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The ATP synthase of *Streptomyces lividans*: characterization and purification of the F_1F_o complex

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Abstract

Everted membrane vesicles of the Gram-positive eubacterium *Streptomyces lividans* were prepared and the ATP synthase (F_1F_o) was characterized in its membrane-bound form. In addition, the F_1F_o complex was solubilized, purified, and functionally reconstituted into phospholipid vesicles. The enzyme complex is similar with respect to subunit composition to those of other eubacterial ATP synthases. Whereas the F_1 part only exhibits ATPase activity in the presence of $CaCl_2$ (Hensel, M., Deckers-Hebestreit, G. and Altendorf, K. (1991) Eur. J. Biochem. 202, 1313–1319), the membrane-bound ATPase is also moderately stimulated by high concentrations of Mg^{2+} ions (20 mM). In contrast, the physiological functions of the ATP synthase, i.e., ATP-driven H^+ translocation and ATP synthesis are strictly dependent on Mg^{2+} ions. The biochemical properties of the ATP synthase of *S. lividans* show distinct similarity to the enzyme complex of rhodobacteria and bacilli. The ATPase activity is inhibited by *N,N'*-dicyclohexylcarbodiimide, venturicidin, and tributyltin, typical inhibitors of F_1F_o-ATPases, which react with the membrane-bound F_o complex. In addition, the ATPase activity is highly sensitive towards oligomycin, a feature which is only shared by the ATP synthase of rhodobacteria and mitochondria.

Keywords: ATP synthase; F_1F_o-ATPase; Everted membrane vesicle; Proteoliposome; (Streptomycetes)

1. Introduction

Streptomycetes are an outstanding group of eubacteria, exhibiting a differentiated cell cycle. Furthermore, due to their secondary metabolism, streptomycetes are producers of an enormous amount of low molecular mass compounds, like antibiotics, immunomodifiers or enzyme inhibitors. Whereas processes linked to the regulation of the cell cycle and biosynthetic pathways of the secondary metabolism of streptomycetes have been studied in detail, the primary and especially the energy metabolism of this group of bacteria is not well characterized.

The membrane-bound ATP synthase (F_1F_o; EC 3.6.1.34) is a key enzyme in cellular energy metabolism. It consists of two distinct moieties, the water-soluble F_1 complex carrying the catalytic activity for ATP synthesis and hydrolysis [1] and the membrane-integrated F_o complex acting as an ion translocator [2,3]. Due to its highly conserved structure, even between distantly related groups of organisms, the ATP synthase complex has been characterized from various sources and is therefore well suited for comparative analyses.

The F_1 portion of the ATP synthase of *Streptomyces lividans* (SLF_1) has been isolated previously and a biochemical characterization revealed that the ATPase activity of SLF_1 was exclusively stimulated in the presence of high concentrations of Ca^{2+}, whereas other divalent cations failed to stimulate SLF_1 [4]. Further studies revealed that SLF_1 could not be cross-reconstituted to F_1-stripped everted membrane vesicles of *Escherichia coli*, and that the immunological cross-reactivity between both species remained limited to subunit β [4].

Using oligonucleotide probes derived from the N-terminal amino acid sequences of SLF_1 subunits, the *atp* operon encoding the subunits of the ATP synthase of *S. lividans*

Abbreviations: ACMA, 9-amino-6-chloro-2-methoxy-acridine; DCCD, *N,N'*-dicyclohexylcarbodiimide; ECF_1F_o, ATP synthase of *Escherichia coli*; MEGA-9, nonanoyl-*N*-methylglucamide; SDS-PAGE, sodium dodecylsulphate/urea/polyacrylamide gel electrophoresis; SLF_1F_o, ATP synthase of *Streptomyces lividans*; TTFB, 4,5,6,7-tetrachloro-2-trifluoromethyl-benzimidazole; U, μmol P_i/min.

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was cloned and sequenced [5]. The arrangement of the genes resembled that of other non-photosynthetic eubacteria like *Bacillus* sp. or *E. coli*. Phylogenetic calculations based on the DNA sequence of the highly conserved subunit β of *S. lividans* and various other bacterial species indicated a high evolutionary distance between *S. lividans*, as a representative of the Gram-positive group with high G + C content of the DNA, and the group of Gram-positive species with low G + C content like *Bacillus* sp. Furthermore, *S. lividans* was found to be in juxtaposition to the rhodobacteria branch.

Throughout this work, methods have been developed and employed for the characterization of membrane-dependent processes in streptomycetes. The availability of everted membrane vesicles allowed the study of bioenergetic processes in streptomycetes. Furthermore, a comprehensive biochemical characterization of the membrane-bound ATP synthase of *S. lividans* (SLF_1F_o), its isolation, and its reconstitution was performed. The biochemical properties of SLF_1F_o revealed distinct similarities to the enzyme complexes of bacilli and rhodobacteria underlining their phylogenetic relationship.

