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# **A Comparison of an ATPase from the Archaeobacterium Halobacterium Saccharovorum with the F<sub>1</sub> Moiety from the Escherichia Coli ATP Synthase**

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## SUMMARY

A purified ATPase associated with membranes from Halobacterium saccharovorum was compared with the F<sub>1</sub> moiety from the Escherichia coli ATP Synthase. The halobacterial enzyme was composed of two major (I and II) and two minor subunits (III and IV), whose molecular masses were 87 kDa, 60 kDa, 29 kDa, and 20 kDa, respectively. The isoelectric points of these subunits ranged from 4.1 to 4.8, which in the case of the subunits I and II was consistent with the presence of an excess of acidic amino acids (20 to 22 Mol%). Peptide mapping of sodium dodecylsulfate-denatured subunits I and II showed no relationship between the primary structures of the individual halobacterial subunits or similarities to the subunits of the F<sub>1</sub> ATPase (EC 3.6.1.34) from E. coli. Trypsin inactivation of the halobacterial ATPase was accompanied by the partial degradation of the major subunits. This observation, taken in conjunction with molecular masses of the subunits and the native enzyme, was consistent with the previously proposed stoichiometry of 2:2:1:1. These results suggest that H. saccharovorum, and possibly, Halobacteria in general, possess an ATPase which is unlike the ubiquitous F<sub>0</sub>F<sub>1</sub>-ATP Synthase.

## INTRODUCTION

The H<sup>+</sup>-translocating ATPase (ATP Synthase) which is found in the membranes from eubacteria, mitochondria, and chloroplasts (ref. 1) consists of two moieties; F<sub>0</sub>, which is integrated into the membrane and is associated with proton translocation and F<sub>1</sub>, which is an extrinsic membrane protein and contains the catalytic unit. In Escherichia coli, F<sub>1</sub> is composed of five different subunits, designated  $\alpha$ - $\epsilon$  in order of decreasing molecular mass. An intriguing question is whether the archaeobacteria, which are phylogenetically distant from both the eubacteria and the eukaryotes (ref. 2), possess an ATP Synthase. Several observations are consistent with the presence of such an enzyme in the archaeobacteria. ATP synthesis at the expense of proton gradients has been suggested to occur in the methanogenic bacteria (ref. 3), Halobacterium halobium (refs. 4, 5), and Sulfolobus acidocaldarius (ref. 6). The methanogenic bacterium strain Gö1 contains a membrane-bound ATPase which morphologically resembles an F<sub>1</sub>F<sub>0</sub>-ATP Synthase and reacts with an antiserum against the  $\beta$  subunit from the ATP Synthase from E. coli (ref. 7). On the other hand, none of the archaeobacterial ATPases have a subunit composition that resembles an F<sub>1</sub> ATPase (EC 3.6.1.34). The enzyme from Methanobacterium barkeri (ref. 8) consists of two subunits whose molecular masses are 62 kDa and 49 kDa. The ATPase from S. acidocaldarius is composed of 65 kDa and 51 kDa subunits which are thought to be analogous to the  $\alpha$  and  $\beta$  subunits found in F<sub>0</sub>F<sub>1</sub>-ATP Synthases (ref. 9). The membrane-bound ATPase from Halobacterium saccharovorum contains four subunits. The major ones, subunits I and II, have molecular masses of 87 kDa and 60 kDa; the molecular masses of the minor ones, subunits III and IV, are 29 kDa and 20 kDa (ref. 10). We have described the purification and several of the properties of this membrane-bound ATPase from H. saccharovorum (refs. 10-13). The enzyme is inhibited by N,N'-dicyclohexylcarbodiimide (DCCD), 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole, and competitively inhibited by ADP (ref. 11). The conditions for DCCD inhibition of the halobacterial enzyme are analogous to those that result in the inhibition of F<sub>1</sub> ATPase activity (ref. 14). In the latter case, inhibition is accompanied by the binding of the inhibitor to a glutamyl residue (ref. 15) in a highly conserved region of the  $\beta$  subunit (ref. 16). DCCD inhibition of the

halobacterial ATPase activity is associated with the binding of DCCD to subunit II and it may be that the  $\beta$  subunit and subunit II are homologous. Recently, Nanba and Mukohata (ref. 17) reported that a membrane-bound ATPase from *H. halobium* is composed of two subunits whose molecular masses are 86 kDa and 64 kDa.

While the halobacterial ATPases appear to be structurally different from  $F_0F_1$ -ATPases, there is evidence to suggest a degree of relatedness. Polyclonal antisera against the  $\beta$  subunit of the ATPase from *S. acidocaldarius*, which reacted with the  $\beta$  subunits from *E. coli*, chloroplast, and beef heart mitochondria and a 62 kDa peptide from *H. halobium* membranes (ref. 18), strongly reacted with subunit II from *H. saccharovorum* (M. Lübben, personal communication). This, and the analogous conditions required to demonstrate DCCD inhibition in the halobacterial and  $F_1$ -ATPases, suggested that, in spite of the apparent differences in subunit molecular masses, the halobacterial enzyme may indeed be a  $F_0F_1$ -type ATP Synthase. The bulk proteins of the Halobacteria, as typified by *Halobacterium salinarum*, are acidic (ref. 19), as are those halobacterial enzymes which have been analyzed (refs. 20, 21), so there was good reason to suspect that the halobacterial ATPase was an acidic protein. Because the mobilities of highly acidic proteins in SDS gels may be anomalous (refs. 22, 23), there was some reason to question the apparent differences in the molecular masses of subunits derived from the halobacterial and  $F_1$  ATPase. Therefore, we reexamined the halobacterial ATPase in more detail and compared it with the  $F_1$  ATPase obtained from *E. coli*. Here we report the results of such experiments which support the earlier notion that the halobacterial enzyme is structurally different from  $F_1$ -type ATPases.

