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Mitochondrial Adenosine Triphosphatase

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Introduction

Because of the molecular complexity of mitochondrial ATPase, and its important role in both oxidative phosphorylation and in ATP-dependent functions, the elucidation of the molecular properties and mechanism of action of this enzyme complex, represent in the author's view one of the most important and challenging problems of modern biochemistry.

The purpose of this review is to give the reader an up-to-date account of work carried out on mitochondrial ATPase, and where possible to compare mitochondrial ATPase with ATPases of bacteria and chloroplasts. At the same time an effort is made to indicate aspects of the ATPase problem that need more intensive investigation. New students in the field should also consult the reviews of Senior [1] and Penefsky [2], and the review summarizing work in the author's laboratory on the mitochondrial ATPase of rat liver [3]. The recent articles of Mitchell [4, 5] are also highly recommended especially for those who are interested in the possible mechanisms(s) of action of energy transducing ATPases.

The Complete ATPase Complex

Mitochondrial ATPase is perhaps the most complex enzyme system known to man (Fig. 1). In its intact form in the mitochondrial inner membrane, the complete ATPase complex is now believed to consist of four functional components: a headpiece which is called Factor 1 (F_1) and catalyzes ATPase activity [6]; a membrane sector which is thought to direct the flow of protons to F_1 during oxidative phosphorylation [4, 5, 7, 8]; a basic peptide or "stalk" which binds F_1 to the membrane sector and confers oligomycin-sensitivity on F_1 (Stalk $\cong$ OSCP or oligomycin-sensitivity conferring peptide) [9]; and an ATPase inhibitor peptide which inhibits ATPase activity during oxidative phosphorylation [10]. As will be described below the F_1 component and membrane sector can be subdivided further into 5 and 4 polypeptides, respectively. The "stalk" represents a tenth polypeptide, and, although there is controversy about the identity of the ATPase inhibitor [11, 12], it may well represent an eleventh component in some ATPase preparations.

The manner in which the complete ATPase complex may participate in oxidative phosphorylation and in ATP-dependent functions is illustrated in simplified form in Fig. 2.

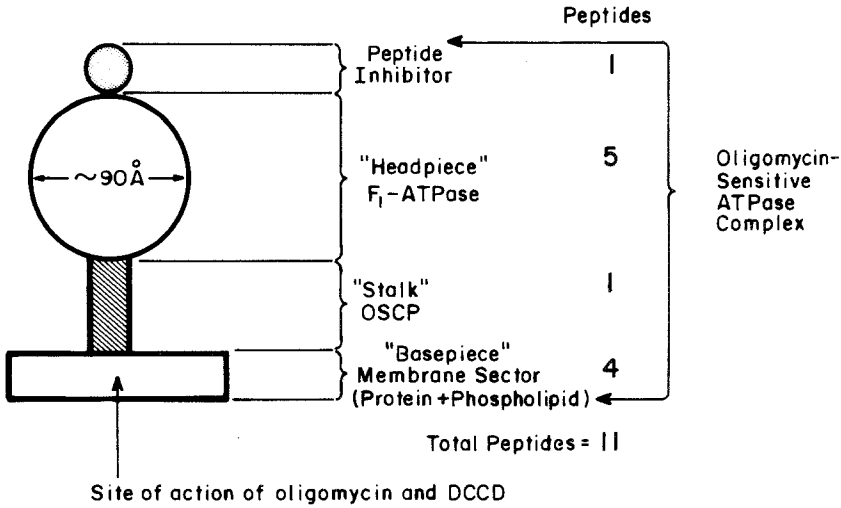

Figure 1. *Diagram of the oligomycin-sensitive ATPase complex showing its four functional components and the site of action of oligomycin and dicyclohexylcarbodiimide (DCCD).* Whether the ATPase inhibitor is an integral part of the F_1 complex or a separate entity is an unsettled question.

Purification

The oligomycin-sensitive ATPase complex. The complete ATPase complex is usually referred to as the "oligomycin-sensitive ATPase" to distinguish it from the F_1 component of the complex which is not inhibited by oligomycin. The complete complex, unlike the F_1 component, is also inhibited by dichlohexylcarbodiimide (DCCD) [13, 14], a covalent inhibitor of oxidative phosphorylation [15].

To date the oligomycin-DCCD-sensitive ATPase complex has not been rigorously purified from any source, although some of the ATPase literature may give this impression. The most highly purified preparation is clearly that of Tzagoloff and Meagher [16] from mitochondria of the yeast strain *Saccharomyces cerevisiae* (Tables I and III). The purification scheme consists of solubilizing the enzyme from submitochondrial particles with 0.25% Triton X-100, centrifuging once at high speed, and then sedimenting the supernatant for 17 hours in a glycerol gradient (5-15% w/v). The purified complex has a specific ATPase activity of 28 μmoles P_i formed/min/mg. Although the preparation still contains phospholipids (~10% by weight) it appears under the electron microscope to be a fairly homogeneous complex of oval shaped globular particles 100 by 150 Å. Sedimentation analysis together with correction for phospholipid content give an upper limit for the molecular weight of

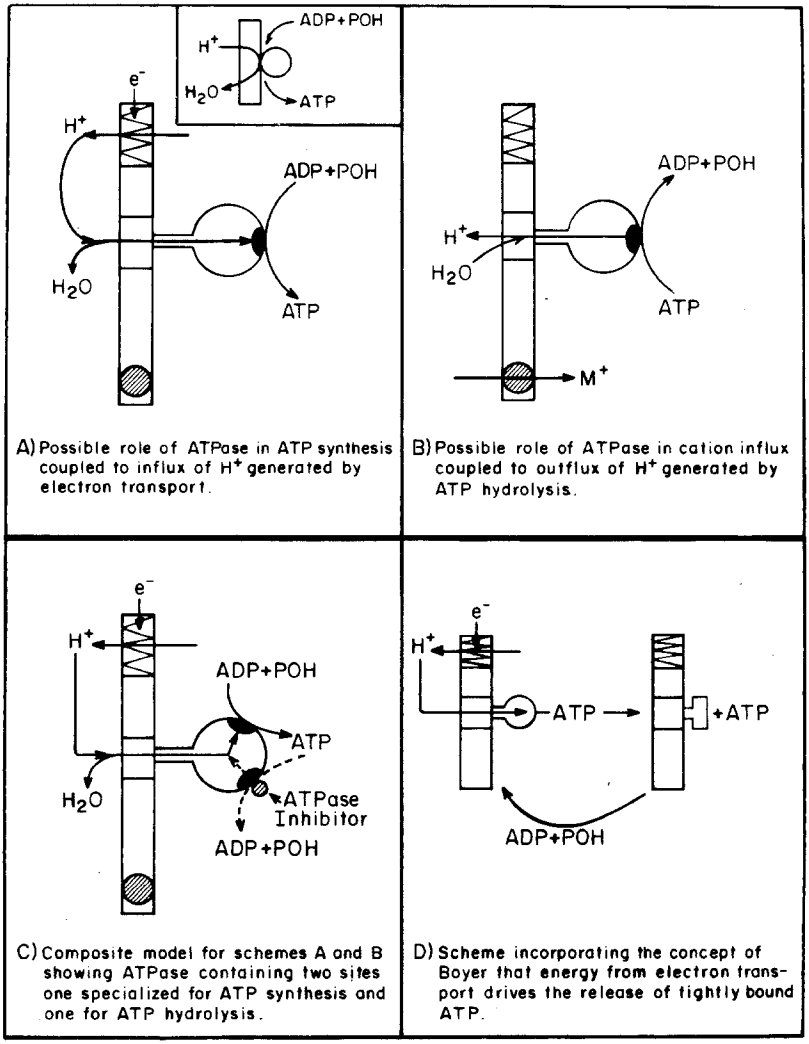

Figure 2. *Models illustrating in simplified form how the oligomycin-sensitive ATPase complex may participate in ATP synthesis and ATP-dependent cation uptake.* Models in *A* and *B* illustrate how a single catalytic site operating in a reversible manner may participate in both activities. The composite model depicted in *C* shows how two sites, one specialized for ATP synthesis and one specialized for ATP hydrolysis, may be involved in these processes. The scheme depicted in *D* incorporates the recent concept of Boyer and colleagues [141, 142] that energy from electron transport drives the release of tightly bound ATP, presumably by causing a conformational alteration at the level of the oligomycin-sensitive ATPase complex.

To be consistent with the observations that protons are released into the external medium during electron transport, and consumed during ATP formation, both the

468,000. As will be noted below most of the peptide components of this complex can be accounted for as components of F_1 (five) or as components (five) necessary for oligomycin-sensitivity of F_1. Unfortunately the degree of contamination of this preparation by other membrane components, e.g. flavoproteins and cytochromes has not been reported.

The rigorous purification and characterization of the oligomycin-sensitive ATPase complex from mammalian sources has not been achieved. Several preparations of low specific activity (< 9 μmoles P_i/min/mg) have been obtained from bovine heart mitochondria by using cholate (or deoxycholate) and ammonium sulfate fractionation [16-19] (Table I). One of these preparations, the Tzagoloff, Byington, MacLennan preparation [16] has been purified more extensively by Capaldi [18] and examined by gel electrophoresis in sodium dodecyl sulfate. It contains polypeptides of similar size to those observed in the yeast preparation together with an additional 73,000 molecular weight component which is assumed to be an impurity.

The apparent success of the Tzagoloff-Meager preparation from yeast [15] may be related to the selectivity of Triton X-100 for the oligmycin-sensitive ATPase complex. In this regard it is interesting to note that the Triton X-100-solubilized preparation recently described by Swaljung *et al.* [19] contains a very low content of cytochromes. It has been noted also in the author's laboratory [20] that under appropriate conditions Triton X-100 solubilizes the oligomycin-sensitive ATPase complex of rat liver mitochondria without solubilizing cytochrome oxidase. Cytochrome *b*, however, is solubilized.

Although oligomycin and/or DCCD-sensitive ATPase preparations have not been obtained as yet from bacterial or chloroplasts membranes, there is good reason to believe that such complexes exist. Similar to mitochondrial ATPase, the membrane ATPases of chloroplasts and bacteria are sensitive to either oligomycin, DCCD, or both agents [21-24].

The oligomycin-insensitive ATPase complex, F_1. The F_1 component of the oligomycin-sensitive ATPase complex has been purified to homogeneity from mitochondria of bovine heart [6, 25-27], rat liver

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electron transport chain (jagged line) and the ATPase system are depicted (after Mitchell [4, 5, 110]) as involving proton translocation.

The inset in *A* indicates that the "active center" of the ATPase may lie at the junction between the F_1 component and the membrane sector of the oligomycin-sensitive ATPase complex as recently suggested by Mitchell [5]. Presumably in this case the "stalk" or oligomycin sensitivity-conferring component is meshed with the membrane sector. The advantage to this model is that it allows the P_i and ADP sites of F_1 to become specifically accessible to H^+ channeled through the membrane sector. In addition, this model allows for the formation of ATP in a low dielectric environment.

