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The F_1-type ATPase in anaerobic *Lactobacillus casei*

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An ATPase from anaerobic *Lactobacillus casei* has been isolated and 100-times purified. The 400 kDa enzyme molecule was found to have a hexagonal structure 10 nm in diameter composed of at least six protein masses. SDS-electrophoresis reveals four or, under certain conditions, five types of subunit, of apparent molecular masses 57 (α), 55 (β), 40 (γ), 22 (δ) and 14 (ε) kDa with stoichiometry of 3α, 3β, γ, δ, ε. The following features resembling F_1-ATPases from other sources were found to be inherent in the solubilized *L. casei* ATPase. (i) Detachment from the membrane desensitizes ATPase to low DCCD concentrations and sensitizes it to water-soluble carbodiimide. (ii) Soluble ATPase is inhibited by Nbf chloride and azide, is resistant to SH-modifiers and is activated by sulfite and octyl glucoside, the activating effect being much stronger than in the case of the membrane-bound ATPase. Substrate specificity of the enzyme is also similar to that of other factors F_1. Divalent cations strongly activate the soluble enzyme when added at a concentration equal to that of ATP. An excess of Mn^{2+}, Mg^{2+} or Co^{2+} inhibits ATPase activity of F_1, whereas that of Ca^{2+} induces its further activation. No other F_1-like ATPases are found in *L. casei*. It is concluded that this anaerobic bacterium possesses a typical F_1-ATPase similar to those in mitochondria, chloroplasts, aerobic and photosynthetic eubacteria.

Introduction

It is generally accepted that $\Delta\tilde{\mu}_{H^+} \leftrightarrow$ ATP interconversion is catalysed by the enzyme which is of the similar structural pattern in mitochondria, chloroplasts and respiring or photosynthesizing eubacteria. The enzyme in question, called H^+-ATP-synthase, is composed of two oligosubunit subcomplexes, i.e., F_0 and F_1, responsible for H^+ transfer and ATP synthesis (hydrolysis), respectively [1–22]. The Na^+-ATP-synthase from anaerobic bacterium *Propionigenum modestum* carrying out ATP synthesis at the expense of the decarboxylase-produced $\Delta\tilde{\mu}_{Na^+}$, proved to be also of the F_0F_1-type [23].

In all the above cases the main function of the F_0F_1 complex is to form ATP at the expense of $\Delta\tilde{\mu}_{H^+}$ (or $\Delta\tilde{\mu}_{Na^+}$). The reverse process (ATP $\rightarrow \Delta\tilde{\mu}_{H^+}$), which can be catalyzed by the same enzyme under certain conditions, is usually regarded as a reaction of small physiological significance.

The situation seems to be opposite in anaerobic glycolyzing bacteria. They utilize the glycolytic ATP to form $\Delta\tilde{\mu}_{H^+}$, which may then be used to support uphill transport of ions and metabolites. Here the ATP $\rightarrow$ $\Delta\tilde{\mu}_{H^+}$ transition is the main (or even the only) function of the enzyme which may be defined as H^+-ATPase [22]. It was shown that enzymes of the same function found in plant and fungi plasmalemma are of a quite different structure and mechanism as compared with an F_0F_1, whereas H^+-ATPases from tonoplast and the animal cell secretory granules are quite similar but not identical to the typical F_0F_1 (for review, see Ref. 22).

The study of H^+-ATPase from the facultatively anaerobic bacterium *Streptococcus faecalis* [24] revealed the subunit composition identical to F_0F_1 of other eubacteria. At the same time, H^+-ATPases of the strictly anaerobic bacterium *Clostridium pasteurianum* [25] and facultatively anaerobic *Lactobacillus casei* [26] were suggested to be of simpler subunit composition. It was reported that the *L. casei* enzyme contains six identical

Abbreviations: Mes, 2-morpholinoethanesulfonic acid; Mops, 3-morpholinopropanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; BSA, bovine serum albumin; DCCD, *N,N'*-dicyclohexylcarbodiimide; $\Delta\tilde{\mu}_{H^+}$, the proton electrochemical gradient; $\Delta\tilde{\mu}_{Na^+}$, the sodium electrochemical gradient; SDS, sodium dodecyl sulfate; Nbf-Cl, 7-chloro-4-nitrobenz-2-oxa-1,3-diazole; DTT, dithiothreitol; TEA, triethanolamine buffer.

