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Structural aspects of proton-pumping ATPases

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[Plate 1]

ATP synthase is found in bacteria, chloroplasts and mitochondria. The simplest known example of such an enzyme is that in the eubacterium *Escherichia coli*; it is a membrane-bound assembly of eight different polypeptides assembled with a stoichiometry of $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1 a_1 b_2 c_{10-12}$. The first five of these constitute a globular structure, F_1-ATPase, which is bound to an instrinsic membrane domain, F_0, an assembly of the three remaining subunits. ATP synthases driven by photosynthesis are slightly more complex. In chloroplasts, and probably in photosynthetic bacteria, they have nine subunits, all homologues of the components of the *E. coli* enzyme; the additional subunit is a duplicated and diverged relation of subunit *b*. The mammalian mitochondrial enzyme is more complex. It contains 14 different polypetides, of which 13 have been characterized. Two membrane components, *a* (or ATPase-6) and A6L, are encoded in the mitochondrial genome in overlapping genes and the remaining subunits are nuclear gene products that are translated on cytoplasmic ribosomes and then imported into the organelle. The sequences of the proteins of ATP-synthase have provided information about amino acids that are important for its function. For example, amino acids contributing to nucleotide binding sites have been identified. Also, they provide the basis of models of secondary structure of membrane components that constitute the transmembrane proton channel. An understanding of the coupling of the transmembrane potential gradient for protons, $\Delta\tilde{\mu}_{H^+}$, to ATP synthesis will probably require the determination of the structure of the entire membrane bound complex. Crystals have been obtained of the globular domain, F_1-ATPase. They diffract to a resolution of 3–4 Å† and data collection is in progress. As a preliminary step towards crystallization of the entire complex, we have purified it from bovine mitochondria and reconstituted it into phospholipid vesicles.

1. Introduction

The ATP synthases (also known as proton translocating ATPase, H^+-ATPases, or F_1F_0-ATPases) found in the cytoplasmic membranes of eubacteria, the thylakoid membranes of chloroplasts and the inner membranes of mitochondria have a common function and closely related structures. They catalyse the synthesis of ATP from ADP and phosphate (Pi) by employing the proton electrochemical potential gradient, $\Delta\tilde{\mu}_{H^+}$, across the membrane to drive the reaction forward. Under normal physiological conditions in aerobic bacteria and mitochondria, $\Delta\tilde{\mu}_{H^+}$ is generated by respiration, and in chloroplasts and anaerobically growing photosynthetic bacteria, by photon capture. In anaerobic bacteria, $\Delta\tilde{\mu}_{H^+}$ is maintained by hydrolysis of ATP generated by glycolysis, and in this case the ATP synthase works in reverse (Mitchell 1966; Nicholls 1982). Related ATP synthases are also present in archaebacteria, and the enzymes in *Halobacterium halobium* (Mukohata *et al.* 1986) and *Sulpholobus acidocaldarius*

† 1 Å = 10^{-10} m = 10^{-1} nm.

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(Anemüller *et al.* 1985) may have simpler subunit compositions. The archaebacterial enzymes are more closely related to H^+-ATPases that are found in secretory vesicles (see Denda *et al.* (1988); Bowman *et al.* (1988)); for example, the enzyme in coated vesicles or the *Neurospora* vacuole, which hydrolyses ATP and establishes a proton gradient across the membrane. In this article, we review structural work that we have carried out on ATP synthase. Extensive sequence analyses have been performed on the subunits of the enzyme and on their genes, in eubacteria, chloroplasts and mitochondria. These have demonstrated close relations between the enzymes from these various sources, and have helped to identify regions of the proteins that are important to the function of the enzyme. To extend these observations and to obtain a fuller understanding of the enzyme, a high resolution three-dimensional structure is needed, and we also present our progress towards this goal.

2. Eubacterial ATP synthases

The Escherichia coli *enzyme*

The *E. coli* ATP synthase, the simplest member of the F_1 F_0 family to be defined, is a complex of eight different polypeptides; five of them (α, β, γ, δ and ε) constitute F_1, the extrinsic membrane domain of the enzyme, and the remainder (*a*, *b* and *c*) make up the membrane sector, F_0. Their stoichiometries are $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1 a_1 b_2 c_{10-12}$ (Fillingame 1981). Basic information concerning the structure of the *E. coli* enzyme has been derived by a combination of protein sequence studies of the subunits (Walker *et al.* 1982*a*), and of DNA sequence studies of the corresponding genes (reviewed in Walker *et al.* (1984)). In *E. coli*, the genes are co-transcribed from the *unc* or *atp* operon. The operon contains two sub-clusters of genes: the promoter proximal sub-cluster contains the three genes for F_0 subunits and is followed by five genes for F_1 components (see figure 1). These clusters are preceded in the operon by a ninth gene, *unc*I, that encodes a membrane protein of unknown function (Gay & Walker 1981; Gay 1984).

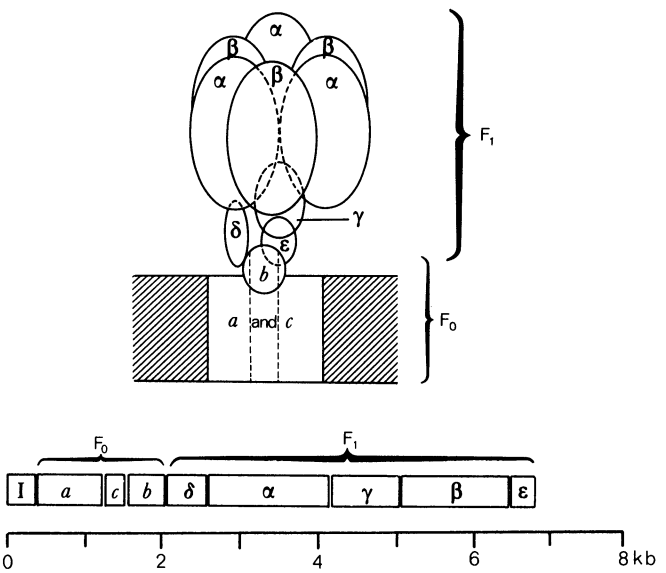

Figure 1. Arrangement of subunits in *E. coli* ATP synthase and of corresponding genes in the *unc* operon.

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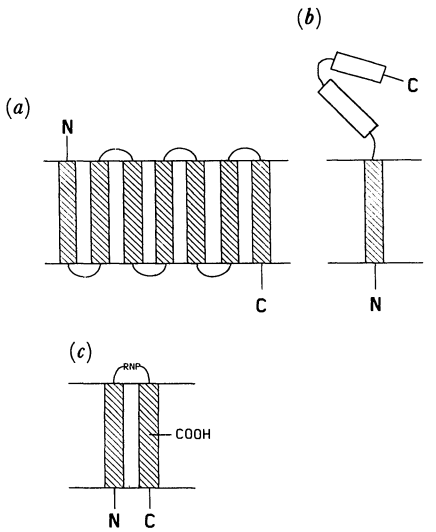

FIGURE 2. Predicted secondary structures of the three membrane components of *E. coli* ATP synthase. The shaded regions represent transmembrane α helices. The second α helical stretch in the *a* subunit is not as hydrophobic as the other six. In the *c* subunit, COOH represents a buried carboxyl moiety, the site of reaction of dicyclohexylcarbodiimide (DCCD).