TABLE I. Summary of purification schemes for oligomycin-sensitive and oligomycin-insensitive ATPase preparations

ATPase preparation	Source	Method solubilization	No. of electrophoretic species in SDS
Oligomycin-sensitive			
Tzagoloff *et al.* [16], Stekhoven *et al.* [11]	Heart	Deoxycholate	14
Tzagoloff *et al.*—Capaldi [18]	Heart	Deoxycholate-NaBr	10
Swanljung *et al.* [19]	Heart	Triton X-100	10-12
Tzagoloff and Meager [15]	Yeast	Triton X-100	10 (2 overlap)
Landry [14]	Yeast	Triton X-100	11
Oligomycin-insensitive (F_1 or F_1-like)			
Catterall and Pedersen [28, 29]	Liver	Extensive washing and sonication	5
Lambeth and Lardy [30]	Liver	Sonication	5
Senior and Brooks [27]	Heart	Sonication	5
Knowles and Penefsky [25]	Heart	Sonication	5
Horstman and Racker [26], Brooks and Senior [12]	Heart	Sonication	6
Kozlov and Mikelsaar [49]	Heart	Sonication (pH 5.2)	3
Andreoli *et al.* [42]	Heart	Sonication	—
Toson *et al.* [105], Lee [106]	Heart	Phospholipid incubation	—
Tzagoloff and Meager [15]	Yeast	Sonication	5
Goffeau *et al.* [31]	Yeast	Sonication	5
Kobayashi and Anraku [36]	*E. coli*	Sonication	—
Hanson and Kennedy [37]	*E. coli*	Triton X-100	4
Bragg and Hou [35]	*E. coli*	Suspension in EDTA Buffer	5
Schnebli and Abrams [36]	*S. faecalis*	Extensive washing in 1 mM Tris, pH 7.5	—
Adolfsen and Moudrianakis [37, 48]	*A. faecalis*	Ionic shock	4
Munoz *et al.* [40]	*M. lysodeikticus*	Washing 4x in 0.03 m Tris, pH 7.5 + osmotic shock	
Mirsky and Barlow [143]	*B. megaterium*	See above	1
Farron [32], Lien and Racker [33]	Chloroplast	Washing 3x in Tris-EDTA-ATP buffer, pH 8.0	5
Howell and Moudrianakis [34], Adolfsen and Moudrianakis [48]	Chloroplast	Washing 3x with water and once with 1 mM EDTA	5

[28-30], and yeast [15, 31] (Table I). Analogous preparations have been purified to homogeneity from chloroplasts [32-34] and from several bacterial strains [35-41]. Unlike preparations of the complete or oligomycin-sensitive ATPase complex, F_1 preparations are lipid free and water soluble.

Hydrodynamic properties of F_1 preparations from mitochondria, bacteria, and chloroplasts are summarized in Table II. These complexes are about 90-100 Å in diameter and range in molecular weight from 325,000 to about 380,000 daltons.

TABLE II. Hydrodynamic properties of F_1 and F_1-like ATPase preparations

Property	Units	ATPase		
		Mitochondrial [3, 28]	*Streptococcus faecalis* [38, 51]	Chloroplast [32]
Molecular weight	Daltons	384,000	385,000	325,000
$s_{20,w}$	Sec^{-1}	12.2	13.4	13.8
$\bar{V}$	cm^3-g	0.74	0.735	0.737
$D_{20,w} \times 10^7$	cm^3-sec^{-1}	3.0	3.2	3.9
f/fo	—	1.5	1.35	1.20

Purification schemes of most F_1 preparations are similar in several respects. They start with membrane fragments, employ sonication to strip the F_1 component from the membrane, and utilize anion exchange and gel filtration chromatography as terminal steps. Since the F_1 component is cold labile when removed from the membrane, most of the purification steps are carried out at room temperature.

There are also some notable differences in the various purification schemes for F_1. The major differences are in the treatments of the membrane prior to sonication, and in the buffer systems used. The procedures of Senior and Brooks [27] and of Knowles and Penefsky [25] for the heart F_1 involve incubating membranes overnight at pH 9.2, a treatment that may release the ATPase inhibitor peptide of Pullman and Monroy [10]. The procedure of Catterall and Pedersen [28, 29] for the rat liver enzyme involves washing the membrane five times in a low ionic strength buffer containing 50 mM EDTA. The washing cycle is not used in the preparation of the enzyme from rat liver membranes in the procedure described by Lambeth and Lardy [30].

Most of the procedures, namely those of Senior and Brooks [27], Knowles and Penefsky [25], Horstman and Racker [26], and Lambeth and Lardy [30] involve buffers containing ATP throughout the purification scheme. The purification scheme of Catterall and Pedersen [28, 29] contains ATP only at one stage of purification. Despite these differences, however, these preparations are all homogeneous as judged by several criteria, and exhibit ATPase specific activities of at least 60 μmoles P_i/min/mg in the appropriate buffer system.

The notable exception to the above is the preparation of Andreoli *et al.* [42] called Factor A which has a very low specific ATPase activity that can be stimulated by exposure to elevated temperatures. This ATPase preparation, unlike the others noted above, is prepared directly

from bovine heart mitochondria rather than from submitochondrial particles [42].

Release of analogous F_1-like ATPases from both bacteria and chloroplasts membranes can be effected without sonication [32-41], usually by repeated washing in low ionic strength buffers or by washing in EDTA containing buffers (Table I). Similar to purified mitochondrial preparations, F_1-like preparations from bacteria and chloroplasts are cold labile [21, 37, 43, 44].

Subunits

Number. It now seems clear from work carried out in a number of laboratories [11, 15, 31, 35, 45-48) that the F_1 component of the complete oligomycin-sensitive ATPase consists of five different polypeptide chains (Table IV), and that at least five more components comprising the "stalk" and membrane sector of the complex are necessary together with phospholipids for conferring oligomycin-sensitivity (Table III). An additional peptide which is a potent inhibitor of ATPase activity may constitute an eleventh component. However, as

TABLE III. Apparent molecular weights of polypeptides observed in oligomycin-sensitive ATPase preparations

ATPase preparation	Swanljung *et al.* [19]	Tzagoloff *et al.*–Capaldi [16, 18]	Tzagoloff and Meager [15]	Landry [14]
Source	Heart	Heart	Yeast	Yeast
Apparent molecular weight of polypeptides[a]	178,000[b]			
	126,000[b]			
	84,000			
		73,000[b]		
	58,000			65,000
				60,500
			58,000	58,000
	55,000	55,000	54,000	55,000
		52,000		
	43,000		38,500	36,000
	30,000	30,000	31,000	32,500
	27,000	29,000	29,000	
	20,000	20,000	22,000	20,000
	15,000	19,000	18,500	17,000
	12,000	12,500	12,000 x 2	13,500
		10,000		
	10,000			9,000
		8,000	7,500	8,000

[a] As determined by gel electrophoresis in sodium dodecyl sulfate.
[b] Considered to be either aggregates or impurities.

will be noted below there is not complete agreement on this point. When F_1 preparations are isolated by one of the two procedures described by Horstman and Racker [26] (the procedure that omits pretreatment of the membranes with Sephadex G-50) the inhibitor protein is firmly attached. Such F_1 preparations show six rather than the usual five polypeptides upon electrophoresis in sodium dodecyl sulfate [12].

F_1-like ATPases prepared from chloroplasts [47, 48] also show five different polypeptide components when examined by gel electrophoresis in sodium dodecyl sulfate, whereas considerable variations exist in the reported subunit compositions of bacterial preparations. The *Streptococcus faecalis* preparation of Schnebli and Abrams [38] contains two major subunits, the *Alcaligenes faecalis* preparation four subunits [48], and the *E. coli* ATPase four or five subunits depending on the method of purification [35, 37]. To date there have been no reports of inhibitor peptides associated with bacterial preparations, but, as indicated in more detail below, one of the five polypeptides of the chloroplast enzyme is thought to be an inhibitor of ATPase activity.

Molecular weight and stoichiometry. The two largest peptide subunits of F_1, designated A and B, α and β, or 1 and 2, have molecular weights between 50,000-62,000 (Table IV). They comprise as much as 88% of

TABLE IV. Apparent molecular weights of polypeptides characteristic of F_1 or F_1-like ATPase preparations

ATPase preparation	Source	Apparent molecular weight[a]				
		A	B	C	D	E
Catterall and Pedersen [28, 45]	Liver	62,500	57,000	36,000	12,000	7,500
Lambeth and Lardy [30, 46]	Liver	53,000	50,000	28,000	12,500	7,500
Knowles and Penefsky [11,25]	Heart	54,000	50,000	33,000	17,300	11,000[c]
Senior and Brooks [27, 46]	Heart	53,000	50,000	25,000	12,500	7,500
Kozlov and Mikelsaar [49]	Heart	54,000	50,000	–	–	11,000
Tzagoloff and Meager [15]	Yeast	58,000	54,000	38,500	31,000	12,000
Goffeau *et al.* [31]	Yeast	60,500	58,000	32,500	13,500	8,000
Bragg and Hou [35]	*E. coli*	57,000	50,800	30,000	21,000	12,000
Hanson and Kennedy [37]	*E. coli*	60,000	56,000	35,000	13,000	–
Adolfsen *et al.* [48]	*A. faecalis*	59,000	54,000	43,000	12,000	–
Schnebli *et al.* [51]	*S. faecalis*	66,000[b]	–	–	–	–
Mirsky and Barlow [143]	*B. megaterium*	69,000	–	–	–	–
Lien and Racker [33]. Lien *et al.* [47]	Chloroplast	59,000	56,000	37,000	17,500	13,000
Howell and Moudrianakis [34], Adolfsen *et al.* [48]	Chloroplast	62,000	59,000	43,000	21,000	14,000

[a] Determined on calibrated polyacrylamide gels in sodium dodecyl sulfate.
[b] Presumably a dimer composed of two different 33,000 molecular weight polypeptides.
[c] Presumably a dimer composed of two 5,700 molecular weight monomers.

the Coomassie blue staining intensity of various preparations. A third peptide designed C, γ or 3 contains about 10% of the total stain and ranges in molecular weight in various laboratories from 25,000 to 36,000. The two smallest peptide designated D and E, δ and ϵ, or 4 and 5, contain less than 3-7% of the total stain and have molecular weights below 20,000. The stoichiometry of these peptides based on staining in gels in that region where stain is proportional to protein concentration has been reported by Catterall, Coty and Pedersen [45] and by Senior [1] to be A_3B_3CDE for the rat liver and bovine heart enzymes, respectively. Under the electron microscope those subunits that can be observed appear to form a hexagonal array which in some profiles appears to have a subunit in the center of the haxagon (Fig. 3) [3].

More recently Kozlov and Mikelsaar [49] have reported that the bovine heart F_1 solubilized by sonication at pH 5.2, rather then at pH values greater than 7.0, lacks the C and D subunits but retains full activity. These investigators point out that the sum of the molecular weights of the C, D, and E subunits is very near the molecular weight of the major subunits A and B. They imply that the three minor subunits observed in most ATPase preparations may comprise a single subunit *in vivo*. An alternate stoichiometry of the ATPase suggested by these workers is A_3B_3C where C in this formulation is a subunit similar in size to the A and B subunits, or about 50,000-60,000 molecular weight. Possible structures of F_1 suggested by these and other investigators are summarized in Fig. 3.

