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Subunit Organization of the Stator Part of the F_0 Complex from *Escherichia coli* ATP Synthase

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Membrane-bound ATP synthases (F_1F_0) catalyze the synthesis of ATP via a rotary catalytic mechanism utilizing the energy of an electrochemical ion gradient. The transmembrane potential is supposed to propel rotation of a subunit *c* ring of F_0 together with subunits γ and ε of F_1, thereby forming the rotor part of the enzyme, whereas the remainder of the F_1F_0 complex functions as a stator for compensation of the torque generated during rotation. This review focuses on our recent work on the stator part of the F_0 complex, e.g., subunits *a* and *b*. Using epitope insertion and antibody binding, subunit *a* was shown to comprise six transmembrane helixes with both the N- and C-terminus oriented toward the cytoplasm. By use of circular dichroism (CD) spectroscopy, the secondary structure of subunit *b* incorporated into proteoliposomes was determined to be 80% α-helical together with 14% β turn conformation, providing flexibility to the second stalk. Reconstituted subunit *b* together with isolated *ac* subcomplex was shown to be active in proton translocation and functional F_1 binding revealing the native conformation of the polypeptide chain. Chemical crosslinking in everted membrane vesicles led to the formation of subunit *b* homodimers around residues *b*Q37 to *b*L65, whereas *b*A32C could be crosslinked to subunit *a*, indicating a close proximity of subunits *a* and *b* near the membrane. Further evidence for the proposed direct interaction between subunits *a* and *b* was obtained by purification of a stable ab_2 subcomplex via affinity chromatography using His tags fused to subunit *a* or *b*. This ab_2 subcomplex was shown to be active in proton translocation and F_1 binding, when coreconstituted with subunit *c*. Consequences of crosslink formation and subunit interaction within the F_1F_0 complex are discussed.

KEY WORDS: ATP synthase; F_1F_0; ab_2 subcomplex; subunit *a*; subunit *b*; circular dichroism; crosslinking; *Escherichia coli*.

INTRODUCTION

One of the most frequently occurring reactions in biology is the synthesis of ATP. The vast majority of ATP is produced by an almost ubiquitous multisubunit enzyme complex, the ATP synthase (F_1F_0). Whether in mitochondria, chloroplasts, or bacteria, ATP synthases catalyze the synthesis of ATP from ADP and inorganic phosphate utilizing the energy of an electrochemical ion gradient generated across the membrane by respiration or photosynthesis. In case of low driving force, e.g., anaerobiosis, bacterial ATP synthases can also serve as primary ion pumps, thereby generating an ion gradient across the membrane at the expense of ATP, which may then be derived from glycolysis. ATP synthases share high homology with respect to the mechanism of catalysis and ion translocation as well as the mode of coupling. In the enterobacterium *Escherichia coli*, the ATP synthase is built up of two different entities, the peripheral F_1 part ($\alpha_3\beta_3\gamma\delta\varepsilon$) and the mem-