From the primary structures of the membrane components *a*, *b* and *c*, secondary structure models, shown in figure 2, can be deduced. These models derive from the presence of stretches of about 22–25 hydrophobic amino acids in the sequences that could be folded into membrane spanning α-helices. These spans are detected by calculations of hydrophobicity made, for instance, with the computer program SOAP (Kyte & Doolittle 1982). So, it appears that the *a* subunit contains six or seven transmembrane segments (Walker *et al.* 1984), that the *c* subunit is folded into a hairpin structure made of α-helices linked by a turn segment (Sebald & Hoppe 1981; Walker *et al.* 1984) and that the *b* subunit is anchored in the membrane by a single α-helix near to its N-terminus (Walker *et al.* 1982*b*); the remainder of the *b* protein is proposed to lie outside the lipid bilayer (see figure 2) and is thought to be important in the transmission to the catalytic sites in F_1 of conformational changes brought about by protons. This model for the *b* subunit is supported by experimental evidence (Hoppe & Sebald 1984). All three constituent chains of F_0 appear to be required for a functional proton channel (Hoppe & Sebald 1984; Schneider & Altendorf 1985), although it has been claimed that the *a* subunit can pump protons (Meyenburg *et al.* 1985). The subunit *c* contains an essential carboxyl group, the site of reaction with dicyclohexylcarbodiimide (DCCD), and the proposed model for this protein (figure 2) places this carboxyl group within the lipid bilayer.

The sequences of the F_1 subunits α, β, γ, δ and ε are typical of globular proteins, and those of α and β are homologous (Walker *et al.* 1982*c*), which suggests that they will be folded in a similar way. The β-subunits have been demonstrated to bind adenine nucleotides, and the sequences of α and β both contain a primary sequence motif that has been shown to be associated with purine binding sites in a wide range of proteins (figure 3; Walker *et al.* 1982*c*; Gorbalenya *et al.* 1985; Higgins *et al.* 1986; Doolittle *et al.* 1986). It is clear that the catalytic sites of the enzyme are found in the β (and possibly the α subunits) and in the bovine enzyme chemical labelling studies have identified specific amino acids in the β-chains that are involved

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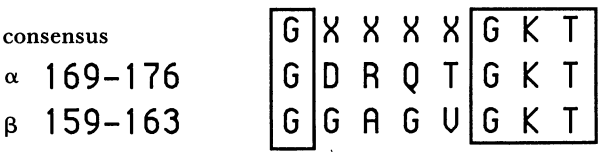

FIGURE 3. The purine nucleotide binding sequence motif in the α- and β-subunits of ATP synthase. The numbering refers to the *E. coli* enzyme, but the residues are completely conserved in all species investigated. The motif has been found in many other nucleotide binding proteins (Walker *et al.* 1982*c*).

TABLE 1. ACTIVE SITE AND NUCLEOTIDE BINDING RESIDUES DETECTED BY CHEMICAL LABELLING STUDIES OF BOVINE F_1-ATPASE

Reagents: Nbf-Cl, 4-chloro-7-nitrobenzofurazan; FSBA, 5′-*p*-fluorosulphonylbenzoyladenosine; FSBI, 5′-*p*-fluorosulphonylbenzoylinosine; DCCD, dicyclohexylcarbodiimide. For references, see Walker *et al.* (1987*c*).

residue	reagent	conservatism
Tyr311	Nbf-Cl[a]	invariant[b]
Lys162		invariant[b]
Lys401		Arg in chloroplasts and some bacteria
Lys301	8-azido-ADP	not conserved
Ile304		invariant
Try311		invariant
Leu342	2-azido-ADP	invariant
Ile344		invariant
Tyr344		invariant
Pro346		invariant
His427	FSBA	not conserved
Tyr368		invariant
Tyr345	FSBI	invariant
Glu199	DCCD[c]	invariant

[a] Under alkaline conditions Nbf migrates from Tyr311 to Lys162 and/or Lys401.
[b] Invariant means completely conserved in all 12 species investigated.
[c] Thermophilic ATPase.

either in the catalytic mechanism or are associated with the substrate binding sites of the enzyme (see table 1).

ATP synthases from photosynthetic bacteria

The genes for subunits of the enzymes have been studied in two members of the Rhodospirillaceae (purple non-sulphur bacteria), *Rhodopseudomonas blastica* and *Rhodospirillum rubrum* and in two unicellular cyanobacteria. In the former group, the five genes for F_1 components form an operon, the order of the genes being the same as in the F_1 cluster of the *unc* operon (figure 4). However, F_0 genes are not associated with them (Tybulewicz *et al.* 1984; Falk *et al.* 1985), and in *R. rubrum* the genes for membrane subunits have been found at a second independent locus. Again they are organized as in the *E. coli* F_0 locus (Falk & Walker 1988), but an important difference is that there are two related but different genes for the *b* subunit arranged in tandem. That implies that, rather than assembling two identical *b* subunits as in the *E. coli* ATPase, the *R. rubrum* enzyme contains one subunit per complex of each of the non-identical homologues *b* and *b*′.

In the unicellular cyanobacteria, *Synechococcus* 6301 and *Synechococcus* 6716, the genes for ATP synthase are grouped at two and three loci, respectively. They code for homologues of all eight

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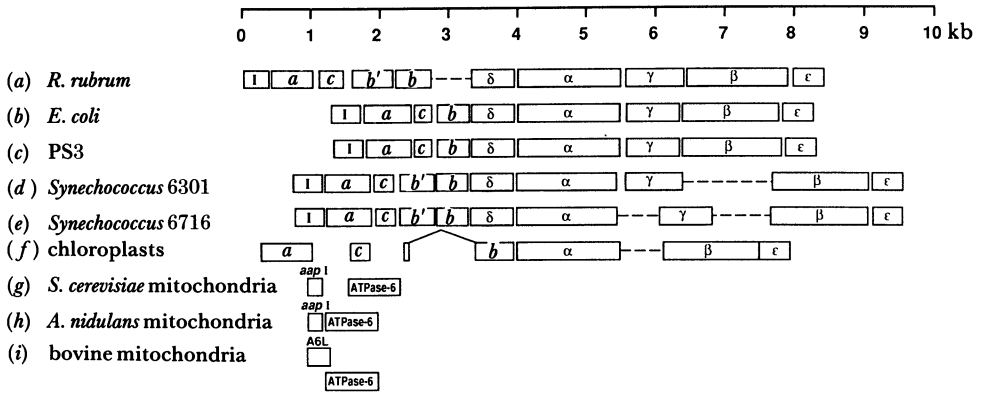

