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PURIFICATION AND PROPERTIES OF THE LATENT F_1-ATPase OF *MICROCOCCUS LYSODEIKTICUS*

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Summary

The latent coupling factor (F_1)-ATPase of *Micrococcus lysodeikticus* has been purified to homogeneity as determined by a number of criteria including, non-denaturing polyacrylamide gel electrophoresis, crossed immunoelectrophoresis and analytical ultracentrifugation. By inclusion of 1 mM phenylmethyl sulfonyl fluoride, a serine protease inhibitor, in the shock-wash step of release of F_1 from the membranes, the spontaneous activation of both crude and purified ATPase by endogenous membrane protease(s) can be prevented, thereby yielding a highly latent ATPase preparation. Equilibrium ultracentrifugation of the latent ATPase gave a molecular weight of 400 000. The ATPase contained five different subunits α, β, γ, δ, and ϵ and their molecular weights determined by SDS-polyacrylamide gel electrophoresis were 60 000, 54 000, 37 000, 27 000 and 9000, respectively. The subunit composition was determined with ^{14}C-labelled, F_1-ATPase prepared from cells grown on medium containing [U-^{14}C]-labelled algal protein hydrolysate. Within the limitations of this method the results tentatively suggest a subunit composition of 3 : 3 : 1 : 1 : 3.

Introduction

The coupling factor F_1-ATPases of mitochondria, chloroplasts and bacteria possess a high degree of structural and functional similarity [1]. Two major subunits in the 50 000—60 000 molecular weight range are present and they usually possess three additional subunits ranging from about 10 000 to 40 000

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daltons. The stoichiometry of the individual subunits remains, however, a controversial matter [1—3].

The ATPase of the Gram-positive, strictly aerobic bacterium *Micrococcus lysodeikticus* has been of particular interest in that its latency closely resembles that of the chloroplast F_1 [4,5], and it has provided us with a valuable biochemical marker in elucidating the molecular architecture of a bacterial membrane system [6]. In common with other F_1-ATPases, the *M. lysodeikticus* enzyme also possesses two major subunits [7,8] but the number and molecular weights of the 'minor' subunits has remained uncertain thus making comparisons with other ATPases difficult. Highly purified bacterial F_1-ATPases have generally been found to possess two major (α and β) and three minor (γ, δ, ϵ) subunits and although subunit compositions of $\alpha_3\beta_3\gamma_1\delta_1\epsilon_1$ have been reported [9—11], there is no unanimity on the stoichiometry [12]. Significant differences in subunit compositions have been observed and in some instances these could probably be attributed to the methods of release and purification, and loss or modification of subunits by dissociation and/or proteolysis [7,18,13]. In the investigations described in this report, we have been able to overcome some of the difficulties in maintaining the latency of *M. lysodeikticus* F_1-ATPase, an important prerequisite for establishing the properties of the native coupling factor. By relatively simple purification procedures we have been able to obtain homogeneous F_1-ATPase with the maintenance of a high level of latency.

Materials and Methods

Preparation of crude ATPase

Micrococcus lysodeikticus (NCTC 2665) was grown in peptone water yeast extract medium and cells harvested as previously described [14]. Membrane fractions were prepared by the standard procedures used in this laboratory [15,16] involving lysozyme lysis of cells in 50 mM Tris-HCl buffer, pH 7.5, treatment of the lysate with DNAase and three consecutive washes of the membranes in 50 mM Tris-HCl. The washed membranes were then suspended in 50 mM Tris-HCl, pH 7.5, containing 0.5 mM disodium EDTA for 12 h at 0—4°C. The membranes were then washed, suspended in 50 mM Tris-HCl, pH 7.5, and diluted with cold distilled H_2O containing 1 mM phenylmethyl sulfonyl fluoride to give a final buffer concentration of 3 mM Tris-HCl. This step, known as the 'shock wash', releases most of the ATPase from the membranes [5]. After removing the membranes by centrifugation, the yellow supernatant was made 10% (v/v) in glycerol. The solution was concentrated under pressure with N_2 in an Amicon apparatus equipped with a UM-50 diaflo membrane (Amicon Corp., Lexington, MA). The concentrated crude ATPase was then centrifuged (150 000 × g, 60 min, 4°C). The clear yellow supernatant fluids were pooled and either concentrated or charged directly onto an A24 DEAE Sephadex® column equilibrated with 0.26 M Tris-HCl, pH 7.5, containing 10% (v/v) glycerol.