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brane-embedded F_0 complex (ab_2c_{12}) (for review, see Altendorf *et al.*, 2000; Deckers-Hebestreit *et al.*, 2000; Fillingame *et al.*, 2000). In the F_1 part, subunits α and β are alternately arranged in an $\alpha_3\beta_3$ hexamer surrounding the centrally located subunit γ (Abrahams *et al.*, 1994), which extends from the lower part of the hexamer to form a 4.5-nm stalk region connecting F_1 to F_0 (Gogol *et al.*, 1987; Wilkens and Capaldi, 1998; Stock *et al.*, 1999). Subunit ε is in tight contact with subunit γ at the lower part of the mushroomlike F_1 complex (Tang and Capaldi, 1996; Watts *et al.*, 1996; Schulenberg *et al.*, 1997), whereas subunit δ is supposed to be located at the upper periphery of the $\alpha_3\beta_3$ hexagon (Lill *et al.*, 1996; Ogilvie *et al.*, 1997; Wilkens *et al.*, 2000). A consistent model exists, in which during catalysis the three β subunits carrying the catalytic sites are forced into asymmetry by eccentric rotation of a centrally located γε-c_{12} rotor subcomplex propelled by protonation/deprotonation of residue *c*D61 (Schulenberg *et al.*, 1999; Sambongi *et al.*, 1999; Pänke *et al.*, 2000; Masaike *et al.*, 2000; Nakamoto *et al.*, 1999). Due to the rotational movement a second stalk is supposed to be necessary for the stabilization of the F_1F_0 complex, which is built up at least of the two copies of subunit *b* (Ogilvie *et al.*, 1997; Rodgers *et al.*, 1997; Rodgers and Capaldi, 1998; Wilkens and Capaldi, 1998; Wilkens *et al.*, 2000; Böttcher *et al.*, 1998, 2000). The intertwining of helixes within subunit γ during rotation is supposed to generate elastic torque, tensing the parallel helixes of the subunit *b* dimer via connections with subunit δ at the top and subunit *a* at the bottom of the enzyme molecule (Wang and Oster, 1998; Junge, 1999; Cherepanov *et al.*, 1999; Greie *et al.*, 2000). Whereas detailed structural information is available for the corpus of the F_1 complex, as well as for subunit *c* and the membrane part of subunit *b* (Abrahams *et al.*, 1994; Stock *et al.*, 1999; Girvin *et al.*, 1998; Dmitriev *et al.*, 1999; Wilkens *et al.*, 1995; 1997; Uhlin *et al.*, 1997), comparatively less is known about the structure of subunit *a* and the hydrophilic part of assembled subunit *b*, as well as the precise organization of the F_0 subunits within the membrane and their interactions with the F_1 complex. Both subunits *a* and *c* are directly involved in proton translocation across the membrane, probably by formation of a transient salt bridge between residues *a*R210 and *c*D61 (Fillingame, 1990; Deckers-Hebestreit and Altendorf, 1996). Whereas structural data on subunit *c* are available from NMR experiments (Girvin *et al.* 1998; Rastogi and Girvin, 1999), in case of subunit *a* far less is known even on the topology of the polypeptide chain within the membrane.

TOPOLOGY OF SUBUNIT *a*

Especially for the N-terminal quarter of subunit *a*, contradictive models exist concerning the orientation of hydrophilic loop regions and the number of transmembrane helixes. Although most of the experiments concerning protein topology were performed by means of accessibility of hydrophilic loop regions from either side of the membrane, even the orientation of the N-terminus is controversially discussed (reviewed and commented in detail in Deckers-Hebestreit *et al.*, 2000). Because of these discrepancies, two different models emerged, one with six transmembrane helixes and the N-terminus facing the cytoplasm (Yamada *et al.*, 1996; Jäger *et al.*, 1998; Deckers-Hebestreit *et al.*, 2000) and another one with five helixes and the N-terminus oriented toward the periplasmic side of the membrane (Long *et al.*, 1998; Valiyaveetil and Fillingame, 1998; Wada *et al.*, 1999). Irrespective of this contradiction, both models are in agreement with former studies (Bjørbæk *et al.*, 1990; Lewis *et al.*, 1990; cf. Deckers-Hebestreit and Altendorf, 1992) concerning the topology of the C-terminal three-quarters of subunit *a* comprising the functionally important residues like *a*R210. Two cytoplasmic loop regions were found, ranging from around *a*K66 to *a*H95 and *a*K169 to *a*E196, respectively, and also two periplasmic domains including residues *a*V110 to *a*E131 and *a*L229 to *a*I246. In both models, the C-terminus is oriented toward the cytoplasm, resulting in four transmembrane helixes beyond the first cytoplasmic loop region starting around *a*K66. Further evidence for the putative penultimate transmembrane helix of subunit *a* can be derived from crosslinking experiments, in which cysteine residues introduced at one face of a putative α helix between residues *a*L207 and *a*I225 were shown to crosslink with cysteines introduced into the second transmembrane helix of subunit *c* with positions ranging from *c*I55 to *c*Y73 (Jiang and Fillingame, 1998), thereby suggesting a parallel paired orientation of both helixes.