The six polypeptides comprising the oligomycin-sensitivity conferring part of the complete oligomycin-sensitive ATPase complex have molecular weights ranging from about 7500 to about 30,000 (Table III). Although the relative amounts of these peptides in the complex have not been accurately determined, they are usually assumed to be present in a 1 : 1 stoichiometry [1].

Of the various nonmitochondrial F_1-like preparations, subunit stoichiometries and/or structures have been suggested for the chloroplast enzyme (CF_1), and for the two bacterial preparations. Berzborn [50] reports that the five polypeptides in CF_1 are in the stoichiometric ratio $\alpha_2\beta_2\gamma_2\delta\epsilon_2$ or in the terminology preferred by the author $A_2B_2C_2DE_2$. Schnebli *et al.* [51] present data which suggest that the two 33,000 molecular weight subunits comprising the *Streptococcus faecalis* F_1 are in the stoichiometry ratio A_6B_6. A hexagonal structure of alternating A and B subunits is proposed where each of the six components of the hexagon consist of an AB dimer of about 66,000 molecular weight.

The stoichiometric ratios of subunits in F_1 have been calculated from molecular weight and relative dye intensity data obtained after electrophoresis in sodium dodecyl sulfate. Such ratios should be considered as tentative until confirmed by independent techniques.

Isolation and amino acid composition. All five subunits of bovine

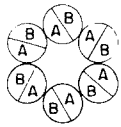
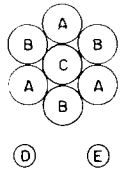
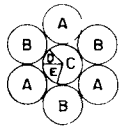
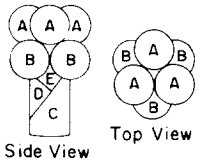
I		Hexagonal model proposed by Schnebli *et al.* (51) for Streptococcus faecalis F_1
II		Hexagonal model proposed by Catterall and Pedersen (3) for rat liver F_1. The small subunits, D and E, are too small to be observed by the electron microscope used. Similar structures have been observed for the Micrococcus lysodeikticus F_1 by Munoz *et al.* (52)
III		Hexagonal model proposed by Kozlov and Mikelsaar (49) for bovine heart F_1. The C, D and E subunits above are depicted as constituting a single, seventh, central subunit.
IV		Alternate "stalk" model proposed by Kozlov and Mikelsaar (49)

Figure 3. *Subunit structures proposed for F_1 or F_1-like preparations from mitochondria and bacteria.* The molecular weights of the A and B subunits in model 1 are near 30,000. In the other models the *A* and *B* subunits are assumed to have molecular weights between 50,000-62,000; the *C* subunit, a molecular weight between 25,000-36,000; and the *D* and *E* subunits molecular weights of about 12,000 and 7,500, respectively.

heart F_1 [11, 53] and the three major subunits of the rat liver enzyme [45] have been isolated in pure form. In general the isolation techniques are quite similar and involve pretreatment of the enzyme with either urea or guanidine HCl followed by chromatography on anion exchange celluloses or Sephadexes. The isolated subunits do not catalyze the hydrolysis of ATP.

Amino acid compositions have been determined for intact F_1 or F_1-like ATPases [28, 32, 38] (Table V), for the five F_1 subunits [11, 53], for the smallest subunit of the oligomycin-sensitivity conferring peptides [54], and for the ATPase inhibitor [12]. Subunits A, B and D

TABLE V. Amino acid compositions of F_1 or F_1-like ATPases

The results are presented as residues of each amino acid per 30,000 g of protein

Amino acid residue	Rat liver mitochondrial ATPase [28]	Bovine heart mitochondrial ATPase [32]	Spinach chloroplast ATPase [32]	*S. faecalis* ATPase [51]
Cysteine	0.7		1.1	0.9
Aspartic acid	21.3	19.8	20.4	28.4
Threonine	13.1	13.3	19.3	19.2
Serine	13.9	12.5	16.1	17.4
Glutamic acid	27.3	30.0	37.5	36.6
Proline	11.3	10.9	11.0	11.0
Glycine	22.3	22.9	22.5	24.7
Alanine	23.5	25.4	24.6	23.8
Valine	21.0	19.6	19.3	19.2
Methionine	4.9	4.7	7.5	6.4
Isoleucine	16.8	16.3	18.2	17.4
Leucine	20.3	21.7	25.7	26.6
Tyrosine	6.6	7.2	7.5	9.2
Phenylalanine	7.3	7.3	7.5	8.2
Lysine	14.2	15.1	11.8	17.4
Histidine	4.0	4.1	2.1	4.6
Arginine	11.3	16.0	17.0	12.8

of the F_1 complex appear to be acidic in nature, whereas subunits C and E are basic. The smallest subunit (7500-9000 molecular weight) of the set of peptides conferring oligomycin-sensitivity on F_1 is highly hydrophobic and soluble in chloroform-methanol [54].

There is some significant disagreement about the amino acid composition of subunits C and B. Knowles and Penefsky [11] report that subunit C of heart F_1 contains 18 residues proline/mole whereas Brooks and Senior [53] do not find detectable quantities. There is also disagreement about the identity of the ATPase inhibitor peptide. Although Brooks and Senior [12] have presented rather convincing evidence that the inhibitor protein is a sixth subunit of F_1, easily removed by heat treatment, Knowles and Penefsky [11] maintain that the fifth and smallest subunit of F_1 (subunit E) is the ATPase inhibitor. Both investigators suggest that the peptide they define as the ATPase inhibitor is identical to the inhibitor protein isolated by Pullman and Monroy [10].

Perhaps relevant to the argument of Knowles and Penefsky [11] are the recent reports by Nelson *et al.* [55] on the chloroplast ATPase (CF_1). The fifth or smallest subunit of this preparation (subunit E) has been shown by these investigators to be a potent inhibitor of ATPase activity. However, its amino acid composition is significantly different from either the fifth subunit of mitochondrial ATPase [11, 53], or the inhibitor preparation of Pullman and Monroy [10].

Impurities or products of proteolysis? The large number of

polypeptides associated with the oligomycin-sensitive ATPase complex, together with the apparent discrepancies in subunit amino acid compositions reported by different laboratories raise the question as to whether one or more of these polypeptide components is an impurity or degradation product of a more intact *in vivo* complex. With respect to F_1, the possibility of impurities seems unlikely, both because all F_1 or F_1-like preparations have been shown to be homogeneous by several criteria, and because most preparations, regardless of phylogenetic origin, contain five different polypeptide chains. The possibility, however, that a specific protease is present in all of these species, which is activated upon sonication or lysis and immediately attacks a more intact oligomeric F_1 complex has not been rigorously excluded. The same argument is applicable to the possible origin of some of the peptides necessary for oligomycin sensitivity.

The possibility that limited proteolysis may be occurring during purification such that the two smaller peptides of F_1 (D and E), and perhaps the ATPase inhibitor, arise from the three larger peptides has not been rigorously excluded either. In fact, in case of the bovine heart ATPase, it is puzzling as to why two groups of investigators [25, 46] using similar techniques report molecular weights for subunit C of 25,000 and 33,000, respectively. If limited proteolysis does occur, it is likely that it occurs to different extents in different preparations. This might explain discrepancies in subunit amino acid compositions reported by different investigators for the bovine heart enzyme.

Biogenesis. Tzagoloff and co-workers [56, 57] have shown that the five polypeptides comprising F_1 are synthesized on cytoplasmic ribosomes. Of the five polypeptides required for oligomycin-sensitivity, the "stalk" or OSCP component is synthesized on cytoplasmic ribosomes, whereas the four components comprising the membrane sector are synthesized in the mitochondrion. These investigators have also presented evidence that the smallest subunit of the oligomycin-sensitive ATPase complex, a hydrophobic peptide of 7500-9000 molecular weight, may exist in the intact mitochondrion as an oligomeric unit of 45,000 molecular weight [54]. It is believed to be converted to the monomer in organic solvents or in alkali.

Knowledge of the biogenesis of the ATPase inhibitor peptide of Pullman and Monroy [10] is lacking. Such information, if it can be derived from the yeast system, would help establish whether this peptide is in fact the fifth sununit of F_1-like preparations as suggested by the work of Knowles and Penefsky [11] and Nelson *et al.* [55], or conversely whether it is a separate and distinct component constituting a sixth component of F_1-like preparations [12], and therefore an eleventh component of the complete oligomycin-sensitive ATPase complex. An ATPase inhibitor peptide of about 6000 molecular weight has been purified from mitochondria of the yeast strain *Candida utilis* by Satre and Jerphanion [58].

Comparison with other ATPases. F_1-like preparations isolated from mitochondria, bacteria and chloroplast are composed of five polypeptide chains of different size (Table IV). There is no obvious relationship between the number and molecular weight of these polypeptides, and the polypeptide subunits of other well-known ATPases that do not participate in ATP synthesis. As noted in Table VI, the ATPase from

TABLE VI. Comparison of subunit composition of F_1 with ATPases not involved in ATP synthesis

ATPase preparation	No. of subunits	Subunit molecular weight	Suggested stoichiometry
Mitochondrial [45]	5	62,500, 57,000, 36,000, 12,000, 7,500	A_3B_3CDE
Myosin [61]	4	220,000, 25,000, 18,000, 16,000	A_2BC_2D
Sodium-potassium [60]	2	135,000, 57,000	A_2B
Sarcoplasmic reticulum [59]	1	102,000	

sarcoplasmic reticulum contains a single polypeptide of about 100,000 molecular weight [59]. The Na^+, K^+ ATPase contains only two polypeptides, one of about 135,000 molecular weight, and a glycopeptide of about 57,000 molecular weight [60]. Myosin ATPase is more complex, and contains four subunits ranging in molecular weight from 16,000 to 220,000 [61]. In no case, however, does there appear to be a consistent pattern suggestive of a common genetic origin of F_1-like and other ATPase molecules.

Catalytic Properties (See Table VII)

Amino acid residues involved. Of the various functional components of the oligomycin-sensitive ATPase, only the F_1 component has been chemically modified. These studies implicate tyrosine residues [9 or 10] as playing an important role in ATP hydrolysis. Reaction of mitochondrial F_1 with agents which react with tyrosine residues such as iodine [62], tetranitromethane [62, 63], iodoacetamide [63], and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBT) [64, 65], inhibit ATPase activity. ATP and ADP protect against inhibition by tetranitromethane and iodoacetamide [63]. Coupling activity in those cases examined, however, is not affected [62, 65]. NBT also inhibits the ATPase activity of the chloroplast enzyme (CF_1).

