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Significant quantities of endogenous GDP and ADP are present on catalytic sites of the F_1-ATPase isolated from *M. lysodeikticus* in the absence of added nucleotides

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The F_1-ATPase from *Micrococcus lysodeikticus* is isolated in the absence of exogenous nucleotides. After removing loosely bound nucleotides from the isolated enzyme by gel permeation chromatography, analysis for tightly bound nucleotides revealed in 14 experiments 0.4 ± 0.1 mol ADP, 0.5 ± 0.2 mol GDP, and 0.8 ± 0.2 mol ATP per mol of F_1. Incubation of the isolated enzyme with Mg^{2+} or Ca^{2+} did not alter the endogenous nucleotide composition of the enzyme, indicating that endogenous ATP is not bound to a catalytic site. Incubation of the enzyme with P_i decreased the amount of tightly bound ADP and GDP but did not affect the ATP content. Hydrolysis of MgATP in the presence of sulfite raised the tightly bound ADP and lowered tightly bound GDP on the enzyme. In the reciprocal experiment, hydrolysis of MgGTP in the presence of sulfite raised tightly bound GDP and lowered tightly bound ADP. Turnover did not affect the content of tightly bound ATP on the enzyme. These results suggest that endogenous ADP and GDP are bound to exchangeable catalytic sites, whereas endogenous ATP is bound to noncatalytic sites which do not exchange. The presence of endogenous GDP on catalytic sites of isolated F_1 suggests that the F_0F_1-ATP synthase of *M. lysodeikticus* might synthesize both GTP and ATP under physiological conditions. In support of this hypothesis, we have found that plasma membrane vesicles derived from *M. lysodikticus* synthesize [^{32}P]GTP from [^{32}P]P_i using malate as electron donor for oxidative phosphorylation.

Introduction

The ATP synthases of energy transducing membranes are composed of an integral membrane complex, F_0, which mediates transmembrane proton conduction, and a peripheral membrane protein complex, F_1, which contains the catalytic sites. When removed from the membrane in soluble form, F_1 hydrolyzes ATP and a number of other nucleotides. The isolated enzyme is usually made up of five different polypeptide chains with the stoichiometry $\alpha_3\beta_3\gamma\delta\epsilon$. The isolated F_1-ATPases bind up to 6 mol of adenine nucleotides per mol [1,2]. Three of the binding sites are potential catalytic sites and are exchangeable during catalytic turnover. The other three, which have a poorly defined functional role, and are thus called noncatalytic sites, do not exchange during turnover. The endogenous nucleotide contents of F_1-ATPases isolated from different sources vary considerably. For instance, the F_1-ATPase isolated from the thermophilic bacterium PS3 contains none [3], whereas the F_1-ATPase isolated from *Escherichia coli* contains about 5 mol of adenine nucleotide per mol [4,5]. These variations might reflect differences in physiological properties of the intact synthases or represent differences in loss of nucleotides during purification of individual enzymes. Although F_1-ATPases from different sources hydrolyze GTP and ITP and bind exogenous GTP, ITP and GDP [6–8] only ATP and ADP have been reported to be present in isolated enzymes. We report here that the F_1-ATPase isolated from *M. lysodeikticus*, which is

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Abbreviations: FPLC, fast protein liquid chromatography; CDTA, *trans*-1,2-diaminocyclohexane-*N,N,N',N'*-tetraacetic acid; DCCD, *N,N'*-dicyclohexylcarbodiimide; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

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purified in the absence of adenine nucleotides, contains tightly bound endogenous GDP in addition to tightly bound ATP and ADP.