Assay of ATPase activity

ATPase activity was measured by the liberation of inorganic phosphorus (P_i)

from ATP and determining the P_i colorimetrically by the method of Fiske and Subbarow [17]. The assay mixture contained 50 μl of sample, 4 μmol disodium ATP, 4 μmol $CaCl_2$ and 45—50 μmol Tris-HCl, pH 7.5, in a total volume of 0.5 ml [5]. To determine trypsin stimulated activity, assay tubes contained 150 μg of twice crystallized pancreatic trypsin (Sigma, St. Louis, MO) as well as the other components in the amounts indicated above [5]. One unit of activity is defined as that amount of enzyme which will liberate 1 μmol P_i/min at 37°C under the standard assay conditions. Specific activity is expressed as units/mg protein. Protein was determined by the method of Lowry et al. [18] using bovine serum albumin as standard.

Polyacrylamide gel electrophoresis

Non-dissociating polyacrylamide gel electrophoresis was performed in cylindrical tubes as described by Davis [19]. SDS-polyacrylamide gel electrophoresis was performed in either the Laemmli [20] or Weber and Osborn [21] buffer system. Both cylindrical and gradient slab gels were employed as indicated in the appropriate figure legend. Gels were stained with Coomassie brilliant blue.

Crossed immunoelectrophoresis

The two-dimensional, crossed immunoelectrophoresis technique used was identical to that described in our recent studies [16]. The purified enzyme (1.2 μg protein) was added to the well of the agarose plate and examined for reactions with membrane antiserum (purified immunoglobulins) in the agarose for the second dimensional electrophoresis. The immunoplates were processed and stained with Coomassie brilliant blue as previously described [16,22].

Analytical ultracentrifugation

The high-speed meniscus depletion method of Yphantis [23] was used to determine the molecular weight of the purified enzyme. Equilibrium runs were performed in a Beckman-Spinco Model E ultracentrifuge equipped with a photoelectric scanner. The speed of the rotor was 10 000 rev./min and the temperature was maintained at 4.2°C with an RTIC temperature control unit.

Amino acid analysis

The purified ATPase was hydrolzyed for 21 h in 6 M HCl under reduced pressure at 110°C. Analysis of the hydrolysate was performed in a Durrum D-500 automatic analyzer. Values were not corrected for partial destruction of amino acids during hydrolysis or for incomplete liberation of amino acids after 21 h of hydrolysis.

Subunit composition

Radioactively labelled cells were prepared by growing *M. lysodeikticus* in 1 : 5 (v/v) diluted peptone water yeast extract medium supplemented with 100 μCi of uniformly ^{14}C-labelled algal protein hydrolysate (Amersham, Arlington Heights, IL). ^{14}C-Labelled crude ATPase was then prepared essentially as described for non-labelled ATPase. The shock wash was concentrated and made 1% (v/v) in Triton X-100 and 0.15 M in NaCl. Antibody to purified

ATPase (a generous gift from Dr. Joel D. Oppenheim, Department of Microbiology, at this School) prepared as previously described [6] was added to precipitate the ATPase. The precipitate was dissolved in 1% SDS and electrophoresed in a cylindrical gel employing the Laemmli [20] buffer system. The gel was sliced in 0.5—1.0 mm sections with a Gilson automatic gel slicer and collector. Counts were determined in a Nuclear Chicago Mark I liquid scintillation counter and were normalized by subtracting the backgrounds and dividing subunits counts by their respective molecular weights.

