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Conformational Transmission in ATP Synthase During Catalysis: Search for Large Structural Changes

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Escherichia coli ATP synthase has eight subunits and functions through transmission of conformational changes between subunits. Defective mutation at βGly-149 was suppressed by the second mutations at the outer surface of the β subunit, indicating that the defect by the first mutation was suppressed by the second mutation through long range conformation transmission. Extensive mutant/pseudorevertant studies revealed that β/α and β/γ subunits interactions are important for the energy coupling between catalysis and H^+ translocation. In addition, long range interaction between amino and carboxyl terminal regions of the γ subunit has a critical role(s) for energy coupling. These results suggest that the dynamic conformation change and its transmission are essential for ATP synthase.

KEY WORDS: ATP synthase; conformation transmission; mutagenesis; pseudorevertant; rotational catalysis.

INTRODUCTION

The ATP synthase (F_0F_1) of bacteria, mitochondria, and chloroplasts has a conserved basic subunit structure with a defined subunit stoichiometry: catalytic sector F_1, $\alpha_3\beta_3\gamma\delta\varepsilon$; and membrane sector F_0, ab_2c_{10-12} (for reviews, see Refs. 1–4). The primary structures of the subunits, especially those of the α and β subunits, are highly conserved among different species. This complicated enzyme synthesizes ATP coupled with a transmembrane electrochemical proton gradient established by the electron transfer chain. F_0F_1 is a reversible enzyme, and can form an electrochemical proton gradient through ATP hydrolysis. Studies on the *E. coli* enzyme have contributed greatly to the understanding of catalysis and energy coupling. The crystal structure of the $\alpha_3\beta_3\gamma$ complex of bovine mitochondrial F_1 was solved recently,[5] and could allow logical interpretation of the results of mutations, chemical modifications, and affinity labelings. The mitochondrial structure may even suggest future experiments on the bacterial enzyme, although it is desirable to have a higher-ordered structure of the *E. coli* enzyme.

The cooperativity between three catalytic sites in the β subunits has been established.[6–9] Thus, mutant enzymes with defects in catalytic cooperativity have been isolated: they are active in unisite (single site) catalysis but defective in multisite (steady state) catalysis.[10–12] Unisite and multisite catalyses can be assayed in the presence of a substoichiometric amount of ATP ($1 > ATP/F_1$) and excess ATP, respectively. In multisite catalysis, ATP binds to all three catalytic sites, the rate being 10^5–10^6-fold faster than that of unisite catalysis. Azide[13] and DCCD[14] are inhibitory toward multisite catalysis by F_1 but have only slight effects on unisite catalysis.

The properties of F_0F_1 briefly summarized above indicate the unique features of the enzyme: the asymmetric multi-subunit membrane enzyme carries out steady-state catalysis coupling between the chemical reaction and the electrochemical proton gradient. The entire catalytic event in ATP synthesis may comprise successive conformational changes of the F_0 and F_1 subunits starting from the downhill proton transport

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through F_0. It may be easily speculated that the conformation transmission includes large subunit structural changes, such as rotation of a subunit or a complex of subunits, when the asymmetric subunit stochiometry of F_0F_1 is considered seriously. Rotation of the γ subunit relative to the three β subunits has been proposed.[5] Evidence for rotation catalysis has been reported[5,15–18] and the results of recent dissociation/reconstitution experiments support γ subunit rotation.[19] The rotation can also be speculated from the structure and function of the F_0 sector: *c*Asp-61 of the *c* subunit (10–12 copies) and the *a* subunit (one copy) residue form a proton pathway. If *c*Asp-61 of all 10–12 *c* subunits interacts with the *a* subunit during the H^+ translocation cycle, a logical model is rotation of either the *a* or entire *c* subunit assembly. Rotation of the inner complex ($ab_2\gamma\delta\varepsilon$) relative to the outer complex ($\alpha_3\beta_3c_{10-12}$) has been proposed.[15] However, no direct results showing actual rotation of F_0F_1 subunits have been presented so far, although rotation catalysis is a fascinating possibility.

In this article we discuss whether or not the results of genetic studies support subunit conformation transmission through large structural changes in subunits. We also discuss possible experiments to show rotational catalysis. Our discussion and speculation refer mainly to *E. coli* F_0F_1, because it has been studied extensively by means of mutagenesis.