Materials and Methods

Enzyme purification. A bacterial culture of *Micrococcus lysodeikticus*, Fleming's strain (2665) was used. The bacteria were grown under aerobic conditions at 30°C for 15 h in a medium containing 1% Bacto-tryptone (Difco), 0.5% yeast extract (Difco), 0.5% NaCl (pH 7.4) and were harvested in early stationary phase. To obtain membranes, the cells were treated with lysozyme and DNAase according to Ref. 9. The membranes were washed first with 0.1 M and then 0.03 M Tris-HCl buffer (pH 7.4) before extracting the ATPase with 0.003 M Tris-HCl (pH 7.4) according to Ref. 9. The ATPase extract was centrifuged at $140000 \times g$ at 15°C for 30 min and the supernatant was concentrated by ultrafiltration in Amicon cells with synthetic membranes (YM 10 Diaflo).

The ATPase was purified by gel-filtration on a column packed with Superose 6 (1 × 100 cm) equilibrated with 50 mM Tris-sulfate buffer (pH 7.8). About 5 ml of protein (1 mg/ml) was loaded and the column was eluted with the same buffer at a rate 1 ml per min. Fractions (1 ml) containing ATPase (Fig. 1A) were pooled, the protein was concentrated to 0.6–0.8 mg/ml by ultrafiltration and stored at −10°C in 50 mM Tris-sulfate buffer (pH 7.8). When the purified ATPase was submitted to polyacrylamide electrophoresis in the presence of 1% SDS according to Laemmli [10], it was clear that it was nearly homogeneous as illustrated in Fig. 1B. Although the ϵ subunit ran off the gel shown in Fig. 1B, it was readily apparent when the pooled fractions were submitted to electrophoresis under different conditions.

Preparation of plasma membrane vesicles. Plasma membrane vesicles from *M. lysodeikticus* were prepared as follows. *M. lysodeikticus* cells (30 g) were treated with 3 mg of lysozyme and 1 mg of DNAse in 300 ml of 10 mM Tris-HCl buffer (pH 7.4), 5 mM $MgSO_4$ and 0.25 M sucrose for 20 min at 37°C. The resulting suspension was centrifuged at $16000 \times g$ and the sediment was resuspended in the same buffer also containing 1 mM ATP and 1 mM malate to a final protein concentration of about 10 mg per ml. The suspension was sonicated for 1 min under cooling in MSE-500 W ultrasonic disintegrator at a frequency of 20 kcycles and a current of 0.5 A. The sonicated mixture was centrifuged at $22000 \times g$ and the sediment discarded. The supernatant was centrifuged at $144000 \times g$. The pellet was washed in 10 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose and 5 mM $MgSO_4$ and then suspended in the same buffer at a final protein concentration of about 20 mg per ml. The vesicles were stored in 1 ml aliquots at −20°C.

Analytical procedures. The protein concentration was determined by the method of Lowry et al. [11] using bovine serum albumin as a standard. The MgATPase activity was assayed with an ATP-regenerating system coupled to oxidation of NADH by pyruvate in the presence of lactate dehydrogenase. A sample of ATPase (10–50 μl) was added to 1 ml of assay reaction mixture which contained 50 mM Tris-HCl buffer (pH 8.0), 2.6 mM $MgSO_4$, 2.6 mM ATP, 50 mM KCl, 1 mM PEP, 0.2 mM NADH, 10 U of pyruvate kinase and 10 U of lactate dehydrogenase at 37°C. For determination of MgGTPase activity, lactate dehydrogenase and pyruvate kinase were increased to 30 U each. CaATPase activity was measured in 50 mM Tris-HCl buffer (pH 8.0) containing 3 mM ATP and 3 mM $CaCl_2$ at 37°C by P_i release was determined according to Rathbun and Betlach [12].

Tightly bound nucleotides were analyzed by the following procedure. Samples of the ATPase were centrifuged through the columns of Sephadex G-50 equilibrated with appropriate buffer. The protein was precipitated by treatment with perchloric acid (final concentration 0.5 N) and separated by centrifugation. The precipitate was dissolved in 0.2 M NaOH and protein was determined according to Lowry et al. [11]. The supernatant was neutralized by $KHCO_3$ (final concentration 0.5 M) and after 30 min at 0°C, $KClO_4$ was removed by centrifugation. The supernatant containing extracted nucleotides (about 0.1–0.2 ml) was diluted to 1 ml with 50 mM Tris-HCl buffer (pH 7.5).