FIGURE 4. Organization of genes for subunits of ATP synthase in mitochondria, chloroplasts and eubacteria. The arrangement of genes for the *a* subunit in mitochondria and chloroplasts. In the operons for ATP synthase subunits in eubacteria, the gene for the *a* subunit is preceded by the *unc*I gene, encoding a 14 kDa protein of unknown function. In mammalian mitochondria, the gene for the homologous *a* subunit (ATPase-6) is preceded by the gene for A6L. A similar arrangement exists in fungal mitochondria with an analogous subunit *aap*I preceding the *a* (ATPase-6) subunit. No analogous gene precedes the *a* subunit in chloroplasts. The data are taken from Grisi *et al.* 1981; Novitski *et al.* 1984; Falk *et al.* 1985; Cozens & Walker 1987; Walker *et al.* 1987*a*, *b*; Falk & Walker 1988; Ohta *et al.* 1988; H. S. van Walraven & J. E. Walker, unpublished work. The scale is kilobases.

of the subunits of the *E. coli* enzyme (figure 4; Cozens & Walker 1987; H. S. van Walraven & J. E. Walker, unpublished work). In both species are found two related genes that encode proteins with similar structures to the *E. coli* F_0 component, subunit *b*, again implying a nine subunit enzyme. The cyanobacterial homologues *b* and *b′* differ from *E. coli b* in so far as the N-terminal hydrophobic domains encoded in the genes are considerably longer. This is reminiscent of the equivalent subunit in chloroplast ATP synthase, subunit I, where it has also been shown to have an extra N-terminal hydrophobic extension relative to the *E. coli b* subunit (Bird *et al.* 1985). Sequence analysis of the gene product isolated from the chloroplast enzyme complex has demonstrated that the extra extension is removed in a post-translational processing event, although the functional significance of this finding is unknown (Bird *et al.* 1985). However, it suggests that the cyanobacterial homologues will be similarly processed. One possibility is that the extra domain serves to direct the pre-protein to the thylakoid membrane in the chloroplast or to the specialized photosynthetic membrane of the cyanobacterium, although it should be emphasized that this process is not thought to involve transfer of the complete protein across a membrane.

At present, these proposed features of the *R. rubrum* and cyanobacterial enzymes lack protein chemical support, as no ATP synthase has been characterized fully from these sources, although the enzyme from the thermophile, *Synechococcus* 6716, has been purified and appears to contain nine subunits as predicted (H. S. van Walraven, R. Lutter and J. E. Walker, unpublished results).

The DNA sequences of the genes for ATP synthase in both *R. rubrum* and the cyanobacteria reveal another interesting feature: in all three cases the subunit *a* gene is preceded by a homologue of *E. coli unc* I and so there is considerable evolutionary pressure to conserve this gene, although its function is obscure.

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Table 2. Equivalent subunits in ATP synthases

Subunits on the same horizontal line are believed to be equivalent in bacteria (exemplified by *E. coli*), in bovine mitochondria and in chloroplasts. A dash indicates absence of a subunit from the complex. For references, see Walker *et al.* 1987*c*).

E. coli	chloroplasts	bovine mitochondria
α	α	α
β	β	β
γ	γ	γ
δ	δ	OSCP
ε	ε	δ
—	—	ε
a	X	*a* (or ATPase-6)
b	I and II	*b*
c	*c*	*c*
—	—	*d*
—	—	inhibitor
—	—	F_6
—	—	A6L
—	—	*e*

Table 3. Comparison matrices for α, β and *c* subunits of ATP synthases

(i) α-subunits	(1) wheat chloroplast	100	—	—	—	—	—	—		
	(2) *Synechococcus* 6301	71	100	—	—	—	—	—		
	(3) *E. coli*	52	53	100	—	—	—	—		
	(4) *R. rubrum*	59	61	57	100	—	—	—		
	(5) *R. blastica*	57	59	54	75	100	—	—		
	(6) bovine mitochondrion	57	59	57	71	69	100	—		
	(7) maize mitochondrion	57	58	53	70	68	71	100		
		(1)	(2)	(3)	(4)	(5)	(6)	(7)		
(ii) β-subunits	(1) tobacco chloroplast	100	—	—	—	—	—	—		
	(2) *Synechococcus* 6301	79	100	—	—	—	—	—		
	(3) *E. coli*	62	63	100	—	—	—	—		
	(4) *R. rubrum*	64	65	66	100	—	—	—		
	(5) *R. blastica*	64	65	65	72	100	—	—		
	(6) bovine mitochondrion	66	63	66	72	72	100	—		
	(7) tobacco mitochondrion	67	64	62	71	71	74	100		
		(1)	(2)	(3)	(4)	(5)	(6)	(7)		
(iii) *c*-subunits	(1) wheat chloroplast	100	—	—	—	—	—	—	—	—
	(2) *Synechococcus* 6301	88	100	—	—	—	—	—	—	—
	(3) *Bacillus acidocaldarius*	40	40	100	—	—	—	—	—	—
	(4) PS3	36	41	38	100	—	—	—	—	—
	(5) *E. coli*	29	33	30	40	100	—	—	—	—
	(6) *R. rubrum*	34	29	26	25	20	100	—	—	—
	(7) yeast mitochondrion	28	23	25	25	18	44	100	—	—
	(8) bovine mitochondrion	33	29	31	28	21	44	56	100	—
	(9) tobacco mitochondrion	20	20	30	23	21	39	44	52	100
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)

3. Chloroplast ATP synthase

Gel electrophoresis, in the presence of urea, of ATP synthase isolated from spinach and bean chloroplasts indicates that the chloroplast enzyme is similar to those in photosynthetic bacteria in so far as it is a complex of nine different polypeptides (Pick & Racker, 1979; Westhoff *et al.* 1985; Süss 1986). Five of them have been identified as the α, β, γ, δ and ε subunits of coupling factor, CF_1 (chloroplast F_1-ATPase), and their sequences show that they are equivalent to their bacterial homonyms (see table 2). Two further components are the homologues of bacterial subunits *b* (known as subunit I in chloroplasts (Bird *et al.* 1985)) and *c* (chloroplast subunit

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III), and a third component with molecular mass of 20 kDa, known as subunit X, has been shown to be equivalent to bacterial subunit *a* (Cozens *et al.* 1986). The ninth component, subunit II, has a molecular mass of 15 kDa and is related to the photosynthetic bacterial subunits *b'*.

So the body of available evidence suggests a rather close similarity between chloroplast and cyanobacterial ATP synthases and this is further illustrated, for example, by binary comparisons of sequences of α, β and *c* subunits from eubacteria, mitochondria and chloroplasts (see table 3). They clearly demonstrate that the cyanobacterial and chloroplast subunits are more closely related to each other than to homologues from other sources. Also, their close evolutionary relation is emphasized by the similarity between the arrangement of the six genes for ATP synthase subunits that are found in chloroplast DNA and the two clusters in the genome of *Synechococcus* 6301 (figure 4). The three remaining subunits γ, δ and II, of the chloroplast enzyme are encoded in nuclear genes.