Results

Purification of ATPase

Crucial to the purification of a stable, latent ATPase was the addition of the serine protease inhibitor, phenylmethyl sulfonyl fluoride to the shock-wash step. The membrane-bound ATPase is relatively stable, and remains latent for long periods of time. However, during the release of ATPase from the membranes, a membrane protease also appears to be released and as with trypsin its presence in the shock wash results in activation of the latent enzyme and alteration of its subunit composition. A summary of the solubilization and initial purification steps prior to column chromatography and percent recoveries is given in Table I. After charging the 150 000 $\times g$ supernatant ATPase fraction onto the DEAE-Sephadex column, two column volumes of 0.26 M Tris-HCl, pH 7.5, containing 10% (v/v) glycerol were allowed to pass through. The ATPase was then eluted with a linear gradient, 0.26—0.40 M Tris-HCl, pH 7.5, containing 10% (v/v) glycerol. The elution pattern is shown in Fig. 1. The ATPase

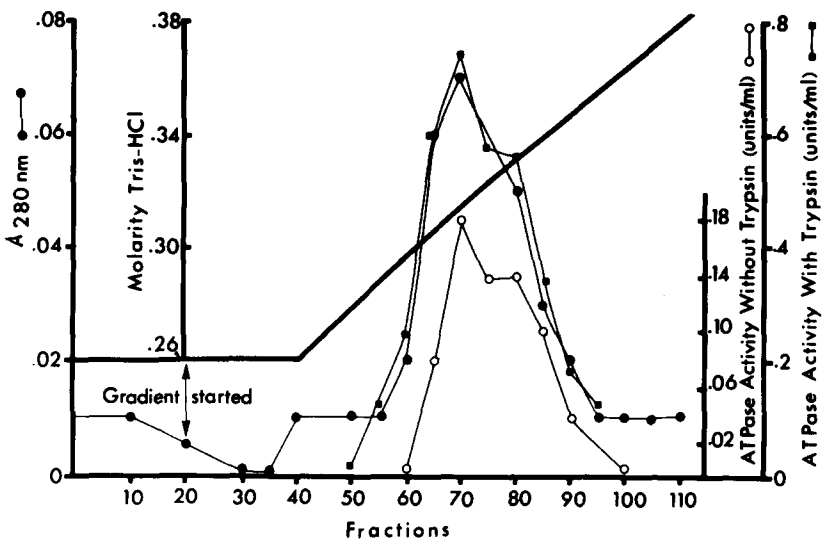

Fig. 1. Chromatography of *M. lysodeikticus* F_1-ATPase on A25-DEAE Sephadex. 180 units of ATPase (S.A. 4 units/mg) from steps outlined in Table I were charged onto a column (2.5 X 100 cm) A25-DEAE Sephadex equilibrated with 0.26 M Tris-HCl, pH 7.5, containing 10% (v/v) glycerol. The ATPase was eluted with a gradient of 0.26—0.40 M Tris-HCl, gradient pH 7.5. Fractions (10 ml) were collected at a flow rate of 5 ml/min. ●———●, *A* 280 nm; ■———■, ATPase activity with trypsin; ○———○, ATPase activity without trypsin.

TABLE I

SOLUBILIZATION AND PURIFICATION OF *M. lysodeikticus* MEMBRANE ATPase

Fractions	Volume (ml)	Protein concentration (mg/ml)	Total protein (mg)	ATPase (units/ml)		Total ATPase		Specific activity * (units/mg)	Recovery of ATPase activity (%)
				—T **	+T **	—T **	+T **		
50 mM Tris, pH 7.5, washed membrane	94	17	1600	—	0.5	—	47	0.03	
EDTA washed membrane	79	15.6	1232	0.1	0.6	7.9	47.4	0.04	
EDTA wash I	225	1.2	270	0.08	0.22	18	49	0.18	
EDTA wash II	195	0.29	52	—	—	—	—	—	
3 mM shock wash	1420	0.30	426	0.20	0.54	284	767	1.80	100
Depleted membrane	31	14.4	446.4	—	—	—	—	—	
UM 10 concentrate	102	3.2	326.4	1.2	5.2	122.4	530.4	1.63	69
150 000 g supernatant	110	1.5	165.0	1.4	5.8	154.0	638	3.87	83

* Trypsin-stimulated activity.