DOMAIN/DOMAIN INTERACTION(S) AMONG CATALYTIC SUBUNITS

Catalytic Site in the β Subunit

Systematic mutational studies on the β subunit indicated that βLys-155, βThr-156, βGlu-181, and βArg-182 are essential catalytic residues.[20–23] Residues βLys-155 and βThr-156 are in the P-loop (Gly-X-X-X-X-Gly-Lys-Thr/Ser, phosphate-binding loop or glycine-rich sequence) of the β subunit[20–22] and βGlu-181 and βArg-182 are in the GER sequence.[23] (Fig. 1). βGlu-185 was shown to be essential for multisite catalysis: all mutations except Glu→Asp at position 185 lowered the multisite catalysis but had only a slight effect on the unisite catalysis.[12] These results are in excellent agreement with the higher-ordered structure of the mitochondrial $\alpha_3\beta_3\gamma$ complex.[5]

The importance of the P-loop conserved in nucleotide-binding proteins has been determined by affinity labeling and mutagenesis. However, some residues in the P-loop are not absolutely essential: the first residue of the P-loop, βGly-149, could be replaced by Ser or Ala.[24] Thus the double mutant method (or mutant/pseudorevertant approach) may be effective for locating other important residue(s) functionally interacting with that in the P-loop: it may be easy to isolate pseudorevertants to suppress defective mutants mapped to nonessential residues. The two amino acid residues (mutation/pseudoreversion) may be functionally or structurally related. For such an approach, we looked for defective mutants in the P-loop, and found that a βGly-149→Cys mutant could not grow by oxidative phosphorylation, and was defective in multisite catalysis and oxidative phosphorylation.[24]

Interaction of the Catalytic Site and the Outer Surface of the β Subunit

The defect of the βCys-149 mutant was suppressed by a second amino acid replacement, βGly-172→Glu, βSer-174→Phe, βGlu-192→Val, or βVal-198→Ala.[25] It is noteworthy that the amino acid changes do not fall into a pattern (e.g., large to small or porlar to nonpolar), suggesting that the βGly-149→Cys mutation does not disrupt a specific interaction between βGly-149 (or P-loop) and other residue(s), and that the second mutation restored such a specific interaction. Bovine residues corresponding to those that suppressed the βCys-149 mutant are located mostly near the outer surface of the β subunit.[5] They are not actually close to βGly-149 but are on the α helix or β sheet extending from the region near the P-loop.

The βSer-174→Phe single mutant exhibited low multi-site catalytic activity and was defective in energy coupling, and both defects were suppressed by the second mutation, βGly-149→Ser or βGly-149→Cys.[24,25] The βSer-174→Phe mutation was also suppressed by a βAla-295→Thr, βAla-295→Pro, or βLeu-400→Gln mutation.[26] Bovine residues corresponding to βAla-295 and βLeu-400 are on the β sheet and loop near the ATP binding site, respectively.[5] These results suggest that the α helix and β sheets move dynamically during conformation transmission between the catalytic residues in the P-loop and other