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## MATERIALS AND METHODS

### Chemicals

The following materials were obtained from the indicated companies: polyoxyethylene sorbitane monopalmitate (Tween 40), cetyltrimethyl ammonium bromide (CTAB), soybean trypsin inhibitor, iodoacetic acid (Sigma Chemical Company); Pharmalyte pH 2.5-5 (Pharmacia); TPCK-trypsin (Worthington);  $\alpha$ -chymotrypsin (Calbiochem); BioRad protein assay reagent and IEF-standards (BioRad); Fluorescamine (Fluram) (Roche Diagnostics); and V8 protease (Miles Laboratories).

### Purification of the Halobacterial ATPase

The ATPase from *H. saccharovorum* (ATCC 29252) was prepared as described previously (ref. 12). Following ammonium sulfate-mediated chromatography on DEAE, the enzyme was centrifuged through a sucrose gradient constructed by layering 1 mL of each 1 M  $(NH_4)_2SO_4$  - 50 mM Tris-HCl- pH 8.0 buffer that was 25, 22.5, 20, 17.5 and 15% with respect to sucrose. After centrifugation at  $210\,000 \times g$  for 18 h at 23°C, 0.4 mL fractions were collected and assayed for ATPase activity. The peak fraction of the sucrose gradient of the halobacterial ATPase had a specific activity of 3.6  $\mu$ Mol  $PO_4$ /min/mg protein. The enzyme at this stage was stable for several weeks when stored at room temperature (22-24°C).

### Purification of F<sub>1</sub> ATPase from E. coli

The F<sub>1</sub>-ATPase from E. coli was prepared as described previously (refs. 24, 25) from strain LE 392, which was kindly provided by Dr. R. Simoni.

**Gel electrophoresis**—Polyacrylamide gel electrophoresis in presence of SDS was done according to the procedure of Laemmli (ref. 26). For polyacrylamide gel electrophoresis in the presence of the cationic detergent CTAB, the method of Weber and Osborn (ref. 27) was used, but was modified by reversing the electrodes and using gels and a running buffer which contained 0.1% CTAB instead of SDS. Isoelectric focusing (IEF) was carried out on vertical slab gels as described previously (ref. 28), except that the IEF gels contained 2% Tween-40 instead of Triton X-100 to avoid streaking of proteins. Gel slices excised after electrophoresis in the first dimension were equilibrated in 4% Tween-40, 8 M urea, 0.5% ampholytes pH 2.5-5, 1% mercaptoethanol. The pH gradient of IEF gels was determined after completion of the run by eluting 1-cm<sup>2</sup> gel slices with 1 mL of deaerated water for 1 h and measuring the pH of the solution. Values for the gradient were also determined using marker proteins with known isoelectric points and these agreed with those obtained by the pH measurements. To obtain peptide maps of the ATPase subunits, regions of the gel containing the subunits were excised and partial proteolysis was carried out for 1 h at room temperature according to the method of Cleveland et al. (ref. 29). Control experiments indicated that no further proteolysis took place upon longer incubation. Gels were stained with Coomassie Blue (ref. 30) or silver (ref. 31). Permanent records of the stained gels were made using Electrophoresis duplicating paper (Kodak) as described in the literature accompanying the paper. Densitometer traces of gels were obtained by scanning vertically sectioned portions of the gels at 590 nm in a Gilford gel scanner.

**Amino acid analysis of subunits**—Following SDS gel electrophoresis with 9% polyacrylamide gel, the regions containing the subunits were located by comparison to samples of fluorescamine-labeled enzyme (see below) which had been applied to the lanes at the edges of the gel. Portions of gel containing the individual subunits were excised and the subunits were recovered by electroelution (Electroelution apparatus, BioRad) at a current of 10 mA per tube using a buffer consisting of 50 mM NH<sub>4</sub>HCO<sub>3</sub>, 0.5 mM dithiothreitol, and 0.01% Tween 40. Detergent was removed by passing the electroeluted fractions through Extractigel-columns (Pierce Chemical Company) equilibrated in 7.5 mM NH<sub>4</sub>HCO<sub>3</sub>. Samples were hydrolyzed with 6N HCl at 110°C, and the amino acid composition was determined using a Waters Picotag system. Procedural controls from blank gels as well as gels containing an acidic standard protein (the main component of soybean trypsin inhibitor) were treated identically and used to correct for some minor peaks eluting from the Picotag column. For the determination of cysteine, samples were carboxymethylated (ref. 32) and dialysed against a buffer containing 7.5 mM NH<sub>4</sub>HCO<sub>3</sub> and 1 mM mercaptoethanol.

**Other methods**—The ATPase from H. saccharovorum was digested with trypsin by incubating TPCK-trypsin and the native enzyme in 50 mM TES-3.5 M NaCl-2.5 mM MgCl<sub>2</sub>-10 mM KCl-pH 8.0 buffer. The ratio of protein to trypsin was 20:1 (w/w). The reaction was stopped by adding a five-fold molar excess of soybean trypsin inhibitor.

