LIFE SCIENCE

THE "SECOND STALK" OF ATP SYNTHASE: DIMERIZATION DOMAIN STRUCTURE

Hydrolysis of adenosine triphosphate (ATP) drives many of the vast range of energy-consuming processes within a cell. The ATP synthase enzyme is a molecular motor that couples proton movement to ATP synthesis. In the rotational model of the enzyme, protons flow down an electrochemical gradient, through the membrane-embedded F_0 portion of ATP synthase, causing a ring of *c* subunits to rotate. The attached "central stalk" rotates in turn and interacts sequentially with three β subunits in the ring-shaped F_1 portion, whose conformational changes catalyze ATP synthesis.

In this model, F_1 must be held against, and stationary relative to, the rotating central stalk. The dimeric *b* subunit, or "second stalk," of F_0 serves as an elongated external scaffold that holds the two portions together in this way. This study focuses on a model for the isolated dimerization domain of the *b* subunit of *Escherichia coli*, derived from solution small-angle x-ray scattering (SAXS) of the dimeric domain and x-ray crystallography of the monomer (Fig. 1) carried out at the Biophysics Collaborative Access Team beamline 18-ID. A right-handed α-helical coiled-coil domain is found to be consistent with the data.

The *E. coli* second stalk is a homodimer of *b* subunits that consists of four domains. This study concentrates on residues 62-122, which make up the dimerization domain. Initial crystallographic data unambiguously showed a single extended helix comprising residues 68-122. SAXS data indicate that the dimer in solution has an extended structure 95 Å long. To construct a model for the homodimer, researchers noted that most of the hydrophobic residues in the monomer are arranged in a strip along the helix surface and assumed that this strip constitutes the dimer interface. Because the strip moves around the helix in a right-handed sense, the model for the dimer has a right-handed superhelical twist.

A parallel, two-stranded, right-handed coiled-coil has never been observed, although natural and designed four-stranded right-handed helical bundles have. To form a right-handed coiled-coil, a polypeptide should contain an 11-residue (undecad) repeating motif with hydrophobic residues at the *"a"* and *"h"* positions. Sequence data show that the dimerization domain has a well-maintained undecad repeat but a poor heptad repeat, which would characterize a left-handed coiled-coil. Mutational data also support the right-handed model. Insertion of alanine (A) for the hydrophilic arginine 83 (R83), which occupies an undecad *"a"* position, strengthened dimerization, according to circular dichroism and sedimentation data. Alanines substituted for arginines at positions 82 (a heptad *"d"* position) and 98 weakened the structure, indicating that the additional alanine itself was not the cause of stronger dimerization. SAXS data taken on the R83A protein fit a right-handed model only slightly better than a left-handed one.

This model satisfies most of the available evidence, and there appears to be no reasonable alternative. The reason for such an uncommon structure is not obvious, but it may provide a mechanism for the proposed elastic energy storage during coupled rotation in ATP synthase.

See: P.A. Del Rizzo, Y. Bi, S.D. Dunn, and B.H. Shilton, "The 'second stalk' of *Escherichia coli* ATP synthase: Structure of the isolated dimerization domain," Biochemistry-US **41**, 6875-6884 (2002).

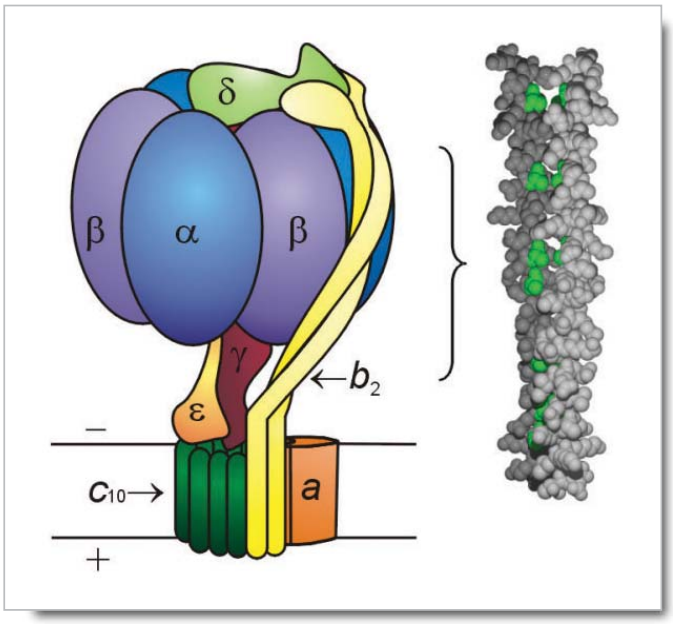

Fig. 1. Function and structure of the b_2 dimerization domain in ATP synthase. ATP synthesis is catalyzed as follows. Proton flow through the membrane-embedded F_0 portion causes the ring of *c* subunits and the γε "central stalk" to rotate, which bridges the F_0 and F_1 complexes. As the central stalk rotates, it drives conformational changes in the β-subunits. The "second stalk" of the complex is composed of the b_2 and δ subunits. Its function is to provide a stator to prevent rotation of F_1. A model for the dimerization domain of b_2, derived from the crystal structure of the $b_{62\text{-}122}$ monomer and SAXS of the solution dimer, is shown to the right of the ATP synthase holoenzyme. The alanines that define the undecad repeat, characteristic of a right-handed coiled-coil, are in green.