As already mentioned above, there are contradictive results concerning the topology of the N-terminal quarter of subunit *a*. In one model, two transmembrane helixes are present, up to the first cytoplasmic loop region ranging from around *a*N33 to *a*S49 and *a*L54 to *a*G70, respectively, together with the N-terminus

oriented toward the cytoplasm, which results in the six-helix model (Yamada *et al.*, 1996; Jäger *et al.*, 1998). In the other model, an N-terminal stretch up to residue *a*T37 is facing the periplasm with only one membrane-spanning helix present up to residue *a*K66 resulting in five transmembrane helixes (Long *et al.*, 1998; Valiyaveetil and Fillingame, 1998; Wada *et al.*, 1999; Patterson *et al.*, 1999). It should be mentioned that the five-helix model, based primarily on cysteine accessibility studies, relies on a very narrow pattern of reciprocal reactivity using different maleimides (only a factor of 1.5 to 2.5 for right-side-out versus inside-out vesicles) (Valiyaveetil and Fillingame, 1998), whereas the use of several monoclonal or peptide-specific antibodies targeted against the N-terminal region of subunit *a* clearly demonstrated a cytoplasmic orientation of the N-terminus (with at least a factor of 10 for reciprocal reactivity in membrane vesicles of different orientation), resulting in the presence of six transmembrane helixes within subunit *a* (Yamada *et al.*, 1996; Jäger *et al.*, 1998; Deckers-Hebestreit *et al.*, 2000).

SECONDARY STRUCTURE AND HOMODIMER FORMATION OF SUBUNIT *b*

Subunit *b* is anchored in the membrane via an N-terminal stretch of hydrophobic amino acids. The remaining hydrophilic portion of the polypeptide chain is oriented toward the cytoplasm and participates in F_1 interaction and in the assembly of the F_1F_0 complex (Steffens *et al.*, 1987; Takeyama *et al.*, 1988). To elucidate the structure of subunit *b*, many experiments were carried out with both subunit *b* assembled within the F_1F_0 complex and with the N-terminally truncated soluble part of subunit *b*, including cross-linking studies and CD spectroscopy. From the results obtained, a consistent model can be deduced, in which the two copies of subunit *b* are supposed to form an elongated dimer essential for F_1 binding, with predominantly α-helical conformation and parallel paired helixes around residues *b*D53 to *b*K66, *b*S84, *b*V124 to *b*S146, and the C-terminal residue *b*L156 (cf. Fig. 1) (Dunn, 1992; McLachlin and Dunn, 1997; Rodgers *et al.*, 1997; Rodgers and Capaldi, 1998; Sorgen *et al.*, 1998a; recently reviewed in detail in Altendorf *et al.*, 2000). In addition, recent experiments revealed a close proximity of the two copies of subunit *b* also within a region near the membrane (S. Konrad, J.-C. Greie, K. Altendorf, and G. Deckers-Hebestreit, unpublished results 2000). Substitutions of residues *b*Q37, *b*G43, and *b*S60 with cysteine led to dimer formation in the presence of the UV-activated crosslinker benzophenone-4-maleimide using everted membrane vesicles. Oxidation of cysteine residues by Cu(1,10-phenanthroline)$_2$SO$_4$ also resulted in the formation of homodimers in case of mutations *b*G43C, *b*A45C, *b*S60C, and *b*L65C. These data suggest region *b*Q37 to *b*L65 also to be involved in formation of the subunit *b* dimer. Crosslinking experiments using genetically introduced cysteine residues within the N-terminal region of subunit *b* in assembled F_1F_0 complexes, together with NMR analysis of the synthetic peptide b_{1-33} comprising the N-terminal membrane-spanning domain, revealed a possible direct interaction of the transmembrane helixes around residues *b*T6 to *b*F17 at an angle of 23° (Dmitriev *et al.*, 1999). Residues *b*N4 to *b*M22 form a continuous α helix, which is interrupted by a bend around *b*K23 to *b*W26 with the helical structure resuming at residue *b*P27 at a 20° angle offset. The helical stretch comprised by residues *b*N2 to *b*W26 is thought to span the core of the lipid bilayer, thereby anchoring subunit *b* in the membrane. The 20° angle offset after the bend seems to compensate the 23° tilt of the helixes and allows the reorientation of the following helical segments at an angle more perpendicular to the membrane (Dmitriev *et al.*, 1999).