Separation of nucleotides was carried out by ion exchange chromatography on a Pharmacia-LKB (HR5/10) Mono-Q column using an FPLC system (Pharmacia-LKB). The column was equilibrated with 50 mM Tris-HCl (pH 7.5). After loading the sample, the column was washed with 5 ml of the same buffer and nucleotides were eluted with a salt gradient of 0 to 0.4 M NaCl in 50 mM Tris-HCl (pH 7.5) at a flow rate 1 ml per min. Fractions of 0.25 ml were collected. The column was then washed with 2 ml of 1 M NaCl followed by 3 ml of 50 mM Tris-HCl. The absorbance of the eluate was monitored at 254 nm. The mM extinction coefficients of 15.0 for ADP and ATP and 13.7 for GDP at 254 nm were used. For reference, separation of AMP, ADP, ATP, GMP, GDP and GTP present in a standard mixture by this method is illustrated in Fig. 2B. Absorption spectra of ADP, ATP and GDP isolated by fractionation illustrated in Fig. 1A were determined between 220 nm and 320 nm using an LKB Ultraspec recording spectrophotometer in a volumn of 0.2 ml. A molecular mass for the ATPase of 380000 was used in calculations. The GTP and ATP synthase activities catalyzed by plasma membrane vesicles from *M. lysodeikticus* were determined