4. Mitochondrial ATP synthase

Subunit composition

The bovine mitochondrial ATP synthase is more complex than either bacterial or chloroplast enzymes and contains 14 different subunits (figure 5). Five of them, α, β, γ, δ and ε, make up the F_1-ATPase complex. Sequence analysis of these polypeptides has shown that the α, β and γ subunits are homologous to bacterial homonyms, and that (confusingly) the bovine δ and ε subunits are not (see table 2). Rather, bovine δ is equivalent to bacterial ε, bovine ε has no bacterial (or chloroplast) counterpart (Walker *et al.* 1982 *d*). The equivalent subunit of bacterial δ is another subunit of bovine ATP-synthase, the OSCP (oligomycin sensitivity conferring protein), which is not released from the membrane complex as a component of the

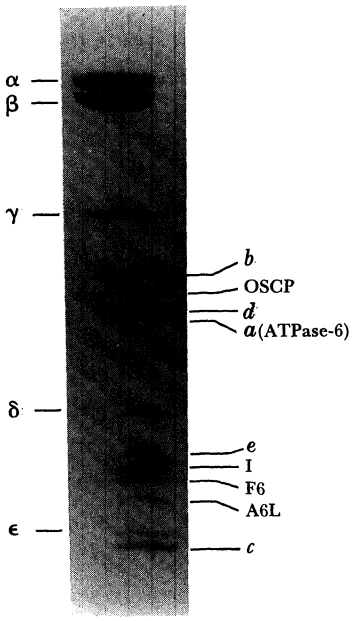

Figure 5. Gel electrophoresis of the bovine mitochondrial ATP synthase complex. The positions of subunits are indicated.

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F_1-ATPase. It is required with a second protein, F_6, for correct binding of F_1 to the membrane sector (MacLennan & Tzagoloff 1986; Knowles *et al.* 1971). Thus OSCP appears to play a functionally similar role to bacterial δ (Hundal *et al.* 1983), as the homology implies (Walker *et al.* 1985).

Homologues of bacterial F_0 subunits *a*, *b* and *c* have been characterized also in the bovine mitochondrial enzyme. Two of them, ATPase-6 (equivalent to bacterial *a*) and bovine *c* (the DCCD-reactive proteolipid; Sebald & Hoppe 1981), are soluble in mixtures of chloroform and methanol and can be extracted with the organic solvent from both mitochondria and the purified ATP synthase complex (Fearnley & Walker 1986). A third component of F_0 with relative molecular mass (M_r) 24570 has been sequenced. It has a hydrophobic profile similar to the *b* subunits in other species, although its amino acid sequence is not evidently homologous to them. The protein is a membrane component of the enzyme and it seems very likely that this is the mitochondrial *b* subunit (Walker *et al.* 1987*a*). This proposed relation is strengthened by the sequence of a protein isolated from yeast ATP synthase, which is related to both bacterial and bovine *b* subunits (Velours *et al.* 1988).

Two additional components also have been isolated from the membrane sector of bovine mitochondrial ATP synthase and their amino acid sequences have been determined. The sequence of the larger, M_r 19000, called subunit *d* (see figure 2), is not particularly hydrophobic, but the protein remains with the membrane sector when F_1 is released (Walker *et al.* 1987*a*). This sequence is not related to any other ATP synthase subunit, and it probably has no counterpart in either bacteria or chloroplasts. The smaller, known as A6L, is the third component of the enzyme complex to have been isolated from the chloroform: methanol extract of mitochondria (Fearnley & Walker 1986). Its function is unknown, but it may be analogous (although it is not significantly homologous) to the *aap*I protein, which is found in yeast mitochondrial ATP synthase (Velours *et al.* 1984), where it is required for assembly of the enzyme complex (Macreadie *et al.* 1983).

The final component to have been characterized from the bovine ATP synthase is the inhibitor protein, a small basic protein that binds to the F_1 sector. It may be important in the physiological regulation of the enzyme (Ernster *et al.* 1979; Pedersen *et al.* 1981). This leaves an unidentified component (M_r 14000) for future investigation. It is referred to as subunit *e*.

Mitochondrial and nuclear genes

As in the case of the chloroplast ATPase, genes for subunits of mitochondrial ATPase are found both in the nucleus and in organellar DNA. In mammals and invertebrates two subunits, A6L and ATPase-6, are encoded by overlapping genes in mt-DNA (figure 4; Fearnley & Walker 1986), and the remainder are nuclear gene products. Both ATPase-6 and *aap*I are also mitochondrial gene products in fungi. In *Saccharomyces cerevisiae* subunit *c* (the DCCD-reactive proteolipid) is a mitochondrial gene product (Macino & Tzagoloff 1979) in addition to ATPase-6 and *aap*I, whereas in all other species studied it is encoded in the nucleus.

cDNAs corresponding to 10 of the 12 nuclear encoded subunits of the bovine ATPase have been characterized (the exceptions are the δ-subunit and subunit *e*). They have been isolated from a bovine cDNA library by using mixed synthetic oligonucleotide probes, 17 or 18 bases in length, designed on the basis of known protein sequences in the subunits. These experiments have confirmed the sequences of the subunits of the enzyme from bovine mitochondria (Gay & Walker 1985; Walker *et al.* 1987; Walker *et al.* 1987*a*, *b*; Walker *et al.* 1989; Dyer *et al.* 1989).