The halobacterial ATPase was labeled with fluorescamine after making the enzyme (0.4 mg/mL) 0.2 M with respect to sodium borate by passing the ATPase through 1-mL Sephadex G-50 columns using the method described by Penefsky (ref. 33). Labeling was accomplished by adding fluorescamine (5 mg/mL in acetonitrile) to a final concentration of 10 µg/mL. After 30 min at 22°C, excess reagent was removed by centrifuging the sample through a Sephadex G-50 gel equilibrated with 50 mM Tris-HCl, pH 8.0, buffer.

ATPase activity was determined using MnATP in the presence of 0.01% Triton X-100 (as described in ref. 11). Protein was determined by the method of Bradford (ref. 34) using bovine  $\gamma$ -globulin as the standard.

## RESULTS

### Amino Acid Composition and Isoelectric Points of Subunits I and II

Following sucrose gradient centrifugation, the peak halobacterial ATPase fraction was dissociated with SDS and the subunits were separated by SDS-electrophoresis (fig. 1). Five components were detected after staining with Coomassie Blue. The molecular masses of four were similar to those previously shown to be associated with the ATPase (ref. 12). They were designated as subunits I-IV in order of decreasing mobility during electrophoresis ( $M_R$  = 87k, 60k, 29k, and 20k). The 49k polypeptide visible in figure 1 (the faint band below subunit II) originated from a persistent contaminant and was not a component of the ATPase (ref. 12).

The amino acid compositions of subunits I and II are shown in table 1. Both subunits were relatively high in glutamic and aspartic acids and low in lysine. The excess of acidic over basic residues in subunits I and II was approximately 20 Mol% and 22 Mol%, respectively (uncorrected for amide content). Extensive sequence homology between subunits I and II and the  $\alpha$  and  $\beta$  subunits from the  $F_1$  ATPase of *E. coli* was not likely given the distinct differences with respect to overall amino acid compositions and relative acidity (table 1).

There was some uncertainty concerning the glutamic and aspartic acid content of subunits I and II because amides were not determined in the analysis. Therefore, the subunits were examined by IEF. This procedure would not affect the amide-nitrogen content and should provide a measure of the relative acidity of the subunits. Figure 2 shows an IEF gel of subunits I and II. Similar IEF gels were run with subunits III and IV (not shown). The isoelectric points of all four subunits were acidic (table 2) and were at least from one to two pH units lower than those of the subunits from the  $F_1$  ATPase of *E. coli*.

### Relative Molecular Masses of the Subunits

The values of the relative molecular masses for subunits I and II are significantly larger than those for the  $\alpha$  and  $\beta$  subunits from  $F_1$  ATPases. Acidic proteins may exhibit reduced mobilities during SDS gel electrophoresis compared to the marker proteins, presumably because of the binding of less SDS (refs. 22, 23), and, as a consequence, their molecular masses may be overestimated. In order to ascertain whether the apparent molecular masses of subunits I and II reflected this phenomenon, we compared the electrophoretic behavior of the halobacterial subunits in the presence of the cationic detergent CTAB, which can obviate this difficulty (refs. 23, 35). The molecular masses of subunits I and II determined in the presence of CTAB were  $89 \text{ kDa} \pm 3 \text{ kDa}$  and  $64 \text{ kDa} \pm 2 \text{ kDa}$ , respectively (fig. 3), values which were, within the limits of experimental error, similar to those derived from SDS gel electrophoresis. In control experiments, values for the molecular masses of the subunits from the *E. coli*  $F_1$  ATPase were identical whether derived from either CTAB (fig. 3) or SDS gel electrophoresis.

## Peptide Mapping of Subunits

Peptide maps of the halobacterial and the *E. coli* ATPase subunits were determined according to the method of Cleveland et al. (ref. 29). The results of a typical experiment following 1 h of chymotrypsin digestion are shown in figure 4. Subunits I and II (lanes 1 and 2) were cleaved into a limited number of large peptides (lanes 5 and 6), whereas smaller and more numerous peptides (lanes 7 and 8) were produced from the  $\alpha$  and  $\beta$  subunits (lanes 3 and 4) of the *E. coli* F<sub>1</sub> ATPase. Subunits III and IV were not degraded by chymotrypsin using these conditions in contrast to the  $\gamma$ ,  $\delta$  and  $\epsilon$  subunits of the *E. coli* F<sub>1</sub> ATPase (data not shown). Numerous peptides were obtained when subunits I and II and the  $\alpha$  and  $\beta$  subunits *E. coli* F<sub>1</sub> ATPase were digested with V8 protease. However, the resulting patterns following electrophoresis were different (not shown).

## Trypsin Inactivation of the Native Halobacterial ATPase

Digestion with trypsin inactivated the halobacterial ATPase in a time-dependent manner (inset, fig. 5). Inactivation was associated with the disappearance of subunits I and II and was accompanied by the appearance of several polypeptides of molecular masses between 32 kDa and 40 kDa (fig. 5). In the experiment described in figure 5, 3 h of incubation resulted in the loss of 90% of the initial ATPase activity although 40% and 33% of subunits I and II were still present.

## DISCUSSION

The ATPase from *H. saccharovorum* shared several properties with F<sub>1</sub>-type ATPase. These included the acidic conditions necessary to demonstrate DCCD inhibition (ref. 10), inhibition by ADP, which was competitive with respect to ATP (ref. 11), and the absence of vanadate inhibition (ref. 11). The competitive inhibition by ADP implied that ATP was hydrolyzed through an enzyme-ADP complex, unlike E<sub>1</sub>E<sub>2</sub>-ATPases which hydrolyze ATP via an enzyme-phosphate intermediate (ref. 36).