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or very similar major subunits of 43 kDa [27] and one 25 kDa subunit [26]. Some peculiarities in catalytic properties of the *L. casei* H^+-ATPase have been mentioned [28].

However, Dunn et al. [29] recently reported that there is an immunological cross-reactivity between (i) α- and β-subunits of the *E. coli* F_1 and (ii) 51 and 60 kDa polypeptides in the *L. casei* SDS extract, respectively.

In this study we reinvestigated the problem of subunit composition and catalytic mechanism of factor F_1 from *L. casei*. To isolate F_1, a procedure was used which was much faster than that in the previous studies [26–28]. It was found that both structural and functional properties of the *L. casei* F_1 obtained this way proved to be very similar to those of other eubacterial H^+-ATPases. For a preliminary communication, see Ref. 30.

Materials and Methods

Chemicals

Mes, Mops, Tris, PMSF, DNAase I, diethylstilbestrol and Coomassie blue were purchased from Sigma (St. Louis, MO, U.S.A.); SDS, bovine serum albumin (BSA) and octyl glucoside were from Serva (Heidelberg, F.R.G.); ATP, ADP, GTP, ITP, UTP, pyruvate kinase, phospho*enol*pyruvate, acrylamide, methylene bisacrylamide were from Reanal (Budapest, Hungary); *N*-ethylmaleimide was obtained from BDH (Poole, U.K.); *N*-cyclohexyl-*N'*-(morpholinium ethyl)carbodiimide was from Merck (Darmstadt, F.R.G.); DCCD was from Fluka (Buchs, Switzerland); Agarose blue was bought from Reachim (Moscow, U.S.S.R.); Toyo-Pearl HW-60 F gel was from Toyo-Soda (Tokyo, Japan).

Bacterium and growth conditions

L. casei ATCC 7469 was kindly provided by Dr. E.I. Mileykovskaya. The culture was originally obtained from the museum of the Institute of Microbiology of the U.S.S.R. Academy of Sciences, Moscow. The culture was preserved by lyophilization. During the experimental work it was maintained with monthly transfer on tryptic soy agar columns as was recommended by Kihara and Snell [31]. *L. casei* was grown for experiments in static cultures at 37 °C in liquid medium containing 1% tryptone (Difco), 0.7% yeast extract (Difco), 0.5% glucose and 0.5% KH_2PO_4 (pH 7.0) in a hermetically plugged 2 litre vessel filled with liquid medium. The cells were harvested by centrifugation (11 000 × *g*, 10 min, 4 °C) and washed with medium containing 10 mM Mes-KOH (pH 6.0), 10 mM $MgSO_4$, 150 mM KCl, 1 mM PMSF. Washed cells were used immediately for isolation of membranes.

Preparation of membranes

The following procedures were carried out at 4 °C. Washed cells were resuspended (0.2–0.5 g of wet weight/ml) in the medium A containing 50 mM Mes-KOH (pH 6.0), 5 mM $MgSO_4$, 0.5 mM EDTA, 1 mM PMSF, DNAase I (20 μg/ml) and homogenized with Potter glass homogenizator. The cell suspension was passed through a French press cell at 700 kg/cm^2 three times. After this, unbroken cells were removed by centrifugation (11 000 × *g*, 10 min). The membrane fragments were sedimented at 200 000 × *g* for 50 min, washed twice in the medium A and resuspended to 5 mg of protein per millilitre in medium A containing 25% glycerol (v/w) above and stored in liquid N_2.