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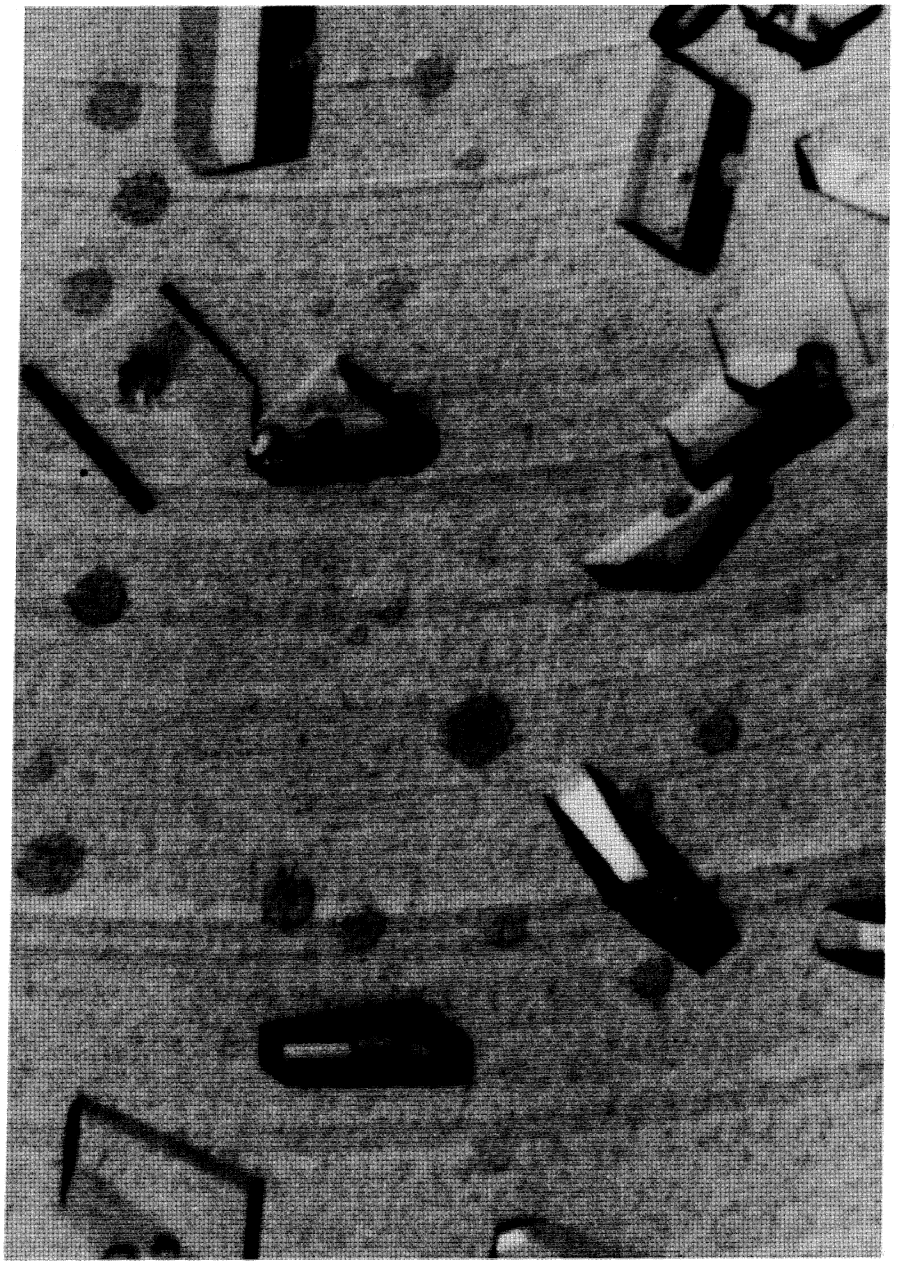

FIGURE 6. Crystals of F_1-ATPase from bovine heart mitochondria. They belong to the orthorhombic spacegroup. They were grown by microdialysis and reached their full size (250–300 × 250–300 × 50–100 μm) within 3–4 weeks.

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5. Towards understanding the molecular mechanism of the enzyme

From the foregoing description, it is evident that the H^+-ATPases have been well studied at a primary structural level. Conserved features of their sequence have been delineated (see Cozens & Walker (1987); Falk & Walker (1988)) and amino acids that are involved in catalysis have been identified. None the less, our knowledge of the structure of the enzyme is still rather superficial, and important basic facts have not been established. For example, the stoichiometries of subunits in the membrane sector of the mitochondrial and chloroplast enzymes have not been established with any certainty, and such estimates as have been made are at variance with those made on homologues in the *E. coli* enzyme. This deficiency needs to be corrected. Progress has been made by electron microscopy in defining the gross arrangement of subunits within the complex (Gogol *et al* 1987; Boekma *et al.* 1988). This shows that α and β subunits alternate in a hexagonal arrangement, with the minor subunits occupying the central region of this assembly; the bulk of the F_1 domain is distanced from the membrane by a stalk. However, it is unlikely that a fuller understanding will come about until a high resolution structure of the enzyme has been determined.

In an attempt to approach this goal, we have been studying the crystallization of F_1-ATPase from bovine heart mitochondria, and crystals have been obtained under a wide range of conditions. Routinely, an orthorhombic and a monoclinic crystal form can be grown by precipitation with polyethylene glycol (PEG 6000). Crystals belonging to the orthorhombic space group grow more reproducibly and overall have better crystallographic properties (see table 4 and figure 6, plate 1). Data collection and the search for suitable heavy atom derivatives are currently in progress. The solution of this structure would provide important information, but would be no more than a first step towards understanding the enzyme. However, it would provide an important framework for mutagenesis experiments on subunits of F_1-ATPase that present attempts lack. Indeed, the lack of a high-resolution structure seriously undermines current attempts to probe the function of the enzyme by mutagenesis (see, for example, Parsonage *et al.* (1988)) as the observed effects of mutations on function cannot be interpreted unambiguously. The main task, however, is to reach a molecular mechanism of the coupling of $\Delta\tilde{\mu}_{H^+}$ to ATP synthesis. Current models propose a binding change mechanism for ATP synthesis in which energy is required to release newly synthesized ATP from the enzyme (Cross 1981). This mechanism proposes that the F_1 domain contains three catalytic sites (called tight, open and loose) that are non-equivalent at any time. Catalysis requires interconversion of these sites and implies a rotation of a particular type of site around the F_1-head. This, it is proposed, is brought about by conformational changes transmitted from the membrane domain via the

Table 4. Properties of crystals of F_1-ATPase from bovine heart mitochondria

property	crystal type: monoclinic	orthorhombic
space group	$P2_1$	$P2_12_12_1$
Unit cell dimensions/Å		
a	141.0	280.3
b	136.8	110.2
c	109.9	138.4
β	101.10°	90°
solvent content (%)	56.0	57.0
maximum diffraction/Å	6.0	3.0

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stalk to the head (F_1). The structure of the proton channel and the stalk region are crucial to understanding this process, and here also a high-resolution structure of the complete complex should help. This is a much more difficult task than structural analysis of F_1, requiring, as it does, the crystallization and structural analysis of a membrane bound complex. Recent successes with analysis of photosynthetic proteins (Deisenhofer *et al.* 1985) provide encouragement that this is an attainable prospect, and as a first step we have purified to homogeneity the bovine mitochondrial complex. The purified enzyme is sensitive to the action of oligomycin and, after reconstitution into phospholipid vesicles, is capable of ATP synthesis. This indicates that the purified protein complex is correctly arranged in the membrane. The critical step is now to crystallize either this complex or an ATPase from another source.

References

Anemüller, S., Lübben, M. & Schäfer, G. 1985 The respiratory system of *Sulfolobus acidocaldarius*, a thermophilic archaebacterium. *FEBS Lett.* **193**, 83–87.

Bird, C. R., Koller, B., Auffret, A. D., Huttly, A. K., Howe, C. J., Dyer, T. A. & Gray, J. C. 1985 The wheat chloroplast gene for CF_0 subunit I of ATP synthase containing a large intron. *EMBO J.* **4**, 1381–1388.

Boekema, E. J., van Heel, M. & Gräber, P. 1988 Structure of the ATP synthase from chloroplasts: studies by electron microscopy. *Biochim. biophys. Acta* **933**, 365–371.