A polypeptide (M<sub>r</sub> 62 K) obtained from *H. halobium* membranes (ref. 18) and subunit II from *H. saccharovorum* (M. Lübben, personal communication) reacted with polyclonal antibodies for the  $\beta$  subunit from the ATPase from *S. acidocaldarius* as did the  $\beta$  subunit from the F<sub>1</sub> ATPase from *E. coli*, which suggested a degree of homology between subunit II and the F<sub>1</sub>-ATPase  $\beta$  subunit. However, as we have shown, the halobacterial enzyme and the *E. coli* F<sub>1</sub>-type ATPase differed structurally. Subunits I and II, the major subunits of the halobacterial enzyme, had larger molecular masses than those for the  $\alpha$  and  $\beta$  subunits from F<sub>1</sub>-type ATPase. The amino acid compositions of subunits I and II, which were remarkably similar to those of a malate dehydrogenase obtained from an extremely halophilic bacterium (ref. 20), the alkaline phosphatase from *H. salinarium* (ref. 21), and the bulk protein from *H. salinarium* (ref. 19), differed from the composition of the  $\alpha$  and  $\beta$  subunits from *E. coli* F<sub>1</sub> ATPase. As is the case with other proteins from extremely halophilic bacteria, subunits I and II had a greater excess of acidic amino acids (table 1). Although the contribution of Gln and Asn to these numbers was not known, this could not be of great significance in view of the acidic isoelectric points of the halobacterial subunits (table 2), which were much lower than the isoelectric points of the  $\alpha$  and  $\beta$  subunits from the F<sub>1</sub> ATPase of *E. coli*. Subunits I and II did not stain with silver using Merrill's procedure (ref. 31), whereas the subunits from the *E. coli* F<sub>1</sub> ATPase were readily stained (ref. 37). This correlated with the low cysteine, methionine, and lysine content present in subunits I and II, amino acids, which have been implicated in the formation of nucleation centers for silver grains (ref. 38).

The peptide maps obtained by the method of Cleveland et al. (ref. 29) failed to demonstrate that the halobacterial and E. coli ATPase subunits shared any related primary structures. The cleavage patterns shown in figure 4 suggested that the distribution of chymotryptic cleavage sites differed. Thus, subunits I and II and the  $\alpha$  and  $\beta$  subunits of E. coli F<sub>1</sub> ATPase were not related with respect to their amino acid compositions or their amino acid sequences.

The subunit stoichiometry of the halobacterial ATPase was previously proposed to be 2:2:1:1 on the basis of the molecular masses of individual subunits and of the holo-enzyme (ref. 12). The almost complete inactivation of the enzyme following treatment with trypsin, but only partial structural degradation of subunits I and II, is consistent with the presence of multiple subunit copies. A subunit stoichiometry of 3:3:1:1 would exceed the molecular mass of 350 k of the halobacterial enzyme (as determined by gel-exclusion chromatography, ref. 12) by nearly 40%.

These results suggested that the ATPase from H. saccharovorum and F<sub>1</sub>-type ATPases do not resemble each other on the basis of subunit structure, amino acid composition, or amino acid sequences. In addition, none of the F<sub>1</sub>-type ATPases, whether from bacteria, chloroplasts, or bovine mitochondria, loses hydrolytic activity upon mild treatment with trypsin (ref. 16). The relationship of the ATPase from H. saccharovorum to the enzyme from H. halobium (ref. 17) is less clear. Although the molecular masses of both native enzymes and their two larger subunits are essentially the same, the enzymes differ in a number of significant properties (summarized in table 3). Antiserum to the ATPase from H. halobium (ref. 17) reacted with the H<sup>+</sup>-ATPase from plant tonoplasts and a membrane-ATPase from the archaebacterium S. acidocaldarius (ref. 39). Conversely, antibodies to the  $\beta$  subunit of the Sulfolobus ATPase, which strongly cross-reacted with the  $\beta$  subunit of F<sub>1</sub> ATPases and a protein of similar molecular mass from several archaebacterial extracts, reacted weakly with a 62 k protein from an extract of H. halobium (ref. 18). It remains to be seen whether these cross reactions reflect the presence of common epitopes in all these ATPases, possibly originating from a nucleotide-binding site, or whether they point to a closer evolutionary relatedness of ATPases than had previously been thought.



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TABLE 1.— AMINO ACID COMPOSITION OF SUBUNITS I AND II OF THE HALOBACTERIAL ATPase AND THE  $\alpha$  AND  $\beta$  SUBUNITS OF THE E. COLI F<sub>1</sub> ATPase.<sup>a</sup>

Amino acid	Mol%		Residues per protein subunit			
	I	II	I	II	$\alpha$	$\beta$
Cys	0.6	0.4	4.6	2.2	4	1
Asp	11.5	11.2	90.4	61.3	44	38
Glu	15.2	18.2	119.1	99.7	59	59
Ser	6.2	6.8	48.6	37.2	32	25
Gly	13.9	12.4	109.2	67.8	48	45
His	1.4	1.0	11.4	5.5	7	7
Arg	3.7	5.6	29.5	30.4	39	26
Thr	5.8	5.5	45.9	29.9	23	24
Ala	8.9	7.7	69.8	42.0	58	34
Pro	5.5	4.7	43.3	25.9	18	20
Tyr	3.6	3.3	28.2	18.0	16	15
Val	8.2	6.5	64.7	35.7	40	47
Met	1.8	1.0	14.5	5.5	10	15
Ile	3.5	5.5	27.8	30.1	40	27
Leu	5.0	5.9	39.3	32.6	46	42
Phe	2.0	2.7	15.8	15.0	14	14
Lys	2.8	1.6	22.2	8.7	24	20
Trp	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	1	1

<sup>a</sup>References 40-44.