2. Materials and methods

2.1. Chemicals

Tryptic soy broth was a product of Difco. Soybean asolectin (phosphatidylcholine type II-S), oligomycins, ACMA, valinomycin, *n*-octyl-β-D-glucopyranoside, and reagents for the ATPase assay were obtained from Sigma. The detergents MEGA-9 and Aminoxid WS-35 (acyl (C11-C17)-amidopropyldimethylaminoxide) were obtained from Oxyl Chemie and Goldschmidt, respectively. The chromatography media Sepharose CL-6B and Fractogel TSK-DMAE were obtained from Pharmacia and Merck, respectively. TTFB was kindly provided by Dr. H.U. Schairer (Universität Heidelberg). Other chemicals were of analytical grade and obtained from Merck or Riedel de Haën.

2.2. Bacterial strains and growth

Streptomyces lividans 66 strain 1326 kindly donated by Dr. H. Schrempf (Universität Osnabrück) and other *Streptomyces* spp. obtained from the 'Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH' (Braunschweig) were grown in tryptic soy broth media (30 g/l) at 30°C with vigorous aeration. For the preparation of protoplasts *Streptomyces* spp. were grown in baffled flasks with an agitation of about 180 rpm. Mycelia were pelleted at $7300 \times g$ for 20 min and washed in 50 mM Tris-HCl (pH 7.5) and 10 mM $MgCl_2$.

2.3. Preparation of membranes

Washed *Streptomyces* spp. mycelia were resuspended in buffer 1 (50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10% (v/v) glycerol, 5 mM 4-aminobenzamidine, 0.2 mM phenylmethylsulfonyl fluoride, 0.2 mM dithiothreitol) containing 1 mg/ml lysozyme and traces of DNase I, and protoplasts were formed at 37°C in 30 min. Protoplasts were collected at $14000 \times g$ for 20 min and washed twice in buffer 1. Subsequently, protoplasts (about 10 mg/ml protein) were disrupted by sonication using the plate of a Branson Sonifier B15 for three periods of a 1-min burst (50% duty cycle at 70% output) in an ice bath under a stream of nitrogen. After disruption cell debris were removed by centrifugation at $9300 \times g$ for 20 min. Subsequently, the membranes were pelleted at $190000 \times g$ for 90 min. The pellet was washed again and stored in liquid nitrogen after resuspension in buffer 1.

2.4. Purification of SLF_1F_o

Membranes of *S. lividans* prepared as described above were pelleted and homogenized in 20 mM MES-Tricine-NaOH (pH 7.5), 5 mM monothioglycerol, 1 mM $MgCl_2$, and 2% (w/v) MEGA-9 at about 30 mg/ml membrane protein and incubated on ice for 30 min. Solubilized material was recovered by centrifugation (30 min, $490000 \times g$) and 500 μl of the supernatant were layered on top of a linear glycerol gradient from 10 to 30% (v/v) in 10 mM MES-Tricine-NaOH (pH 7.5), 1 mM $MgCl_2$, 1 mM monothioglycerol, 0.2% (w/v) MEGA-9. The gradient was developed for 5 h at $370000 \times g$. Fractions of 250 μl were recovered from the gradient, assayed for ATPase activity, checked by SDS-PAGE, and stored in liquid nitrogen.

An alternative method used for the purification of SLF_1F_o involved gel filtration and ion exchange chromatography on an FPLC system (Pharmacia). Membranes of *S. lividans* were homogenized at a protein concentration of 20 mg/ml in 50 mM Tris-HCl (pH 7.0), 100 mM NaCl, 20% (v/v) glycerol, 6 mM 4-aminobenzamidine, 1 mM $MgCl_2$, 2 mM β-mercaptoethanol, 1% (v/v) Aminoxid WS-35, 100 μg/ml soybean asolectin and stirred for 30 min at room temperature. After removal of unsolubilized material (30 min, $490000 \times g$), the supernatant (20 ml) was applied to a Sepharose CL-6B column (1.6 × 100 cm) equilibrated with 50 mM Tris-HCl (pH 7.0), 100 mM NaCl, 20% (v/v) glycerol, 1 mM 4-aminobenzamidine, 1 mM $MgCl_2$, 2 mM β-mercaptoethanol, 0.1% (v/v) Aminoxid WS-35, 100 μg/ml soybean asolectin. The column was developed with a constant flow rate of 20 ml/h at 4°C. Fractions containing ATP-hydrolyzing activity were pooled and applied onto a Fractogel TSK-DMAE column (15 × 1 cm; Merck, Darmstadt) equilibrated with the same buffer. An NaCl gradient from 100 to 400 mM in the same buffer was applied and the SLF_1F_o complex was

eluted at about 300 mM NaCl with a constant flow rate of 1 ml/min at room temperature. The purified ATP synthase was stored in liquid nitrogen.