** T, trypsin.

—, denotes activity too low for accurate determination.

activity begins to elute at 0.29 M Tris-HCl. These fractions containing ATPase activity were pooled, concentrated and then dialyzed against 50 mM Tris-HCl, pH 7.5, containing 20% (v/v) glycerol. 70—80% of the ATPase activity could be recovered from the column and the specific activity was 7.1 units/mg when assayed in the presence of trypsin. The basal activity (assayed without trypsin) was 1.14 units/mg. Thus the level of latency in our purified F_1-ATPase is considerably higher than the 2-fold increase in the trypsin-stimulated ATPase activity reported by Carreira et al. [24]. The latency and ATPase activity of our preparations was stable for at least one month at 4°C.

Criteria of purity

The homogeneity of the ATPase preparations was ascertained by a number of criteria including non-dissociating, polyacrylamide gel electrophoresis and crossed immunoelectrophoresis [16]. As shown in Fig. 2A only a single broad band of protein was detected by regular polyacrylamide gel electrophoresis at a high loading of 100 μg protein (one narrow band was seen at lower protein levels). A single precipitation peak was obtained (Fig. 2B) by crossed immunoelectrophoresis of the purified enzyme reacted against antibody to isolated

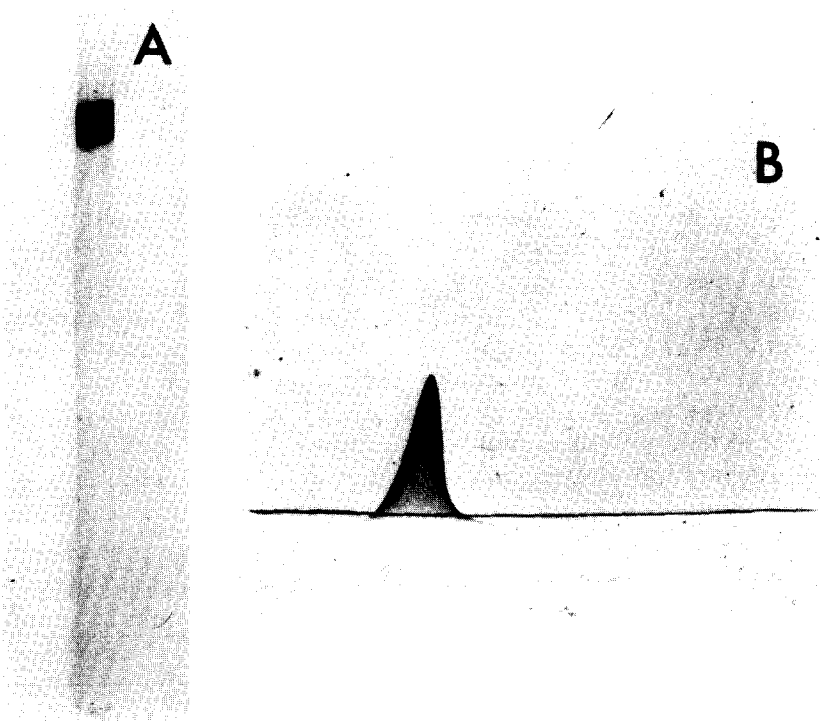

Fig. 2. Homogeneity of F_1-ATPase preparation determined by: (A) electrophoresis of 100 μg protein of the purified ATPase under non-denaturing conditions in 7.5% polyacrylamide gel as described by Davis [19], and (B) crossed, two-dimensional electrophiresis of 2 μl purified ATPase solution (0.6 mg protein/ml) electrophoresed for 65 min at 15 mA (anode to left) in the first direction and into antimembrane immunoglobulins (50 μl, 17.5-fold concentrated with respect to original serum) in the second direction (55 V constant voltage, 18 h, anode at top). Both gel (A) and immunoplate (B) were stained with Coomassie brilliant blue, the latter after processing the plate as previously described [16,22].

membranes. The presence of minor contaminants (e.g. succinylated lipomannan, NADH dehydrogenase) would have been readily detectable by this sensitive technique [25]. Sedimentation analysis in the analytical ultracentrifuge using the schlieren optical system gave only one sedimenting boundary. By these criteria we judge the ATPase preparation to be at least 95% homogeneous.