<sup>b</sup>nd, not determined.

TABLE 2.— ISOELECTRIC POINTS OF THE SUBUNITS FROM THE ATPase FROM H. SACCHAROVORUM AND THE F<sub>1</sub> ATPase FROM E. COLI

Isoelectric focusing gels contained 8 M urea, 2% Tween 40, and 2% ampholytes pH 2.5-5. Individual subunits were separated by SDS gel electrophoresis prior to IEF. For details see Methods and reference 28.

<u>H. sacchrovorum</u>		<u>E. coli</u>	
Subunit	Isoelectric point	Subunit	Isoelectric point <sup>a</sup>
I	4.1	α	6.4
II	4.3	β	5.3
III	4.8	γ	>7
IV	4.1	δ	5.2
		ε	6.0

<sup>a</sup>References 45, 46.

TABLE 3.— COMPARISON OF THE HALOBACTERIAL ATPases

Property	ATPase from	
	<u>H. saccharovororum</u>	<u>H. halobium</u> <sup>a</sup>
pH Optimum	9.0	5.8
Activation by		
Cl <sup>-</sup>	yes	no
SO <sub>4</sub> <sup>2-</sup>	no	yes
Detergents	yes	no

<sup>a</sup>References 17, 47.

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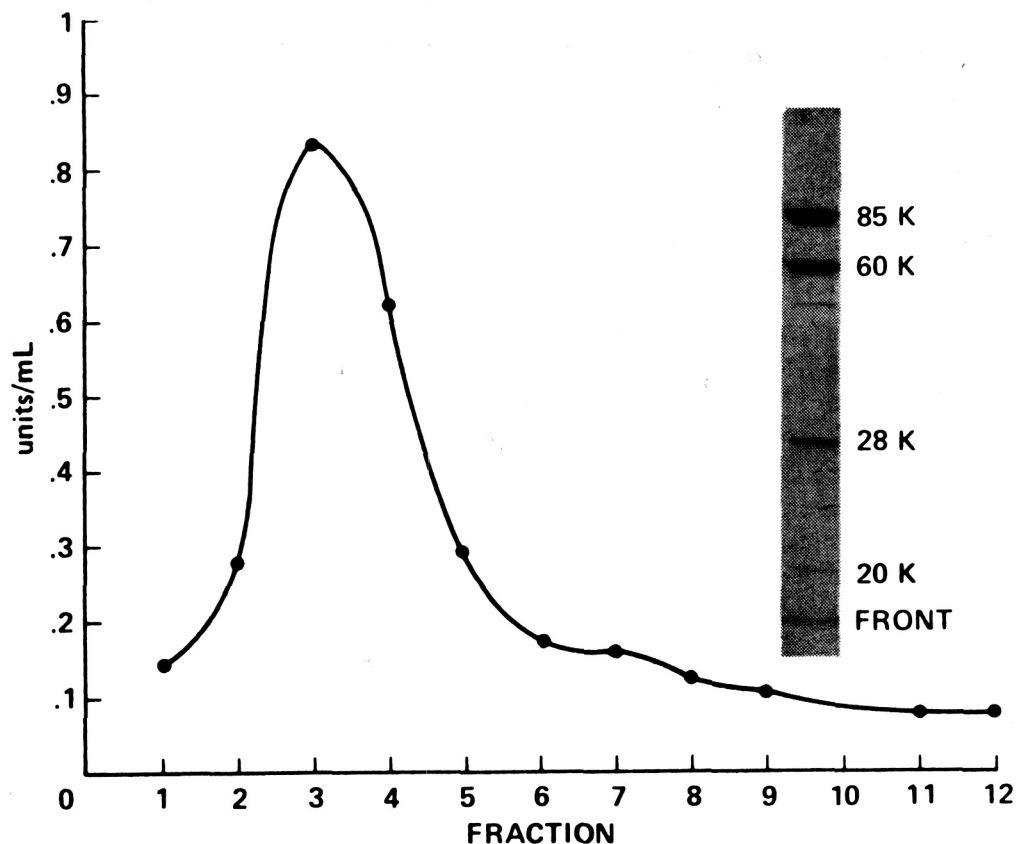


Figure 1.— Purification of the ATPase from *H. saccharovorum* on a sucrose gradient. The ATPase activity, expressed as  $\mu\text{mol PO}_4/\text{min/mL}$  of fraction, is shown following centrifugation of the ATPase from *H. saccharovorum* on a sucrose gradient (see Methods). Inset is an SDS gel (ref. 26) of sucrose gradient fraction 3, following staining with Coomassie Blue. Protein (8  $\mu\text{g}$ ) was applied on the gel and the acrylamide concentration was 11%. The molecular masses of the subunits are indicated.