2.5. Reconstitution of SLF_1F_o

For the formation of liposomes, soybean asolectin (purified as described [6]) was dissolved at a concentration of 50 mg/ml in 50 mM Tricine-KOH (pH 7.5), 5 mM $MgCl_2$, 1 mM dithiothreitol, 1% (w/v) octylglucoside by sonication in an ice bath under a stream of nitrogen. Fractions of purified SLF_1F_0 (maximally 500 μl, about 1 mg/ml protein) were added to 500 μl of the phospholipid solution and incubated on ice for 20 min with occasional shaking. For formation of proteoliposomes the suspension was diluted with 70 ml of the same buffer without detergent and stirred for 5 min at room temperature. Subsequently, the proteoliposomes were recovered by centrifugation (2 h, 190000 × g) and resuspended in 500 μl of 50 mM Tricine-KOH (pH 7.5), 1 mM $MgCl_2$, 1 mM dithiothreitol.

2.6. Assays

The ATP-hydrolyzing activities and protein concentrations were determined as described [4,7]. ATP synthesis with everted membrane vesicles of *S. lividans* was essentially measured as described [8].

ACMA fluorescence quenching was measured as described [9]. Everted membrane vesicles of *S. lividans* (200–500 μg membrane protein), and proteoliposomes (0.5–1 mg phospholipid) were suspended in 20 mM Tricine-KOH (pH 7.5), 200 mM KCl, and various amounts of $MgCl_2$ or $CaCl_2$ to a final volume of 2 ml. ACMA was added to a final concentration of 2 μM and the assay was allowed to equilibrate at 37°C. For energization of membranes different substrates were added as indicated and, subsequently, the proton gradient was dissipated by addition of 2 μM TTFB (in ethanol).

3. Results

3.1. Preparation and characterization of membrane vesicles

A method involving the sonic disruption of protoplasts allowed the preparation of everted membrane vesicles of *Streptomyces* spp. An indication that these vesicles represent closed compartments is given by analysis of ATP synthesis rates (not shown) and generation of an electrochemical proton gradient ($\Delta\tilde{\mu}_{H^+}$) upon energization with various substrates (compare Fig. 3). It should be mentioned that the preparation of everted membrane vesicles by this method is applicable to a variety of *Streptomyces* species, as *S. coelicolor, S. griseus, S. viridochromogenes*, and *S. diastatochromogenes* (not shown).

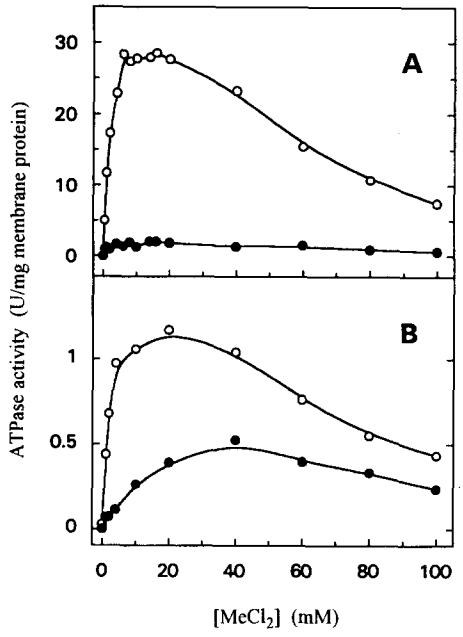

Fig. 1. Ion requirement of the ATPase activity. The specific ATPase activities of SLF_1 (A) and of everted membrane vesicles of *S. lividans* (B) were determined in the presence of different amounts of $MgCl_2$ (●) and $CaCl_2$ (○). The assay was performed under standard conditions with an ATP concentration of 1 mM as described [4].

3.2. Ion requirement of ATPase activity

For the initial characterization of the ATP synthase of *S. lividans*, the ATPase activity of everted membrane vesicles was determined. Specific activities of 1.2 U/mg membrane protein and 0.4 U/mg were determined in the presence of 10 mM $CaCl_2$ and 10 mM $MgCl_2$, respectively. However, the stimulation of the membrane-bound ATPase activity of *S. lividans* by divalent metal ions showed unusual characteristics. Whereas the ATPase activity of isolated SLF_1 was strictly dependent on the presence of $CaCl_2$ (Fig. 1A), membrane-bound ATPase activity was also observed in the presence of $MgCl_2$, with a broad maximum between 20 mM and 60 mM $MgCl_2$ (Fig. 1B). The Mg^{2+}-dependent ATPase activity was found to be only 30–50% of the activity determined using $CaCl_2$ as stimulating metal ion (Fig. 1B). Other divalent metal ions did not stimulate the ATP hydrolysis of the membrane-bound ATPase activity (data not shown).