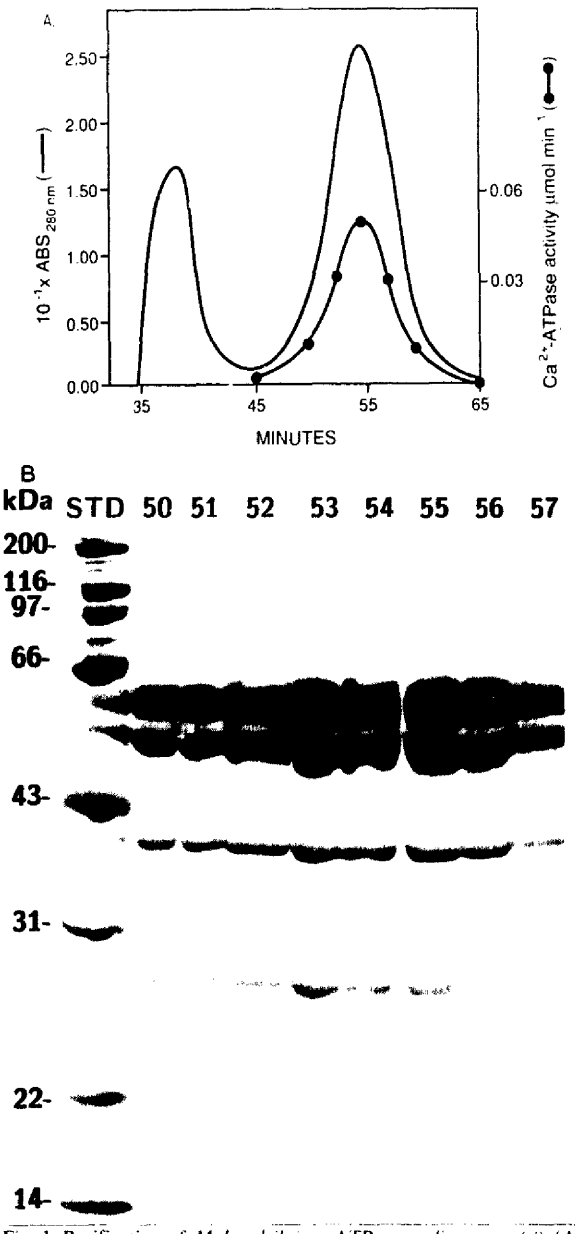

Fig. 1. Purification of *M. lysodeikticus* ATPase on Superose 6.0. (A) 5 ml of crude ATPase extract (about 2 mg/ml) were loaded onto a column (1 × 100 cm) of Superose 6 which was equilibrated and eluted with 50 mM Tris-HCl (pH 7.8) with a flow rate 1 ml per min. The Ca-ATPase of 10 µl of each fraction was assayed as described in 'Materials and Methods'. (B) Pattern of stained proteins obtained after submitting fractions 50–57 (Fig. 1A) to polyacrylamide gel electrophoresis according to Laemmli [10] on 12% gels in the presence of 1% sodium dodecyl sulfate. 50 µl of each fraction were loaded into the wells of the designated lanes. The molecular weight standards were: rabbit skeletal muscle myosin, 200000; *E. coli* β-galactosidase, 116250; rabbit muscle phosphorylase *b*, 97400; bovine serum albumin, 66200; hen egg white ovalbumin, 42699; bovine carbonic anhydrase, 31000; soybean trypsin inhibitor, 21500; and hen egg white lysozyme 14400.

by the incorporation of $[^{32}P]P_i$ into GTP and ATP. Reaction mixtures (0.5 ml) contained 20 mM Hepes (pH 7.4), 5 mM $MgSO_4$, 0.25 M sucrose, $H^{32}PO_4$ ($2.5 \cdot 10^6$ cpm) 1 mM KH_2PO_4, 1 mM GDP or ADP and plasma membrane vesicles (40 µg of protein). Syntheses were initiated by addition of 10 mM malate and were carried out at 20°C with intensive aeration. At 2 min intervals, samples, 50 µl each, were removed from the reaction mixtures and injected into 0.5 ml of 2% activated charcoal suspended in 0.5 M HCl. After 5 min, the charcoal containing $[^{32}P]$GTP or $[^{32}P]$ATP was collected on glass microfiber filters (Whatman GF/F) and submitted to liquid scintillation counting.

Results

The ATPase purified from the membranes of *Micrococcus lysodeikticus* hydrolyzes CaATP with a specific activity of about 3–5 µmol min^{-1} (mg protein)$^{-1}$ at 37°C. The ATPase activity observed when F_1 was removed from the membrane, where the ATPase is latent in the absence trypsin digestion, might be caused by partial proteolytic digestion of a small inhibitory subunit by endogenous proteinases [13,14]. As presented in Table I, Mg^{2+}-dependent ATPase activity of the soluble ATPase in a coupled ATP-regenerating system was 1.9 µmol min^{-1} (mg protein)$^{-1}$ and was stimulated 2-fold by sulfite. The same level of activity and sulfite stimulation were observed for the hydrolysis of MgITP. However, MgGTP was hydrolyzed more slowly with a specific activity of 0.85 µmol min^{-1} mg^{-1} but exhibited 3.5-fold activation with sulfite. The high level of stimulation of GTPase activity by sulfite is a characteristic of the *M. lysodeikticus* ATPase that distinguishes it from the mitochondrial ATPase [15].

The tightly bound, endogenous nucleotides present in a typical preparation of *M. lysodeikticus* ATPase is shown in Fig. 2. From comparison of Fig. 2A with Fig. 2B it appears that the enzyme contains three different tightly bound nucleotides, ADP, GDP and ATP. To verify that the peaks do indeed contain these nucleotides, samples of the different peaks were submitted to scanning spectrophotometry. The spectra of

TABLE I

ATPase and GTPase activity of F_1-ATPase from Micrococcus lysodeikticus

Enzyme activity was measured with an ATP or GTP regenerating system containing 50 mM Tris-HCl buffer (pH 8.0), 50 mM KCl, 1 mM PEP, 0.2 mM NADH, 10 or 30 U of lactate dehydrogenase and pyruvate kinase at 37°C.