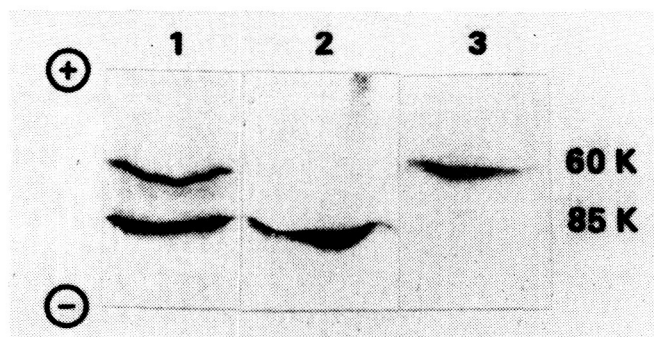


Figure 2.— Isoelectric focusing of the ATPase from *H. saccharovorum*. The ATPase was dissociated by 1% SDS in a buffer according to reference 26, followed by addition of Tween 40 to a final concentration of 4% and urea to a final concentration of 8 M. Subunits were separated as in reference 28, except for the modifications described in Methods. The IEF gel contained 2% Tween 40, 8 M urea, and 2% ampholytes, pH 2.5 to 5. Proteins were stained with Coomassie Blue (ref. 28). Only the major subunits are shown. Lane 1, 22.5  $\mu\text{g}$  ATPase; lane 2, 11  $\mu\text{g}$  of subunit I; and lane 3, 10  $\mu\text{g}$  of subunit II.

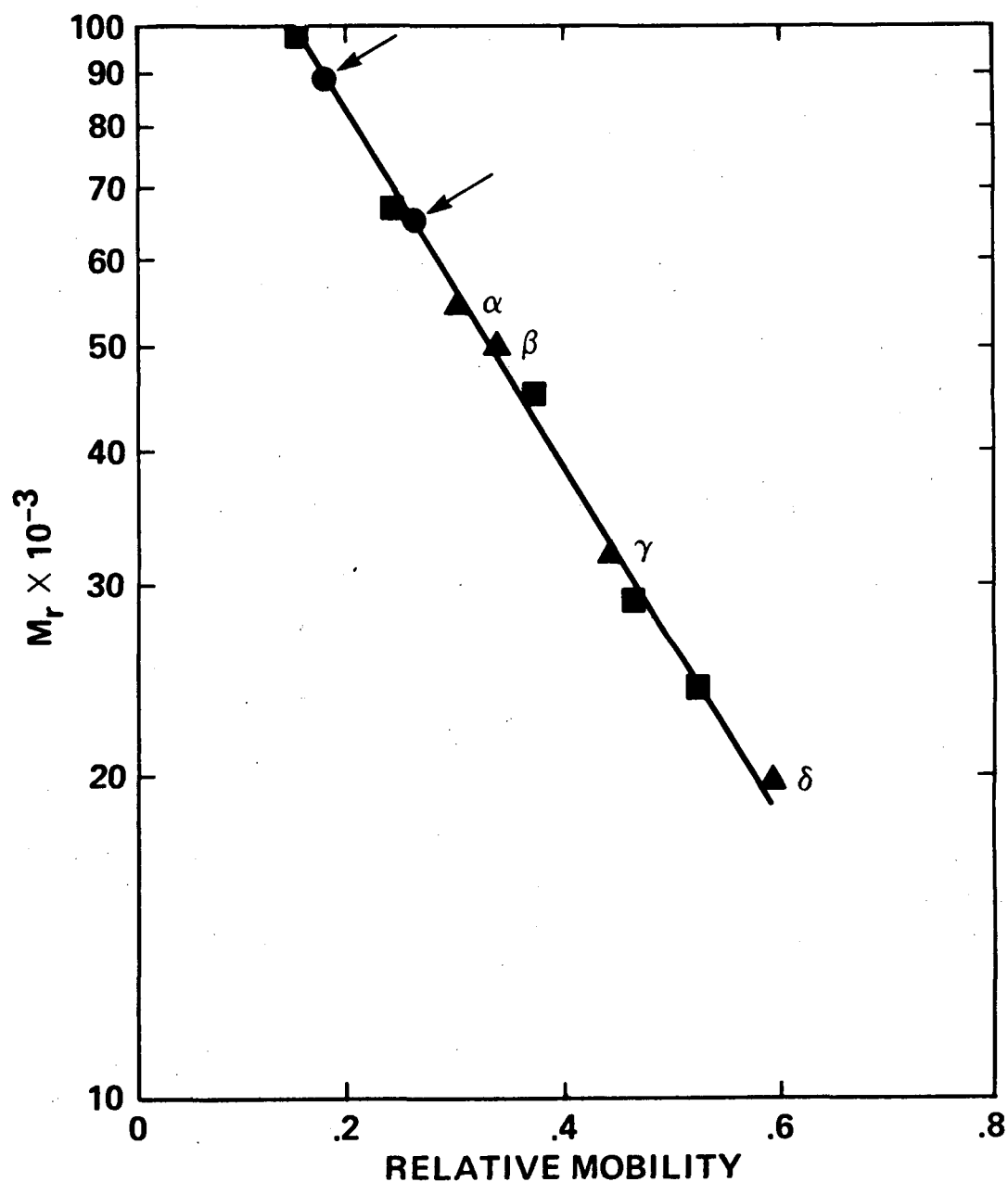


Figure 3.— Polyacrylamide gel electrophoresis in the presence of cetyltrimethyl ammonium bromide. The gel contained 0.1% CTAB (see Methods). Acrylamide concentration was 12%. Greek letters indicate subunits of the  $F_1$  ATPase from *E. coli*. Standard proteins, designated by squares, were (in the order of decreasing molecular mass): Phosphorylase b (97.4 kDa), bovine serum albumin (67 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), and trypsinogen (24 kDa). Arrows indicate subunits I and II of the ATPase from *H. saccharovororum*.