Extraction and purification of the F_1-ATPase

Extraction of ATPase was performed according to Beechey et al. [32]. The thawed membrane fragments washed in medium A at 4 °C were resuspended in the medium containing 1 mM Mops-KOH (pH 7.0), 1 mM EDTA, 1 mM PMSF to a final protein concentration 1 mg/ml. A half-volume of chloroform was added and the mixture was shaken vigorously for 30 s. The resulting emulsion was spun (2000 × *g*, 2 min) to divide chloroform and aqueous layers. The upper aqueous layer containing soluble ATPase was pipetted off and centrifuged at 200 000 × *g* for 1 h at 4 °C to remove membrane contamination. All the following procedures were performed at room temperature. The resulting supernatant (the crude F_1 extract) was adjusted to 5 mM $MgSO_4$ (final concentration) just prior to loading onto a Blue-agarose column (5 ml syringe containing 3 ml of gel) previously equilibrated with medium containing 2 mM Mops-KOH (pH 7.0), 5 mM $MgSO_4$. After loading the extract (at a rate of 3 ml/min), the column was washed with three bed volumes of equilibrating medium and the ATPase activity was eluted with the medium containing 2 mM Mops-KOH (pH 7.0), 10 mM EDTA at a flow rate of 3 ml/min. 9 vol. of medium containing 1 mM Mops-KOH (pH 7.0), 6 mM $MgSO_4$ were added to the fractions with the highest ATPase activity and fractionation was repeated with the second Blue-agarose column as described above. Fractions with eluted ATPase activity were combined, the pH of the partially purified enzyme solution was adjusted to 7.5 by adding 1 M KOH and then loaded onto a DEAE-Sephacel column (20 ml syringe containing 10 ml of gel) at a rate of 3 ml/min. This column was preequilibrated with the medium containing 10 mM Mops-KOH (pH 7.4), 0.5 mM EDTA and 10 mM Na_2SO_4. The column with the associated enzyme was washed with the equilibrating medium. The enzyme was eluted by a step gradient of Na_2SO_4 in equilibrating medium (50 mM to 1 M ammonium sulfate) at a flow rate of 0.1 ml/min. Peak ATPase fractions (0.4–0.5 M Na_2SO_4) were combined, dialyzed against the medium containing 10 mM Mops-KOH (pH 7.0), 1 mM EDTA and concentrated by ultrafiltration. The obtained F_1-solution (1 ml containing about 0.5–1 mg protein) was loaded onto a

column with Toyo-Pearl HW-60 F gel (2.5 × 90 cm) equilibrated with medium B (2 mM Mops-KOH (pH 7.0)/0.5 mM EDTA). The column was eluted with medium B at a flow rate of 66 ml/h. Fractions of the highest ATPase activity were combined, concentrated by ultrafiltration and stored at 4°C as an ammonium sulfate precipitate (corresponds to 60% of saturation).

ATPase assay

Hydrolysis of ATP was measured by liberation of P_i in medium C (50 mM Mes-KOH (pH 6.1)) or in medium D (50 mM Mops-KOH (pH 7.0)). The additional components are indicated in the figure or table legends. As an ATP-regenerating system, pyruvate kinase (25 μg/ml), 1 mM phospho*enol*pyruvate and 40 mM KCl were added to the measuring medium. The ATPase reaction was initiated by enzyme addition and the incubation was allowed to proceed for 10 min at 37°C. Reactions were terminated by the addition of SDS and EDTA to a final concentration of 1% and 5 mM, respectively. After this the P_i formed was determined according to Sumner [33].

Other methods

SDS-polyacrylamide gel electrophoresis with 13.5% polyacrylamide gel was performed according to the procedure of Laemmli [34]. For 7% polyacrylamide gel electrophoresis in non-dissociating conditions, the method of Davis was used [35]. The ATPase activity in polyacrylamide gels was revealed by the method [36]. The protein was assayed according to Lowry et al. [37] with BSA as standard. For electron microscopy, the preparations of isolated soluble F_1-ATPase (0.1 mg protein/ml) were negatively stained with 5% ammonium molybdate or 1% uranyl acetate solutions in water. The grids were examined in a Philips-400 electron microscope (80 kV).