Although mercurial agents and other sulfhydryl blocking agents inhibit neither the ATPase activity of the rat liver [66] nor bovine heart

TABLE VII. Catalytic properties of mitochondrial ATPase

Property	General comments
Subunits involved	F_1: Not known for certainty. CF_1: The two largest subunits appear to be necessary for both coupling and ATPase activity.
Specificity for NTP's	In the membrane: ATP > ITP > GTP > UTP > CTP. For F_1: ATP, ITP, and GTP hydrolytic rates are equal or nearly equal. UTP and CTP rates are very low.
Specificity of metal ions	Mg^{++}, Co^{++}, Mn^{++}, Ca^{++} and Fe^{++} support hydrolysis. The relative specificity of cations depends on both the K^+ and ATP concentration used.
Activity in absence of added metal	Low but significant rates of ATP hydrolysis is reported.
K_m for ATP	In the membrane: K_m = 0.1-0.3 mM. For F_1: K_m = 0.79-1.25 mM. The membrane bound enzyme appears to have a significantly higher affinity for ATP than does F_1.
Inhibition by ADP	K_i values for the membrane bound enzyme range from 3 μM to 4mM, and for F_1 from 30 μM to 300 μM. The reason for these apparent discrepancies is not known.
Inhibition by ATPase inhibitor and by AMP-PNP	Both inhibit ATP-dependent activities but not oxidative phosphorylation. The ATPase inhibitor is non-competitive with ATP whereas AMP-PNP is competitive with ATP.
Inhibition by inhibitors of oxidative phosphorylation	*Inhibitors of membrane bound enzyme:* Oligomycin, rutamycin, aurovertin B, DCCD, tri-n-butyltin, venturicidin, quercetin, spegazzine, and ATPase antibody. *Inhibitors of F_1:* aurovertin, azide, quercetin, spegazzine, ATPase antibody, and in some cases Dio 9.
Activation by anions	Two-fold or more. Bicarbonate, bisulfite, borate, maleate, terepthlate, dichromate, and chromate are particularly effective. Appear to alter K_m(ATP). May act as Lewis bases.
Effect of phospholipids	Not required for activity of F_1: Required for maximal activity of oligomycin-sensitive ATPase. Lysolecithin is most effective. Phospholipids such as cardiolipin will induce release of F_1 from the membrane.
Exchange reactions	F_1 and oligomycin-sensitive ATPase preparations have not been shown as yet to catalyze any of the partial reactions of oxidative phosphorylation other than ATPase activity.

F_1 preparation [6, 63], 0.1 mM *p*-mercuribenzoate has been shown to inhibit by 50% the ATPase activity catalyzed by the oligomycin-sensitive ATPase [16]. The significance of this finding remains to be established, but it may indicate differences in the conformation of F_1 in the two preparations and therefore the accessibility of reactive sulfhydryl groups.

Subunits involved. Two studies suggest that the three small subunits, C, D and E, may not be required for ATPase activity. In the author's laboratory significant variations have been noted in the content of the two smallest subunits, D and E, from different preparations without corresponding variations in ATPase activity [29]. More recently, Kozlov and Mikelsaar [49] report that sonication of bovine heart submitochondrial particles at pH 5.2 releases an F_1 preparation which lacks the C and D subunits but which retains ATPase activity and competitive product inhibition by ADP. These workers point out however that the C and D subunits may be present in their preparation in the form of a larger subunit comprised of C, D and E polypeptides.

Studies of both bacterial ATPase [67] and the chloroplast enzyme (CF_1) [65] show that the three smallest subunits can be removed without dramatically altering ATPase activity. Coupling activity is affected, however, when either the three smallest peptides are removed [67], or in the case of the chloroplast enzyme [68], when the A and B (α and β) subunits are complexed with specific antibodies.

The ATPase activity of the chloroplast enzyme is inhibited about 80% when the tyrosine reactive agent NBT is used [65]. This agent reacts rather specifically with the B or β subunit.

The possibility that the two larger subunits constitute the "active centre" of the enzyme, and are directly responsible for ATPase and coupling activity, whereas the smaller subunits are required for coupling only (perhaps to simply bind the enzyme to the membrane) is suggested by the above studies. Provided the smallest subunit is in fact an inhibitor of ATPase activity as suggested by the data of Knowles and Penefsky [11] and Nelson *et al.* [55], a regulatory role may be associated with this subunit.

Specificity for nucleoside triphosphates. Results reported from three different laboratories [16, 69, 70] are in agreement that the nucleoside triphosphate specificity of the membrane bound ATPase of heart and liver mitochondria is in the order ATP > ITP > GTP > UTP > CTP. In these cases the rate of ATP hydrolysis is about twice that of ITP. The nucleoside triphosphate specificity of both the oligomycin-sensitive ATPase, and of F_1 preparations from bovine heart differ in some respects from the specificity of the membrane bound enzyme, and from each other. F_1 preparations examined in two different laboratories [6, 16] were shown to catalyze equal or near equal rates of hydrolysis with ATP, ITP, and GTP; whereas, the one oligomycin-sensitive ATPase preparation studied was found to be rather specific for ATP [16]. Similar to the membrane bound enzyme, both F_1 and oligomycin-sensitive F_1 catalyze hydrolysis of the pyrimidine nucleotides, UTP and CTP, at the lowest rates. This is also the case for ATPases from chloroplast and bacteria which preferentially catalyze hydrolysis of purine nucleoside triphosphates [21, 36, 37, 44].

Almost without exception, care has not been taken to measure initial rates, and to vary NTP concentration. Usually, the specificity data are reported at one time point and at one concentration of substrate. This may account for some of the differences observed among the various preparations.

Requirement for metal ions. Membrane bound ATPase and F_1 preparations of heart and liver catalyze high rates of ATP hydrolysis in the presence of Mg^{++}, Co^{++}, Mn^{++} and Fe^{++} [6, 70, 71]. Ca^{++} is significantly less effective under the conditions tested. Some apparent differences are associated with activation by Ca^{++}. Whereas Pullman *et al.* [6] find that Ca^{++} is about half as effective as Mg^{++} in activating the ATPase activity of F_1, Tzagoloff *et al.* [16] report that Ca^{++} is essentially without effect on the oligomycin-sensitive ATPase. Conversely, Vambutas and Racker [21] report that the ATPase of chloroplast membranes is preferentially activated by Ca^{++}, and that Mg^{++} has little effect.

The most thorough study of the interaction of metal ions with F_1-like ATPases, which might shed some light on the differences just noted, has been carried out by Adolfsen and Moudrianakis [72]. These investigators have shown that the bacterial ATPase from *Alcaligenes faecalis*, and the bovin heart F_1, are preferentially activated by K^+ at low ATP concentrations and by Mg^{++} at high ATP concentration. They have pointed out that it is probably improper to call F_1-like preparations either Ca^{++} or Mg^{++} ATPases because the degree of activation of the various enzymes depends on monovalent cation concentration. Thus, the *Alcaligenes faecalis* enzyme is preferentially activated by Ca^{++} in the absence of monovalent cations and by Mg^{++} in the presence of monovalent cations [72].

Consistent with the observations of Adolfsen and Moudrianakis [72], Davis and Bragg [44] report that the purified *E. coli* ATPase is activated equally well with either Ca^{++} or Mg^{++}.

Activity in the absence of metal ions. F_1-like preparations from rat liver [73], bovine heart [74, 75], *Streptococcus faecalis* [76], *Micrococcus lysodeikticus* [40], and *Alcaligenes faecalis* [72] have all been shown to catalyze low rates of ATP hydrolysis in the absence of added divalent cation. The hydrolytic activity has been noted in some cases when attempts were made to measure the binding of ATP to the enzyme [73, 74, 76]. There are indications that limited hydrolysis may occur under conditions used to study binding [73, 76]. These findings are of interest and need to be examined in greater detail to establish whether hydrolysis takes place at a site separate from the site normally activated by addition of cations.

K_m *for ATP.* F_1 preparations from both rat liver [3] and bovine heart mitochondria [77, 78] have been reported to have K_m(ATP) values between 0.79 and 1.25 mM in the pH range between 7.4 and 8.0. These

values were obtained either by following the release of ADP with a coupled enzyme assay [3] or by following initial rates of proton release [77, 78]. The K_m (ATP) of the purified, F_1-like, *Streptococcus faecalis* ATPase determined by following the release of P_i is 2.5 mM [38], whereas the same enzyme from *E. coli* also assayed by a P_i release assay, has a K_m between 0.29 and 0.60 mM ATP [36, 37].

The membrane bound F_1 has a significantly lower K_m for ATP [79, 80]. Results obtained by Mitchell and Moyle [79] using the proton release assay and by our laboratory [80] using a coupled assay indicate a K_m (ATP) of about 100 μM for the rat liver system. Using the proton release assay, Hammes and Hilborn [78] report a K_m (ATP) at pH 8.0 of 0.315 mM for F_1 bound to heart membranes. The significantly lower values for K_m (ATP) for the membrane bound F_1 suggest that binding of F_1 to the membrane enhances its affinity for ATP. It is possible that this is related to different conformational states of the enzyme when on and off the membrane. In contrast to these observations Hanson and Kennedy [37] report a K_m of 0.29 mM ATP for the purified *E. coli* ATPase and a similar K_m of 0.23 mM ATP for the membrane bound enzyme.

Although in general most investigators have obtained typical Michaelis-Menten kinetics for the hydrolysis of ATP catalyzed by both the membrane bound and purified F_1, there is not complete agreement on this point. Ebel [81] reports for rat liver F_1 that reciprocal plots of initial velocity data with varying MgATP concentration are curved in the absence of activating anions (see below) with Hill coefficients of about 0.5. In the presence of activating anions, however, there is no evidence of curvature in the reciprocal plots. In addition, Rossi and co-workers [82] report triphasic velocity *vs* ATP plots corresponding to K_m (ATP) values of 12.5, 75, and 400 μM for the membrane bound ATPase of heart mitochondria.

Inhibition by ADP. ADP is a potent product inhibitor of the ATPase reaction catalyzed by membrane bound ATPase [70, 79, 83], the oligomycin-sensitive ATPase [16], F_1 [3, 6, 78] and F_1-like preparations from chloroplast [21] and bacteria [38]. In those preparations examined, the inhibition appears to be competitive with ATP and to be rather specific for ADP [3, 78]. dADP is also a potent inhibitor of the ATPase reaction [80]. Other nucleoside diphosphates are much less effective but do inhibit to some extent [84].

There is considerable disagreement about the effectiveness of ADP as a product inhibitor. In rat liver membranes, Cooper and Lehninger [70] using a P_i release assay report that 4 mM ADP provides only about 50% inhibition of enzyme activity. In heart membranes, 3 mM or 10 mM ADP is required for 50% inhibition depending on whether the membrane fraction is prepared by disrupting the mitochondria with digitonin or by sonication [83]. Mitchell and Moyle [79], using a proton release assay,

report values for the K_i(ADP) ranging from 3 μM to 9 μM. They find that activating anions (see below) increase the apparent K_i(ADP). Van de Stadt *et al.* [77] and Hammes and Hilborn [78], also using a proton release assay, report values of 150 μM and 80 μM, respectively, for inhibition of the ATPase activity of heart membranes.

There is also significant disagreement among K_i(ADP) values reported for purified ATPase preparations. Using a phosphate release assay Pullman *et al.* [6] report that the heart F_1 has an apparent K_i(ADP) for inhibition of ATP hydrolysis of $\sim$ 1.0 mM, whereas the apparent K_i (ADP) for inhibition of the hydrolysis of ITP is $\sim$ 0.1 mM. Using the proton release assay, Hammes and Hilborn [78] report a K_i(ADP) of 30 μM for the same enzyme. The K_i(ADP) of the rat liver enzyme using a phosphate release assay and measuring initial rates is 0.24-0.31 mM [3]. The only such study of the oligomycin-sensitive ATPase [16] shows that 10 mM ADP is required for half-maximal inhibition.