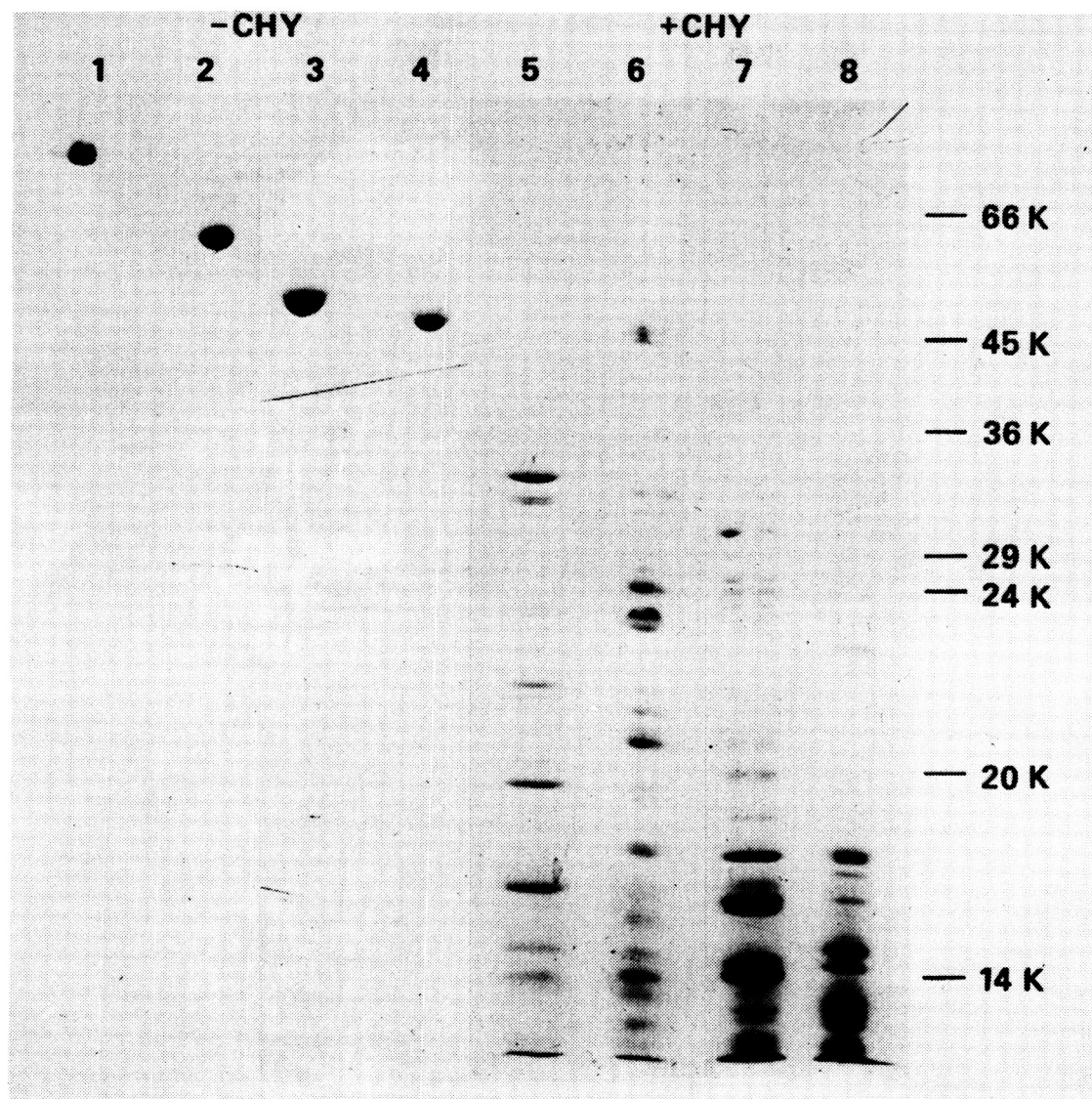


Figure 4.— Peptide mapping of the two major subunits of the ATPase from *H. saccharovorum* and the F<sub>1</sub> ATPase from *E. coli*. The proteolytic cleavage patterns of the ATPase subunits were analyzed on a 13% SDS gel. Conditions were as described in reference 29; also see Methods. Gels were stained with Coomassie Blue. Lanes 1-4, untreated subunits and lanes 5-8, subunits treated with 2  $\mu$ g chymotrypsin. Lanes 1, 5, subunit I of the ATPase from *H. saccharovorum*; lanes 2, 6, subunit II of the ATPase from *H. saccharovorum*; lanes 3, 7,  $\alpha$  subunit of the F<sub>1</sub> ATPase from *E. coli*; and lanes 4, 8,  $\beta$  subunit of the F<sub>1</sub> ATPase from *E. coli*. Molecular mass standards are indicated on the right.