Results

Purification and subunit composition of the factor F_1 from L. casei

Chloroform treatment of the *L. casei* membranes after Beechey's method [32] was found to solubilize about 70% of the membrane-bound ATPase activity. The rest was solubilized by means of the repeated chloroform treatments. 100-times purification of ATPase was achieved with Blue-agarose pseudo-affinity chromatography and subsequent DEAE-Sephacel chromatography (Table I). The obtained enzyme samples contained few contaminations (Fig. 1) which were removed with the polyacrylamide gel electrophoresis under non-denaturating conditions. The resulting prepara-

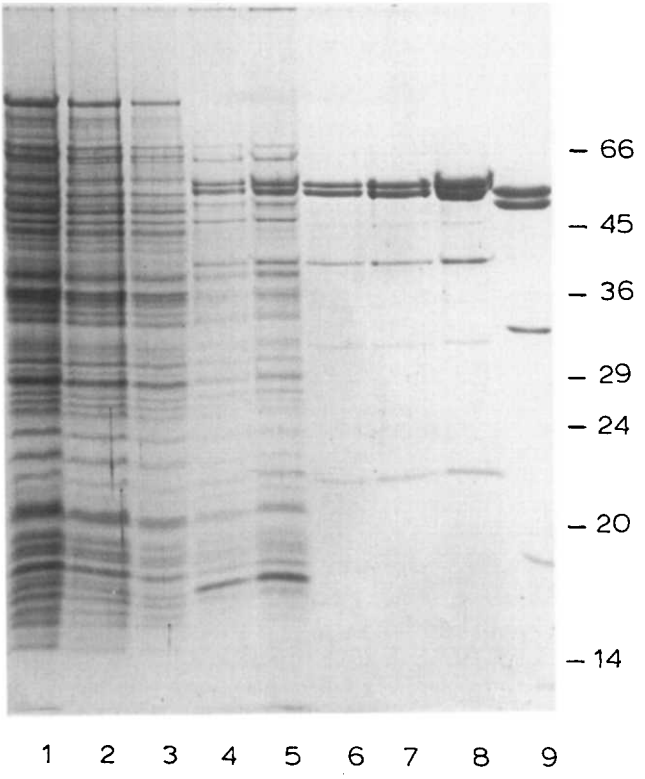

Fig. 1. SDS-13.5% polyacrylamide gel electrophoresis of the *L. casei* F_1-ATPase at different stages of purification. The *L. casei* culture used for purification F_1-ATPase was grown to the late logarithmic phase. Lanes 1–3, the *L. casei* membranes, 30, 20, 10 μg protein respectively. Lanes 4, 5, the crude F_1-extract (chloroform treatment), 5, 10 μg protein respectively. Lanes 6–8, the solubilized ATPase after the first blue-agarose column and following DEAE-Sephacel column, 1, 2, 4 μg protein, respectively. Lane 9, purified F_1-ATPase from bovine heart mitochondria, 3 μg protein.

TABLE I

Purification of the L. casei F_1-ATPase

Step	Protein (mg)	Activity [a] (μmol/min)	Specific activity (μmol/min per mg)	Yield (%)	Purification (fold)
1 Membrane fragments	80.8	42	0.52	100	1
2 Crude extract of the chloroform-released ATPase	9.65	30	3	71	5.8
3 Blue-agarose column	0.5	17	34	40.5	65
4 DEAE-Sephacel column	0.22	11.8	55	28	105

[a] The ATPase activity was measured in medium C containing 5 mM ATP, 5 mM $MgSO_4$, 10 mM Na_2SO_3 (membranes) or in medium D containing 5 mM ATP, 2 mM $MgSO_4$, 10 mM Na_2SO_3 (F_1).

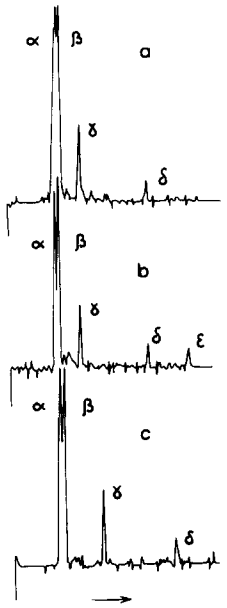

Fig. 2. Comparison of subunit composition of the *L. casei* F_1-ATPase obtained using different methods. Photometric scans at 580 nm of SDS-13.5% polyacrylamide gel electrophoregrams with the *L. casei* soluble ATPase stained with Coomassie blue G-250. (a) After purification of the ATPase on a Blue-agarose column followed by 7% polyacrylamide gel electrophoresis in non-dissociating conditions. (b) After double purification on a Blue-agarose column. (c) The purified F_1-ATPase from bovine heart mitochondria. In the cases (b) and (c) 2.5 and 3 μg protein were loaded on lanes respectively.