Crosslink formation using genetically introduced cysteine residues revealed possible intersubunit interactions of the subunit *b* dimer over the full span of the polypeptide chain (cf. Fig. 1). A close proximity was shown for residue *b*N2 and a C-terminal stretch of residues including *c*V74, *c*M75, and *c*V78 within subunit *c* (Fillingame *et al.*, 2000; Jones *et al.*, 2000), as well as residues *a*G227 and *a*L228 of subunit *a* (Fillingame *et al.*, 2000). Substituted *b*R36 could be crosslinked to subunit *a* (McLachlin *et al.*, 2000). Mutant *b*A92C was shown to crosslink with both subunit β and region αI464 to αM483 of subunit α, whereas *b*I109C could only be crosslinked to subunit α (McLachlin *et al.*, 2000). The C-terminus of subunit *b* is supposed to make contact with both the N-terminus of subunit α and the C-terminus of subunit δ (Rodgers and Capaldi, 1998; McLachlin *et al.*, 1998; McLachlin and Dunn, 2000). Characterization of a *b*L156C/αC90 crosslink product together with calculations based on the F_1 X-ray structure and electron microscopy of the central stalk indicate that subunit *b* spans at least 11 nm, protruding from the membrane surface to the top of F_1 (Rodgers and Capaldi, 1998). The secondary structure composition of this intersubunit-spanning segment is not yet clear and there are several indica-

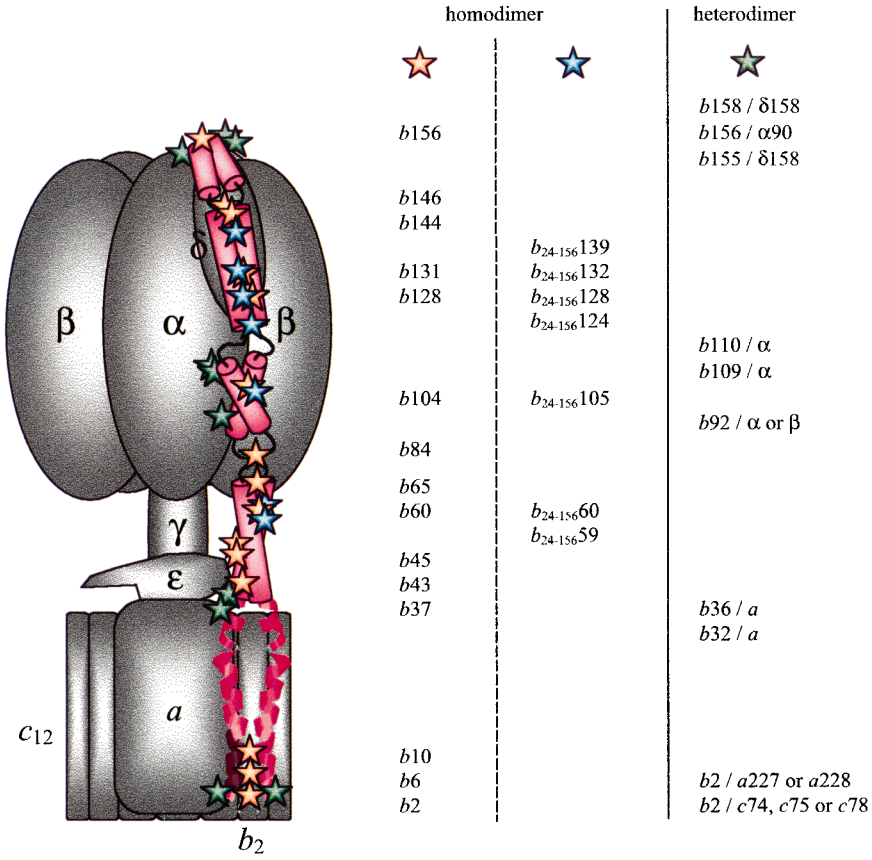

Fig. 1. Organization of the subunit *b* dimer within the ATP synthase. Crosslink products obtained for $b_{24–156}$ or assembled subunit *b* resulting either in the formation of subunit *b* homodimers or heterodimers with other F_1F_0 subunits are indicated by stars. Position b_{158} refers to a genetically elongated subunit *b* (McLachlin *et al.*, 1998). The structure of $b_{1–33}$ is derived from NMR data (Dmitriev *et al.*, 1999). The content and length of α helixes within the hydrophilic part of subunit *b* are derived from CD spectroscopic data (Greie *et al.*, 2000), whereas the positioning of helixes is speculative.