Molecular weight

Equilibrium sedimentation, performed as described in Materials and Methods, gave a molecular weight 400 000 using a $\bar{v}$ of 0.73, which was estimated from the amino acid analysis by the method of Cohn and Edsall [26]. This $\bar{v}$ is likely an underestimate of the true value which is probably closer to 0.74 as has been reported for mitochondrial ATPase [27]. Schnebli et al. [28] have calculated a $\bar{v}$ of 0.742 for *Streptococcus faecalis* ATPase by comparison of sedimentation equilibrium in ^2H_2O and H_2O by the method of Edelstein and Schachman [29]. Using a $\bar{v}$ of 0.74 would give a molecular weight of 426 000 for our ATPase.

The plot of ln c versus r^2 throughout the cell did not deviate significantly from linearity indicating a monodisperse system and providing further evidence for the homogeneity of the *M. lysodeikticus* F_1-ATPase preparation.

Amino acid analysis

The amino acid analysis presented in Table II shows a similar amino acid composition to mitochondrial and other bacterial ATPases [1,30].

Subunit composition

SDS-polyacrylamide gel electrophoresis of the purified ATPase is shown in Fig. 3 and reveals five subunits, two major subunits α and β having molecular weights of 60 000 and 54 000 and three minor subunits γ, δ, and ϵ with molecular weights of 37 000, 27 000 and 9000, respectively. A minor band below the β subunit can be seen in Fig. 3 and it may possibly originate from limited proteolysis since it was not invariably present. Similar bands below the β subunit have been reported by other investigators [31,32]. In Fig. 4 a plot of radioactive counts versus gel slice number is shown for the radioactively labelled ATPase preparation electrophoresed under denaturing conditions as

TABLE II

AMINO ACID COMPOSITION OF LATENT *M. lysodeikticus* F_1-ATPase

Amino acid analysis	mol%	Amino acid analysis	mol%
Lys	3.5	Gly	9.8
His	4.3	Ala	11.2
Arg	5.1	Val	8.9
Asp	10.0	Met	1.5
Thr	6.0	Ile	5.4
Ser	4.3	Leu	8.3
Glu	12.9	Tyr	0.92
Pro	4.6	Phe	2.9