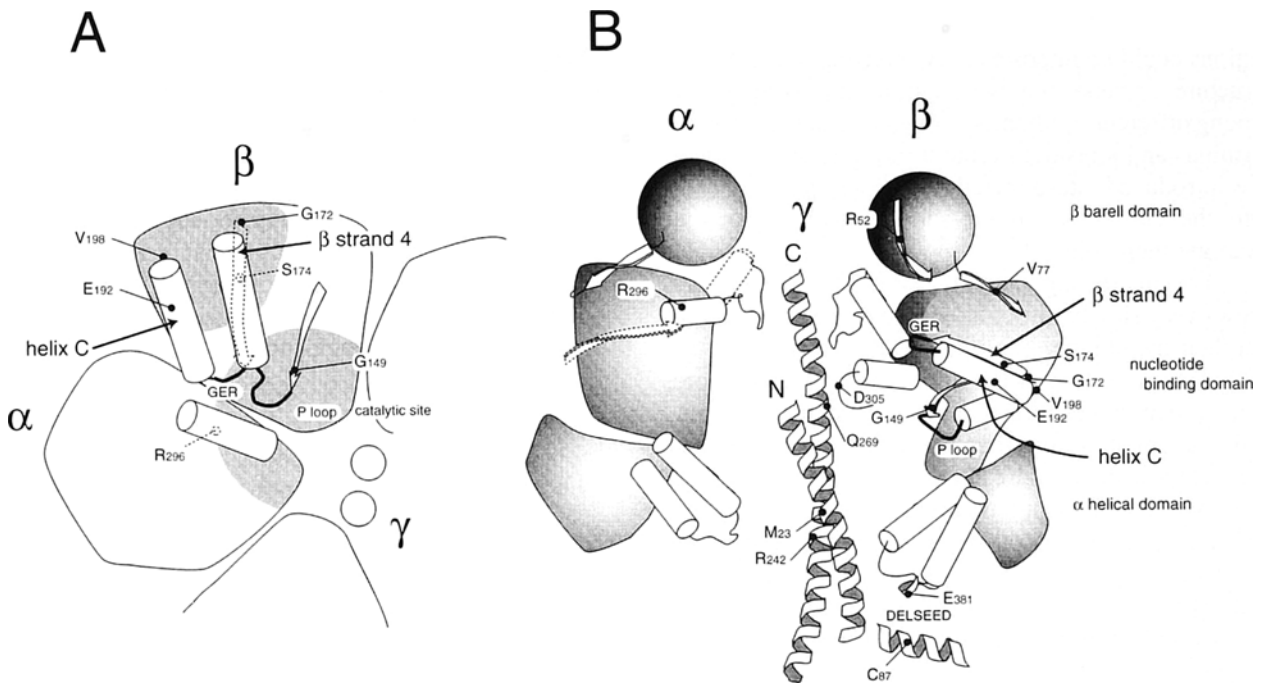

Fig. 1. Domain/domain interactions in F_1 sector during catalysis. The defective βGly149→Cys mutation was suppressed by the second mutations mapped at helix C or β sheet 4 of the β subunit and the αArg296→Cys mutation. We propose that the movement of helix C during catalysis is essential for energy coupling and catalysis. The γ subunit regions required for energy coupling are shown schematically together with the β subunit domains that interact with the γ subunit. Catalytic residues are in P-loop and GER sequence. This model is based on the results discussed in the text and the crystal structure of the bovine $\alpha_3\beta_3\gamma$ complex.[5] (A) Bottom view; (B) side view.

domains, and eventually between different catalytic sites. Consistent with this interpretation, the orientations of the α helix containing the corresponding residues between βGlu-192 and βVal-198 are different in the three bovine β subunits. Multisite catalysis was inhibited on chemical modification of βGlu-192[27,28] (mitochondrial βGlu-199) by DCCD. These results suggest that the movement of the α helix may be critical for multisite catalysis.

The α/β subunit conformation transmission was clearly shown by a similar mutant/pseudorevertant approach. The defective energy coupling of the βSer-174→Phe mutation was suppressed by the second mutation in the α subunit, αArg-296→Cys substitution.[29] The bovine residue corresponding to αArg-296 is located at the interface of the α/β subunit.[5] As discussed above, the βGly-149→Cys mutation was suppressed by βSer-174→Phe. Thus it may be possible that catalytic conformational changes around βGly-149 in the P-loop may be transmitted directly to the α subunit domain containing αArg-296 (Fig. 1).

ROLES OF THE γ SUBUNIT CONFORMATION TRANSMISSION IN CATALYSIS AND ENERGY COUPLING

Amino and Carboxyl α Helical Domains of the γ Subunit

The amino and carboxyl terminal α helices of the γ subunit (*E. coli*, total 286 amino acid residues) occupy the central position of the $\alpha_3\beta_3\gamma$ complex, as shown by the higher-ordered structure.[5] Furthermore, the positions of the three β subunits relative to the two helices are different: this structure suggests that at any given time during catalysis, the three β subunits and also the three α subunits may be asymmetrically arranged with respect to the γ subunit.

In contrast to the high homology of the α or β subunit, the identical residues among the γ subunits so far sequenced amount to about 10% when they are aligned to obtain maximal identity.[30] However, the results of studies on the amino and carboxyl terminal

regions could be interpreted by referring to the bovine structure because the two regions are homologous among different γ subunits: 18 residues in the carboxyl terminus and 4 residues around γMet-23 are identical. We introduced site-directed mutations systematically into the conserved amino and carboxyl regions to examine their roles.[31,32]

