ESCHERICHIA COLI ATP SYNTHASE (F-ATPase): CATALYTIC SITE AND REGULATION OF H+ TRANSLOCATION

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Summary

We discuss our recent results on the *Escherichia coli* F-ATPase, in particular its catalytic site in the β subunit and regulation of H+ transport by the γ subunit. Affinity labelling experiments suggest that βLys-155 in the glycine-rich sequence is near the γ-phosphate moiety of ATP bound at the catalytic site. The enzyme loses activity upon introduction of missense mutations in βLys-155 or βThr-156 and changes catalytic properties upon introduction of other mutations. By analysis of mutations and their pseudo revertants, residues βSer-174, βGlu-192 and βVal-198 were found to be located near the glycine-rich sequence. The combined approaches of chemical labelling and genetics have been fruitful in visualizing the structure of the catalytic site. Analysis of mutations in the γ subunit suggests that this subunit has an essential role in coupling catalysis with proton translocation.

Introduction

The ATP synthase (or F-ATPase) catalyzes ATP synthesis or hydrolysis coupled with proton translocation (for reviews, see Fillingame, 1990; Futai et al. 1989, 1991; Senior, 1990). The catalytic sector F₁ (F₁-ATPase) is composed of five different subunits: α, β, γ, δ and ε. The β and α subunits have homologies with the A (72×10³ Mr) and B (54×10³ Mr) subunits, respectively, of the V-ATPase (for a review, see Forgac, 1989). Furthermore the F₀ sector c subunit, which forms the proton pathway in the F-ATPase, is similar to the proteolipid of the V-ATPase (Mandel et al. 1988; Hanada et al. 1991). Thus, the two types of ATPase are likely to have many common structural and mechanistic features (Hanada et al. 1990).

The F-ATPase of *Escherichia coli* is similar to those found in mitochondria and chloroplasts. Over the past 10 years, structure–function relationships of the bacterial enzyme have been extensively studied by a combination of affinity labelling (chemical modification) and mutational analysis. These approaches identified many of the residues making up the catalytic site in the β subunit and the proton pathway. More recently, the role of the γ subunit in coupling ATP hydrolysis/synthesis to proton translocation has been recognized. In this article we discuss our studies in defining the catalytic site near the ATP γ-phosphate moiety and the role(s) of the γ subunit.

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Key words: ATP synthase, glycine-rich sequence, active transport, coupling.
The glycine-rich sequence

The glycine-rich sequence, Gly-X-X-X-X-Gly-Lys-Thr (Fig. 1A), is found in many nucleotide binding proteins, including the β subunit of F-ATPase, the A subunit of V-ATPase, adenylate kinase and p21 ras protein (Walker et al. 1984). Crystallographic studies of adenylate kinase and ras proteins have shown that this sequence forms a loop structure between an α helix and a β sheet (Dreusicke et al. 1988; Milburn et al. 1990). In the E. coli β subunit, the glycine-rich sequence corresponds to positions 149–156 (Gly-Gly-Ala-Gly-Val-Gly-Lys-Thr). We have been interested in the roles of the sequence in the catalytic mechanism of F₁ ever since we obtained the primary structure of the β subunit (Kanazawa et al. 1982).

Binding of adenosine triphosphopyridoxal to βLys-155

The F₁-ATPase was shown to lose its activity after binding 1 mole of adenosine triphosphopyridoxal (AP₃-PL), and this inhibition was prevented by adding ATP (Noumi et al. 1987). Approximately 60% of AP₃-PL was in the α subunit and 40% in the β subunit. Lys-201 of the α subunit and Lys-155 of the β subunit were target residues, indicating that binding of AP₃-PL to either residue inhibited activity (Tagaya et al. 1988). Addition of Mg²⁺ decreased the AP₃-PL concentration required for inhibition and shifted the labelling predominantly to the β subunit (βLys-155 and βLys-201) (Ida et al. 1991). These results suggest that, like the βLys-201 and αLys-201 residues, the βLys-155 residue is near the γ-phosphate moiety of ATP. It should be noted that the corresponding lysine residues in the glycine-rich sequences of adenylate kinase (Tagaya et al. 1987) and p21 ras protein (Ohmi et al. 1988) were labelled with adenosine diphosphopyridoxal and guanosine triphosphopyridoxal, respectively. The pyridoxal nucleotide derivatives have proved useful as affinity analogues for labelling the lysine residue in the glycine-rich sequence of nucleotide binding proteins.

The results of our labelling experiments stimulated us to introduce mutations into αLys-201 (Ida et al. 1991). The α subunit mutants (αLys-201→Glu or αLys-201 deletion) were active in oxidative phosphorylation. The purified mutant enzymes had lower multisite catalysis than the wild-type enzyme but were similar to it in single-site (uni-site) catalysis. These results suggest that αLys-201, or residues in its vicinity, is important for catalytic cooperativity but not for catalysis itself.

