ATP by submitochondrial particles with various levels of control by the inhibitor protein

Mg-ATP particles were prepared by sonication of bovine heart mitochondria in the presence of Mg-ATP as described in ref. 38. All or most of the ATP synthases are complexed with the inhibitor protein. From these particles, 'state 3' particles were prepared according to ref. 24 following the procedure described by Van de Stadt and Van Dam [72]; this procedure partially relieved the inhibitory action of the inhibitor protein. Also, from Mg-ATP particles 'noncontrolled' particles were prepared as described in ref. 38; these are particles in which most of the inhibitory action of the inhibitor protein has been abolished. Synthesis of ATP was measured in an incubation medium that contained 25 mM Tris-HCl, pH 7.4, 20 mM succinate, 10 mM $MgCl_2$, 15 mM $^{32}P_i$ phosphate, 30 mM glucose and 10 U hexokinase, the incubation time being 4 min. Temperature, 30 °C [38]. Hydrolysis was measured in the presence of an ATP-regenerating system and 1 μM FCCP as described in ref. 73.

Particles	Activity (μmol min^{-1} mg^{-1})	
	Synthesis	Hydrolysis
'Mg-ATP'	0.16	0.3
'State 3'	0.19	2.2
'Noncontrolled'	0.15	7.1

arise from the hydrolysis of ATP under conditions in which binding of medium ADP is prevented (O.B. Martins et al., unpublished results). All the observations strongly suggest that at least one of the adenine nucleotide binding sites of F_1 has a regulatory function.

4. Energy

The function of the ATP synthase is to transform energy of electrochemical H^+ gradients into the β-γ pyrophosphate bond of ATP. Thus, the central question concerning this enzyme is how this is brought about. As a first approach, it would be of importance to ascertain the step or steps of the catalytic cycle at which energy is required. Boyer and co-workers [29,30,54] have thoroughly studied the exchange in F_1 that occurs between the γ-phosphoryl oxygens of ATP and the oxygens of water during hydrolysis of ATP. From the characteristics of the reaction, it was concluded that reversal of hydrolysis may occur at the catalytic site. In other words, at the catalytic site synthesis and hydrolysis of ATP may take place before ADP and P_i are released into the medium. Along this line, under conditions for single-site catalysis, it was possible to determine the equilibrium constant for ATP hydrolysis at the catalytic site; the value obtained was near unity [55]. These observations indicate that at the catalytic site no input of energy is required for synthesis of ATP. This is in marked contrast to the overall process of ATP hydrolysis which occurs with a large energy change [56,57].

More recent considerations [58,59] of the free energy changes during hydrolysis of ATP and other compounds that belong to the class of 'high energy' indicated that intramolecular effects such as opposing resonance and electrostatic repulsion are of secondary importance, and that the differences in solvation energy of reactants and products were the predominant free-energy contributions to the large negative free energy of hydrolysis of these molecules. More recently, De Meis [60], in an impressive work on the free energy of hydrolysis of pyrophosphate in media in which the structure of water was altered by cosolvents, showed that the free energy of hydrolysis reached values of 5 kcal/mol lower than in totally aqueous media. Thus, it would seem that the energy required for synthesis of the β-γ pyrophosphate bond of ATP is critically dependent on the characteristics of the environment in which the reaction is carried out.

Of great significance to ascertaining the role of water in the process of energy transduction in biological membranes was the observation that phosphorylation of the acyl residue at the catalytic site of the Ca^{2+}-ATPase of sarcoplasmic reticulum by P_i is largely facilitated by cosolvents that alter the aqueous structure of the media [61]. Similar data have been obtained with other ATPases (for a review, see ref. 62). Therefore, it has been proposed [60,61] that the main energy barrier for synthesis of 'high-energy phosphates' from medium phosphate would be the partitioning of the highly polar phosphate into the hydrophobic pocket of the catalytic site. Once phosphate is in such environment spontaneous synthesis of acyl phosphates would take place in the ATPases that function through a phosphorylated intermediate. For F_1 in which no phosphorylation of the enzyme takes place, an analogous situation would prevail, except that the species to be phosphorylated would be ADP that lies at a catalytic site.