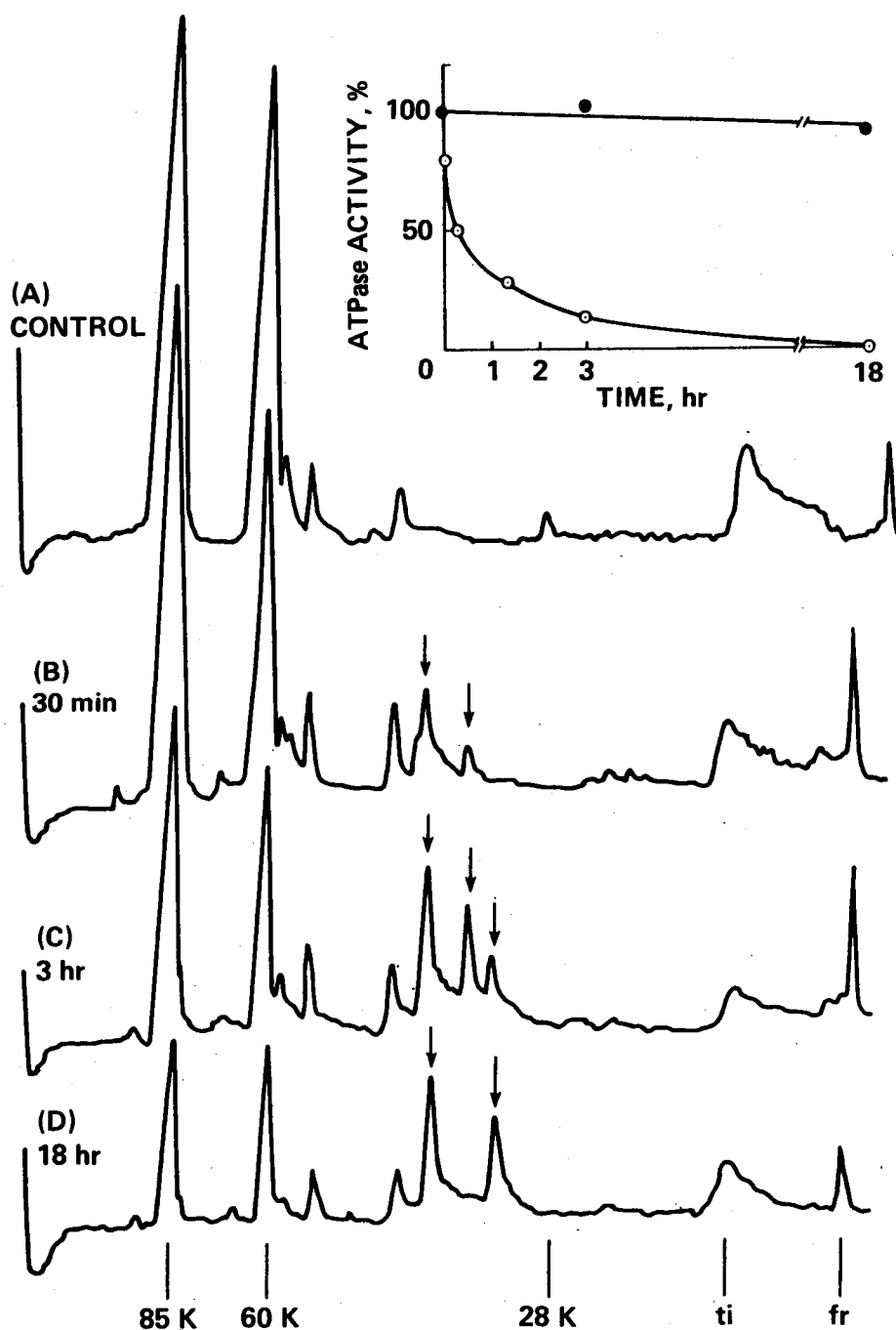


Figure 5.— Trypsin treatment of the ATPase from *H. saccharovorum*. The ATPase was treated with trypsin for the indicated times. Soybean trypsin inhibitor was added to stop the reaction. ATPase activity of the digested enzyme (—) is shown in the inset as percent activity of control enzyme (—), to which trypsin inhibitor was added prior to trypsin. ATPase samples were examined on SDS gels containing 11% acrylamide. Following staining with Coomassie Blue, gels were scanned at 590 nm. A, control ATPase; B-D, ATPase treated with trypsin for 30 min (B), 3 h (C), and 18 h (D), respectively. The major subunits of the halobacterial ATPase are denoted. Several trypsin cleavage products are indicated by arrows; ti, trypsin inhibitor; fr, dye front.



## Report Documentation Page

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16. Abstract  A purified ATPase associated with membranes from <u>Halobacterium saccharovororum</u> was compared with the F <sub>1</sub> moiety from the <u>Escherichia coli</u> ATP Synthase. The halobacterial enzyme was composed of two major (I and II) and two minor subunits (III and IV), whose molecular masses were 87 kDa, 60 kDa, 29 kDa, and 20 kDa, respectively. The isoelectric points of these subunits ranged from 4.1 to 4.8, which in the case of the subunits I and II was consistent with the presence of an excess of acidic amino acids (20 to 22 Mol%). Peptide mapping of sodium dodecylsulfate-denatured subunits I and II showed no relationship between the primary structures of the individual halobacterial subunits or similarities to the subunits of the F <sub>1</sub> ATPase (EC 3.6.1.34) from <u>E. coli</u> . Trypsin inactivation of the halobacterial ATPase was accompanied by the partial degradation of the major subunits. This observation, taken in conjunction with molecular masses of the subunits and the native enzyme, was consistent with the previously proposed stoichiometry of 2:2:1:1. These results suggest that <u>H. saccharovororum</u> , and possibly, Halobacteria in general, possess an ATPase which is unlike the ubiquitous F <sub>0</sub> F <sub>1</sub> -ATP Synthase.					
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