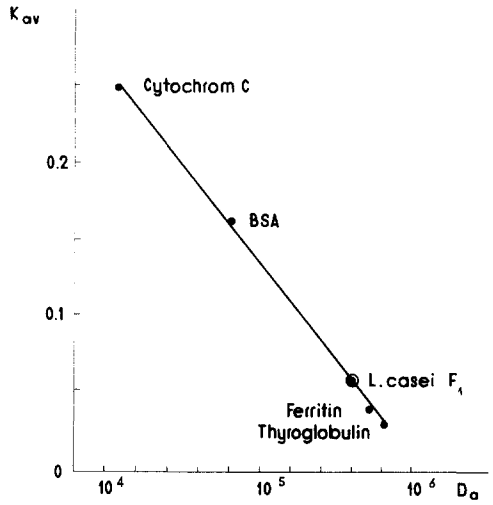

Fig. 3. Estimation of the molecular mass of the *L. casei* soluble ATPase by gel-filtration on a Toyo-Pearl HW-60 F gel column.

tion was found to contain four main subunits of the following apparent molecular masses (SDS-electrophoresis data): 57(α), 55(β), 40(γ) and 22(δ) kDa. A rough estimation of the subunit stoichiometry in the SDS-electrophoresis densitogram gave values 3α, 3β, γ, δ (Fig. 2a). Gel-filtration of the F_1-ATPase showed a single peak of about 400 kDa (Fig. 3). Electron microscopy of the isolated F_1 revealed standard hexagonal particles about 10 nm in diameter typical for soluble H^+-ATPase from other sources. No particles of other sizes were found (Fig. 4).

The above data indicate that a typical F_1-ATPase can be solubilized from the *L. casei* membranes. In the next series of experiments we studied possible existence of other F_1-like ATPases in the same system. As can be seen in Fig. 5, the ATPase activity was eluted as a single symmetric peak when any of the three methods was employed, i.e., gel-filtration, pseudoaffinity chromatography and ion-exchange chromatography. No ATPase

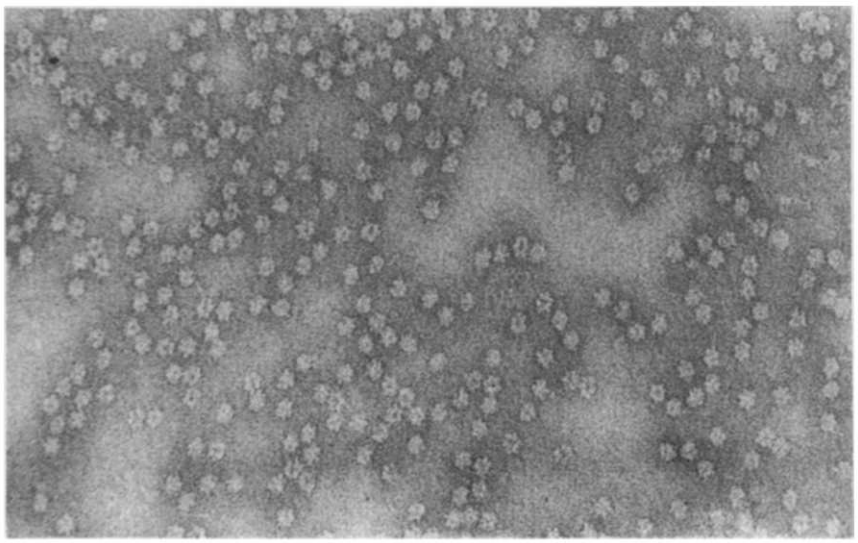

Fig. 4. Electron micrograph of negatively stained preparation of the *L. casei* purified F_1-ATPase.