tions that this segment is not a rigid α helix, but rather provides inherent flexibility resulting from structural elements other than α helixes. Secondary structure predictions suggest β turn conformation around residues *b*R82 to *b*Q85 (Walker *et al.*, 1982; Senior, 1983). The analysis of deletion mutants ranging from residues *b*A50 to *b*I75 revealed a tolerance of up to 11 amino acid residues in length, which corresponds to a distance of 1.6 nm (Sorgen *et al.*, 1998b), indicating an inherent stretching flexibility that cannot be explained by just one rigid rodlike α helix protruding from the membrane to the top of F_1. The secondary structure composition of isolated $b_{25–156}$ has been determined by CD spectroscopy to be highly α-helical (Dunn, 1992; Rodgers *et al.*, 1997). Recently, we were successful in determining the secondary structure of full-length subunit *b* reconstituted into *E. coli* lipid vesicles (Greie *et al.*, 2000). Reconstituted subunit *b* recovered its native conformation, which was shown by coreconstitution with *ac* subcomplex, resulting in an F_0 complex functional in both proton translocation and coupled ATPase activity after F_1 binding. CD spectroscopic analysis of reconstituted subunit *b* revealed an α-helical content of 80% together with 14% β turn conformation, suggesting additional structural elements other than α helixes, which, as a consequence, would turn a proposed rigid α helix into shorter α-helical stretches flexibly connected by β turn motifs (Greie *et al.*, 2000). With respect to the model of a rotary ATP synthase, in which subunit *b* as part of the stator has to compensate torque generated by the rotation of the subunit *c* oligomer, an increased flexibility within the hydrophilic

portion of subunit *b* seems reasonable, since in this model elastic energy is supposed to be stored within the stator components, thereby causing energy transmission between proton translocation and ATP synthesis/hydrolysis (Cherepanov *et al.*, 1999; Junge, 1999). Within this elastic coupling, the sequential loading of *c* subunits with protons followed by rotation of the subunit *c* oligomer would then lead to an intertwining of the two helixes of subunit γ. Subunit γ, being in a fixed position relative to the *c* oligomer, would then serve as a torsional spring responding to the torque generated by rotation. Until its discharge for the release of tightly bound ATP, the sequential accumulation of elastic energy within the rotor part requires a compensatory element. The subunit *b* dimer with its α-helical segments is supposed to create a parallelogramlike elastic counterbearing held together by interactions with subunits δ and α at the top of F_1 and subunit *a* within the membrane. Whereas experimental evidence for these intersubunit interactions have already been given for the F_1 part (Aris and Simoni, 1983; Rodgers *et al.*, 1997; Dunn and Chandler, 1998; Rodgers and Capaldi, 1998; McLachlin *et al.*, 2000; McLachlin and Dunn, 2000), the so far only postulated interaction of subunits *a* and *b* within the F_0 complex has recently been demonstrated.