Substrate	Activity (µmol min^{-1} mg^{-1})	Sulfite activation
2.5 mM MgATP	1.9	
2.5 mM MgATP, 20 mM sulfite	4.7	2.5-fold
2.5 mM MgGTP	0.85	
2.5 mM MgGTP, 20 mM sulfite	3.0	3.5-fold

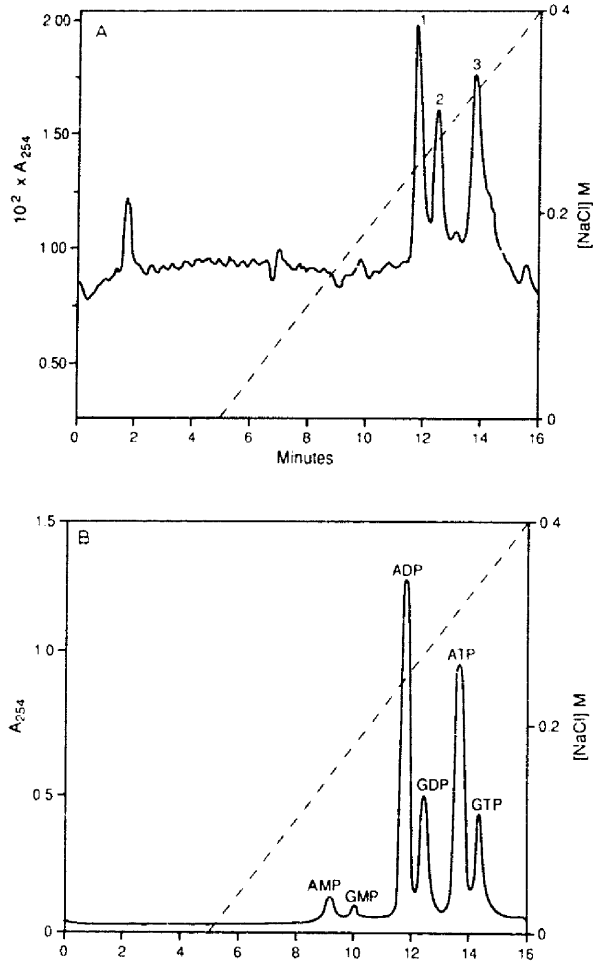

Fig. 2. (A) Separation of tightly bound endogenous nucleotides removed from the *M. lysodeikticus* ATPase by anion exchange chromatography. Loosely bound nucleotides were removed from a 0.2 ml sample of the ATPase (about 0.5 mg/ml) by centrifugation through a 1.0 ml Sephadex G-50 column equilibrated with 50 mM Tris-HCl (pH 7.5). The protein was precipitated with perchloric acid. The protein content of the precipitate was determined according to Lowry et al. [11]. The supernatant was neutralized with $KHCO_3$, recentrifuged to remove $KClO_4$, and then submitted to ion exchange chromatography on a Mono-Q column which was eluted with a NaCl gradient from 0 to 0.4 M in 50 mM Tris-HCl (pH 7.5) at a flow rate 1 ml per min. (B). Position of AMP, ADP, ATP, GMP, GDP and GTP when eluted from the MonoQ column using a gradient of 0.4 M NaCl in 50 mM Tris-HCl (pH 7.5) into the same buffer at a flow rate of 1 ml per min. The concentrations of nucleotides were: AMP, 1.9 μM; ADP, 27 μM; ATP, 23 μM; GMP, 0.58 μM; GDP, 12.5 μM, and GTP, 8.6 μM.

samples from peaks 1, 2 and 3 are presented in Fig. 3. Comparison of retention times of peaks in Fig. 2 and these spectra with those of authentic samples of ADP and GDP confirmed that peak 2 contained GDP. The spectrum of the material examined in peak 3 was that of an adenine nucleotide (curve 3 in Fig. 3). Therefore this peak is concluded to be ATP. The small peak eluting at 2 min before the salt gradient was applied probably represents traces of protein not precipitated after PCA treatment which were detected by the sensitive optical system employed. From the data of fourteen experiments, the mol of ADP, GDP and ATP found per mol of ATPase were 0.4 ± 0.1, 0.5 ± 0.2 and 0.8 ± 0.2, respectively.

