Isolation of Subunits from *Methanosarcina barkeri* ATPase: 
Nucleotide-Binding Site in the α Subunit 

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The α (62,000-dalton) and β (49,000-dalton) subunits of *Methanosarcina barkeri* ATPase were purified to homogeneity. The subunits and ATPase complex were trypsinized in the presence of various nucleotides. ATP and ADP changed the trypsin sensitivity of the α subunit in the complex and isolated forms, suggesting the presence of a nucleotide-binding site in the α subunit.

Methanogenic bacteria are considered to synthesize ATP by a chemiosmotic mechanism (1, 3, 13). Acid-induced ATP synthesis observed in *Methanosarcina barkeri* cells is inhibited by *N,N'-dicyclohexylcarbodiimide* (13), a specific inhibitor of ATP synthase (4). We previously found *N,N'-dicyclohexylcarbodiimide-sensitive ATPase in membranes of *M. barkeri* and purified this ATPase after solubilization with dilute buffer containing EDTA (7). The properties of the ATPase were similar to those of F₁ ATPase, but its subunit composition and inhibitor sensitivities were different from those of typical F₁ ATPase (4). The ATPase of *M. barkeri* consisted of only two types of subunits, with molecular weights of 62,000 (α subunit) and 49,000 (β subunit), and it was not inhibited by NaN₃ or 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole.

In this study, we compared the trypsin sensitivities of the α and β subunits in their isolated forms and within the ATPase complex and found that the conformations of both subunits changed upon nucleotide binding. Our results also suggest that at least part of the nucleotide-binding site is present in the α subunit.

**Isolation of the α and β subunits from purified ATPase.** *M. barkeri* MS (DSM 800) was grown in medium containing methanol (5). The ATPase was solubilized from membranes and purified as described (7), except that the enzyme was concentrated by high-performance liquid chromatography on a DEAE-5PW column. The enzyme solution (5 mg of protein per 1.3 ml of 50 mM Tris hydrochloride [pH 7.2]-1 M NaCl) was rapidly frozen at −50°C. After 20 min, the frozen sample was thawed at 25°C, concentrated to 50 μl by centrifugation in a Centrifloc-10 tube (Amicon Corp., Lexington, Mass.), and diluted 10-fold with distilled water. The dissociated subunits were separated by high-performance liquid chromatography on a TSK DEAE-5PW column (7.5 by 75 mm). Essentially equimolar amounts of the α and β subunits were eluted from the column with 0.40 and 0.45 M NaCl, respectively (Fig. 1). Total recovery of the protein was about 65%.

The purified subunits gave a single bands on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). The amino-terminal residues of the α and β subunits were methionine and valine, respectively. Neither the individual subunits nor the two in combination showed any ATPase activity, and no reconstituted αβ complex could be detected by gel filtration chromatography. Since no ATPase activity could be restored after freeze-thawing of the crude

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FIG. 1. Isolation of α and β subunits by high-performance liquid chromatography on a DEAE-5PW column. Subunit dissociation is described in the text. Subunits were eluted with a linear gradient of 0 to 60% of solution B (0.8 M NaCl in 20 mM Tris hydrochloride [pH 7.2]) in solution A 20 mM Tris hydrochloride (pH 7.2) in 40 min at 25°C (flow rate, 0.5 ml/min). The amount of protein in each fraction was determined (11). Portions (50 μl each) of fractions 47 through 66 were subjected to SDS-PAGE (inset).

FIG. 2. SDS-PAGE of the isolated α subunit treated with trypsin in the presence of various nucleotides. The α subunit (10 μg) was incubated with 1 μg of TPCK-trypsin in 30 μl of 50 mM Tris hydrochloride buffer (pH 7.2) for 3 h at 28°C. The mixture was incubated at 100°C for 3 min with 1% SDS and 2-mercaptoethanol (2 mM) and applied to a 14% polyacrylamide gel containing 1% SDS. Protein bands were stained with Coomassie brilliant blue. Lanes: 1, α subunit before digestion; 2, α subunit digested with trypsin in the absence of nucleotide; 3 through 10, α subunit digested with trypsin in the presence of 1 mM nucleotide (3, ATP; 4, ATP plus Mg [2 mM]; 5, ADP; 6, ADP plus Mg [2 mM]; 7, AMP; 8, UTP; 9, CTP; 10, GTP). The position of trypsin (23,300 daltons) is indicated by an arrow. The 62,000- and 54,000-dalton fragments are indicated by 62K and 54K.

FIG. 3. SDS-PAGE of M. barkeri ATPase treated with trypsin in the presence of nucleotides. The enzyme (10 μg) was incubated with 1 μg of TPCK-trypsin in 30 μl of 50 mM Tris hydrochloride buffer (pH 7.2). Lanes: 1, ATPase; 2, ATPase digested with trypsin in the absence of nucleotides; 3 through 5, ATPase digested in the presence of 1 mM ATP (lane 3), ATP plus 2 mM Mg²⁺ (lane 4), and ADP (lane 5). The position of trypsin is indicated by an arrow. The 62,000- 54,000-, 49,000-, and 46,000-dalton fragments are indicated by 62K, 54K, 49K, and 46K.

FIG. 4. SDS-PAGE of M. barkeri ATPase treated with trypsin in the presence of nucleotides. The enzyme (10 μg) was incubated with 1 μg of TPCK-trypsin in 30 μl of 50 mM Tris hydrochloride buffer (pH 7.2). Lanes: 1, ATPase; 2, ATPase digested with trypsin in the absence of nucleotides; 3 through 5, ATPase digested in the presence of 1 mM ATP (lane 3), ATP plus 2 mM Mg²⁺ (lane 4), and ADP (lane 5). The position of trypsin is indicated by an arrow. The 62,000- 54,000-, 49,000-, and 46,000-dalton fragments are indicated by 62K, 54K, 49K, and 46K.

The addition of Mg²⁺ and of less trypsin did not alter the proteolytic pattern. On the other hand, the β subunit in the ATPase complex was less sensitive to trypsin (Fig. 3, lane 2), whereas in the presence of Mg-ATP a 46,000-dalton fragment was produced, possibly from the β subunit (Fig. 3, lane 4). These results indicate that the β subunit became
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LITERATURE CITED