Bowman, E. J., Tenney, K. & Bowman, B. J. 1988 Isolation of genes encoding the *Neurospora* vacuolar ATPase: analysis of *vma*-1 encoding the 67 kDa subunit reveals homology to other ATPases. *J. biol. Chem.* **263**, 13994–14001.

Cozens, A. L., Walker, J. E., Philips, A. L., Huttly, A. K. & Gray, J. C. 1986 A sixth subunit of ATP synthase, an F_0 component, is encoded in the pea chloroplast genome. *EMBO J.* **5**, 217–222.

Cozens, A. L. & Walker, J. E. 1987 The organization and sequence of the genes for ATP synthase subunits in the cyanobacterium *Synechococcus* 6301: support for an endosymbiotic origin of chloroplasts, *J. molec. Biol.* **194**, 359–383.

Cross, R. L. 1981 The mechanism and regulation of ATP synthesis by F_1ATPases. *A. Rev. Biochem.* **50**, 681–714.

Deisenhofer, J., Epp, O., Miki, R., Huber, R. & Michel, H. 1985 Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3 Å resolution. *Nature, Lond.* **318**, 618–624.

Denda, K., Konishi, J., Oshima, T., Date, T. & Yoshida, M. 1988 The membrane associated ATPase from *Sulfolobus acidocaldarius* is distantly related to F_1-ATPase as assessed from the primary structure of its α subunit. *J. biol. Chem.*, **263**, 6012–6015.

Doolittle, R. F., Johnson, M. S., Hussain, I., van Houten, B., Thomas, D. C. & Sancar, A. 1986 Domainal evolution of a prokaryotic DNA repair protein & its relationship to active-transport proteins. *Nature, Lond.* **323**, 451–453.

Dyer, M. R., Gay, N. J., Powell, S. J. & Walker, J. E. 1989 ATP synthase from bovine mitochondria: complementary DNA sequence of the mitochondrial import precursor of the γ subunit and the genomic sequence of the mature protein. *Biochemistry* **28**, 3670–3680.

Ernster,. L., Carlsson, C., Hundal, T. & Nordenbrand, K. 1979 Mitochondrial ATPase inhibitor: properties and applications. *Meth. Enzymol.* **55**, 399–407.

Falk, G., Hampe, A. & Walker, J. E. 1985 Nucleotide sequence of the *Rhodospirillum rubrum atp* operon. *Biochem. J.* **228**, 391–407.

Falk, G. & Walker, J. E. 1988 DNA sequence of a gene cluster coding for subunits of the F_0 membrane sector of ATP synthase in *Rhodospirillum rubrum*. *Biochem. J.* **254**, 109–122.

Fearnley, I. M. & Walker, J. E. 1986 Two overlapping genes in bovine mitochondrial DNA encode membrane components of ATP synthase. *EMBO J.* **5**, 2003–2008.

Fillingame, R. H. 1981 Biochemistry and genetics of bacterial H^+-translocating ATPases. *Curr. Top. Bioenerget.* **11**, 35–106.

Gay, N. J. 1984 Construction and characterization of an *Escherichia coli* strain with an *unc*I mutation, *J. Bact.* **158**, 820–825.

Gay, N. J. & Walker, J. E. 1981 The *atp* operon: nucleotide sequence of the promoter and the genes for the membrane proteins and the δ subunit of *Escherichia coli* ATP synthase. *Nuc. Acids Res.* **9**, 3919–3926.

Gay, N. J. & Walker, J. E. 1985 Two genes encoding the bovine mitochondrial ATP synthase proteolipid specify precursors with different import sequences and are expressed in a tissue-specific manner. *EMBO J.* **4**, 3519–3524.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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Gogol, E. P., Lücken, U. & Capaldi, R. A. 1987 The stalk connecting the F_1 and F_0 domains of ATP synthase visualized by electron microscopy of unstained specimens. *FEBS Lett.* **219**, 274–278.

Gorbalenya, A. E., Blinov, V. M. & Koonin, E. V. 1985 Prediction of nucleotide binding properties of virus specific proteins from their primary structure. *Molek. Genetika* **11**, 30–36.

Grisi, E., Brown, T. A., Waring, R. B., Scazzocchio, C. & Davies, R. W. 1982 Nucleotide sequence of a region of the mitochondrial region of *Aspergillus nidulans* including the gene for ATPase subunit 6. *Nucl. Acids Res.* **10**, 3531–3539.

Higgins, C. F., Hiles, I. D., Salmond, G. P. C., Gill, D. R., Downie, J. A., Evans, I. J., Holland, I. B., Gray, L., Buckel, S. D., Bell, A. W. & Hermodson, M. A. 1986 A family of related ATP-binding subunits coupled to many distinct biological processes in bacteria. *Nature, Lond.* **323**, 448–450.

Hoppe, J. & Sebald, W. 1984 The proton conducting F_0 portion of bacterial ATP synthases. *Biochim. biophys. Acta* **768**, 1–27.

Hundal, T., Norling, B. & Ernster, L. 1983 Lack of ability of trypsin-treated mitochondrial F_1-ATPase to bind to the oligomycin sensitivity conferring protein (OSCP). *FEBS Lett.* **162**, 5–10.

Knowles, A. F., Guillory, R. J. & Racker, E. 1971 Partial resolution of the enzymes catalysing oxidative phosphorylation XXIV. A factor required for the binding of mitochondrial adenosine triphosphatase to the inner mitochondrial membrane. *J. biol. Chem.* **246**, 2672–2679.

Kyte, J. & Doolittle, R. F. 1982 A simple method for displaying the hydropathic character of a protein. *J. molec. Biol.* **157**, 105–132.

Macino, G. & Tzagoloff, A. 1979 Assembly of the mitochondrial membrane system: Partial sequence of a mitochondrial ATPase gene in *Saccharomyces cerevisiae*. *Proc. natn. Acad. Sci. U.S.A.* **76**, 131–135.

MacLennan, D. H. & Tzagoloff, A. 1968 Studies of the mitochondrial adenosine triphosphatase IV: purification and characterization of the oligomycin sensitivity conferral protein. *Biochemistry* **7**, 1603–1610.

Macreadie, I. G., Novitski, C. E., Maxwell, R. J., John, U., Ooi, B. G., McMullen, G. L., Lukins, H. B., Linnane, A. W. & Nagley, P. 1983 Biogenesis of mitochondria: the mitochondrial gene (*aap* 1) coding for the mitochondrial ATPase subunit 8 in *Saccharomyces cerevisiae*. *Nucl. Acids Res.* **11**, 4435–4451.

Meyenburg, K. v., Jørgenson, B. B., Michelson, O., Sørensen, L. & McCarthy, J. E. G. 1985 Proton conduction by subunit *a* of the membrane-bound ATP synthase of *Escherichia coli* revealed after induced overproduction. *EMBO J* **4**, 2357–2363.