Other properties of the nucleotide binding sites were examined. The results of these experiments are summarized in Table II. The ATPase was incubated under different conditions, and then separated from the incubation mixture by centrifugation through the Sephadex G 50 column. Tightly bound nucleotides were then analyzed.

As presented in Table II incubation of the ATPase with Mg^{2+} or Ca^{2+} for 30 min did not change the content of tightly bound ADP, GDP or, most importantly, ATP. Hydrolysis of tightly bound ATP was not observed under these conditions. Incubation of the ATPase with P_i resulted in a decrease of the level of tightly bound ADP and GDP but did not affect the

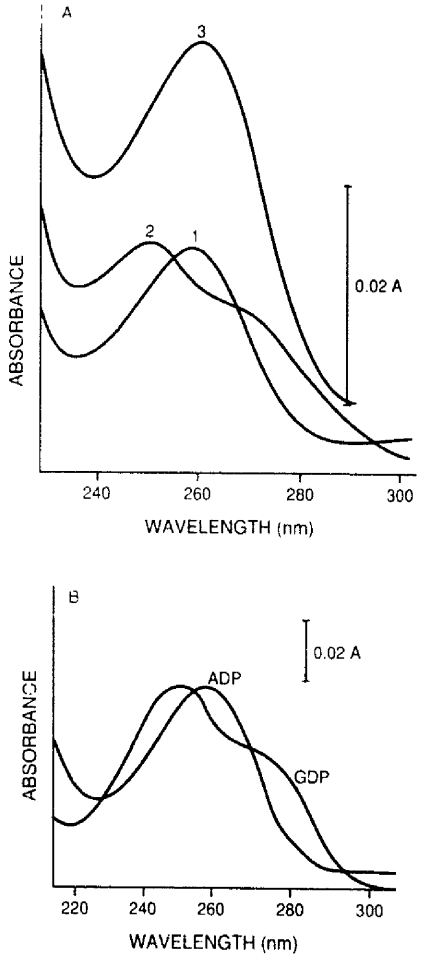

Fig. 3. Absorbtion spectra of the tightly bound endogenous nucleotide of the *M. lysodeikticus* ATPase. (A) Absorption spectra of peak 1(1) and peak 2(2) and peak 3(3) from Fig. 2. (B) Absorption spectra of 4.7 μM ADP and 5 μM GDP in 50 mM Tris-HCl buffer (pH 7.8).

TABLE II

Effects of Mg^{2+}, Ca^{2+}, P_i and PP_i on tightly bound endogenous nucleotides on the M. lysodeikticus F_1-ATPase

Samples, 0.2 ml each, of *M. lysodeikticus* ATPase preparations (0.2–0.7 mg of protein/ml) were centrifuged through a 2 ml column of Sephadex G-50 equilibrated in 50 mM Tris-HCl (pH 7.5) or 20 mM sodium phosphate (pH 6.3), and then incubated under the different conditions indicated. Unless indicated otherwise, samples were treated in 50 mM Tris-HCl (pH 7.5). Samples marked with an asterisk (*) were passed through a second centrifuge column equilibrated with 50 mM Tris-HCl before analysis for nucleotides as described in Materials and Methods.

Additions	Bound nucleotides (mol/mol F_1)		
	ADP	GDP	ATP
None	0.4	0.4	0.9
None	0.4	0.4	1.0
None	0.5	0.3	1.0
1 mM Mg^{2+}; 20°C; 1 min	0.6	0.5	0.8
10 mM Mg^{2+}; 20°C; 1 min	0.5	0.4	1.0
10 mM Mg^{2+}; 37°C; 30 min	0.5	0.4	0.9
5 mM Ca^{2+}; 37°C; 30 min	0.4	0.3	1.0
* 20 mM P_i (pH 6.3); 37°C; 30 min	0.1	0.2	1.0
* 10 mM PP_i, 1 mM CDTA; 37°C; 30 min	0.4	0.6	0.8
20 mM P_i (pH 6.3), 1 mM Mg^{2+}; 37°C; 30 min	0.6	0.5	0.8

ATP content. The addition of 1 mM $MgSO_4$ to the incubation mixture prevented loss of ADP and GDP promoted by P_i. Inorganic pyrophosphate in combination with CDTA did not promote release of tightly bound nucleotides from the enzyme.