The wide disagreement among various laboratories for the same or similar ATPase preparations may well reflect different states of the enzyme or differences in assay conditions. Since ADP is a product inhibitor it is essential in studies of its inhibitory effects that ATP solutions free of ADP be used, and that initial rates be measured. Also, the finding that activating anions affect the apparent K_i (ADP) may explain some of the apparent discrepancies noted above, and should be taken into account when studying inhibition by ADP.

Inhibition by ATPase inhibitor and by AMP-PNP. The bovine heart ATPase inhibitor of Pullman and Monroy [10] inhibits ATPase activity catalyzed by the membrane bound ATPase [10], the oligomycin-sensitive ATPase [16] and F_1 [26]. It is evidently specific for mitochondrial ATPase and does not inhibit myosin ATPase, Na^+, K^+ATPase or a number of phosphatases [10]. For maximal inhibition of F_1, preincubation with Mg^{++} and ATP at pH values between 5.5 and 7.0 are required [26]. The molecular weight of the inhibitor was reported originally to be about 15,000 [10], but more recent estimates put its values closer to 10,500 [12]. Assuming the latter value and a molecular weight of the bovine heart ATPase of 360,000, it can be calculated from the inhibition data of Pullman and Monroy [10] that about 0.6 mole inhibitor binds with one mole F_1. Van de Stadt *et al.* [77] have shown the inhibitor protein is noncompetitive with ATP.

It is significant to note that the ATPase inhibitor inhibits ATP-dependent activities without inhibiting oxidative phosphorylation [85]. Asami *et al.* [85] suggest that one natural role of the inhibitor might be to inhibit ATP-dependent activities and thus control the back-flow of energy from ATP to the mitochondrial electron- and ion-transport systems. The results of Van de Stadt, De Boer, and Van Dam [77] which show that succinate oxidation (energization) favors dissociation of the inhibitor from submitochondrial particles (presumably dissociation from

the ATPase), whereas MgATP favors association, are in basic agreement with the proposal of Asami *et al.* [85]. As yet, this type of association-dissociation phenomenon has not been observed in intact mitochondria during state 4 → state 3 transitions, nor has it been observed in systems other than heart. However, recent work in the author's laboratory suggests that an inhibitor-like protein may be present in rat liver mitochondria [71].

As emphasized earlier in this review there is considerable controversy over the actual identity of the ATPase inhibitor of Pullman and Monroy [10]. Some investigators claim it is the smallest or fifth subunit of F_1; others claim it is attached to some F_1 preparations as a sixth subunit. Before the inhibitor's regulatory role can be more fully understood in molecular terms, it seems essential to resolve the identity problem.

The action of the ATPase inhibitor protein is mimicked to some extent by the ATP analog AMP-PNP [71, 74]. Similar to the inhibitor protein, AMP-PNP inhibits ATP-dependent functions in submitochondrial particles but is without effect on oxidative phosphorylation. Unlike the ATPase inhibitor protein, however, AMP-PNP is competitive with ATP for the hydrolytic site of both the membrane bound and purified F_1 [71, 74].

Inhibition by inhibitors of oxidative phosphorylation. Oligomycin [86], rutamycin (oligomycin D) [87], aurovertin B [88, 89], DCCD [13, 90, 91], tri-n-butyltin chloride [87, 92], venturicidin [93], azide [94], and an antibody to F_1 [95], are all inhibitors of oxidative phosphorylation [86-95], the membrane bound ATPase [86-95], and in those cases studied, the oligomycin-sensitive ATPase [16]. Of these various inhibitors, only aurovertin [87, 96], azide [71], and the F_1 antibody [95] inhibit the ATPase activity of F_1 [71, 87, 95, 96]. Another inhibitor Dio-9 is a potent inhibitor of F_1 from yeast but it has little effect on the heart enzyme [97].

Chang and Penefsky [98] report that aurovertin is uncompetitive with ATP and that two sites on F_1 interact with this inhibitor, one of which is observed only in the presence of ATP. In the absence of ATP, or in the presence of ADP or Mg^{++}, one mole of aurovertin/mole F_1 is bound [98, 99]. Catterall and Pedersen [73] show that, in addition to inhibiting ATPase activity, aurovertin also inhibits the high affinity binding of ADP to the rat liver F_1 in the absence of added Mg^{++}. Unlike inhibition of oxidative phosphorylation by aurovertin, however, neither inhibition of ATPase activity ($\sim$ 65%) nor high affinity ADP binding ($\sim$ 76%) to F_1 is complete. The same applies to azide, which inhibits ATPase activity of purified F_1 no more than 60% [71].

Recently, two other inhibitors of the membrane bound and purified ATPase have been reported. One of these, quercetin (3,3′,4′,5,7-pentahydroxyflavone), is reported by Lang and Racker [100] to be similar in its action to the ATPase inhibitor peptide [10]. At low concentrations

quercetin inhibits soluble and membrane bound ATPase but has no effect on oxidative phosphorylation in submitochondrial particles. The other inhibitor, spegazzine, a dihydroindole alkaloid, is reported by Roveri and Vallejos [101] to be similar to aurovertin in its action. Spegazzine inhibits oxidative phosphorylation almost completely, has a lesser inhibitory effect on ATP-dependent reactions, and partially inhibits the membrane bound and soluble ATPase.

Atractyloside, a specific inhibitor of the ADP/ATP transport system of mitochondria, has been shown to be without effect on Mg^{++}-stimulated ATPase activity of submitochondrial particles [69, 102]. However, the recent reports that F_1 contains tightly bound nucleotides (see page 25) together with the genetic study of Griffiths and co-workers [103] implicating the oligomycin-sensitive ATPase as containing part of the atractyloside sensitive locus, suggest that the interaction of atractyloside with F_1 and oligomycin-sensitive F_1 preparations should be re-examined.

Activation by anions. A number of oxyanions have been shown to markedly activate the ATPase activity of the membrane bound ATPase of rat liver [79], and the ATPase activity catalyzed by rat liver [30, 71, 81] and bovine heart F_1 [30, 104]. Bicarbonate, bisulfite, borate, maleate, terephthalate, dichromate, chromate, and PP_i usually activate such preparations by at least two-fold.

The mechanism by which these anions activate the ATPase, and the functional significance of this activation, if any, remains to be established. However, several observations seem noteworthy. First, the activating anions contain oxygen and might be considered Lewis bases. Secondly, the structure of the anion is important. Fumarate and maleate are cis-trans isomers but only maleate activates the ATPase [71]. Third, the enhanced ATPase activity that appears in the presence of anions is sensitive to aurovertin indicating that it does not arise from autovertin-insensitive sites on the enzyme which are normally not participating in ATP hydrolysis [30]. It is possible that anions may be participating in the hydrolytic event simply as general base type catalysts, or conversely, by inducing conformational changes in the enzyme.

In submitochondrial particles of rat liver, Mitchell finds that activating anions do not alter the K_m(ATP), but do decrease the K_i(ADP). In contrast, Ebel [81] working with the purified F_1 of rat liver reports that activating anions affect the general shape of Lineweaver-Burke plots (see previous section, page 18) converting them from a nonlinear to a linear form. He also notes that activating anions have little or no effect on the hydrolysis of nucleoside triphosphates other than ATP [81]. Consistent with the observations of both of these investigators the author finds that activating anions have no effect on the K_m(ATP) of the membrane bound ATPase, but that they lower the apparent K_m of the purified F_1 [66].

It would be of considerable interest to know whether the activation of ATPase activity by anions has physiological significance. Activation by bicarbonate is of particular interest since the concentration of this anion is likely to fluctuate in the matrix depending on whether or not Krebs Cycle intermediates are being utilized.

Effect of phospholipids. Phospholipids are not required for ATPase activity of purified F_1 or F_1-like preparations. The *Streptococcus faecalis* enzyme, however, has been reported by Redwood [105] to readily interact with phospholipids without effect on ATPase activity. The enzyme can be incorporated into egg phosphatidylcholine liposomes (PC) in a ratio of PC/ATPase of 11 : 1 (w/w). The intrinsic association constant for the interaction determined from Scatchard plots is about $7.4 \times 10^{-7}\ M^{-1}$ at 4°.

Oligomycin-sensitive ATPase preparations contain phospholipids (10-30% by weight), and may or may not be activated by further addition of phospholipids. The oligomycin-sensitive ATPase preparation of Tzagoloff *et al.* [16] from bovine heart contains as much as 30% (w/w) phospholipid and is not activated by further addition. The analogous preparation from yeast mitochondria contains 10-31% (w/w) phospholipid but its interaction with additional phospholipids has not been reported [15].

Perhaps the most detailed study of the effect of phospholipids on oligomycin-sensitive ATPase preparations is that by Swanljung *et al.* [19]. Working with a lipid depleted preparation of very low specific ATPase activity (0.49 μmoles ATP hydrolyzed/min/mg), these investigators showed that the activity is activated up to 18-fold by lysolecithin and to a lesser extent by cardiolipin, phosphatidylinositol, and phosphatidylethanolamine. About 1.1 μmole lysolecithin/mg protein was required for half-maximal inhibition. These investigators also note in their report that ADP and Mg^{++} enhance the activation of suboptimal amounts of phospholipids, and that the addition of phospholipids significantly lowers the K_m (ATP). In the presence of cardiolipin the K_m (ATP) is lowered from 0.71 mM to 0.14 mM ATP.

Mechanistically, it is puzzling as to why phospholipids are necessary for maximal activity of lipid deficient, oligomycin-sensitive ATPase preparations but not for F_1 preparations. This would suggest that in the absence of phospholipids the oligomycin-sensitivity conferring peptides induce a less active state or conformation of F_1 that is normally counteracted by phospholipids. The possibility that phospholipids may serve, at least to some extent, as the cement for bridging F_1 to the oligomycin-sensitivity conferring peptides is suggested by the studies of Toson *et al.* [106] and Lee [107] which show that F_1 can be released from the inner membrane by addition of certain phospholipids, particularly cardiolipin.

Exchange reactions. Submitochondrial particles have been shown to

catalyze ATP-ADP, ATP-P_i, ATP-HOH, and P_i-HOH exchange reactions (for a review, see ref. 108). These exchange reactions are inhibited by oligomycin [108], and aurovertin [88, 108, 109], and are thought to involve directly the F_1 component of the ATPase in the membrane. To date none of the F_1 or oligomycin-sensitive F_1 preparations discussed in this review have been shown to catalyze these exchange reactions. It seems likely that a vesiculated membrane-like structure is required to observe these exchanges in accordance with the predictions of Mitchell [110]. Should this be the case, one might predict that oligomycin-sensitive ATPase preparations would catalyze these exchanges if appropriately dialyzed to remove detergent so that vesicle formation can take place. It is noteworthy that the respiration deficient, vesicular membrane system of Kagawa and Racker [111], the respiration deficient yeast mutant of Groot *et al.* [112], and the recent complex V of Hatefi and Hanstein [113] catalyze both an ATP-P_i exchange reaction and oligomycin-sensitive hydrolysis of ATP. However, the molecular composition of these various systems relative to the oligomycin-sensitive ATPase has not been rigorously established, making it difficult to deduce whether polypeptides other than those associated with oligomycin-sensitive ATPase preparations are necessary to observe the ATP-P_i exchange. Certainly electron transport components do not seem to be necessary.