The interesting mutant, γMet-23→Arg or Lys, grows very slowly through oxidative phosphorylation, but their membranes exhibited ATPase activity essentially similar to that of the wild type.[32] Furthermore, mutant membranes formed a much lower electrochemical gradient of protons than the wild-type one, indicating that these mutants are defective in energy coupling. The γMet-23→Lys mutation was suppressed by γArg-242→Cys, and seven other mutations between γGln-269 and γVal-280.[33] Other mutants, γGln-269→Glu and γThr-273→Val, had exhibited reduced ATPase activity and coupling efficiency.[34] These mutant phenotypes were suppressed by replacement of the amino terminal residues at positions 18, 34, and 35, and those near carboxyl terminus positions 236, 238, 242, and 246. These results clearly established that the conserved amino and carboxyl termini are involved in energy coupling.[34]

As expected from the results of the double mutant studies, the amino and carboxyl terminal helices of the γ subunit are close to each other in the mitochondrial crystal structure.[5] γMet-23 in the amino terminal helix is actually near the residues corresponding to γArg-242 in the carboxyl terminal α helix. Thus it is reasonable to conclude that the γMet-23→Lys mutation was suppressed by γArg-242→Cys because these two residues are in close proximity: the helix–helix interaction between domains near γMet-23 and γArg-242 was disrupted by the γMet-23 →Lys mutation but suppressed by the second one γArg-242→Cys. However, the carboxyl terminal residues between γGln-269 and γVal-280 are not in the domain interacting directly with γMet-23, although the γMet-23→Lys mutation was suppressed by the carboxyl terminal mutations. The α carbon of the γMet-23 residue in the amino terminal helix is more than 20 Å away from that of the γGln-269 or γVal-280 located in the carboxyl terminal helix. These results suggest that the two regions defined by mutagenesis interact through long-range conformational transmission, which includes movement of the two helices.[34] This interpretation is consistent with the results of the double mutant studies for the following reasons.[33,34] First, the same mutation (γLys-23) was suppressed by different amino acid changes in the carboxyl terminal helix: Arg→Cys, Gln→Arg, Ala→Val, Ile→Phe, etc. Thus the carboxyl terminal mutation restabilized the interactions between γ subunit helices, and possibly also those between the γ and β subunits. Second, single mutants as to positions 23, 269, and 277 were temperature-sensitive in energy coupling, but double mutants could synthesize ATP at higher temperature. These results suggest that the structural integrity, possibly for subunit movement, was perturbed in a single mutant but stabilized in a double mutant.

β/γ Subunit Interaction

The results of mutational studies suggest that the γ subunit is essential for energy coupling.[24–32] The γ subunit mutation also often lowered the catalytic activity.[31] Thus it became of interest to know the domains of the β subunit that interact with the γ subunit. It is also important to know which interaction is essential for catalysis and energy coupling.

For the three different β subunits participating in multisite catalysis, the γ subunit interacts with each β subunit. The crystal structure suggests that three regions of each β subunit can potentially interact with two γ subunit helices.[5] Carboxyl terminal γGln-269 is suggested to form a hydrogen bond with βAsp-302 in a loop structure of the β subunit having no bound-nucleotide.[5] This hydrogen-bonded interaction seems to be essential for normal multisite catalysis: mutant enzymes with replacement of γGln-269 by Leu, Arg, and Glu exhibited 15, 35, and 2.4%, respectively, of the wild-type activity.[31–33] Furthermore, Ala insertion into the amino terminal side of γGln-269 lowered the ATPase activities and energy coupling, indicating that the direction of the 269 residues is also important (Fujie, K., Omote, H., and Futai, M., in preparation). Amino acid replacements at position β301–β305 (DDLTD) also lowered multisite catalysis and energy coupling (Omote, H., Tainaka, K., and Futai, M., in preparation).

The loop region formed from the DELSEED sequence (between βAsp-380 and βAsp-386) of the β subunit interacts with the γ subunit. The two glutamate residues, βGlu-381 and βGlu-384, in DELSEED are close to γCys-87: βCys-381 and βCys-384 mutants could form disulfide bonds with γCys-87.[17]. The DELSEED region is also the binding site for inhibitors such as quinacrine mustard.[35] This region is also implicated in the binding of the ε subunit.[36,37]. εSer-

108→Cys forms a crosslink with βCys-381 or βCys-383.[17,38] The β/γ or β/ε subunit crosslinking is nucleotide dependent, suggesting a large conformational change of DELSEED, and domains including γCys-87 and εSer-108. It is noteworthy that the positions of the βAsp-305 loop and DELSEED relative to the γ subunit helices differ with the β subunit form (ATP-bound, ADP-bound, or empty form): DELSEED of ATP-bound β is the closest to γ80 ~ γ90, and the βAsp-305 loop of empty β is the closest to γGln-269. These results indicate that the three β subunits are different with respect to their interactions with a single-copy γ subunit. Thus the γ subunit changes in conformation possibly through rotation during catalysis to provide asymmetry as to the three β subunits, and such conformational changes were defective in the γMet-23→Lys mutant discussed above.

