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Letter to the Editor: Backbone ^{1}H, ^{15}N and ^{13}C assignments for the subunit *a* of the *E. coli* ATP synthase

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Abstract

The structure of the 30 KDa subunit *a* of the membrane component (F_0) of *E. coli* ATP synthase is investigated in a mixture of chloroform, methanol and water, a solvent previously used for solving the structure of another integral membrane protein, subunit *c*. Near complete backbone chemical shift assignments were made from a set of TROSY experiments including HNCO, HNCA, HN(CA)CB, HN(CO)CACB and 4D HNCOCA and HNCACO. Secondary structure of subunit *a* was predicted from the backbone chemical shifts using TALOS program. The protein was found to consist of multiple elongated α-helical segments. This finding is generally consistent with previous predictions of multiple transmembrane α-helices in this polytopic protein.

Biological context

ATP synthase catalyzes formation of ATP in the cytoplasm of bacterial cells and inner compartments of mitochondria and chloroplasts of eukaryotes at the expense of transmembrane electrochemical gradient of protons or, in some bacteria, Na^{+}-ions. ATP synthesis takes place in the multisubunit F_1-complex attached to the ion-conducting F_0-complex, which is embedded in the membrane. The structure of the F_1-complex from bovine mitochondria has been solved by X-ray crystallography (Abrahams et al, 1994). A high-resolution structure of the F_0-complex is not known. The basic subunit composition of the F_0-complex, found in *E. coli* and most eubacteria, is ab_2c_{10-11} (Vonck et al., 2002).

The flow of protons through a channel proposed to be located at the interface of the subunits *a* and *c* is believed to drive the rotation of the cylindrical *c*-oligomer. This rotation is coupled to rotation of an elongated subunit γ of the F_1 inside the $\alpha_3\beta_3$ core of the F_1 complex. Alternating conformational changes in the catalytic sites at the α/β interfaces induced by the rotating γ-subunit lead to release of the newly synthesized ATP molecules.

In the absence of a high-resolution structure of the complete enzyme, we are investigating the structures of individual subunits of the F_0-complex from *E. coli* by multidimensional solution NMR. To date, the structure of subunit c has been solved in a monophasic mixture of chloroform, methanol and water (Girvin et al., 1998). The modeled structure of the subunit c oligomer calculated from the NMR structure of subunit *c*, and a set of inter-subunit distance constraints derived from Cys-Cys cross-link analysis, agrees well with a low-resolution structure of subunit *c* oligomer from *Ilyobacter tartaricus*, solved by electron microscopy (Vonck et al., 2002).

Subunit *a* is a 271 residue polytopic membrane protein, predicted to form five transmembrane α-helices.

The combination of size, hydrophobic nature and largely α-helical secondary structure of subunit *a* present major challenges for solution NMR. Aside from short peptides, β-barrel proteins of about 150 residues are the only membrane proteins which have yielded high-resolution NMR structures in aqueous detergent solution.

Preliminary NMR experiments with subunit *a* (Dmitriev, unpublished) in detergent solutions indicated that the quality of the spectra would not be sufficient to obtain backbone chemical shift assignments. An alternative solvent for membrane proteins is a monophasic mixture of chloroform, methanol and water, a solvent which has been used to solve the structure of the ATP synthase subunit *c* (Girvin et al., 1998).

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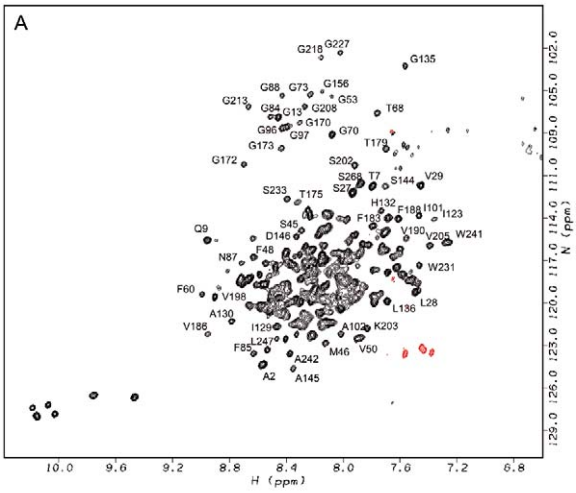

Figure 1. (A) TROSY spectrum of subunit *a* with some of the sequence-specific assignments indicated in the uncrowded regions of the spectrum. (B) Amino acid sequence of subunit *a* with segments of α-helical secondary structure predicted by TALOS from the backbone chemical shifts shown in blue lettering.

The correlation time of the protein in the low-viscosity chloroform-methanol-water solvent is much shorter than in the aqueous detergent solutions, where each protein molecule is associated with dozens of detergent molecules in a micelle. While it is not possible to test protein activity in solution, following purification in chloroform-methanol-water solvent, subunit *a* was successfully reconstituted with subunits *b* and *c* and phospholipids into a functional F_0-complex (Dmitriev et al., 2004) . This result indicates that the protein is not denatured by the chloroform-methanol-water solvent.

We report backbone chemical shift assignments for the subunit *a* of the *E. coli* ATP synthase.

Methods and experiments

Subunit *a* was purified from a strain overproducing complete ATP synthase operon from a pBR322-derived plasmid. The subunit *a* gene (*atpB*) was modified to include a hexahistidine tag immediately after the N-terminal methionine. The *E. coli* culture was grown on a minimal medium made in ∼95% D_2O containing 0.3% U-[$^{13}C_6$]-glucose and 15 mM $^{15}NH_4Cl$ as sole sources of carbon and nitrogen respectively. Subunit *a* was extracted into a mixture of chloroform, methanol and water and purified by Ni-NTA chromatography (Dmitriev et al., 2004). The NMR sample contained 0.3 mM subunit *a* in $CDCl_3/CD_3OH/H_2O$ (4:4:1) with 25 mM CD_3COONa, pH 5.5. NMR experiments were performed on a Bruker DMX-750 spectrometer equipped with triple-axis pulse field gradients at 300 K. Proton chemical shifts are referenced to internal 3-(methylsilyl)propane-1,1,2,2,3,3,-d_6-sulfonic acid sodium salt (DSS). The ^{13}C and ^{15}N chemical shifts are referenced indirectly to DSS using the absolute frequency ratios. Sequence-specific assignments were obtained using TROSY versions of HNCO, HNCA, HN(CA)CB, HN(CO)CACB and 4D HNCOCA and HNCACO experiments (Yang et al., 1999). The combination of 4D HNCOCA and 4D HNCACO experiments was essential for resolving multiple assignment ambiguities in the three-dimensional data sets. Spectra were processed using FELIX 2000 (Accelrys). Automated peak picking was performed using CHIFIT 2.0 (Chylla et al., 1995). Backbone chemical shift assignments were done with assistance of AutoAssign (Moseley et al., 2001) and Mapper (Güntert et al., 2000). Some of the assignments are shown in Figure 1. Secondary structure prediction from chemical shifts was done using TALOS (Cornilescu et al., 1999).

Extent of assignments and data deposition

Approximately 95% of all CA, CB, CO, HN and N chemical shifts in subunit *a* has been assigned. Assignments are missing for residues 112-114, 159-163, 222-223 and 256-257.

The ^1H, ^{13}C and ^{15}N-chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the BMRB accession number 6021.

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