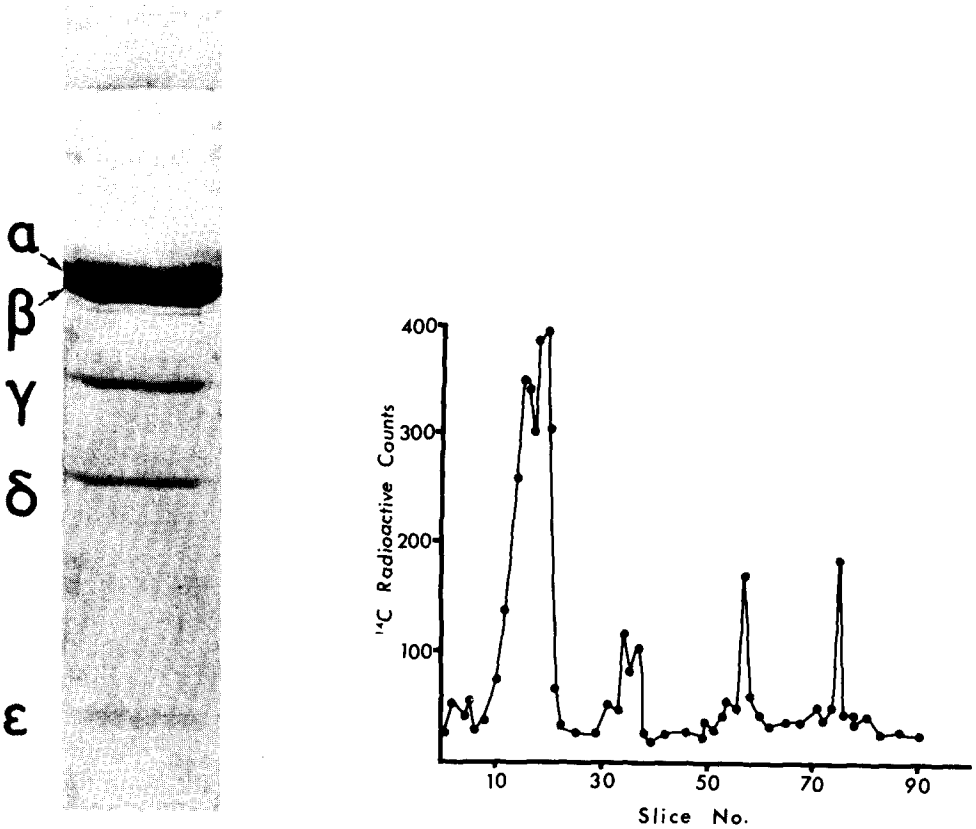

Fig. 3. SDS-polyacrylamide gel electrophoresis of purified F_1-ATPase (25—30 μg protein) in a 10—13% polyacrylamide, gradient slab gel using the Laemmli [20] buffer system. The gel was stained with Coomassie brilliant blue.

Fig. 4. SDS-polyacrylamide gel electrophoresis of ^{14}C-labelled F_1-ATPase prepared as described in Materials and Methods. The subunits of the ^{14}C-labelled ATPase were separated in 10% polyacrylamide cylindrical gels with the Laemmli [20] buffer system. Gel slices were cut and counted for radioactivity as described in Materials and Methods.

described in Materials and Methods. The dip in the peak of the γ subunit seen in the scan was probably due to the concavity of the band. This peak corresponded to a single band on Coomassie blue staining (see also slab gel in Fig. 3). The molar ratio of the subunits in the ATPase molecule was deter mined by integrating the areas under each peak and dividing by the molecular weight of the respective subunit. The values were found to be 3.10 : 2.64 : 1 : 1.03 : 2.99 for α, β, γ, δ, and ε subunits, respectively. The calculated molecular weight based on this subunit stoichiometry ($\alpha_3\beta_3\gamma_1\delta_1\epsilon_3$) is 433 000, a value in good agreement with the molecular weight determined by equilibrium sedimentation.

Discussion

The F_1-ATPase constitutes a fundamental part of the membrane coupling complexes involved in reversible proton translocation as first proposed in the

chemiosmotic hypothesis by Mitchell [33]. In common with chloroplast and mitochondrial F_1-ATPases, many bacterial F_1-ATPases exhibit latency and are believed to catalyze in vivo phosphorylation of ADP [1,2]. The degree of latency varies from one bacterial species to another and would appear to be indicative of the poise of the equilibrium between the ATP synthesizing and utilizing reactions of the membrane. Thus in *M. lysodeikticus* [4,5] as in other strict aerobes such as *Mycobacterium phlei* [34] and *Azotobacter vinelandii* [35] the ATPases are probably almost completely latent, catalyzing the synthesis of ATP by oxidative phosphorylation. In contrast, the ATPase of the facultative anaerobe *Escherichia coli* is less latent [36] and able to utilize the hydrolytic reaction to energize the membrane. The ATPase of the fermentative *Streptococcus faecalis* is apparently non-latent [37] and would rely solely on ATPase activity to generate a membrane potential. The high latency of the membrane-bound ATPase of *M. lysodeikticus* [4,5] provides a suitable system in which to investigate this property and the retention of latency during purification has been used as a valuable indicator of the native state of the F_1 complex in our present studies.