3.3. Effects of inhibitors on ATPase activity

Specific inhibitors allow the classification of F-, V-, and P-type ATPases [10]. In general, the ATPase activity of the membrane-bound ATP synthase of *S. lividans* was inhibited by typical F-type inhibitors (Table 1). The sensitivity of the membrane-bound ATPase activity of *S. lividans* to

Table 1
Effects of inhibitors on the membrane-bound ATPase activity

Inhibitor	Inhibitor concentration (mM)	Residual ATPase activity (%)
N-ethylmaleimide	0.01	86
7-chloro-4-nitrobenz-2-oxa-1,3-diazole	0.10	69
quercetin	0.01	18
fluorescein isothiocyanate	0.10	30
erythrosin B	0.01	36
p-hydroxymercurybenzoate	0.01	28
tributyltin chloride	0.10	25
o-vanadate (sodium salt)	0.01	100
azide (sodium salt)	1.00	55
N,N'-dicyclohexylcarbodiimide	0.04	15
nitrate (sodium salt)	10.00	62
bafilomycin A_1	0.05	58
venturicidin	0.01	20
tentoxin	0.01	100
aurovertin	0.01	94

Everted membrane vesicles of *S. lividans* were preincubated with different amounts of ATPase inhibitors for 20 min at room temperature. Subsequently, the ATPase activity was determined under standard conditions in the presence of 10 mM $CaCl_2$. Inhibitors dissolved in dimethylsulfoxid or methanol were added in aliquots of less than 1% of the volume of the preincubation mixture. Activities were expressed as percentage of activity without inhibitor (100% activity = 1.1 $U \times min^{-1} \times (mg\ membrane\ protein)^{-1}$). Controls contained the same volume of the solvents. Only activities determined in the presence of relevant concentrations of inhibitors are shown.

inhibitors, which reacted with the F_1 part of ATP synthase complexes, resembled that of isolated SLF_1 [4]. However, the ATP synthase of *S. lividans* was not inhibited by aurovertin and tentoxin, although ECF_1F_o and mitochondrial F_1F_o were significantly sensitive towards aurovertin [11], whereas tentoxin is a typical inhibitor of chloroplast F_1F_o [12]. In its membrane-bound form the ATP synthase of *S. lividans* was sensitive towards typical F_o inhibitors like DCCD, venturicidin, and tributyltin. Venturicidin was a very effective inhibitor. The ATPase activity (Table 1), the ATP synthesis reaction (not shown), as well as the ATP-dependent H^+ translocation (not shown) were inhibited by venturicidin concentrations as low as 0.1 μg/ml. Furthermore, SLF_1F_o was found to be highly sensitive towards all oligomycins (Fig. 2; compare also Fig. 3) determining a K_i value of about 10–20 pg/ml (not shown). This sensitivity towards oligomycin was also found for the ATPase activity of other *Streptomyces* spp. (not shown). As expected, the ATPase activity was insensitive towards *ortho*-vanadate, a typical inhibitor of P-type ATPases. However, nitrate and bafilomycin A_1, common inhibitors for V-type ATPases, had a weak inhibitory effect on SLF_1F_o (Table 1). This is in contrast to ECF_1F_o, which is completely insensitive towards bafilomycins even in the presence of high concentrations (up to 1 mM) [13].

3.4. Energy-dependent H^+ translocation

The energization of everted membrane vesicles of *S. lividans* was assayed by ACMA fluorescence quenching (Fig. 3). The energization of membrane vesicles could be performed by ATP (Fig. 3A), as well as by NADH (Fig. 3C) and succinate (not shown), but not with lactate (not shown). Whereas ATPase activity could be observed in the presence of Mg^{2+} and Ca^{2+} ions, ATP-dependent H^+ translocation was strictly dependent upon the presence of $MgCl_2$. After substitution of $MgCl_2$ by $CaCl_2$ (1 to 10 mM), no ATP-dependent ACMA fluorescence quenching could be detected (Fig. 3B). However, the presence of Ca^{2+} in addition to Mg^{2+} in the assay buffer did not inhibit the H^+ translocation into the vesicles (not shown). The ATP-dependent H^+ translocation could be inhibited by the addition of inhibitors specific for F-type ATPases like oligomycin (Fig. 3D), DCCD, and venturicidin (not shown).