This possibility has been experimentally examined, and it was found that in soluble F_1 synthesis of ATP is achieved [16–19] by incubation of F_1 that has bound ADP with P_i, Mg^{2+} and an organic cosolvent such as dimethyl sulfoxide (Me_2SO). The ATP thus formed is not released into the medium, but remains bound to the enzyme. In the synthesis of ATP in the presence of Me_2SO the K_m value for P_i is approx. 1 mM, whereas in totally aqueous media is above 100 mM [16,19]. Thus, by diminishing the difference in hydrophobicity between the media and the catalytic site, by means of organic cosolvents, the entrance of P_i into the catalytic site, and the subsequent phosphorylation of ADP are largely facilitated. Phosphorylation of the Ca^{2+}-ATPase of sarcoplasmic reticulum by P_i shows a similar behavior [61]. These overall findings are considered to be strong evidence in favor of the postulate that the major energy barrier for ATP

synthesis is the partitioning of polar substrates from an aqueous medium into the hydrophobic catalytic site. In this respect, it is of interest that the establishment of electrochemical H^+ gradients significantly increases the affinity of the enzyme for P_i and ADP [27,63]. However, this does not exclude the possibility that in addition to the binding of substrates the release of product ATP is not an energy-requiring process.

As mentioned before, F_1 has three catalytic sites, each localized to each of the three β-subunits of the enzyme. Gromet-Elhanan and co-workers [64,65] have succeeded in the isolation of the β-subunit of chromatophores from *Rhodospirillum rubrum* [64,65]. The isolated subunit can reconstitute photophosphorylation of chromatophores depleted of the subunit. This research group has also shown that the β-subunit possesses two binding sites for adenine nucleotides [66] and another for P_i [67]. Along the same lines, Harris et al. [68] reported that the purified β-subunit exhibits the ability to hydrolyze ATP, albeit at rates much lower than those of the whole enzyme.

We have purified the β-subunit from chromatophores of *R. rubrum*, as well as that from F_1 of mitochondria of bovine heart following the established techniques [65,67]. The purpose was to determine whether the isolated subunit could catalyze synthesis of ATP under conditions similar to those in which soluble F_1 catalyzes the synthesis of tightly bound ATP [16–19]. Although, in agreement with Harris et al. [68], our preparation of the β-subunit from *R. rubrum* exhibited ATPase activity, in repeated attempts under a number of conditions we failed to detect synthesis of ATP by β-subunits from either heart or chromatophores (table 2). We consider these negative findings of importance in the sense that they indicate that the separation of the β-subunit from the rest of the structure abolishes the capacity of the catalytic site to form ATP, but not to hydrolyze it.

Even though it has been described that the isolated β-subunit possesses two binding sites for adenine nucleotides [66], it has been shown that the α-subunit also has a site for adenine nucleotides [9,69]. This evidence was obtained from binding data with isolated subunits and affinity labeling measurements. Taking all of the various findings into consideration, it is likely that in the whole enzyme, at least one binding site for adenine nucleotides lies in the interphase between α- and β-subunits. This type of arrangement has been previously proposed [65,70,71]. Our findings with the isolated β-subunit indicate that in the isolated form the subunit does not have a hydrophobic pocket where spontaneous synthesis of ATP may take place. Therefore, it would appear that only through the interaction of a β- with an α-subunit can such molecular arrangement be achieved.

Table 2

ATP hydrolysis and ATP synthesis by β-subunits from *Rhodospirillum rubrum* and bovine heart mitochondria

β-subunit from *R. rubrum* was prepared as described in refs. 65 and 67 while that of bovine heart mitochondria was prepared according to ref. 74. The assay media for hydrolysis contained 50 mM Tricine, pH 8.0, 1 mM $MgCl_2$, 0.1 mM [γ-^{32}P]ATP (13.6 Ci/mol) and 25 μg β-subunit. For the synthesis of tightly bound ATP the mixture contained 50 mM Mops, pH 7.0, 5 mM $MgCl_2$, 40% DMSO, 2 mM $^{32}P_i$ (45 Ci/mol), 0.2 mM ADP and 100 μg β-subunit (see ref. 19). Both reactions were carried out over a period of 60 min incubation at 30 °C.

β-subunit source	Hydrolysis (nmol min^{-1} mg^{-1})	Synthesis (mol ATP/ mol β-subunit)
R. rubrum	6.8	< 0.01
Mitochondria	14.0	0.03

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References

1 R.H. Fillingame, Annu. Rev. Biochem. 49 (1980) 1079.
2 A.E. Senior and J.G. Wise, Annu. Rev. Biochem. 50 (1981) 681.
3 Y. Hatefi, Annu. Rev. Biochem. 54 (1985) 1015.
4 P.L. Pedersen and E. Carafoli, Trends Biochem. Sci. 12 (1987) 146.