As shown in Table III, incubation of the enzyme with MgATP in the presence of sulfite (at pH 7.5) raised the ADP content and decreased the tightly

TABLE III

Exchange of tightly bound endogenous ATP and GDP promoted by exogenous nucleotides

Samples, 0.2 ml each, of *M. lysodeikticus* ATPase (0.2–0.7 mg of protein/ml) were centrifuged through a 2 ml column of Sephadex G-50 equilibrated with 50 mM Tris-HCl buffer (pH 7.5) and then incubated under the different conditions indicated. After incubation, samples were passed through two centrifuge columns, 1 ml each, equilibrated with the same buffer.

Additions	Bound nucleotides (mol/mol enzyme)		
	ADP	GDP	ATP
In Tris-HCl (pH 7.5)			
None	0.3	0.6	0.6
0.1 mM MgATP; 15 mM sulfite; 37°C; 30 min	1.2	0.2	0.5
0.1 mM MgGTP; 15 mM sulfite; 37°C; 30 min	0.15	0.8	0.6

TABLE IV

Inactivation of M. lysodeikticus ATPase by 40 μM quinacrine mustard with and without exogenous natural ligands

The ATPase (0.2 mg/ml) was incubated in 50 mM Tris-HCl buffer (pH 7.8) with 40 μM quinacrine mustard in the presence or absence of ligands. Samples of the reaction mixtures were withdrawn at 5 min intervals to determine MgATPase activity using the coupled ATP-regenerating system. Pseudo-first-order rates of inactivation, k', were determined from Guggenheim plots [25].

Additions	$10^2 \times k'$ (min^{-1})
None	8.25
5 mM Mg^{2+}	7.8
0.1 mM MgADP	4.5
0.1 mM ATP	4.4
0.1 mM MgATP	2.0
0.1 mM MgGTP	3.2

bound GDP content. On the other hand, incubation with MgGTP and sulfite lowered the level of tightly bound ADP, whereas the level of tightly bound GDP increased. Thus, ADP and GDP can exchange with each other during turnover conditions. The amount of tightly bound ATP did not change under any of the conditions examined.

Quinacrine mustard which has been shown to inactivate the mitochondrial F_1-ATPase by derivatizing the β subunit, [16,17] is a potent inactivator of *M. lysodeikticus* ATPase as shown in Table IV. As has been observed for the mitochondrial enzyme (Bullough, D.A. and Allison, W.S. (1988), unpublished data), adenosine and guanosine di- and triphosphates protect the *M. lysodeikticus* ATP against inactivation with quinacrine mustard. However, inactivation of the *M. lysodeikticus* ATPase with quinacrine mustard did not affect the tightly bound nucleotide content of the enzyme. This observation is consistent with evidence presented on the mitochondrial F_1-ATPase which suggests that the site in the β subunit that reacts covalently with quinacrine mustard is not part of a nucleotide binding site [17,18]. Therefore, binding of nucleotides to their specific sites appears to decrease the affinity or reactivity of quinacrine mustard for its specific site in the β subunit by indirect effects.