Mitchell, P. 1966 Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol. Rev.* **41**, 455–502.

Mukohata, Y., Isoyama, M. & Fuke, A. 1986 ATP synthesis in cell envelope vesicles of *Halobacterium halobium* driven by membrane potential and/or base-acid transition. *J. Biochem.* **99**, 1–8.

Nicholls, D. G. 1982 Bioenergetics. An introduction to the chemiosmotic theory. New York & London: Academic Press.

Novitski, C. E., Macreadie, I. G., Maxwell, R. J., Lukins, H. B., Linnane, A. W. & Nagley, P. 1984 Biogenesis of mitochondria: genetic and molecular analysis of the *oli*2 region of mitochondrial DNA in *Saccharomyces cerevisiae*. *Curr. Top. Genet.* **8**, 135–146.

Ohta, S., Yohda, M., Ishizuka, M., Hirata, H., Hamamoto, T., Otawara-Hamamoto, Y., Matsuda, K. & Kagawa, Y. 1988 Sequence and overexpression of subunits of adenosine triphosphate synthase in thermophilic bacteria, *Biochim. biophys. Acta*, **933**, 141–155.

Parsonage, D., Wilke-Mounts, S. & Senior, A. E. 1988 *E. coli* ATPase: site directed mutagenesis of the β subunit. *FEBS Lett.* **232**, 111–114.

Pedersen, P. L., Schwerzmann, K. & Cintron, N. 1981 Regulation of the synthesis and hydrolysis of ATP in biological systems: role of peptide inhibitors of H^+-ATPases. *Curr. Top. Bioenerget.* **11**, 149–199.

Pick, U. & Racker, E. 1979 Purification and reconstitution of the *N,N'*-dicyclohexylcarbodiimide-sensitive ATPase complex from spinach chloroplasts. *J. biol. Chem.* **254**, 2793–2799.

Schneider, E. & Altendorf, K. 1985 All three subunits are required for the reconstitution of an active proton channel (F_0) of *Escherichia coli* ATP synthase (F_1F_0). *EMBO J.* **4**, 515–518.

Sebald, W. & Hoppe, J. 1981 On the structure and genetics of the proteolipid subunit of the ATP synthase complex. *Curr. Top. Bioenerget.* **12**, 2–64.

Süss, K. H. 1986 Neighbouring subunits of CF_0 and between CF_1 and CF_0 of the soluble chloroplast ATP synthase (CF_1–F_0) as revealed by chemical protein cross-linking. *FEBS Lett.* **201** 63–68.

Tybulewicz, V. L. J., Falk, G. & Walker, J. E. 1984 *Rhodopseudomonas blastica atp* operon: nucleotide sequence and transcription, *J. molec. Biol.* **179**, 185–214.

Velours, J., Esparza, M., Hoppe, J., Sebald, W. & Guérin, B. 1984 Amino acid sequence of a new mitochondrially synthesized proteolipid of the ATP synthase of *Saccharomyces cerevisiae*. *EMBO J.* **3**, 207–212.

Velours, J., Durrens, P., Aigle, M. & Guérin, B. 1988 ATP4, the structural gene for yeast F_0F_1 ATPase subunit 4. *Eur. J. Biochem.* **170**, 637–642.

Walker, J. E., Auffret, A. D., Carne, A., Gurnett, A., Hanisch, P., Hill, D. & Saraste, M. 1982*a* Solid phase sequence analysis of polypeptides eluted from polyacrylamide gels: an aid to interpretation of DNA sequences exemplified by the *Escherichia coli unc* operon and bacteriophage λ. *Eur. J. Biochem.* **123**, 253–260.

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Walker, J. E., Saraste, M. & Gay, N. J. 1982*b* *E coli* F_1-ATPase interacts with a membrane protein component of a proton channel. *Nature, Lond.* **298**, 867–869.

Walker, J. E., Saraste, M., Runswick, M. J. & Gay, N. J. 1982*c* Distantly related sequences in the α and β subunits of ATP synthase, myosin, kinases and other ATP requiring enzymes and a common nucleotide binding fold. *EMBO J.* **1**, 945–951.

Walker, J. E., Runswick, M. J. & Saraste, M. 1982*d* Subunit equivalence in *Escherichia coli* and bovine heart mitochondrial F_1F_0 ATPases. *FEBS Lett.* **146**, 393–396.

Walker, J. E., Saraste, M. & Gay, N. J. 1984 The *unc* operon: nucleotide sequence, regulation and structure of ATP synthase. *Biochim. biophys. Acta* **768**, 164–200.

Walker, J. E., Fearnley, I. M., Gay, N. J., Gibson, B. W., Northrop, F. D., Powell, S. J., Runswick, M. J., Saraste, M. & Tybulewicz, V. L. J. 1985 Primary structure and subunit stoichiometry of F_1-ATPase from bovine mitochondria. *J. molec. Biol.* **184** 677–701.

Walker, J. E., Runswick, M. J. & Poulter, L. 1987*a* ATP synthase from bovine mitochondria: characterization and sequence analysis of two membrane associated subunits and of their corresponding c-DNAs. *J. molec. Biol.* **197**, 89–100.

Walker, J. E., Gay, N. J., Powell, S. J., Kostina, M. & Dyer, M. R. 1987*b* ATP synthase from bovine mitochondria: sequences of imported precursors of oligomycin sensitivity conferral protein, factor 6, and adenosine triphosphatase inhibitor protein. *Biochemistry* **26**, 8613–8619.

Walker, J. E., Cozens, A. L., Dyer, M. R., Fearnley, I. M., Powell, S. J. & Runswick, M. J. 1987*c* Studies of the genes for ATP synthases in eubacteria, chloroplasts and mitochondria: implications for structure and function of the enzyme. *Chem. Script.* **27B**, 97–105.

Walker, J. E., Powell, S. J., Viñas, O. & Runswick, M. J. 1989 ATP synthase from bovine mitochondria: complementary DNA sequences of the import precursor of a heart isoform of the α subunit. *Biochemistry* **28**, 4702–4708.

Westhoff, P., Alt, J., Nelson, N. & Herrmann, R. G. 1985 Genes and transcripts for the ATP synthase CF_0 subunits I and II from spinach thylakoid membranes. *Molec. gen. Genet.* **199**, 290–299.