Mutations in the glycine-rich sequence

The glycine-rich sequence of the β subunit could be replaced by that of the ras protein, even though three residues in the sequences are different (Takeyama et al. 1990); the mutant enzyme with the ras protein sequence (Gly-Ala-Gly-Gly-Val-Gly-Lys-Ser, residues 10–17) had approximately 40% of the wild-type ATPase activity and was active in ATP synthesis. These results suggested that the glycine-rich sequences of the two proteins are similar in structure and function. In contrast, the sequence of adenylate kinase (Gly-Gly-Pro-Gly-Ser-Gly-Lys-Gly-Thr, residues 15–23) could not function in the β subunit. Significant differences between the sequences of the two proteins are apparent; in contrast to the β subunit, adenylate kinase has a glycine insertion between the
lysine and threonine residues, which probably changes the position and projection of the side chain of the $\beta$Thr-156 residue.

The above results suggest that $\beta$Thr-156 itself is essential for catalysis. As expected, $\beta$Thr-156$\rightarrow$Cys and $\beta$Thr-156$\rightarrow$Ala mutant enzymes had neither steady-state (multi-site catalysis) nor single-site (uni-site catalysis) ATPase activities (Iwamoto et al. 1991; Omote et al. 1992). Conversely, $\beta$Thr-156 could be replaced by Ser without loss of activity, consistent with the activity of the ras-like $\beta$ mutant ($\beta$Thr-156 corresponds to ras Ser residue). Furthermore, the $\beta$Lys-155$\rightarrow$Ala or $\beta$ mutant had no uni- and multi-site activity, indicating that this residue is also essential for catalysis (H. Omote, M. Maeda and M. Futai, in preparation).

The $\beta$Gly-149$\rightarrow$Ser and $\beta$Gly-150$\rightarrow$Ser mutant enzymes had essentially normal activities (Iwamoto et al. 1991), whereas $\beta$Ala-151$\rightarrow$Val (Hsu et al. 1987) and $\beta$Ala-151$\rightarrow$Pro (Takeyama et al. 1990) mutants had approximately 6 and 200%, respectively, of the wild-type membrane ATPase activity. Replacement of the $\beta$Ala-151 residue may affect the orientation of catalytically essential residues such as $\beta$Lys-155 and $\beta$Thr-156 and change the kinetic properties of the enzyme.

The V-ATPases and F-ATPases so far studied can be differentiated simply by their inhibitor sensitivities (Forgac, 1989): azide specifically inhibits F-ATPase, whereas N-ethyl maleimide specifically inhibits V-ATPases. However, after a single amino acid substitution in the glycine-rich sequence, the inhibitor sensitivity of the F-ATPase became similar to that of the V-ATPase. Strikingly, a $\beta$Gly-149$\rightarrow$Ser mutation increased the apparent $K_i$ for azide more than 100-fold; the concentrations of azide required for 50% inhibition of the ATPase activity of the wild type and $\beta$Ser-149 mutant were 0.034 and 4.3 mmol l$^{-1}$, respectively (Iwamoto et al. 1991). The $\beta$Ser-150 mutant enzyme was not as azide-resistant, being about fourfold less sensitive than the wild type. The $\alpha$ subunit of V-ATPase has a cysteine residue corresponding to position 153 of the $\beta$ subunit of F-ATPase. The glycine-rich sequences of the $\alpha$ subunits of Neurospora crassa and carrot are Gly-Ala-Phe-Gly-Cys-Gly-Lys-Thr (Bowman et al. 1988; Zimniak et al. 1988). The $\beta$Val-153$\rightarrow$Cys mutant of F-ATPase is as sensitive to N-ethyl maleimide as is the wild type of V-ATPase. A simple but important lesson from these results is that unknown enzymes cannot be classified simply by their sensitivity to inhibitors, since inhibitor sensitivities can be altered by single amino acid substitutions.

Amino acid residues located near the glycine-rich sequence

Residues near the binding site for the $\gamma$-phosphate moiety of ATP can be mapped by genetic procedures: pseudo revertants of the mutants in the glycine-rich sequence may yield two closely related amino acid residues (the first from the original mutation and the second from a pseudo reversion). In contrast, two residues can also be functionally or structurally related if one mutation is suppressed by the second mutation in the glycine-rich sequence.