3.5. ATP synthesis

Everted membrane vesicles of *S. lividans* were also capable of ATP synthesis obtaining an ATP synthesis rate of 22 nmol $ATP \times min^{-1} \times mg^{-1}$ membrane protein. After energization of the membranes with succinate or, to a lesser extent, with NADH, a proton motive force ($\Delta\tilde{\mu}_{H^+}$) was generated by the respiratory chain, which could be utilized for the synthesis of ATP by SLF_1F_o. Again, this function of the ATP synthase was strictly dependent upon the presence of $MgCl_2$. No synthesis occurred when only $CaCl_2$ was present, whereas the addition of both divalent cations reduced the synthesis activity. The presence of ATP synthase inhibitors significantly diminished the ATP

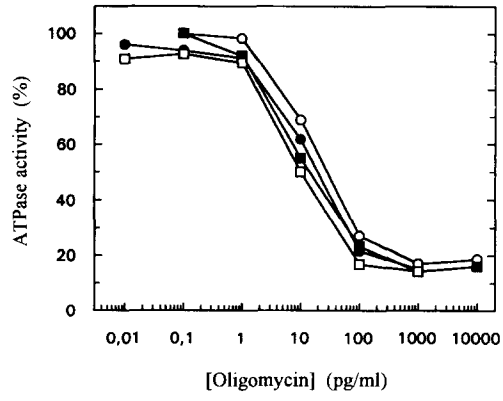

Fig. 2. Inhibition of the membrane-bound ATPase activity by oligomycin. The ATPase activity of *S. lividans* everted membrane vesicles was determined in the presence of different concentrations of oligomycins as described in the legend of Table 1. ATPase activities were expressed as percentage of activity without addition of oligomycin (100% activity = 1.2 $U \times min^{-1} \times (mg\ membrane\ protein)^{-1}$). (●) a mixture of oligomycins A, B, and C, (○) oligomycin A, (■) oligomycin B, and (□) oligomycin C.

synthesis rate. Furthermore, disintegration of $\Delta\tilde{\mu}_{H^+}$ by addition of the protonophore TTFB resulted in an inhibition of ATP synthesis (not shown).

3.6. Purification of SLF_1F_o

The application of a procedure recently published by Moriyama et al. [14] allowed the purification and the reconstitution of a functional ATP synthase of *S. lividans*. In contrast to the protocol developed for the ATP synthase of *E. coli*, 2% (w/v) MEGA-9 were used for the solubilization of high amounts of intact ATP synthase complexes (Fig. 4, lane C). The yield of the purification procedure is summarized in Table 2. The purification protocol did not allow the complete removal of contaminating proteins of about 66–70 kDa.

Whereas the N-terminal amino acids of the SLF_1 subunits have been determined [5], N-terminal sequencing of the SLF_o subunits was not possible so far. Therefore, the designation of SLF_o subunits suggested in Fig. 4, especially that of subunit *a*, remains tentative. Due to the highly hydrophobic character of subunit *a*, an abnormal migration with respect to the apparent molecular mass in SDS-PAGE and a weak staining by Coomassie brilliant blue might be expected. This has also been observed for the corresponding subunit of other species [15–17].

An alternative procedure for the purification of SLF_1F_o involving solubilization with Aminoxid WS-35, gel chromatography, and FPLC ion exchange chromatography allowed the purification of the ATP synthase without contaminating proteins (Fig. 4, lane B). The presence of 20% (v/v) glycerol as stabilizing agent in this procedure was indispensable to prevent the disintegration of the SLF_1F_o complex, but interferred with the chromatography due to the viscosity of the buffers. However, with these SLF_1F_o preparations a successful reconstitution of the enzyme complexes into phospholipid vesicles could not be achieved. Nevertheless, the analysis of the isolated SLF_1F_o complexes indicated a subunit composition for the F_o complex similar to that of *E. coli* and other eubacteria consisting of subunits *a*, *b*, and *c*.

3.7. Reconstitution of SLF_1F_o

SLF_1F_o purified by glycerol gradient centrifugation was reconstituted into phospholipid vesicles by means of the detergent dilution technique [18,19]. Proteoliposomes obtained after reconstitution were analysed by ATP-driven H^+ translocation via ACMA fluorescence quenching (Fig. 5). As in native membranes, no H^+ translocation of the reconstituted SLF_1F_o complex could be measured when only $CaCl_2$ (1 to 20 mM tested) was present (Fig. 5B). However, an ACMA fluorescence quenching of 70% could be observed in a buffer containing 10 mM $MgCl_2$ (Fig. 5A). The additional presence of $CaCl_2$ in the assay buffer resulted in an inhibition of the H^+ translocation ($CaCl_2$ >