Bacterial F_1-ATPases have molecular weights in the range of 350 000—400 000 and it is generally agreed that they possess two major (α and β) and three minor (γ, δ, ϵ) subunits [1,2,30]. Although Carreira et al. [8] have reported four subunits α (60 000), β (50 000), γ (30 000) and ϵ (25 000) for a partially latent ATPase from *M. lysodeikticus*, our investigations have shown that the number of subunits and molecular weights conform to those generally seen for other bacterial species as well as the chloroplast and mitochondrial F_1-ATPases [1,2,30]. The importance of controlling endogenous protease activities in studying subunit compositions and molecular weights is abundantly evident from a number of investigations of ATPases [1,13,38].

Despite the general agreement that bacterial and eukaryotic coupling factor ATPases contain five different polypeptide subunits, the stoichiometry of the subunits remains a controversial matter [1—3]. Until recently it was thought that bacterial and mitochondrial ATPases possessed a similar subunit composition of 3α, 3β, 1γ, 1δ, 1ϵ [1—3,30,39,40]. However several recent investigations on mitochondrial and chloroplast ATPases support a dimeric stoichiometry of 2α, 2β, 2γ, 2ϵ with some uncertainty about the molar ratio of δ [3,41—43]. Our data on the subunit composition of the *M. lysodeikticus* F_1-ATPase based on ^{14}C-labelled ATPase prepared from cells grown in medium containing [U-^{14}C]-labelled algal protein hydrolysate suggest a stoichiometry of $\alpha_3\beta_3\gamma_1\delta_1\epsilon_3$. This subunit composition must remain a tentative one due to the limitations of the labelling of whole cells in which the specific activities of the amino acid pools and the levels of incorporation of individual amino acids into each subunit have not been determined. Similar methods of labelling have been used for subunit studies by Bragg and Hou [9] and Kagawa et al. [11] and the stoichiometries of our and their results would have to take into account the consequences of differences in the amino acid compositions of the individual subunits. It should be noted, however, that analytical data for the α and β subunits of *M. lysodeikticus* [44] and all five purified subunits of chloroplast F_1 [43] indicate that the predominant amino acids (i.e. Ala, Gly, Leu, Val, Asp, Glu) are represented in similar proportions in the various subunits and that the

major differences exist for the less abundant amino acids. The subunit composition suggested from our labelling experiments would give a molecular weight of 433 000 based on the molecular weights of the five subunits determined by SDS-polyacrylamide gel electrophoresis. This estimate is fully compatible with the molecular weight of 400 000 determined by equilibrium sedimentation and would be difficult to reconcile with the dimeric structure proposed for chloroplast and mitochondrial ATPases [3,41—43]. However, further investigations are clearly needed to resolve these differences before final conclusions on the comparative structures of the F_1-ATPases can be deduced.

