Structural and functional analysis of the coupling subunit F in solution and topological arrangement of the stalk domains of the methanogenic A₁A₀ ATP synthase

Ingmar Schäfer · Manfred Rössle · Goran Biuković · Volker Müller · Gerhard Gruber

Original Paper

Abstract The first low-resolution shape of subunit F of the A₁A₀ ATP synthase from the archaeon Methanosarcina mazei Gö1 in solution was determined by small angle X-ray scattering. Independent to the concentration used, the protein is monomeric and has an elongated shape, divided in a main globular part with a length of about 4.5 nm, and a hook-like domain of about 3.0 nm in length. The subunit-subunit interaction of subunit F inside the A₁A₀ ATP synthase in the presence of 1-ethyl-3-(dimethylaminopropyl)-carbodiimide EDC was studied as a function of nucleotide binding, demonstrating movements of subunits F relative to the nucleotide-binding subunit B. Furthermore, in the intact A₁A₀ complex, crosslinking of subunits D-E, A-H and A-B-D was obtained and the peptides, involved, were analyzed by MALDI-TOF mass spectrometry. Based on these data the surface of contact of B-F could be mapped in the high-resolution structure of subunit B of the A₁A₀ ATP synthase.

Keywords A₁A₀ ATP synthase · A₁ ATPase · Methanosarcina mazei Gö1 · Small angle X-ray scattering · F₁F₀ ATP synthase · V₁V₀ ATPase

Abbreviations BSA: bovine serum albumin · CD: circular dichroism · EDC: 1-ethyl-3-(dimethylaminopropyl)-carbodiimide · IPTG: isopropyl-β-D-thio-galactoside · NTA: nitritotriacetic acid · PAGE: polyacrylamide gel electrophoresis · PCR: polymerase chain reaction · SAXS: small angle X-ray scattering · SDS: sodium dodecyl sulfate · Tris: Tris-(hydroxymethyl)aminomethane

Introduction

Archaea are a heterogeneous group of microorganisms that often thrive at harsh environmental conditions such as high temperatures, extreme pH’s, and high salinity. Like other living cells, they use chemiosmotic mechanisms along with substrate level phosphorylation to conserve energy in form of ATP. Because some archaea are rooted close to the origin in the tree of life, these unusual mechanisms are consid- ered to have developed very early in the history of life and, therefore, may represent first energy conserving mechanisms (Ide et al., 1999; Schäfer et al., 1999). A key compo- nent in cellular bioenergetics is the ATP synthase (Pedersen et al., 2000; Senior et al., 2000; Capaldi and Aggeler, 2002). The enzyme from archaea represents a new class of ATPases, the A₁A₀ ATP synthases. They catalyze the for- mation of ATP at the expense of the transmembrane electrochemical ion gradient and are related to the F₁F₀ and V₁V₀ ATP synthases/ases (Inatomi et al., 1989; Stan-Lotter and Hochstein, 1989; Dirmeier et al., 2000). The A₁A₀ ATP synthases are structurally similar to V₁V₀ ATPases but synthesize ATP like the F-type ATPases. The membrane-integrated enzyme consists of subunits A–K in the stoi- chiometry of A₁:B₂:C:D:E:F:H:IK₅. As its bipartite name implies the A₁A₀ ATP synthases is divided into two parts: a water-soluble A₁ ATPase and an integral membrane sub- complex, A₀. ATP is synthesized or hydrolyzed on the A₁ headpiece, consisting of an A₁:B₃ domain, and the energy
provided for or released during that process is transmitted to the membrane-bound AO domain. The energy coupling between the two active domains occurs via the so-called stalk part (Müller and Gruber, 2003).

The structure of the chemically-driven motor (A1) from Methanosarcina mazei Gø1, which is made up of the five different subunits A1B3CDF, was solved by small angle X-ray scattering in solution (Gruber et al., 2001) and image processing of electron micrographs of the negatively stained particles (Coskun et al., 2004a). The data show that the A1 ATPase is rather elongated, with an A1:B3 headpiece and an elongated stalk (Gruber et al., 2001). A comparison of the central stalk of this A1 complex with bacterial F1 and eukaryotic V1 ATPases indicates different lengths of the stalk domain (Gruber et al., 2001). Further insights into the topology of the A1 ATPase were obtained by differential protease sensitivity (Coskun et al., 2002), and cross-linking studies (Coskun et al., 2002, 2004a). These studies resulted in a model in which the subunits C, D (partly), and F form the central stalk domain (Coskun et al., 2002, 2004a). The first structure of the complete methanogenic A1AO ATP synthase was obtained recently by single particle analyses (Coskun et al., 2004b). These studies revealed novel structural features such as a second peripheral stalk, and a collar-like structure. In addition, the membrane-embedded electrically-driven motor AO is very different in archaea with sometimes novel, exceptional subunit composition and coupling stoichiometries that may reflect the differences in energy conserving mechanisms as well as adaptation to temperatures (Muller and Gruber, 2003).