INTERACTIONS BETWEEN SUBUNITS *a* AND *b* AS PART OF THE STATOR

Since both subunits *a* and *b* are thought to participate in the formation of the stator part of the F_1F_0 holoenzyme and are, therefore, supposed to withstand torque generated by rotation of the γε-c_{12} rotor part (cf. Junge, 1999), the formation of stable subunit interactions within the stator subcomplex seems to be a prerequisite for the current theory of catalysis (reviewed in Nakamoto *et al.*, 1999). Although a sturdy interaction between subunits *a* and *b* is, therefore, reasonable, a steady protein–protein contact has not yet been demonstrated. Some experimental indications for a possible proximity of subunits *a* and *b* were derived from crosslinking experiments (Aris and Simoni, 1983; Hermolin *et al.*, 1983) as well as from characterization of the suppressor mutant *a*P240A or L/*b*G9D (Kumamoto and Simoni, 1986). In the latter case, the proposed positions of the corresponding mutations seem to be contradictive, since residue *a*P240 is located near the ultimate periplasmic loop region of subunit *a* (cf. Deckers-Hebestreit *et al.*, 2000), whereas *b*G9 was shown to be part of the transmembrane anchor of subunit *b* (Dmitriev *et al.*, 1999). In addition, the analysis of second-site revertants was demonstrated to be not a reliable method for the determination of distances in membrane proteins. Hence, the space between corresponding suppressor side chains within yeast cytochrome *c* oxidase was shown to extend up to 3 nm (Meunier and Rich, 1998). However, crosslinking studies using genetically introduced cysteines revealed disulfide bond formation between residues *b*N2 and *a*G227 or *a*L228 (Fillingame *et al.*, 2000), arguing for a close proximity of the N-terminal region of subunit *b* and the periplasmic part of the penultimate transmembrane helix of subunit *a*. Although of great functional interest, crosslink formation between putative rotor and stator subunits seems to be less meaningful regarding the characterization of a stable protein–protein interaction, since crosslink formation was also observed between *b*N2C and the C-terminal portion of subunit *c* (Fillingame *et al.*, 2000; Jones *et al.*, 2000). Recently, we were successful in purification and characterization of a stable and functional ab_2 subcomplex (W.-D. Stalz, J.-C. Greie, G. Deckers-Hebestreit, and K. Altendorf, unpublished results 2000). An ab_2 subcomplex was purified via an N-terminal His-tagged subunit *a*, which proved to be functional in proton translocation and F_1 binding after coreconstitution with purified subunit *c* into *E. coli* lipid vesicles. Fusion of the His tag to the N-terminus of subunit *b* as well as to the C-terminus of subunit *a* also led to the purification of an ab_2 subcomplex, providing further evidence for a steady interaction of subunits *a* and *b* regardless of the respective position of the affinity tag. Since the formation of the subcomplex is not triggered by any cross-linking reagent and proved to be stoichiometric and functional, it reflects subunit interactions occurring within the F_0 complex *in vivo*.

Further attempts for the characterization of contact sites between subunits *a* and *b* have recently been reported. By use of the heterobifunctional crosslinker benzophenone-4-maleimide, a genetically introduced cysteine residue at position *b*R36 was shown to crosslink with subunit *a* (McLachlin *et al.*, 2000). Concomitantly, a homologous approach revealed crosslink formation between subunits *a* and *b* in case of a *b*A32C substitution (S. Konrad, J.-C. Greie, K. Altendorf, and G. Deckers-Hebestreit, unpublished results 2000), arguing in favor of residues *b*A32 and *b*R36 representing one face of an α-helical stretch, which is probably in contact with subunit *a*. In both sets of experiments

the exact site of crosslink formation within subunit *a* remains to be elucidated, although protein interaction seems to be restricted to just a few amino acid residues within this region (S. Konrad, J.-C. Greie, K. Altendorf, and G. Deckers-Hebestreit, unpublished results 2000), possibly through interactions of subunit(s) *b* with hydrophilic loop regions of subunit *a*, which would contribute to maintainance of the integrity of the stator complex (McLachlin *et al.*, 2000). One good candidate for interaction with the subunit *b* dimer would be the stretch of hydrophilic residues preceding the penultimate transmembrane helix of subunit *a*, since crosslinking between introduced cysteine residues at positions *b*N2 and *a*G227 or *a*L228 indicate a close proximity of this helix and at least one copy of subunit *b* (Fillingame *et al.*, 2000). In addition, both the N-terminus of subunit *b* and the penultimate transmembrane helix of subunit *a* can be crosslinked to the C-terminal region of subunit *c* (Fillingame *et al.*, 2000; Jones *et al.*, 2000), suggesting a quarternary assembly of subunits *a*, b_2, and *c* within this region. Furthermore, residues *b*A32 and *b*R36 are supposed to be located outside the lipid bilayer, which renders an interaction with residues located directly at the membrane surface rather unlikely. The bend around *b*P27 amounts only up to 20°, which would not be enough for a reorientation of the subsequent amino acid residues toward the membrane. Provided that the NMR structure of b_{1-33} reflects the structure of subunit *b* *in vivo*, crosslink formation between *b*A32 or *b*R36 and subunit *a* can only occur with cytoplasmic loop regions of subunit *a* spanning the distance toward the site of crosslinking. In both the five- and the six-helix model of subunit *a*, the cytoplasmic loop region preceding the penultimate transmembrane helix is 40 to 45 residues in length, also favoring it to be the potential site of crosslink formation. Nevertheless, with the purification and characterization of the ab_2 subcomplex at hand, new insights in the organization of the stator part within the F_0 complex should be possible. Whether both *b* subunits interact with subunit *a* or whether only one copy of the dimer is in contact with subunit *a* whereas the other interacts with the subunit *c* barrel remains to be established. The latter case seems to be attractive, since formation of the subunit *b* dimer would then structurally link subunit *a* to the propelling subunit *c* annulus, thereby stabilizing possible dynamic interactions of helical interfaces between subunits *a* and *c* (Fillingame *et al.*, 2000).