5 R.L. Cross and C.M. Nalin, J. Biol. Chem. 257 (1982) 2874.
6 M.E. Pullman and G.C. Monroy, J. Biol. Chem. 238 (1963) 3762.
7 F.A.S. Kironde and R.L. Cross, J. Biol. Chem. 262 (1987) 12544.
8 F.A.S. Kironde and R.L. Cross, J. Biol. Chem. 262 (1987) 3488.
9 P.V. Vignais and J. Lunardi, Annu. Rev. Biochem. 54 (1985) 977.
10 K. Schwerzman and P.L. Pedersen, Arch. Biochem. Biophys. 250 (1986) 1.
11 W. Sebald, T. Graf and H.B. Lukins, Eur. J. Biochem. 93 (1979) 587.
12 D.L. Foster and R.H. Fillingame, J. Biol. Chem. 257 (1982) 2009.
13 H. Sigrist, K. Sigrist-Nelson and C. Gitler, Biochem. Biophys. Res. Commun. 74 (1977) 178.
14 A.F. Knowles and H.S. Penefsky, J. Biol. Chem. 247 (1972) 6617.
15 M. Tuena de Gómez-Puyou and A. Gómez-Puyou, Arch. Biochem. Biophys. 182 (1983) 82.
16 J. Tonomura and Y. Tonomura, J. Biochem. (Tokyo) 93 (1983) 1601.
17 J. Tonomura, J. Biochem. (Tokyo) 96 (1984) 475.
18 M. Yoshida, Biochem. Biophys. Res. Commun. 114 (1983) 907.
19 A. Gómez-Puyou, M. Tuena de Gómez-Puyou and L. de Meis, Eur. J. Biochem. 159 (1986) 133.
20 D.H. McLennan and A. Tzagaloff, Biochemistry 76 (1968) 1603.
21 A.F. Knowles, R. Guillory and E. Racker, J. Biol. Chem. 246 (1971) 2672.
22 Y. Huang, L. Kantham and D.R. Sanadi, J. Biol. Chem. 262 (1987) 3007.
23 Yu.A. Ovchinnikov, N.N. Modyanov, V.A. Grinkevich, G.I. Belugrudov, T. Hundal, B. Norling, G. Sandri, L. Wojtczak, and L. Ernster, in: Achievements and perspectives of mitochondrial research, vol. 1, Bioenergetics eds. E. Quagliarello, E.C. Slater, F. Palmieri, C. Saccone and A.M. Kroon (Elsevier, Amsterdam, 1985) p. 223.
24 M. Tuena de Gómez-Puyou, U. Müller, G. Dreyfus, G. Ayala and A. Gómez-Puyou, J. Biol. Chem. 258 (1983) 13680.
25 F. Guerrieri, R. Scarfo, F. Zanotti, Y.W. Che and S. Papa, FEBS Lett. 213 (1987) 67.
26 G. Kelin, M. Satre, A.C. Dianoux and P.V. Vignais, Biochemistry 20 (1981) 1339.
27 P.D. Boyer, R.L. Cross, and W. Momsen, Proc. Natl. Acad. Sci. U.S.A. 70 (1973) 2837.
28 P.D. Boyer, FEBS Lett. 58 (1975) 1.
29 J. Rossing, C. Kayalar and P.D. Boyer, J. Biol. Chem. 252 (1977) 2478.
30 C. Kayalar, J. Rosing and P.D. Boyer, J. Biol. Chem. 258 (1977) 2486.
31 C. Grubmeyer and H.S. Penefsky, J. Biol. Chem. 256 (1981) 3718.
32 C. Grubmeyer and H.S. Penefsky, J. Biol. Chem. 256 (1981) 3728.
33 K.S. Soong and J.H. Wang, Biochemistry 23 (1984) 136.
34 J.H. Wang, Biochemistry 23 (1984) 6350.
35 L.M. Amzel, M. McKinney, P. Narayanan and P.L. Pedersen, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 5852.
36 J.E. Walker, I.M. Fearnley, N.J. Gay, B.W. Gibson, F.D. Northrop, S.J. Powell, M.J. Runswick, M. Sarastre and V.L.J. Tybulewicz, J. Mol. Biol. 184 (1985) 677.
37 M. Tommasino and R.A. Capaldi, Biochemistry 24 (1985) 3972.
38 C. Beltrán, M. Tuena de Gómez-Puyou, A. Darszon and A. Gómez-Puyou, Eur. J. Biochem. 160 (1986) 163.
39 N. Nelson, H. Nelson and E. Racker, J. Biol. Chem. 247 (1972) 7657.
40 N.M. Cintron and P.L. Pedersen, J. Biol. Chem. 254 (1979) 3439.
41 J.B. Smith, P.C. Sternweiss and L.A. Hoppel, J. Supramol. Struct. 3 (1975) 248.
42 G. Dreyfus and M. Satre, Arch. Biochem. Biophys. 229 (1984) 212.
43 M. Satre, M.B. de Jerphanion, J. Huet and P.V. Vignais, Biochim. Biophys. Acta 387 (1975) 241.
44 J. Power, R.L. Cross and D.A. Harris, Biochim. Biophys. Acta 724 (1983) 128.
45 A. Gómez-Puyou, M. Tuena de Gómez-Puyou and L. Ernster, Biochim. Biophys. Acta 547 (1979) 252.
46 D.A. Harris, V. Von Tscharner and G.K. Radda, Biochim. Biophys. Acta 548 (1979) 72.
47 K. Schwerzmann and P.L. Pedersen, Biochemistry 20 (1981) 6305.
48 E.C. Slater, J. Rosing and A. Hol, Biochim. Biophys. Acta 292 (1973) 534.
49 G. Letko, U. Kuster, J. Duszinski and W. Kunz, Biochim. Biophys. Acta 593 (1980) 196.
50 D.A. Harris, Biochim. Biophys. Acta 463 (1978) 245.
51 E.A. Vasilyeva, A.F. Fitin, I.B. Minkov and A.D. Vinogradov, Biochem. J. 188 (1980) 807.
52 A. di Pietro, F. Penin, C. Godinot and D.C. Gautheron, Biochemistry 19 (1980) 5671.
53 I.Y. Drobinskaya, I.A. Kozlov, M.B. Murateliev and E.N. Vulfson, FEBS Lett. 182 (1985) 419.
54 G.L. Choatz, R.L. Hutton and P.D. Boyer, J. Biol. Chem. 254 (1979) 286.
55 C. Grubmeyer, R.L. Cross, and H.S. Penefsky, J. Biol. Chem. 257 (1982) 12092.
56 H.M. Kalckar, Chem. Rev. 28 (1941) 71.
57 T.L. Hill and M.F. Morales, J. Am. Chem. Soc. 73 (1951) 1606.
58 P. George, R.J. Witonsky, M. Trachtman, C. Wu, W. Dormost, L. Richman, W. Richman, F. Shurayh and B. Lentz, Biochim. Biophys. Acta 223 (1970) 1.
59 D.M. Hayes, G.L. Kenyon and P.A. Kollman, J. Am. Chem. Soc. 100 (1978) 4331.
60 L. de Meis, J. Biol. Chem. 259 (1984) 6090.
61 L. de Meis, O.B. Martins and E.W. Alves, Biochemistry 19 (1980) 4252.
62 P.L. Pedersen and E. Carafoli, Trends Biochem. Sci. 12 (1987) 186.