The $\beta$Ser-174$\rightarrow$Phe mutant is one of the early mutants isolated in our laboratory, and has about 6% of the wild-type membrane ATPase activity (Kanazawa et al. 1980). We found that the effect of this mutation was suppressed by a second mutation in the $\beta$ gene,
Gly149→Ser (Iwamoto et al. 1991). To confirm the suppression, the two mutations were introduced separately or together into pBWU13, which carries the entire *unc (atp)* operon. Other replacements at residue 149 could suppress the βPhe-174 mutation; the βPhe-174/βAla-149 mutant was similar in activity to the wild type and the βPhe-174/βCys-149 mutant had a low but significantly higher membrane ATPase activity than the βPhe-174 mutant alone (A. Iwamoto, M.-Y. Park, M. Maeda and M. Futai, in preparation). It is also noteworthy that the single βGly-149→Cys mutation resulted in a defective enzyme. In contrast, the βGly-149→Thr or βGly-150→Ser mutations could not suppress the effect of the βPhe-174 mutation. It would appear that suppression of the effect of the βSer-174→Phe mutation requires residues smaller than Thr. These results strongly suggest that the βGly-149 and βSer-174 residues interact functionally and that both are located near the γ-phosphate moiety of ATP.

We have also isolated pseudo revertants of the βCys-149 mutant, and found that either the βVal-198→Ala or the βGlu-192→Val mutation could suppress the effect of the βCys-149 mutation. Thus, the βGlu-192 and βVal-198 residues also interact functionally with the βGly-149 residue. It is reasonable to conclude that βGly-149 and βVal-198 are located close together, because βLys-201 (three residues downstream of βVal-198) was found to be near the binding site of the γ-phosphate moiety of ATP together with βLys-155, as shown by labelling with AP3-PL (Ida et al. 1991). Consistent with the location of the βGlu-192 residue being near the catalytic site, binding of dicyclohexylcarbodiimide (DCCD) to this residue completely inhibited enzyme activity (Yoshida et al. 1982). These results are summarized in a model of the catalytic site near the γ-phosphate moiety of ATP (Fig. 1B).

**Role of the γ subunit in the regulation of H⁺ transport**

Like other ion-motive ATPases, ATP hydrolysis/synthesis at the catalytic site of F-ATPase is tightly coupled with proton transport through the F₉ membrane sector. The mechanism of this coupling is not fully understood, but we think that the γ subunit may have regulatory functions in coupling. The roles of the γ subunit and its amino acid residues are less well understood than those of the β subunit. The chloroplast γ subunit has a unique domain containing two cysteine residues in which a disulfide to sulfhydryl transition activates the enzyme (Miki et al. 1988; Inohara et al. 1991), whereas such domains are not found in the subunits from mitochondria or bacteria. However, the *E. coli* γ subunit may have regulatory role(s) because the subunit is required, along with the α and β subunits, to reconstitute the minimal ATPase complex. We introduced mutations in the carboxyl terminal region between γGln-269 and γThr-277 because the termination mutant (γThr-277→end) was similar in activity to the wild type, whereas the γGln-269→end mutant had no ATPase activity (Miki et al. 1986; Iwamoto et al. 1990). The results of replacing the γGln-269, γThr-273 and γGlu-275 residues suggested that they are required for normal catalytic activity.

Interestingly, the ATPase activities of mutant membranes did not correlate with their abilities for ATP-driven H⁺ translocation: membranes of the γGln-269→Leu, γGlu-275→Lys, γThr-277→end mutants and a frameshift (downstream of γThr-277) mutant
Fig. 1. Glycine-rich sequence and catalytic site of F-ATPase. (A) Alignment of the glycine-rich sequence from the β subunit of *E. coli* F-ATPase (β), the A subunit of yeast V-ATPase (A), the ras protein (ras) and adenylate kinase (Adk). The secondary structures deduced by crystallography (adenylate kinase, Dreusicke et al. 1988; and ras protein, Milburn et al. 1990) are indicated. (B) A model of the catalytic site near the γ-phosphate moiety of ATP in the β subunit. The combined approaches of affinity labelling and analysis of random and directed mutants and pseudo revertants suggest the amino acid residues shown in or near the catalytic site. See text for details.

had similar low ATPase activities, but formed different degrees of electrochemical gradient of protons. The four mutations had different effects on the coupling between ATP hydrolysis and H⁺ translocation. We recently found that the γMet-23→Lys or Arg mutation resulted in an uncoupled enzyme: both enzymes showed membrane ATPase activities similar to that of wild type, but demonstrated substantially lower ATP-dependent H⁺ translocation and *in vivo* ATP synthesis (Shin et al. 1992). Furthermore, it is of interest that the effect of the γLys-23 mutation was suppressed by mutations within
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43 residues of the carboxyl terminus. These results clearly suggest that the two ends of the γ subunit participate in coupling between ATP synthesis/hydrolysis and proton translocation.

This study was supported in part by research grants from the Ministry of Education, Science and Culture of Japan and the Human Frontier Science Program.

References