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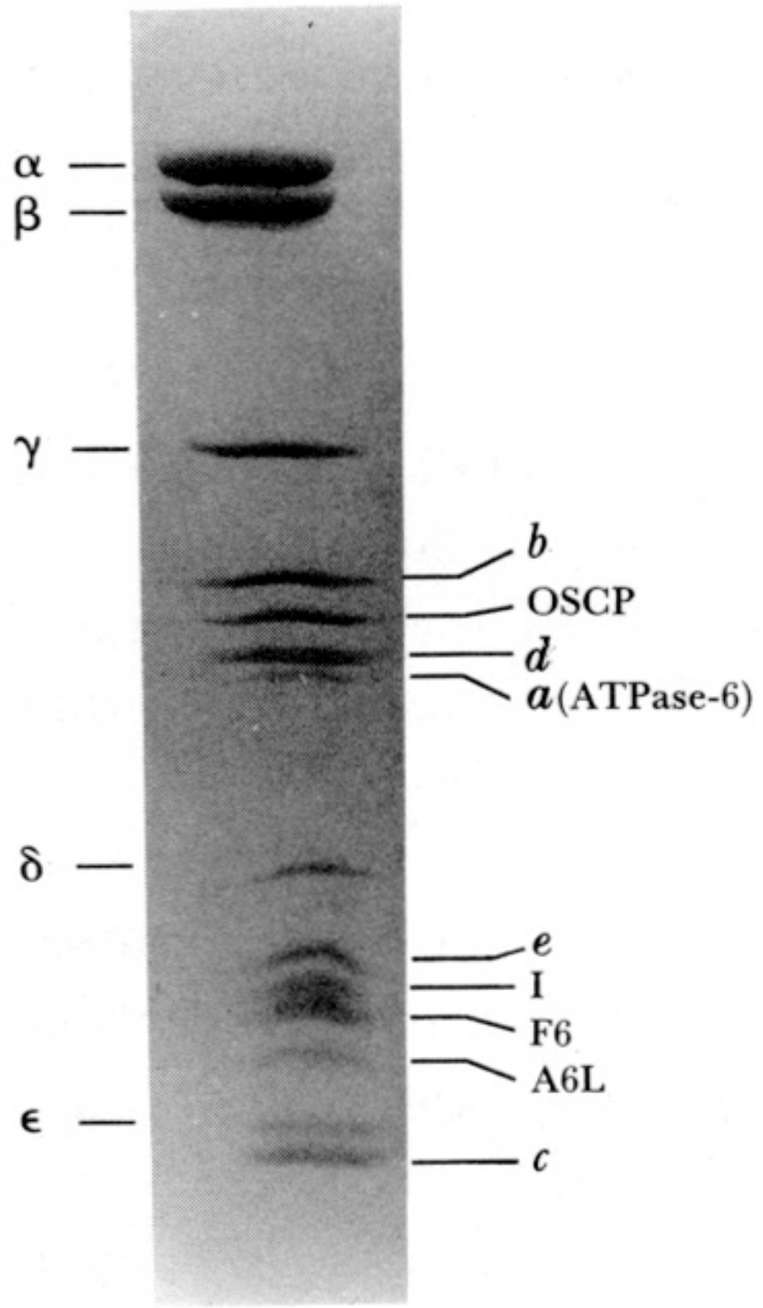

FIGURE 5. Gel electrophoresis of the bovine mitochondrial ATP synthase complex. The positions of subunits are indicated.

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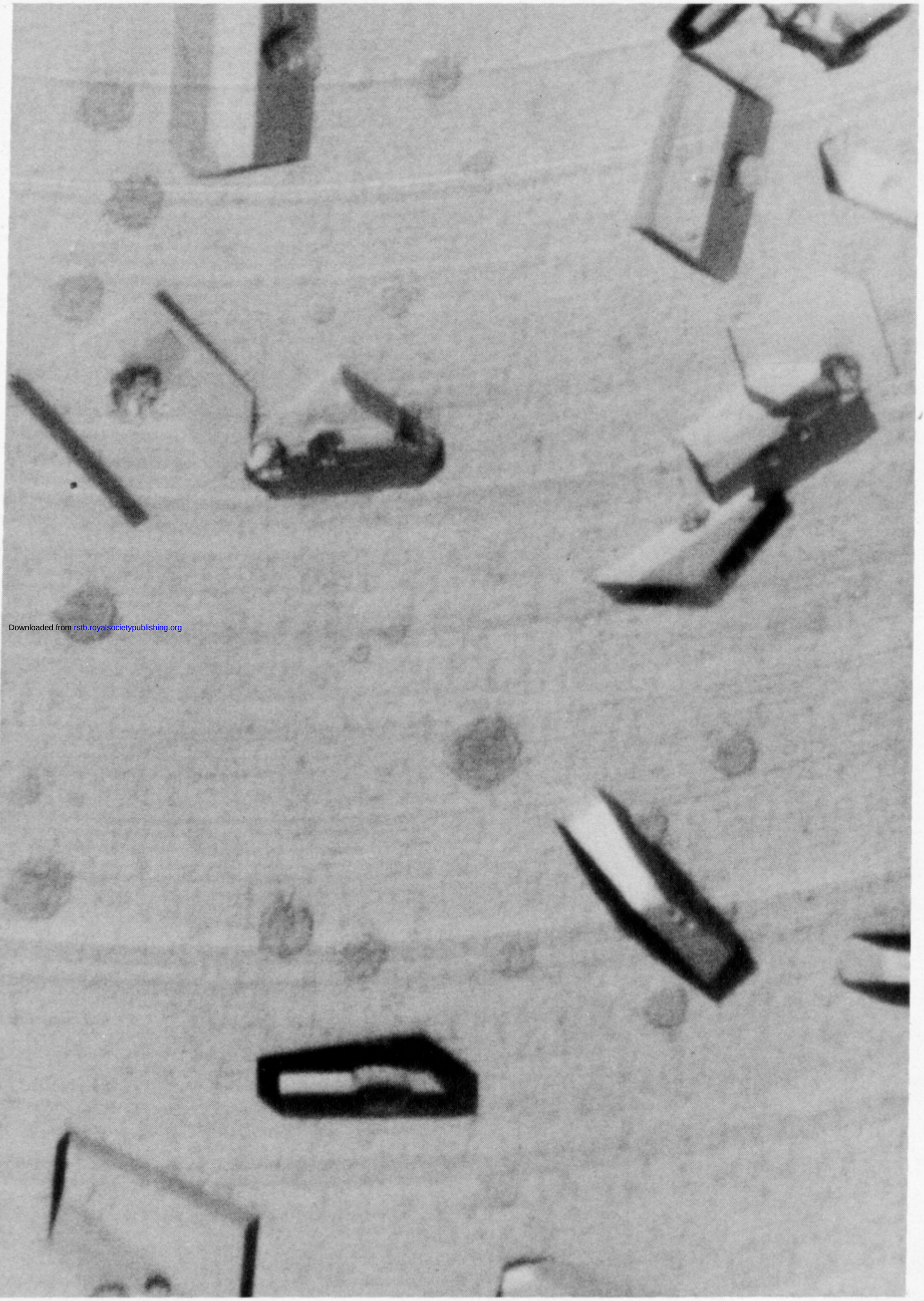

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FIGURE 6. Crystals of F_1-ATPase from bovine heart mitochondria. They belong to the orthorhombic spacegroup. They were grown by microdialysis and reached their full size (250–300 × 250–300 × 50–100 μm) within 3–4 weeks.