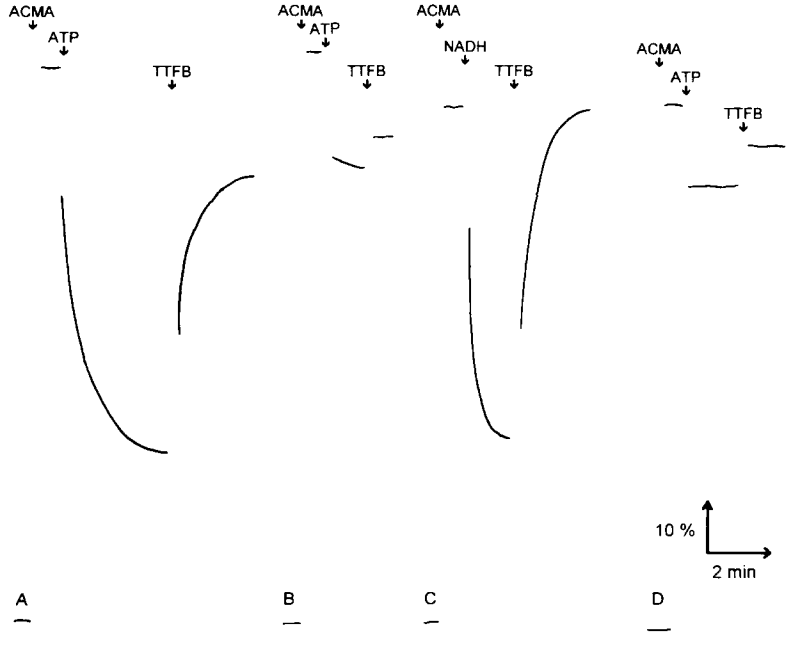

Fig. 3. ACMA fluorescence quenching assay of everted membrane vesicles of *S. lividans*. Everted membranes of *S. lividans* (about 200 μg membrane protein) were assayed for the energy-dependent ACMA fluorescence quenching as described in Section 2. Energization of the membranes was accomplished by the addition of 2 mM ATP (A, B, D) or 5 mM NADH (C). The assay contained 10 mM $MgCl_2$ (A, C, D) or 10 mM $CaCl_2$ (B). The effect of the ATP synthase inhibitor oligomycin (1 μg/ml) was assayed after a preincubation time of 5 min at room temperature (D). The addition of the same aliquot of solvent as used for the addition of the inhibitor had no effect on the ACMA fluorescence.

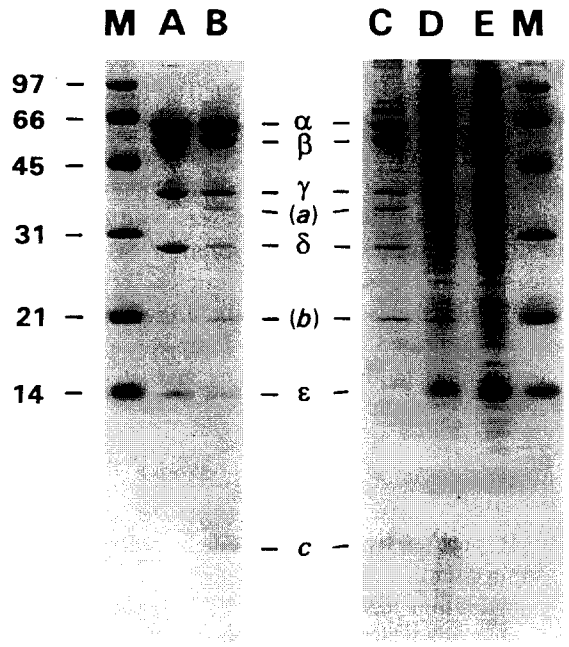

Fig. 4. SDS-PAGE of SLF_1F_o. The isolated SLF_1F_o prepared by both purification methods were subjected to gel electrophoresis on 13% polyacrylamide gels in the presence of 6 M urea [36] and stained with Coomassie brilliant blue G250. The individual lanes represent: Molecular mass marker (M), isolated SLF_1 (A), SLF_1F_o (ion exchange chromatography) (B), SLF_1F_o (glycerol density gradient centrifugation) (C), crude solubilizate (D), and everted membrane vesicles of *S. lividans* (E). The designation of the bands corresponding to subunits *a* and *b* of SLF_o remains tentative. The molecular masses ($\times 10^3$) of the marker proteins is indicated.