Nucleotide Binding Properties (See Table 8)

Reversible binding of ADP. F_1 or F_1-like preparations from rat liver [3, 73], bovine heart [74, 114, 115], *Streptococcus faecalis* [76. 116] and chloroplast [117, 118] bind ADP reversibly and very tightly ($K_d \geqslant 1\ \mu M$). In the case of the rat liver enzyme purified by Catterall

TABLE VIII. Nucleotide binding properties of F_1 or F_1-like preparations

Source of ATPase	Max. no. of sites detected to date	Comments
Liver (3, 71, 73, 80)	3	Two are reversible and at least one is tightly bound to freshly isolated preps.
Heart (74, 84, 114, 115, 122, 123)	5	Not readily exchangeable. Not complete agreement between Harris *et al.* (123) and Penefsky (74, 115)
S. faecalis (76)	2	—
Chloroplast (117, 144)	2	Two ADP bind and are converted to ATP and AMP (117). Two ATP bind (144).

and Pedersen [3, 28, 29, 71, 73] the data strongly suggest that this tight or high affinity binding site is distinct from the site or sites normally involved in ATP hydrolysis. Thus, high concentrations of sucrose, prior incubation with Mg^{++}, and AMP-PNP inhibit ATPase activity without markedly affecting high affinity ADP binding. The tight binding site of the rat liver enzyme (K_d = 0.96 μM) and of the bovine heart enzyme (K_d = 0.28 μM) are kinetically distinct from the site involved in competitive ADP inhibition of ATPase activity. K_i(ADP) for the purified heart and liver F_1 have been reported to be 30 μM and 240 μM, respectively, and are therefore 200-300 times the K_d(ADP) of the tight binding site.

Although binding of ADP to F_1 preparations of heart and liver is evidently not accompanied by transphosphorylation, this is not the case for the chloroplast enzyme. Roy and Moudrianakis [117] report that ADP binds very strongly to two sites on CF_1. Once bound the molecule carries out a stoichiometric transphosphorylation to give AMP and ADP which are not readily released into solution.

Specificity of reversible ADP binding. As noted earlier in this review (page 18) inhibition of ATPase activity of F_1 by ADP is rather specific for ADP and dADP [3, 78]. However, it is not completely specific for adenine nucleotides because if sufficiently high concentrations of other nucleoside diphosphates are used inhibition will be observed. For example, Hilborn and Hammes [84] note that IDP is a weak inhibitor of ATPase activity with a K_i of about 2 mM. The same applies to the specificity of the tight, reversible, ADP binding site on F_1. Although ATP, IDP, and UDP do not bind tightly to this site, some binding does take place [84].

The lack of complete specificity of the two ADP binding sites may be relevant to the report of Gregg [119], Low *et al.* [120] and Vallin [121] that oxidative phosphorylation in submitochondrial particles is not completely specific for ADP, but will phosphorylate IDP, GDP, and UDP as well. Although it is not known whether one, or both of the reversible ADP binding sites is involved in oxidative phosphorylation, there is sufficient lack of specificity in both cases to predict that at high concentrations, IDP and perhaps other nucleoside diphosphates would be phosphorylated to some extent.

Reversible binding of ATP. The reversible binding of ATP to F_1 or F_1-like preparations is more difficult to study. Most investigators find that even in the absence of a cation there is significant residual ATPase activity such that ATP binding cannot be followed directly. To avoid the problem of ATP hydrolysis Garrett and Penefsky [115] use AMP-PNP which is not hydrolyzed. They find two apparently equivalent binding sites (K_d(AMP-PNP) = 1.3 μM). Another 0.4 moles AMP-PNP is bound that cannot be removed by ammonium sulfate precipitation.

ATP and ADP bound to F_1-preparations as isolated. Sanadi and collaborators [122] first noticed nucleotide tightly bound to freshly isolated Factor A preparations from bovine heart mitochondria. One mole of Factor A was shown to contain about one mole of ADP. These workers later showed that Factor A binds another mole of ADP in a reversible manner [114]. Consistent with these observations Harris *et al.* [114] find that F_1 isolated from bovine heart by the procedure of Knowles and Penefsky [25] has tightly associated with it 1.5-2.0 moles ADP/mole F_1. In addition, they show that 2.7-3.0 moles of ATP is bound to the freshly isolated preparations. This tightly bound nucleotide cannot be removed readily by gel filtration or by treatment with charcoal, but can be released by cold denaturation. In confirmation of these observations Catterall and Pedersen report that the rat liver F_1 also has tightly bound ATP and ADP as isolated [66], but a significantly lower level than the heart enzyme ($<$ 2 mole/mole F_1). This may be related to basic differences in heart and liver ATPases, or to differences in methods of enzyme preparation (see page 7).

AMP binding. AMP does not bind reversibly to rat liver F_1 [73], and it is not tightly bound to bovine heart F_1 as isolated [123]. However, as noted above the chloroplast enzyme does appear to have bound AMP derived from the transphosphorylation of ADP.

Conformational Changes

As deduced from optical rotatory dispersion studies. Sanadi and co-workers [122] first examined an F_1-like preparation for conformational changes. The Sanadi preparation (called Factor A), unlike most mammalian F_1 preparations, catalyzes low rates of ATP hydrolysis when freshly isolated, but when heated to 45°, it has hydrolytic rates comparable to other F_1 preparations [42, 122].

Using changes in optical rotation at 232 nm as an index of conformational changes, these workers show that Factor A undergoes significant changes in conformation when heated from 5° to 45° [122]. Changes in ORD are rapid and complete in 2 minutes. In contrast, however, maximal ATPase activity is not observed until 10-20 minutes later. This suggests that changes in the structure of Factor A, too subtle to be detected by ORD measurements, are required together with a gross conformational change in the molecule to provide the conformation optimal for catalyzing hydrolysis of ATP.

Also noted in these studies is the finding that heat treatment of Factor A does not alter its coupling properties. Thus, F_1-like preparations have the capacity to undergo conformational alterations which very markedly affect ATPase activity but have no effect on coupling activity in the membrane.

The changes observed in Factor A upon heating are strikingly similar to those observed for the chloroplast ATPase (CF_1) by Farron and Racker [124]. CF_1 can be isolated as a homogeneous protein with latent ATPase activity. Transformation of CF_1 to an active ATPase by mild heat treatment is accompanied by the appearance of titrable SH groups. The authors conclude that conversion from latent into manifest ATPase represents a conformational change which may proceed via disulfide interchange.

As deduced from aurovertin binding studies. Another approach to determining whether F_1 undergoes changes in conformation has been to examine the fluorescence intensity associated with binding of aurovertin to F_1. Chang and Penefsky [98] have shown that this fluorescence is partially quenched by ATP or Mg^{++}. Moreover, these workers have shown that addition of succinate to a complex of aurovertin and submitochondrial particles gives rise to an enhancement of fluorescence which is dependent on the maintenance of the energized state. They suggest that the fluorescence changes observed might be reflections of conformational changes in F_1.

Van de Stadt *et al.* [125] carried out an analogous set of experiments with submitochondrial heart particles, and presented evidence for two types of aurovertin binding sites in the membrane. P_i and ATP decrease the "apparent" affinity of the particles for aurovertin. Similar to Chang and Penefsky [98] they also interpret their data in terms of conformational changes at the level of F_1.

Continuing these studies Yeates [75] reported that binding of aurovertin to bovine heart F_1 is accompanied by a biphasic fluorescence enhancement. P_i quenched the slow phase; ADP increased the extent of the slow phase; ATP temporarily quenched fluorescence; and Mg^{++}, in contrast to the observations of Chang and Penefsky [98] had no effect. The results are interpreted by assuming that subsequent to binding of aurovertin a slow conformational change in F_1 occurs. Presumably, the effects of P_i, ADP, and ATP on the fluorescence process are reflections of F_1-ligand interactions which either promote or inhibit this conformational change.

Studies with aurovertin probably give a more reliable indication of conformational changes associated with F_1 alone, than of F_1 in the membrane. Interpretations of studies of the interaction of aurovertin with the membrane bound F_1 are based on the assumption that aurovertin interacts only with F_1, and that ligand induced changes result directly from interaction with F_1 or the oligomycin-sensitivity conferring complex, rather than with other ligand binding components of the membrane.

Function

Although this review summarizes much about our knowledge of the structure and catalytic properties of ATPases, it leaves us in the dark as

to how this class of enzymes participate in oxidative phosphorylation and ATP-dependent activities. Unfortunately details of mechanism relevant to function cannot be given at this time because all of the facts are not in yet. What information is lacking? This, of course, is a matter of opinion, but in the author's view we would be much closer to understanding how ATPases participate in oxidative phosphorylation and ATP-dependent activities if we knew the following:

Activities catalyzed by the intact oligomycin-sensitive ATPase. Despite the fact that the oligomycin-DCCD-sensitive ATPase is regarded as the functional unit in the terminal enzymatic step of oxidative phosphorylation, little effort has been expended in studying the properties of *purified* forms of this enzyme complex. Certainly, a great amount of effort has gone into studies of reconstituted inner membrane-like vesicles which catalyze oligomycin-sensitive ATPase activity [126], P_i-ATP exchange activity [110], and in some cases which establish proton gradients that can be coupled to ion transport [127] or ATP formation [128]. The importance of this work should not be minimized, but at the same time we should realize that such reconstructed systems do not provide us with details of mechanism. Moreover, mechanistic details are unlikely to be forthcoming from many of the systems studied to date because they still contain "factors" of undefined composition.

In contrast, *purified* oligomycin- and/or DCCD-sensitive ATPase preparations of defined subunit and phospholipid composition should provide us with the ideal model, both with which to test predictions of the chemiosmotic and other hypotheses, and to derive important information about mechanisms as they relate to structural-functional relationships within the complex and within reconstructed systems. In particular it seems important to establish under what conditions *purified* oligomycin-sensitive ATPase complexes will form vesicles, or be incorporated into phospholipid vesicles, under what conditions they will catalyze partial reactions of oxidative phosphorylation; and under what conditions they will catalyze the formation of a transmembrane potential and support ion uptake. Are the peptides and phospholipids intrinsic to *purified* oligomycin-sensitive ATPase complexes sufficient to catalyze these activities? If not, what additional components of *defined* composition are necessary?

The role of oligomycin-sensitivity conferring peptides. According to the predictions of Mitchell [4, 5, 110], the complex which confers oligomycin and DCCD sensitivity on F_1 may be responsible for directing the flow of protons to the catalytic site of F_1. The experiments of Hinkle and Horstman [7] which demonstrate that oligomycin and DCCD inhibit proton permeability in F_1 deficient particles are consistent with this view. Similarly, the observations by a number of investigators [129-135] that certain energy-linked properties can be restored in bacterial mutants or bacterial vesicle preparations by the addition of

DCCD, and the more recent findings of Altendorf, Harold, and Simoni [136] that this agent inhibits proton permeability in vesicles prepared from an ATPase deficient strain of *E. coli,* are also consistent with the predictions of Mitchell [4, 5, 110]. However, direct proof of the involvement of the oligomycin-sensitivity conferring complex in proton permeability, and the identification of the peptides involved, is lacking. Such information must await purification of the complex and of its individual subunits, followed by their insertion into liposomal membranes. The peptide which is of particular interest is the DCCD binding protein [17, 137] which has become known simply as the "Beechey protein" after one of its discoverers, Dr. R. Brian Beechey.