INTERACTION OF SUBUNITS *a* AND *b* WITHIN THE F_1F_0 COMPLEX

A possible interaction between subunits *b* and *c* is supposed to be restricted to only a short stretch of residues, based on the following observations. Although disulfide bond formation between the N-terminus of subunit *b* and the C-terminus of subunit *c* indicate a close proximity of both subunits (Fillingame *et al.*, 2000), so far, only *ac* or *ab* subcomplexes have been purified (Schneider and Altendorf, 1984; Altendorf *et al.*, 2000; Deckers-Hebestreit *et al.*, 2000; W.-D. Stalz, J.-C. Greie, G. Deckers-Hebestreit, and K. Altendorf, unpublished results 2000). The isolation of a possible *bc* subcomplex has not yet been achieved, arguing for only a weak interaction between stator and rotor at the *b*–*c* interface. Since both subunits *a* and *c* are directly involved in proton translocation, the area of dynamic interactions between rotor and stator subunits was shown to extend up to a complete helix–helix contact (Jiang and Fillingame, 1998; recently reviewed in Fillingame *et al.*, 2000), thereby provoking the formation of a stable *ac* subcomplex. The formation of a stable ab_2 subcomplex seems reasonable, since both subunits belong to the stator part of the F_0 complex and are supposed to withstand mechanical torque built up during rotational catalysis.

There are several hints that subunit *b* alone cannot be held responsible for the formation of an F_1F_0 complex stable under catalytic conditions. First, the interaction between b_{24-156} and subunit δ is reported to have a K_d value in the μM range (Dunn *et al.*, 2000), which seems to be three orders of magnitude too low to withstand a torque of 40 pN nm generated during rotary catalysis (Yasuda *et al.*, 1998). Second, functional F_1 binding is strictly dependent on the presence of subunit *c* as shown by the reconstitution of subunit *b* or ab_2 subcomplex into proteoliposomes (Schneider and Altendorf, 1984, 1985; Greie *et al.*, 2000; W.-D. Stalz, J.-C. Greie, G. Deckers-Hebestreit, and K. Altendorf, unpublished results 2000), which renders it likely that the formation of the putative γε-c_{12} rotor part plays a crucial role in the functional binding of F_1 to F_0. This view is also supported by the fact that mutations in the highly conserved loop region of subunit *c* prevent the formation of a coupled F_1F_0 complex (Mosher *et al.*, 1985; Miller *et al.*, 1989; Fraga and Fillingame, 1991; Fraga *et al.*, 1994). In addition, the formation of crosslinks between genetically introduced cysteine residues indicates a close interaction of subunits γ, ε, and *c* (Schulenberg *et al.*, 1999; Hermolin *et al.*, 1999).

Therefore, another aspect in F_1F_0 interaction would be long-range conformational changes involving a number of subunits of both F_1 and F_0. The dynamic model of coupled proton translocation (Cherepanov *et al.*, 1999; Junge, 1999), as well as the complex pattern of contact sites between F_1 and F_0 as derived from cross-linking data and site-directed mutagenesis (recently reviewed in Deckers-Hebestreit *et al.*, 2000; Altendorf *et al.*, 2000) together with the flexibility of subunit *b* would support a dynamic induced fit interaction between both entities instead of a simple docking procedure involving single subunits. Therefore, a stable F_1 interaction would only be achieved in the presence of fully assembled F_0 complexes providing all possible contact sites for triggering the formation of a stable, functional F_1F_0 ATPase complex.

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