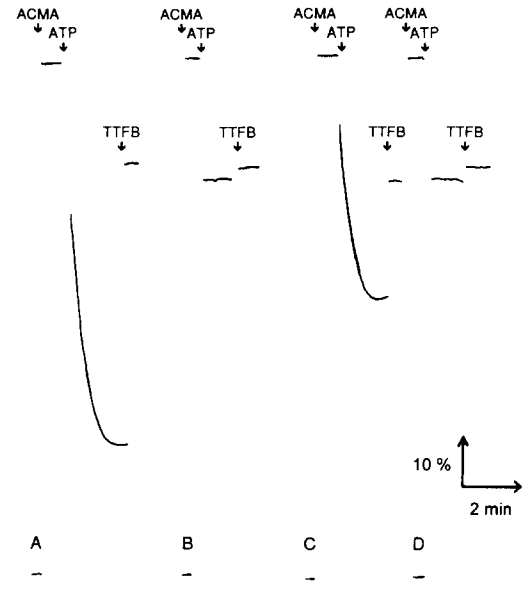

Fig. 5. ACMA fluorescence quenching assay of SLF_1F_o proteoliposomes. Liposomes reconstituted with SLF_1F_o purified by glycerol density gradient centrifugation were assayed for ATP-dependent H^+ translocation. Proteoliposomes prepared as described in Section 2 (about 0.5 mg phospholipid) were suspended in ACMA buffer and preincubated for 5 min at room temperature in the presence of 100 ng/ml valinomycin. The measurements were performed as described in the legend of Fig. 3 in the presence of divalent cations: 10 mM $MgCl_2$ (A, C, D) or 10 mM $CaCl_2$ (B). The effect of ATPase inhibitors was studied in the presence of 10 μg/ml oligomycin (C) or 1 μg/ml venturicidin (D). After the addition of the inhibitors, the mixtures were preincubated for 5 min at room temperature. Preincubations in the presence of the same amounts of solvent had no effect on the ACMA fluorescence quenching rates. H^+ translocation was started by the addition of 2 mM ATP as indicated.

10 mM) (not shown). This observation is consistent with the influence of $CaCl_2$ on the ATP synthesis reaction with everted membrane vesicles.

The Ca-ATPase activity of reconstituted SLF_1F_o was in the range of 1–2 U/mg, whereas the Mg-ATPase activity was about 0.5 U/mg SLF_1F_o. The difference of both activities was comparable to that of native membranes, although the activities are considerably low compared to the activities of isolated SLF_1F_o (compare Table 2 for Ca-ATPase activity). Nevertheless, the ATP-driven, H^+-translocating activity (Fig. 5) indicated the integrity of the reconstituted SLF_1F_o complexes.

After reconstitution the sensitivity of SLF_1F_o to inhibitors, like venturicidin (Fig. 5D) and DCCD (not shown), resembled that observed in everted membrane vesicles. However, a significant loss of the oligomycin sensitivity of reconstituted SLF_1F_o was observed. Significant inhibition of the ATP-dependent H^+ translocation (about 50% reduction of ACMA quenching) was obtained using concentrations of 10 μg/ml oligomycin (Fig. 5C), a 10-fold higher concentration compared to native membranes. However, a similar decrease in oligomycin sensitivity was also observed during the purification of F_1F_o from bovine heart mitochondria [20].

Table 2
Purification of SLF_1F_o by glycerol density gradient centrifugation

Source	Activity (U)	Protein (mg)	Specific activity (U/mg)	Recovered activity (%)	Purification (-fold)
Membranes	112	140	0.8	-	-
MEGA-9 extract	63	37	1.7	56	2.1
Glycerol gradient	40	10	4.0	36	5.0

For the quantification of recovered activity, the Ca-ATPase activity was determined as described [4].

4. Discussion

ATPase activity of *S. lividans* everted membrane vesicles could be detected in the presence of $CaCl_2$ and, to a lesser extent, in the presence of $MgCl_2$. Both ATP-hydrolysing activities were due to the ATP synthase (SLF_1F_o) as revealed by studies with specific inhibitors. In the presence of 10 mM $CaCl_2$ the hydrolytic activity of *S. lividans* membranes has the same order of magnitude as that of *E. coli* in the presence of 1 mM $MgCl_2$ (specific activity: about 1 U/mg membrane protein; compare [21,22]). This result is consistent with previous descrip-

tions of Ca^{2+}-activated membrane ATPase activities of *S. griseus* [23] and *S. viridochromogenes* [24]. However, ATP-driven H^+ translocation and ATP synthesis were strictly dependent upon the presence of Mg^{2+}. In contrast, the Mg-ATPase activity of the ATP synthase in its membrane-bound form was lost in isolated SLF_1, whereas the Ca-ATPase activity remained unaltered [4]. Other characteristics, e.g., the substrate specificity or the effect of stimulating compounds and F_1 inhibitors were found to be similar for isolated SLF_1 as well as for the membrane-bound SLF_1F_o complex.