The relationship between Mg^{++} ATPase activity and oxidative phosphorylation. What one would like to know with certainty is whether the site involved in catalyzing cation-dependent ATPase activity of F_1 or F_1-like preparations is the same site that participates in oxidative phosphorylation in the membrane. Much of the data tabulated in this review is difficult to interpret by assuming that the same site catalyzes both activities. For example, both the inhibitor protein of Pullman and Monroy [10] and AMP-PNP inhibit Mg^{++} ATPase activity of F_1 and membrane bound F_1 but do not inhibit oxidative phosphorylation [10, 71, 74]. (Both of these agents inhibit ATP-dependent functions [74, 85].) Forms of ATPase preparations which lack sufficient ATPase activity, as isolated, i.e. Factor A, and CF_1 are perfectly competent in reconstituting oxidative phosphorylation in F_1 or CF_1 deficient membranes [21, 34, 42, 138]. Estimates of the K_i(ADP) for inhibition of Mg^{++} ATPase activity of F_1 are usually significantly greater than estimates of the K_m(ADP) for oxidative phosphorylation (see page 19). To account for these observations some investigators suggest that F_1 may contain two catalytic sites on the enzyme which are specialized respectively for ATP synthesis and ATP utilization [3, 71, 74]. (See Fig. 2C.)

The rather general acceptance of the Mg^{++}-ATPase site as the site involved in oxidative phosphorylation has probably evolved to some extent from the finding that DNP activates Mg^{++}-ATPase activity of isolated F_1 preparations [6, 30, 71, 139], and binds directly to the enzyme [140]. These findings together with the fact that DNP stimulates ATPase activity of mitochondria when acting as an uncoupler of oxidative phosphorylation, do suggest that perhaps the site catalyzing Mg^{++} ATPase activity is related to the site involved in catalyzing oxidative phosphorylation. However, it is now known that the activating effect of F_1 by DNP is not given by more potent uncoupling agents such as FCCP [71]. Moreover, the concentrations of DNP necessary to maximally activate Mg^{++} ATPase activity of isolated F_1 preparations are usually one to two orders of magnitude greater than those necessary to uncouple oxidative phosphorylation. It seems, therefore, that the

activation of the Mg^{++}-ATPase activity of F_1 by DNP is most likely unrelated to its property as an uncoupler of oxidative phosphorylation, but rather to its property as an activating anion [30 71].

The role of tightly bound nucleotides. The finding that both ATP and ADP are tightly bound to freshly isolated F_1 preparations is of considerable interest, particularly in regard to the mechanism of action of F_1. Boyer and his colleagues [141, 142] have suggested recently on the basis of studies of exchange reactions that, in oxidative phosphorylation, energy from electron transport may cause release of preformed ATP from the catalytic site. Presumably, energy conserved during respiration, either as an electrochemical gradient, as a high energy chemical intermediate, or as an energized membrane conformation, is used to induce a conformational change in a protein containing tightly bound ATP. The return of the protein to its original conformation in the presence of ADP and P_i is coupled to the re-synthesis of another molecule of bound ATP (Fig. 2D).

The nature of the catalytic site is not specified by Boyer and his colleagues, but it seems reasonable to assume that in this hypothesis the catalytic site must reside at the level of F_1 or the oligomycin-sensitive ATPase complex. Whether tightly bound ATP is released from membrane bound ATPase when respiration is initiated remains to be established.

It is equally possible that nucleotides which bind tightly to F_1 play regulatory roles *in vivo,* similar perhaps to that postulated for the ATPase inhibitor [77, 85]. It is of interest to note that the ATPase of *Streptococcus faecalis,* which is involved in ATP-dependent functions, but not in oxidative phosphorylation, has at least one tight binding site for ATP [76].

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References

1. A. E. Senior, *Biochem. Biophys. Acta,* **301** (1973) 249.
2. H. S. Penefsky, in: *The Enzymes* (P. D. Boyer, ed.) Vol. X, 3rd ed.; Academic Press, New York (1974) 375.
3. W. A. Catterall and P. L. Pedersen, in: *Membrane ATPases and Transport Processes* (R. J. Bronk, ed.) Biochem. Soc. Spec. Publ. 4, Biochemical Society, London, in Press (1974).
4. P. Mitchell, *FEBS Letters,* **33** (1973) 267.
5. P. Mitchell, *FEBS Letters,* **43** (1974) 189.
6. M. E. Pullman, H. S. Penefsky, A. Datta and E. Racker, *J. Biol. Chem.,* **235** (1960) 3322.

7. P. Hinkle and L. L. Horstman, *J. Biol. Chem.*, 246 (1971) 6024.
8. L. Ernster, K. Nordenbrand, O. Chude and K. Juntti, in: *Membrane Proteins in Transport and Phosphorylation* G. F. Azzone, M. Klingenberg, E. Quagliariello and N. Siliprandi, eds); Elsevier, North-Holland, in Press (1974).
9. D. H. MacLennan and A. Tzagoloff, *Biochem.*, 7 (1968) 1603.
10. M. E. Pullman and G. C. Monroy, *J. Biol. Chem.*, 238 (1963) 3762.
11. A. F. Knowles and H. S. Penefsky, *J. Biol. Chem.*, 247 (1972) 6624.
12. J. C. Brooks and A. E. Senior, *Arch. Biochem. and Biophys.*, 147 (1971) 467.
13. B. Bulos and E. Racker, *J. Biol. Chem.*, 243 (1968) 3891.
14. Y. Landry, *Ph. D. Thesis*, Université, de Nancy (1973).
15. A. Tzagoloff and P. Meager, *J. Biol. Chem.*, 246 (1971) 7328.
16. A. Tzagoloff, D. H. MacLennan and K. H. Byington, *Biochem.*, 7 (1968) 1596.
17. F. S. Stekhoven, R. F. Waitkus and H. T. B. Van Moerkerk, *Biochem.*, 11 (1972) 1145.
18. R. A. Capaldi, *Biochem. Biophys. Res. Commun.*, 53 (1973) 1331.
19. P. Swanljung, L. Frigeri, K. Ohlson and L. Ernster, *Biochem. Biophys. Acta*, 305 (1973) 519.
20. J. W. Soper and P. L. Pedersen, manuscript in preparation (1974).
21. V. Vambutas and E. Racker, *J. Biol. Chem.*, 240 (1965) 2660.
22. E. Racker, in: *Membranes of Mitochondria and Chloroplasts* (E. Racker, ed.); Van Nostrand Reinhold Company, New York (1970) 127.
23. I. C. West and P. Mitchell, *FEBS Letters*, 40 (1974) 1.
24. F. M. Harold, J. R. Baarda, C. Baron and A. Abrams, *J. Biol. Chem.*, 244 (1969) 2261.
25. A. F. Knowles and H. S. Penefsky, *J. Biol. Chem.*, 247 (1972) 6617.
26. L. L. Horstman and E. Racker, *J. Biol. Chem.*, 245 (1970) 1336.
27. A. E. Senior and J. C. Brooks, *Arch. Biochem. Biophys.*, 140 (1970) 257.
28. W. A. Catterall and P. L. Pedersen, *J. Biol. Chem.*, 246 (1971) 4987.
29. W. A. Catterall, *Ph.D. Thesis*, Johns Hopkins University (1972).
30. D. O. Lambeth and H. A. Lardy, *Eur. J. Biochem.*, 22 (1971) 355.
31. A. Goffeau, Y. Landry, F. Foury, M. Briquet and A. M. Colson, *J. Biol. Chem.*, 248 (1973) 7097.
32. F. Farron, *Biochem.*, 9 (1970) 3823.
33. S. Lien and E. Racker, *Methods in Enzymol.*, 23 (1971) 547.
34. S. H. Howell and E. N. Moudrianakis, *Proc. Nat. Acad. Sci., U.S.A.*, 58 (1967) 1261.
35. P. D. Bragg and C. Hou, *FEBS Letters*, 28 (1972) 309.
36. H. Kobayashi and Y. Anraku, *J. Biochem.*, 71 (1972) 387.
37. R. L. Hanson and E. P. Kennedy, *J. of Bact.*, 114 (1973) 772.
38. H. P. Schnebli and A. Abrams, *J. Biol. Chem.*, 245 (1970) 1115.
39. R. Adolfsen, J. A. McLung and E. N. Moudrianakis, *Biochem.* (1974), in Press.
40. E. Munoz, M. J. R. Salton, M. H. Ng and M. T. Schor, *Eur. J. Biochem.*, 7 (1969) 490.
41. A. Hachimori, N. Muramatsu and Y. Nosoh, *Biochim. Biophys. Acta*, 206 (1970) 426.
42. T. E. Andreoli, K. W. Lam and D. R. Sanadi, *J. Biol. Chem.*, 240 (1965) 2644.
43. A. Abrams, *J. Biol. Chem.*, 240 (1965) 3675.
44. P. L. Davies and P. D. Bragg, *Biochim. Biophys. Acta*, 266 (1972) 273.
45. W. A. Catterall, W. A. Coty and P. L. Pedersen, *J. Biol. Chem.*, 248 (1973) 7427.
46. A. E. Senior and J. C. Brooks, *FEBS Letters*, 17 (1971) 327.
47. S. Lien, R. J. Berzborn, E. Racker, *J. Biol. Chem.*, 247 (1972) 3520.
48. R. Adolfsen and E. N. Moudrianakis, *Fed. Proc. Abs.*, 33 (1974) 1330.