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References

1 Pedersen, P.L. (1975) Bioenergetics 6, 243—275
2 Haddock, B.A. and Jones, C.W. (1977) Bacteriol. Rev. 41, 47—99
3 Amzel, L.M. and Pedersen, P.L. (1978) J. Biol. Chem. 253, 2067—2069
4 Muñoz, E., Nachbar, M.S., Schor, M.T. and Salton, M.R.J. (1968) Biochem. Biophys. Res. Commun. 32, 539—546
5 Muñoz, E., Salton, M.R.J., Ng, M.H. and Schor, M.T. (1969) Eur. J. Biochem. 7, 490—501
6 Oppenheim, J.D. and Salton, M.R.J. (1973) Biochim. Biophys. Acta 298, 297—322
7 Salton, M.R.J. and Schor, M.T. (1972) Biochem. Biophys. Res. Commun. 49, 350—357
8 Carreira, J., Andreu, J.M., Nieto, M. and Muñoz, E. (1976) Mol. Cell. Biochem. 10, 67—76
9 Bragg, P.D. and Hou, C. (1975) Arch. Biochem. Biophys. 167, 311—321
10 Abrams, A., Jensen, C. and Morris, D.H. (1976) Biochem. Biophys. Res. Commun. 69, 804—811
11 Kagawa, Y., Sone, N., Yoshida, M., Hirata, H. and Okamoto, H. (1976) J. Biochem. (Tokyo) 80, 141—151
12 Vogel, G. and Steinhart, R. (1976) Biochemistry 15, 208—216
13 Risi, S., Höckel, M., Hulla, F.W. and Dose, K. (1977) Eur. J. Biochem. 81, 103—109
14 Salton, M.R.J. and Freer, J.H. (1965) Biochim. Biophys. Acta 107, 531—538
15 Salton, M.R.J. (1976) in Methods in Membrane Biology, Vol. 6, (Korn, E.D., ed.), p. 101, Plenum Press, New York, NY
16 Owen, P. and Salton, M.R.J. (1977) J. Bacteriol. 132, 974—985
17 Fiske, C.H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375—400
18 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
19 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404—427
20 Laemmli, U.K. (1970) Nature 227, 680—685
21 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406—4412
22 Weeke, B. (1973) in Quantitative Immunoelectrophoresis (Axelsen, N.H., Krøll, J. and Weeke, B., eds.), Scand. J. Immunol. 2, Suppl. 1, 15—35
23 Yphantis, D.A. (1964) Biochemistry 3, 297—317
24 Carreira, J., Muñoz, E., Andreu, J.M. and Nieto, M. (1976) Biochim. Biophys. Acta 436, 183—189
25 Owen, P. and Smyth, C.J. (1977) in Immunochemistry of Enzymes and Their Antibodies (Salton, M.R.J., ed.), p. 147, Wiley, New York, NY
26 Cohn, E.J. and Edsall, J.T. (1943) in Proteins, Amino Acids, and Peptides, p. 370, Reinhold Publishing Co., New York, NY

27 Penefsky, H.J. and Warner, R.C. (1965) J. Biol. Chem. 240, 4694—4702
28 Schnebli, H.P., Vatter, A.E. and Abrams, A. (1970) J. Biol. Chem. 245, 1122—1127
29 Edelstein, S.J. and Schachman, H.K. (1967) J. Biol. Chem. 242, 306—311
30 Abrams, S. and Smith, J.B. (1974) in The Enzymes, Vol. X, (Boyer, D., ed.), p. 404, Academic Press, New York, NY
31 Catterall, W.A. and Pedersen, P.L. (1971) J. Biol. Chem. 246, 4987—4994
32 Gomez-Puyou, M.T. and Gomez-Puyou, A. (1977) Arch. Biochem. Biophys. 182, 82—86
33 Mitchell, P. (1961) Nature 191, 144—148
34 Higashi, T., Kalra, V.K., Lee, S.H., Bogin, E. and Brodie, A.F. (1975) J. Biol. Chem. 250, 6541—6548
35 Bhattacharyya, P. and Barnes, E.M., Jr. (1976) J. Biol. Chem. 251, 5614—5619
36 Carreira, J., Leal, J.A., Rojas, M. and Muñoz, E. (1973) Biochim. Biophys. Acta 307, 541—556
37 Harold, F.M. (1972) Bacteriol. Rev. 36, 172—230
38 Ryrie, I.J. (1975) Arch. Biochem. Biophys. 184, 464—475
39 Catterall, W.A., Coty, W.A. and Pedersen, P.L. (1973) J. Biol. Chem. 248, 7427—7431
40 Senior, A.E. (1973) Biochim. Biophys. Acta 301, 249—277
41 Senior, A.E. (1975) Biochemistry 14, 660—664
42 Verschoor, G.J., Van der Sluis, P.R. and Slater, E.C. (1977) Biochem. Soc. Trans. 5, 1514—1516
43 Binder, A., Jagendorf, A. and Ngo, E. (1978) J. Biol. Chem. 253, 3094—3100
44 Andreu, J.M., Carreira, J. and Munoz, E. (1976) FEBS Lett. 65, 198—203