The β/γ subunit interaction was also shown by mutant/pseudorevertant studies.[39] A frameshift mutant had a carboxyl terminus comprising 16 residues different from those of the wild-type; the frameshift γ had a longer altered sequence downstream of γThr-277 (7 additional residues at the carboxyl terminus and 9 altered residues downstream of γThr-277). The two methods predicted that the frameshift γ forms an extended β-strand about 60 Å from γThr-277. This extended β-strand may interact with the upper β-barrel domain of the β subunit. Surprisingly, the βArg-52→Cys or βGly-150→Asp mutation could suppress the deleterious effect of the frameshift mutation, suggesting that the altered β/γ interaction in the γ frameshift was restored by the β mutation. βArg-52→Cys located in the β-barrel suppressed the γ frameshift, possibly by overcoming the deleterious interactions between the carboxyl terminus of the γ frameshift and the β-barrel of the β subunit. The defective coupling of the frameshift mutation was also suppressed by the βGly-150→Asp mutation of the P loop, possibly through changes in the mode of conformation transmission between the β catalytic site and the γ subunit. These results may suggest that the frameshift γ subunit rotation may be inhibited by the interaction of the long γ subunit carboxyl terminus with the β subunit, and that such rotation became possible again in the double mutants.

SHOWING THE LARGE CONFORMATIONAL CHANGE OF F_0F_1

As discussed above, F_0F_1 is an enzyme with asymmetric subunits. The higher-ordered structure of the $\alpha_3\beta_3\gamma$ complex clearly indicated that the three catalytic β subunits are asymmetric relative to the γ subunit and the catalytic sites (ATP-bound, ADP-bound, or empty forms). The results of genetic and kinetic studies are consistent with a large conformational change during catalysis including rotation.

Evidence supporting rotation within F_1 and F_0 has been obtained.[5,15-18] However, they are mostly indirect, and consistent if the enzyme subunit (s) rotates. A critical test for the γ subunit rotation reported recently utilizes β-γ crosslinking and dissociation/reconstitution of the F_1 sector. Duncan *et al.*[19] showed that crosslinking between γCys-87 and βAsp-380→Cys inactivates ATPase and ATP driven H^+ pumping. They dissociated the crosslinked enzyme and reconstituted it with the dissociated radiolabeled enzyme. The hybrid enzyme (one β crosslinked with γ, two radioactive β) was reduced with dithiothreitol, subjected to catalytic turnover, and then reoxidized. The hybrid enzyme had a radioactive or nonradioactive β subunit crosslinked similarly with the γ subunit. The results of this experiment support the rotation mechanism strongly but do not actually show rotation in physical terms.

Could we show rotation of the γ subunit(s) retained inside the $\alpha_3\beta_3$ complex like the flagella motor driven by an electrochemical H^+ gradient? The γ subunit rotation may be shown if the γ subunit carboxyl terminal helix is longer and can be labeled with a fluorescent probe. The results of the γ frameshift mutation suggest possible experiments.[39,40] The enzyme with the γ frameshift and the second β subunit mutation was active in catalysis and energy coupling. The carboxyl terminal of the mutant had 16 unrelated amino acid residues. Furthermore, the carboxyl terminal 10 residues of the γ subunit are not essential for catalysis.[31] It may be possible to make the carboxyl or amino terminal helix extend out of the $\alpha_3\beta_3$ assembly, although the frameshift γ was suggested to have an extended β-strand. Thus it may be possible to introduce fluorescent probe at the carboxyl terminus of the extended β-strand or helix, fix the labeled enzyme to the artificial surface, and follow the movement of the fluorescent probe during catalysis. This approach may be possible because an image of a single fluorescence myosin molecule and ATP turnover can be obtained in the aqueous phase.[41]

A similar approach may be possible using F_0F_1 with the β subunit labeled with a fluorescent probe. As it has become possible to introduce a fluorescent probe to a defined position of the outer surface of F_1

many experiments are possible to prove rotation of the F_0F_1 molecule, applying techniques developed for bacterial flagella motors. This may be of interest even if the diffusional movement of F_0F_1 molecules in artificial planar phospholipid membranes changes during catalysis.

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