49. I. A. Kozlov and H. N. Mikelsaar, *FEBS Letters*, 43 (1974) 212.
50. R. J. Berzborn, *Hoppe-Seyler's Z. Physiol. Chem.*, 353 (1972) 693.
51. H. P. Schnebli, A. E. Vatter and A. Abrams, *J. Biol. Chem.*, 245 (1970) 1122.
52. E. Munoz, J. H. Freer, D. J. Ellar and M. R. J. Salton, *Biochim. Biophys Acta*, 150 (1968) 531.
53. J. C. Brooks and A. E. Senior, *Biochem.*, 11 (1972) 4675.
54. M. F. Sierra and A. Tzagoloff, *Proc. Nat. Acad. Sci., U.S.A.*, 70 (1970) 3155.
55. N. Nelson, H. Nelson and E. Racker, *J. Biol. Chem.*, 247 (1972) 7657.
56. A. Tzagoloff, M. S. Rubin and M. F. Sierra, *Biochim. Biophys. Acta*, 301 (1973) 71.
57. A. Tzagoloff, A. Akai and M. S. Rubin, in: *The Biogenesis of Mitochondria* (A. M. Kroon and C. Saccone, eds); Academic Press, New York (1974) 405.
58. M. Satre and M-B. Jerphanion, *Fed Proc. Abs.*, Budapest (1974).
59. D. H. MacLennan, *J. Biol. Chem.*, 245 (1970) 4508.
60. J. Kyte, *J. Biol. Chem.*, 247 (1972) 7642.
61. S. Lowey and D. Risby, *Nature*, 191 (1971) 81.
62. H. S. Penefsky, *J. Biol. Chem.*, 242 (1967) 5789.
63. A. E. Senior, *Biochem.*, 12 (1973) 3622.
64. S. J. Ferguson, W. J. Lloyd and G. K. Radda, *FEBS Letters*, 38 (1974) 234.
65. D. W. Deters, E. Racker, N. Nelson and H. Nelson, *J. Biol. Chem.*, in Press (1974).
66. P. L. Pedersen, manuscript in preparation (1974).
67. P. D. Bragg, P. L. Davies and C. Hou, *Arch. Biochem. Biophys.*, 159 (1973) 664.
68. N. Nelson, D. W. Deters, H. Nelson and E. Racker, *J. Biol. Chem.*, 248 (1973) 2049.
69. T. L. Chan, J. W. Greenawalt and P. L. Pedersen, *J. Cell Biol.*, 45 (1970) 291.
70. C. Cooper and A. L. Lehninger, *J. Biol. Chem.*, 224 (1957) 547.
71. P. L. Pedersen, H. Levine, III and N. Cintrón, in: *Membrane Proteins in Transport and Phosphorylation* (G. F. Azzone, M. Klingenberg, E. Quagliariello and N. Siliprandi, eds); Elsevier, North-Holland (1974) 43.
72. R. Adolfsen and E. N. Moudrianakis, *Biochem.*, 12 (1973) 2926.
73. W. A. Catterall and P. L. Pedersen, *J. Biol. Chem.*, 247 (1972) 7969.
74. H. S. Penefsky, *J. Biol. Chem.*, 249 (1974) 3579.
75. R. A. Yeates, *Biochim. Biophys. Acta*, 33 (1974) 173.
76. A. Abrams, E. A. Nolan, C. Jensen, J. B. Smith, *Biochem. Biophys. Res. Commun.*, 55 (197) 22.
77. R. J. Van de Stadt, B. L. de Boer and K. Van Dam, *Biochim. Biophys. Acta*, 292 (1973) 338.
78. G. G. Hammes and D. W. Hilborn, *Biochim. Biophys. Acta*, 233 (1971) 580.
79. P. Mitchell and J. Moyle, *Bioenergetics*, 2 (1971) 1.
80. P. L. Pedersen, unpublished observation.
81. R. E. Ebel, *Fed. Proc. Abs.*, 33 (1974) 1399.
82. C. R. Rossi, A. Alexandre, G. Carignani and C. S. Rossi, in: *Membrane Proteins in Transport and Phosphorylation* (G. F. Azzone, M. Klingenberg, E. Quagliariello and N. Siliprandi, eds); Elsevier, North-Holland (1974) 171.
83. F. L. Bygrave and A. L. Lehninger, *Proc. Nat. Acad. Sci.*, U.S.A., 57 (1967) 1409.
84. D. A. Hilborn and G. G. Hammes, *Biochem.*, 12 (1973) 983.
85. K. Asami, K. Juntti and L. Ernster, *Biochim. Biophys. Acta*, 205 (1970) 307.
86. H. A. Lardy, D. Johnson and W. C. McMurray, *Arch. Biochem. Biophys.*, 78 (1958) 587.
87. Y. Kagawa and E. Racker, *J. Biol. Chem.*, 241 (1966) 2461.
88. H. A. Lardy, J. L. Connelly and D. Johnson, *Biochem.*, 3 (1964) 1961.

89. J. L. Connelly and H. A. Lardy, *Biochem.*, 3 (1964) 1969.
90. R. B. Beechey, V. Williams, C. T. Holloway, I. G. Knight, *Biochem.*, 6 (1967) 3867.
91. E. Racker and L. L. Horstman, *J. Biol. Chem.*, 242 (1967) 2547.
92. R. L. Williamson and R. L. Metcalf, *Science*, 158 (1967) 1694.
93. P. Walter, H. A. Lardy and D. Johnson, *J. Biol. Chem.*, 242 (1967) 5014.
94. E. C. Slater, *Biochem. J.*, 59 (1955) 392.
95. J. M. Fessenden and E. Racker, *J. Biol. Chem.*, 241 (1966) 2483.
96. A. M. Robertson, R. B. Beechy, C. T. Holloway and I. G. Knight, *Biochem. J.*, 104 (1967) 54c.
97. G. Schatz, H. F. Penefsky and E. Racker, *J. Biol. Chem.*, 242 (1967) 2552.
98. T.-M. Chang and H. S. Penefsky, *J. Biol. Chem.*, 248 (1973) 2746.
99. R. M. Bertina, P. I. Schrier and E. C. Slater, *Biochim. Biophys. Acta*, 305 (1973) 503.
100. D. R. Lang and E. Racker, *Biochim. Biophys. Acta*, 333 (1974) 180.
101. O. A. Roveri and R. H. Vallejos, *Biochim. Biophys. Acta*, 333 (1974) 187.
102. A. Bruni, in: *Regulation of Metabolic Processes in Mitochondria* (J. M. Tager, S. Papa, E. Quagliariello and E. C. Slater, eds), BBA Library Vol. 7; Elsevier-Amsterdam (1966) 275.
103. D. E. Griffiths, R. L. Houghton and W. E. Lancashire, in: *The Biogenesis of Mitochondria* (A. M. Kroon and C. Saccone, eds); Academic Press, New York (1974) 215.
104. E. Racker, *Fed. Proc. Abs.*, 21 (1962) 1962.
105. W. R. Redwood, *Fed. Proc. Abs.*, 33 (1974) 1253.
106. G. Toson, A. R. Contessa and A. Bruni, *Biochem. Biophys. Commun.*, 48 (1972) 341.
107. C. P. Lee, C. H. Huang and B. I. T. Cierkosz, in: *Membrane Proteins in Transport and Phosphorylation* (G. F. Azzone, M. Klingenberg, E. Quagliariello and N. Siliprandi, eds); Elsevier, North-Holland (1974) 161.
108. P. D. Boyer, in: *Current Topics in Bioenergetics* (D. R. Sanadi, ed.); Academic Press, New York, 2 (1967) 99.
109. P. L. Pedersen and C. A. Schnaitman, *J. Biol. Chem.*, 244 (1969) 5065.
110. P. Mitchell, *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation*, Glynn Res. Ltd., Bodmin, Cornwall, England (1966).
111. Y. Kagawa and E. Racker, *J. Biol. Chem.*, 246 (1971) 5477.
112. G. S. P. Groot, L. Kováč and G. Schatz, *Proc. Nat. Acad. Sci., U.S.A.*, 68 (1971) 308.
113. Y. Hatefi and W. G. Hanstein, *Biochem. Biophys. Res. Commun.*, 61 (1974) 313.
114. D. R. Sanadi, B. P. Sani, R. J. Fisher, O. Li and W. V. Taggart, in: *Energy Transduction in Respiration and Photosynthesis* (E. Quagliariello, S. Papa, C. S. Ross, eds); Adriatic Editrice, Bari, Italy (1971) 89.
115. N. E. Garret and H. S. Penefsky, *Fed. Proc. Abs.*, 33 (1974) 1399.
116. A. Abrams and E. A. Nolan, *Biochem. Biophys. Res. Commun.*, 48 (1972) 982.
117. H. Roy and E. N. Moudrianakis, *Proc. Nat. Acad. Sci., U.S.A.*, 68 (1971) 464.
118. H. Roy and E. N. Moudrianakis, *Proc. Nat. Acad. Sci., U.S.A.*, 68 (1971) 2720.
119. C. T. Gregg, *Biochim. Biophys. Acta*, 74 (1963) 573.
120. H. Löw, I. Vallin and B. Alm, in: *Energy-linked Functions of Mitochondria* (B. Chance, ed.); Academic Press, New York (1963) 5.
121. I. Vallin, in: *Energy Transduction in Respiration and Photosynthesis* (E. Quagliariello, S. Papa, C. S. Rossi, eds); Adriatica Editrice, Bari, Italy (1971) 851.
122. J. B. Warshaw, K. W. Lam, B. Nagy and D. R. Sanadi, *Arch. Biochem. Biophys.*, 123 (1968) 385.

123. D. A. Harris, J. Rosing, R. J. Van de Stadt and E. C. Slater, *Biochim. Biophys. Acta*, 314 (1973) 149.
124. F. Farron and E. Racker, *Biochem.*, 9 (1970) 3829.
125. R. J. Van de Stadt, K. Van Dam and E. C. Slater, *Biochim. Biophys. Acta*, 347 (1974) 224.
126. E. Racker, in: *Membrane Proteins*, Proceedings of a Symposium sponsored by the New York Heart Association, Little, Brown and Company, Boston (1969) 38.
127. A. A. Jasàitis, I. B. Nemeček, I. I. Severina, V. P. Skulachev and S. M. Smirnova, *Biochim. Biophys. Acta*, 275 (1972) 485.
128. E. Racker and W. Stoeckenius, *J. Biol. Chem.*, 249 (1974) 662.
129. R. D. Simoni and M. K. Schallenberger, *Proc. Nat. Acad. Sci., U.S.A.*, 69 (1972) 2663.
130. D. L. Gutnick, B. I. Kanner and P. W. Postma, *Biochim. Biophys. Acta*, 283 (1972) 217.
131. P. D. Bragg and C. Hou, *Biochem. Biophys. Res. Commun.*, 50 (1973) 729.
132. G. Van Thienen and P. W. Postma, *Biochim. Biophys. Acta*, 323 (1973) 429.
133. E. A. Berger, *Proc. Nat. Acad. Sci., U.S.A.*, 70 (1973) 1514.
134. B. P. Rosen, *J. Bacteriol.*, 116 (1973) 1124.
135. B. P. Rosen, *Biochem. Biophys. Res. Commun.*, 53 (1973) 1289.
136. K. Altendorf, F. M. Harold and R. D. Simoni, *J. Biol. Chem.*, 249 (1974) 4587.
137. K. J. Cattell, C. R. Lindop, I. G. Knight and R. B. Beechey, *Biochem J.*, 125 (1971) 169.
138. M. Avron, *Biochim. Biophys. Acta*, 77 (1963) 699.
139. N. Sone, E. Furuya and B. Hagihara, *J. Biochem. (Tokyo)*, 65 (1969) 935.
140. L. C. Cantley, Jr. and G. G. Hammes, *Biochem.*, 12 (1973) 4900.
141. P. D. Boyer, Ninth International Congress of Biochem., Stockholm, Sweden, *Abs.* (1973) 211.
142. P. D. Boyer, R. L. Cross and W. Momsen, *Proc. Nat. Acad. Sci., U.S.A.*, 70 (1973) 2837.
143. R. Mirsky and V. Barlow, *Biochim. Biophys. Acta*, 241 (1971) 835.
144. A. Livne and E. Racker, *J. Biol. Chem.*, 244 (1969) 1332.