The presence of tightly bound GTP on the enzyme and the observation that sulfite stimulates GTPase activity, suggested that the membrane-bound ATP synthase of *M. lysodeikticus* can catalyze GTP as well as ATP synthesis. To test this hypothesis, the capacity of membrane vesicles to phosphorylate GDP and ADP with [^{32}P]P_i under conditions of oxidative phosphorylation was determined as described under Materials and Methods. The results of this comparison showed that 33.6 nmol GTP and 39.5 nmol ATP were synthesized per mg protein per min under the same conditions in different experiments. As shown in Table V, GTP-syn-

TABLE V

GTPase synthase activity of M. lysodeikticus plasma membrane vesicles

GTP-synthase activity was measured as described in Materials and Methods. Plasma membrane vesicles (160 μg of protein/ml) were treated by 0.5 mM DCCD in the buffer containing 20 mM Hepes (pH 7.4), 5 mM $MgSO_4$, 0.25 M sucrose at 20°C for 20–25 min. The reaction mixture was diluted 2-fold with the same buffer not containing DCCD but containing GDP (or ADP) and $KH_2^{32}PO_4$. Synthesis was initiated by addition of malate. Ethanol at 0.5% did not inhibit oxidative phosphorylation in the absence of DCCD.

	nmol [^{32}P]GTP min^{-1} (mg protein)$^{-1}$
None	33.6
KCN, 0.5 mM	2.7
FCCP, 45 μM	0.0
DCCD, 0.5 mM	8.7
Ethanol, 0.5%	33.6

thase activity was inhibited by 92% in the presence of 0.5 mM KCN, by 74% after treating the membrane vesicles with 0.5 mM DCCD, and by 100% in the presence of 4.5 μM FCCP.

Discussion

From the results presented it is clear that *M. lysodeikticus* ATPase contains tightly bound ATP, ADP and GDP. Incubation of the enzyme with Mg^{2+}, sulfite and GTP (turnover conditions) did not release ATP from this binding site, these results strongly suggest that ATP is located in a nonexchangeable, noncatalytic site of the enzyme. The observation that endogenous nucleotides were exchanged during hydrolysis of ATP or GTP shows that both ADP and GDP are located in exchangeable sites on the enzyme. Although, it was not shown that the rate of exchange observed is equivalent to that of turnover, the results presented suggest that tightly bound endogenous ADP and GDP are on catalytic sites.

The observation that stimulation of the Mg^{2+}-GTPase by sulfite was greater than stimulation of the Mg^{2+}-ATPase activity by sulfite distinguishes the bacterial ATPase from the mitochondrial ATPase, since sulfite has little effect on GTP or ITP hydrolysis by mitochondrial ATPase [15]. Evidence has been presented showing that sulfite accelerated the release of ADP from the catalytic site of the spinach chloroplast ATPase under turnover conditions [19]. Thus, the fact that sulfite stimulated GTPase activity of the *M. lysodeikticus* ATPase supports our contention that endogenous GDP is located on a catalytic site of the enzyme.

The F_1-ATPases from bovine heart mitochondria and yeast [8,20] and spinach chloroplasts [6] have been shown to bind exogenous GTP or GDP. Harris et al. [21] have reported that hydrolysis of GTP or ITP by submitochondrial particles is relatively inefficient in driving energy dependent reactions. However, in contrast to this contention, Matsuno-Yagi and Hatefi [22] have recently reported that submitochondrial particles catalyze cooperative GTP synthesis. The phosphorylation of GDP and IDP was also shown to be catalyzed by chloroplast [23] and *E. coli* membranes [24].

The results presented establish that tightly bound, endogenous GDP is present on the *M. lysodeikticus* F_1-ATPase when isolated in the absence of exogenous nucleotides. Since other F_1-ATPases are usually prepared in the presence of ATP, which would exchange with endogenous GDP on catalytic sites, it is not clear whether this property is unique to the *M. lysodeikticus* ATPase, or this is a property common to F_1-ATPases in general. The presence of tightly bound endogenous ADP and GDP on a catalytic site of F_1 may indicate that GTP as well as ATP are synthesized by the F_0F_1-ATP synthase of *M. lysodeikticus* under physiological conditions. This possibility is strongly supported by the demonstration that plasma membrane vesicles isolated from *M. lysodeikticus* catalyze ATP and GTP synthesis with equal facility using malate as electron donor for oxidative phosphorylation.

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