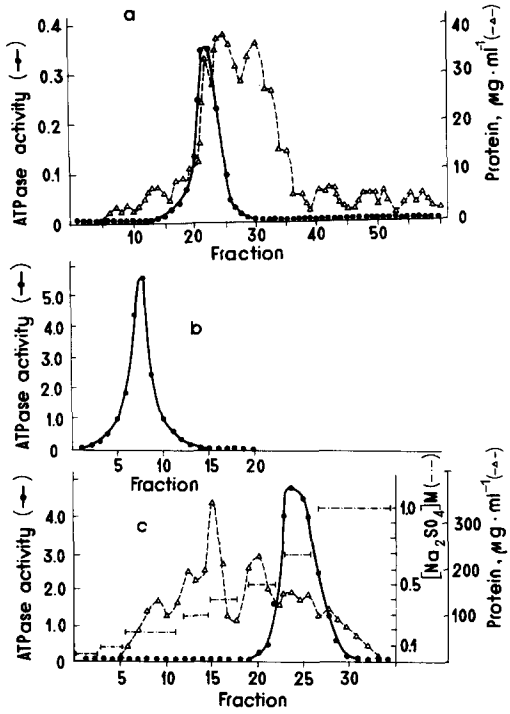

Fig. 5. The use of different methods for purification of the *L. casei* solubilized ATPase. (a) Gel-filtration on a Toyo-Pearl HW-60 F gel column. (b) Pseudoaffinity chromatography on a Blue-agarose column. (c) Ion-exchange chromatography on DEAE-Sephacel column. ATPase activity, $\mu mol \cdot min^{-1} \cdot ml^{-1}$.

activity was revealed in the protein fractions which were not bound to sorbents.

In Fig. 6 we compared the enzyme isolated from the membranes stored in liquid nitrogen or at $-20\,^{\circ}C$. It is seen that in the former case there is only one band with ATPase activity in polyacrylamide gel. At the same time, in the second case this single band tends to be split into two bands, the major one being somewhat lower than that of the enzyme stored in liquid nitrogen.

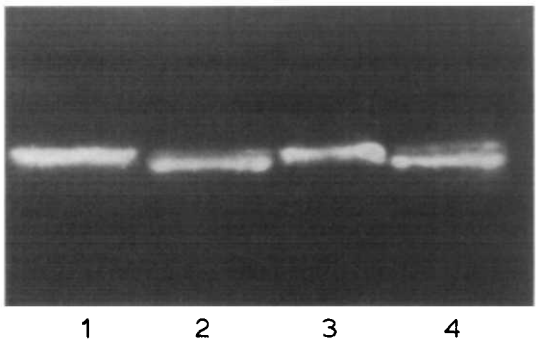

Fig. 6. 7% polyacrylamide gel electrophoresis of the *L. casei* soluble ATPase under non-dissociating conditions. Staining of gels for activity was performed as indicated in Materials and Methods. The *L. casei* soluble ATPase was obtained from membrane fragments stored for 1 month in liquid N_2 (lanes 1, 3), and at $-20\,^{\circ}C$ (lanes 2, 4). Lanes 1, 2, the purified *L. casei* soluble ATPase. Lanes 3, 4, the crude *L. casei* extract before purification.

SDS-electrophoresis of the factor F_1 from the membranes stored at $-20\,^{\circ}C$ failed to reveal any additional subunits. One may assume that the splitting of the band shown in Fig. 6 is due either to dissociation of minor subunit(s) from some of F_1 molecules or to proteolytic cleavage of small part(s) of major subunit(s).

In further experiments, the factor F_1 was extracted and purified from bacteria at various stages of their growth. In all the cases only one type of F_1 was observed. At the same time it was revealed that the amount of contaminations lacking any ATPase activity was lower at earlier stages of the growth. This fact allowed the method of F_1 purification to be simplified in such a way that pure F_1 was obtained by means of the 30 min procedure including only two Blue agarose fractionations. The resulting F_1 was shown to contain one additional (fifth) 14 kDa polypeptide. Stoichiometry of γ, δ and 5th polypeptide was 1 : 1 : 1 (Fig. 2b). The fifth band apparently corresponding to ε subunit was found to disappear during storage of isolated F_1 at room temperature, which was accompanied by a small increase in the ATPase activity calculated per mg of protein.

Catalytic properties of the L. casei F_1-ATPase

Both membrane-bound and solubilized *L. casei* ATPases were shown to hydrolyze ATP and, at a lower rate, GTP, ITP and UTP but not CTP and PP_i (Table II).