By comparing data available for ATP synthases of various other species with the data presented here, striking similarities could be found between the ATP synthases of *S. lividans*, the photosynthetic bacterium *Rhodospirillum rubrum*, and *Bacillus* spp. The effects of divalent metal ions on the ATP synthase of *R. rubrum* and *Bacillus* spp. have recently been described [19,25–30]. In all cases the isolated F_1 complex and the membrane-bound ATP synthase exhibited high Ca-ATPase activities, whereas the Mg-ATPase activities were very low unless stimulated or 'unmasked' by the addition of detergents, hydrophobic compounds or methanol. Furthermore, the Ca-ATPase activity of the enzymes was inhibited by the addition of Mg^{2+}, whereas the ATP-driven H^+ translocation was strictly dependent on the presence of Mg^{2+} [25,26,30]. Most of the above-mentioned features apply also to the ATP synthase of *S. lividans*. The effect of divalent cations on the stimulation of the ATPase activity is very similar, although the concentrations required for the stimulation of SLF_1F_o are higher. As for the ATP synthases of *R. rubrum* and *Bacillus* strains, the ion required for the physiological functions (ATP synthesis and ATP-dependent H^+ translocation) of SLF_1F_o is clearly Mg^{2+}. However, none of the various compounds tested was capable of unmasking the Mg-ATPase activity of SLF_1, or to stimulate the Mg-ATPase activity of membranes (not shown; compare Ref. [4]). Nevertheless, the enzymatic characteristics of the ATP synthase of *S. lividans* revealed a close relationship to the ATP synthases of rhodobacteria and bacilli.

It is still difficult to evaluate the physiological significance of the remarkable effect of divalent cations on the ATP synthases of *R. rubrum* and *Bacillus* spp., as well as of *S. lividans*. Norling et al. [26] discussed the change in the cation specificity of the ATP synthase of *R. rubrum* as a result of conformational changes in the catalytic centre of the enzyme due to the different ion radii of Ca^{2+} and Mg^{2+}. The binding of the Ca-ATP complex to the nucleotide binding sites in F_1 promotes ATP hydrolysis, but fails to allow a conformational change required for H^+ translocation after ATP hydrolysis. Therefore, an intrinsic uncoupling of the ATP synthase by Ca^{2+} has been postulated. A similar explanation may also be valid for the ATP synthase of *S. lividans*. However, a further observation should be taken into consideration. Compounds interacting with the F_o complex like DCCD or oligomycin inhibited the Mg^{2+}-dependent, ATP-driven H^+ translocation as well as the Ca^{2+}-stimulated ATPase activity. This lends support to the notion that conformational changes induced by inhibitor binding to F_o (compare Refs. [31–33]) affect both the Mg-ATPase and the Ca-ATPase activity of F_1, whereas only the Mg-ATPase activity of F_1 give rise to conformational changes required for the translocation of protons through the F_o complex.

Another significant similarity of SLF_1F_o to the ATP synthase of rhodobacteria was detected by analysis of the inhibitor sensitivity. The enzyme of *S. lividans* was found to be susceptible to oligomycin inhibition. Oligomycin sensitivity has been described for the ATP synthases of mitochondria and rhodobacteria, but not for the ATP synthase of other bacteria and chloroplasts (see Ref. [11] and references therein). The mode of inhibition is not fully understood, but it is likely that a hydrophobic interaction between oligomycin and subunits of the F_o complex occurs. For the mitochondrial ATP synthase, an involvement of subunits 6 and 9 (equivalent to bacterial subunits *a* and *c*, respectively) was inferred from the analyses of oligomycin-resistant mutants of yeast (for review, see Ref. [34]). In case of *S. lividans*, preliminary experiments revealed that oligomycin inhibits the germination of *S. lividans* spores if applied to agar plates in a concentration of 1 $\mu g/ml$, demonstrating the relevance of the observed inhibitory effects in vitro. However, so far we were unable to isolate oligomycin-resistant mutants of *S. lividans*, which exhibit a change in the oligomycin sensitivity of their ATPase activity (S. Tötemeyer, M. Hensel, G. Deckers-Hebestreit and K. Altendorf, unpublished results).

The ATP synthase of *S. lividans* was found to be resistant to aurovertin. Lee et al. [35] reported that the amino acid residue Arg398 in subunit β of ECF_1 is involved in this effect. However, alignments of β subunits from various species indicated that the corresponding residue in subunit β of SLF_1 is also an arginine (compare Ref. [5]). This is remarkable, since Hicks and Krulwich [19] reported that the enzyme complex of bacilli is aurovertin-resistant, and pointed out that in the sequence of subunit β of this species phenylalanine was found at the position corresponding to Arg398 of *E. coli*.

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