63 J.E.G. McCarty and S.J. Ferguson, Eur. J. Biochem. 132 (1983) 425.
64 A. Philosoph, A. Binder and Z. Gromet-Elhanan, J. Biol. Chem. 252 (1977) 8747.
65 D. Khananshvili and Z. Gromet-Elhanan, J. Biol. Chem. 257 (1982) 11377.
66 Z. Gromet-Elhanan and D. Khananshvili, Biochemistry 23 (1984) 1022.
67 D. Khananshvili and Z. Gromet-Elhanan, Biochemistry 24 (1985) 2482.
68 D.A. Harris, J. Boork and M. Baltscheffsky, Biochemistry 24 (1985) 3876.
69 M. Futai and H. Kanazawa, Microbiol. Rev. 47 (1983) 285.
70 N. Williams and P.S. Coleman, J. Biol. Chem. 257 (1982) 2834.
71 H.J. Schafer and K. Dose, J. Biol. Chem. 259 (1984) 15301.
72 R.J. van de Stadt and K. van Dam, Biochim. Biophys. Acta 347 (1974) 240.
73 M.E. Pullman, H.S. Penefsky, A. Datta and E. Racker, J. Biol. Chem. 235 (1960) 3322.
74 G.J. Verschoor, P.R. van der Sluis and E.C. Slater, Biochim. Biophys. Acta 462 (1977) 438.