Mn^{2+}, Mg^{2+}, Ca^{2+} and Co^{2+} strongly activated the ATPase, Mn^{2+} being the most efficient. The cations other than Ca^{2+} inhibited the ATPase activity when added in excess to ATP. Excess of Ca^{2+} induced further activation of F_1-ATPase, but not of membrane-bound ATPase. The K_m of the F_1-ATPase measured in the presence of Mg^{2+} proved to be 0.15 mM. The pH

TABLE II

Substrate specificity of the membrane-bound and solubilized L. casei ATPase

The activity was measured in the medium containing 40 mM Mes-KOH (pH 6.1), 5 mM $MgSO_4$ (membranes) or 40 mM Mops-KOH (pH 7.0), 2 mM $MgSO_4$ (F_1). Substrate concentration, 5 mM.

Substrate	Activity (%)	
	membranes	F_1-ATPase
ATP	100 [a]	100 [b]
ADP	0	0
AMP	0	0
GTP	42	42
ITP	57	26
UTP	24	7
CTP	0	0
PP_i	0	0

[a] 0.4 μmol/min per mg protein.
[b] 30 μmol/min per mg protein.

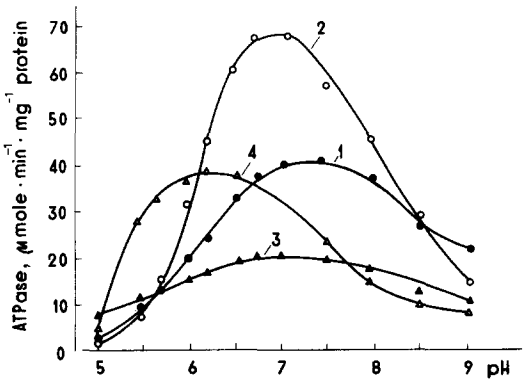

Fig. 7. The effect of pH on the *L. casei* isolated soluble ATPase. The activity was measured in the medium containing 40 mM Mes/40 mM Mops-Tris, 5 mM ATP and 2.5 mM $MgSO_4$ (1, 2), 10 mM $MgSO_4$ (3, 4), 10 mM Na_2SO_3 (2, 4).

optimum of F_1 was found to be shifted to the alkaline region as compared with the membrane-bound ATPase. A reverse shift was induced by sulfite, which moreover increased the ATPase activity at the optimal pH (Fig. 7 and Table III).

TABLE III

Effect of some ATPase inhibitors and activators on the L. casei ATPase

n.m., not measured.

Compound	Concentration	ATPase activity (%)	
		membranes [a]	F_1-ATPase [c]
–	–	100	100
N-Ethylmaleimide [e]	1 mM	66	90
N-Cyclohexyl-*N'*-(methyl morpholinium ethyl)carbodiimide [e]	2 mM	40	10
Nbf-Cl [g]	1 mM	n.m.	0
p-Chloromercuribenzoate	100 μM	n.m.	93
Oligomycin	100 μg/mg protein	70	n.m.
DCCD [f]	100 μM	15	90
NaN_3	2 mM	15 [b]	10 [d]
NaF	10 mM	15 [b]	10 [d]
Diethylstilbestrol	22 nM/mg protein	50	n.m.
Na_2SO_3	10 mM	150	300
Octyl glucoside	30 mM	240	400

The activity was measured in the medium containing 5 mM ATP and 7 mM $MgSO_4$ [a] or 20 mM $MgSO_4$ [b] (100% of activity, 0.3 μmol/min per mg protein), 2 mM $MgSO_4$ [c], or 10 mM $MgSO_4$ [d] (100% of activity, 30 μmol/min per mg protein and 23 μmol/min per mg protein, respectively). [e] The enzyme was preincubated in the presence of a reagent in Mops-Tris buffer (pH 7.0) during 30 min at 20 °C. [f] The same as in [e] but at 4 °C. [g] The enzyme was preincubated in the presence of TEA-buffer (pH 8.0) during 30 min at 20 °C. 10 mM DTT being added to the preincubation mixture was shown to restore 40% ATPase activity.