We have turned our attention to the examination of subunit F of the methanogenic A1AO ATP synthase and describe the structural features of this stalk subunit in solution. The location of F and the related stalk subunits D, E and H in the absence and presence of nucleotides have been explored in the intact A1AO complex.

**Experimental procedures**

**Materials**

ProofStar™ DNA Polymerase and Ni2+-NTA-chromatography resin were received from Qiagen (Hilden, Germany); restriction enzymes were purchased from MBI Fermentas (St. Leon-Rot, Germany). The expression vector pET9d-His6 was provided by G. Stier, EMBL (Heidelberg, Germany). Chemicals for gel electrophoresis were received from Serva (Heidelberg, Germany). Bovine serum albumin was purchased from GERBU Biochemicals (Heidelberg, Germany). All other chemicals were at least of analytical grade and received from BIOMOL (Hamburg, Germany), Merck (Darmstadt, Germany), Roth (Karlsruhe, Germany), Sigma (Deisenhofen, Germany), or Serva (Heidelberg, Germany).

**Constructs and proteins**

The gene, encoding subunit F from *M. mazei* Gø1 was amplified using the oligonucleotide primers 5'-CTTTTCCATGGAGTTACGATGAT-3' (forward primer) and 5'-TTTGAGCTTCTATTCACAGATCAA-3' (reverse primer), incorporating NcoI and SacI restriction sites, respectively (underlined). As template the multicopy vector pTL2 (Lenkker et al., 2003) coding for the A1AO ATP synthase gene F was obtained from Escherichia coli strain DK8 using the standard Nucleobond Plasmid Midi Kit. Following digestion with NcoI and SacI, the PCR product was ligated into the pET9d-His6 vector. The cloned pET9d-His6 vector containing the DNA fragment, encoding subunit F plus six His-residues at the N-terminus, was transformed into *E. coli* cells (strain BL21) and grown on 30 µg/ml kanamycin-containing Luria-Bertoni (LB) agar-plates. To express His6-subunit F, liquid cultures were shaken in LB medium containing kanamycin (30 µg/ml) for about 20 h at 30°C until an optical density OD600 of 0.6–0.7 was reached. To induce production of His6-subunit F, the cultures were supplemented with isopropyl-β-D-thio-galactoside (IPTG) to a final concentration of 1 mM. Following incubation for another 4 h at 30°C, the cells were harvested at 10,000 × g for 20 min, 4°C. Subsequently, they were lysed on ice by sonication for 3 × 1 min in buffer A (50 mM Tris/HCl, pH 8.5, 100 mM NaCl, 4 mM Pefabloc SC (BIOMOL). The lysate was cleared by centrifugation at 10,000 × g for 30 min at 4°C, the supernatant was passed through a filter (0.45 µm pore-size) and supplemented with Ni2+-NTA resin. The His-tagged protein was allowed to bind to the matrix for 90 min at 4°C and eluted with an imidazole-gradient (25–200 mM) in buffer A by mixing on a sample rotator (Neolab). Fractions containing His6-subunit F were identified by SDS-PAGE1 (Laemmli, 1970), pooled, concentrated using Centriprep YM-10 (3 kDa molecular mass (MM) cut off) spin concentrators (Millipore), and subsequently applied on an ion-exchange column (Resource Q (6 ml), Amersham Biosciences), equilibrated in a buffer of 50 mM Tris/HCl (pH 8.5) and 100 mM NaCl. The purity of the protein sample was analyzed by SDS-PAGE (Laemmli, 1970). The SDS-gels were stained with Coomassie Brilliant Blue G250. Protein concentrations were determined by the bicinchoninic acid assay (BCA; Pierce, Rockford, IL, USA).

The methanogenic A1AO ATP synthase of *Methanococcus jannaschii* was purified by sucrose density centrifugation and anion exchange chromatography (DEAE-Sepharose) as described previously (Lingl et al., 2003). ATPase active fractions were pooled and concentrated on Centricon