Inhibitors of the L. casei ATPase

Effects of some inhibitors and activators of the *L. casei* ATPase are shown in Table III. It is seen that this ATPase, like other enzymes of the F_0F_1-type, is sensitive to the low DCCD concentrations in the membrane-bound form but not in the solubilized form. At the same time, solubilization sensitizes the ATPase to a water-soluble carbodiimide derivative. The *L. casei* F_1-ATPase is insensitive to SH-reagents but sensitive to azide and Nbf-Cl, resembling in this respect other F_1-ATPases. The azide inhibition is promoted by the Mg^{2+} concentration increase.

Discussion

The above data seem to indicate that *L. casei* possesses a typical F_1-ATPase. Procedures developed to isolate F_1 were shown to result in purifying an approx. 400 kDa protein composed of two types of major subunit and two or three minor subunits. Apparent molecular masses of the subunits were 57, 55, 40, 22 and 14 kDa, subunit stoichiometry being 3α, 3β, γ, δ, ε. Electron micrographs of the negatively stained *L. casei* F_1-ATPase (Fig. 4) proved to be very similar to those described earlier for F_1 from mitochondria [38], chloroplasts [39] and eubacteria [40–42], i.e., the hexagonal structure (10 nm in diameter) was composed of at least six protein masses.

The fifth F_1 subunit was present only when a fast procedure was used to isolate F_1-ATPase from *L. casei* at an early logarithmic growth stage. At late phases of the growth some contaminations appeared which cannot be removed by the fast method of F_1 isolation. More complicated purification procedures resulted in that the ε subunit was lost, as was already described in studies on some other bacterial F_1-ATPases [40,41,43,44].

In the membrane-bound form the ATPase proved to be sensitive to low DCCD concentrations which were almost without effect upon solubilized enzyme, a property typical for F_0F_1-ATPases just as some other changes accompanying F_1 detachment from the membrane (stimulation of ATPase by an excess of Ca^{2+} and inhibition by that of Mg^{2+}, increase in the stimulating effects of sulfite and octyl glucoside, etc.) [22,45–47].

Our data are in agreement with the above-mentioned results of Dunn et al. [29] that the *L. casei* SDS extracts contains two proteins showing immunological cross-reactivity with two major subunits of the *E. coli* F_1. The larger *L. casei* protein proved to be immunologically related to the β-subunit of *E. coli* F_1, whereas the smaller one was related to the *E. coli* α-subunit [29]. Such an inversion of electrophoretic mobility was also described for major subunits of *B. subtilis* [29,48] and *M. luteus* [29] F_1-ATPases.

At the same time both our data and those of Dunn disagree with previous observations [26–28] on the ex-

istence in *L. casei* of an unusual F_1-like ATPase composed of six identical or very similar major subunits of 43 kDa and one minor subunit. In this connection three explanations may be considered. (i) There are two different F_1-type ATPases in *L. casei*. (ii) 43 kDa subunits are proteolytic products of larger molecular mass subunits. (iii) 43 kDa subunits belong to some other protein accompanying F_1 when it was isolated from bacterial membranes.

The first possibility is hard to exclude completely, but all of our attempts to reveal one more F_1-like ATPase in *L. casei* failed (Figs. 5 and 6).

The second explanation seems more probable, since we used much faster methods to isolate F_1 than in the above-mentioned study [26–28]. Moreover, it was found that storage of isolated F_1 under certain conditions indeed resulted in partial degradation of the enzyme (Fig. 6).

At the same time there are some data that support the third explanation. The catalytic activity of the F_1-ATPase in our experiments was 10–15-times higher than in Refs. 26–28. Nevertheless, other properties of F_1 in these two studies proved to be similar. We mean pH optimum (this paper, Fig. 7 and Ref. 27), relative efficiency of various cations in activation of ATP hydrolysis (this paper and Ref. 26), sensitivity to a water-soluble carbodiimide and Nbf-Cl and resistance to SH-Modifiers (Table III and Refs. 27, 28). As to the low azide sensitivity of the *L. casei* F_1 mentioned by Mileykovskaya et al. [28], this might be due to a rather low concentration of Mg^{2+} used by those authors. In our experiments the maximal azide inhibition was found to require 10 mM of Mg^{2+} at 5 mM ATP concentration. Moreover, we found that storage of the frozen F_1 samples for the long time resulted in decrease in the azide sensitivity.

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