Localization of a Conformational Energy-coupling Determinant near the C Terminus of the β Subunit of the F1F0-ATPase*

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Escherichia coli mutants in the β subunit of the F1F0-ATPase can be complemented with the β subunit from the obligate aerobe Bacillus megaterium. It has been shown that cells carrying such hybrid ATPases have an unusual energy-coupling phenotype. Although they are able to grow on minimal succinate medium, and therefore carry a functional ATP synthase, they are defective in the ability to grow anaerobically, indicating some defect in ATP-driven proton pumping (Scarpetta, M., Hawthorne, C. A., and Brusilow, W. S. A. (1991) J. Biol. Chem. 266, 18567–18572). In this study, chimeric β subunits were constructed consisting of the E. coli or the B. megaterium β subunit carrying the C-terminal 18% of the other's β subunit. The phenotypes of an E. coli β mutant complemented with these chimeric subunits showed that the energy-coupling defect was located in this C-terminal region. The E. coli β subunit carrying the B. megaterium C-terminal region displayed the energy-coupling defect, while the B. megaterium β subunit carrying the E. coli C-terminal region did not. In ATP-dependent fluorescence quenching assays, membranes isolated from cells displaying the energy-coupling defect also pumped protons less well than membranes isolated from cells that were able to grow anaerobically. These results demonstrate that the C terminus of the β subunit is involved in the conformational coupling pathway, which, through the polypeptide backbone of the β subunit, physically links ATP synthesis or hydrolysis to the energy of proton translocation.

Proton-translocating F1F0-ATP synthases, or ATPases, are a class of large multimeric, membrane-bound enzymes that utilize a transmembrane electrochemical gradient of protons to synthesize ATP from ADP and Pi. These enzymes have essentially the same structure and function in bacterial cytoplasmic membranes, in mitochondrial inner membranes, and in chloroplast thylakoid membranes (for recent reviews, see Refs. 1–3). The structure and function of these enzymes has been studied biochemically for decades, and two x-ray structures for the catalytic F1 portion have been published (4, 5). The more recent structure of Abrahams et al. (5) shows a hexamer of alternating α and β subunits surrounding a single γ subunit. According to the binding-change mechanism (6), the energy of proton translocation is transmitted to the catalytic site in the β subunit by conformational changes in the polypeptide backbone of the complex. It has been proposed that the energy from proton movement is transmitted to the catalytic sites of the F1 by a rotation of one or more of the minor subunits relative to the αβ hexamer (6–9). Recent studies by Duncan et al. (10) and by Sabbert et al. (11) have shown that the catalytic cycle of the ATPase appears to involve rotation of the γ subunit relative to the αβ hexamer, supporting such rotational catalysis hypotheses. The interactions of γ with β and/or α, seen in the x-ray structure (5), are probably involved in transmitting energy between the site where energy is released during proton movement and the β subunit. This energy is then transmitted within the polypeptide backbone of the β subunit to the site where ATP is synthesized. The nature of these conformational changes is not known.

Our studies on the β subunits of Escherichia coli and the obligate aerobe Bacillus megaterium have demonstrated that replacement of the E. coli β subunit with the B. megaterium β subunit produces an ATPase with a physiological defect in ATP-driven proton pumping while still allowing protonotive force (pmf)-dependent ATP synthesis (12). Cells carrying such hybrid complexes can grow aerobically on a nonfermentable carbon source such as succinate, but, despite having significant measurable membrane-bound ATPase activity, are unable to grow anaerobically on certain media containing glucose (12). Since the sequence of the β subunit has been highly conserved during evolution, there are a limited number of amino acid differences between the two β subunits (13). The goal of these studies is to define the conformational coupling pathway within the polypeptide backbone of the β subunit, which connects the catalytic site for ATP synthesis/hydrolysis to the site or sites of transmembrane proton movement. We have constructed a variety of chimeric β subunits consisting of regions from both the E. coli and B. megaterium subunits. Our initial studies indicated that the defect might be localized to the C-terminal 39% of the B. megaterium β subunit (13). For the studies reported here, we created more specific chimeras and conducted more extensive enzymatic analyses on the resultant ATPases in order to further define the region of the β subunit responsible for defective energy coupling.

MATERIALS AND METHODS

Bacterial Strains and Growth Media—All experiments were performed using the β deletion strain JP17 (14). Genetic and biochemical results for this mutant transformed with various β plasmids were compared to the same assays done on wild type Unc+ E. coli strain LE392 (15).

Membranes were prepared from cells grown in LB medium (16) containing 40 mg/liter of chloramphenicol. Minimal glucose or minimal succinate plates consisted of 60 mg KH2PO4 and 40 mg NaH2PO4 adjusted to pH 7.0, 1 g/liter (NH4)2SO4, 1 mM MgSO4, 1 mM MgSO4, 0.2% B1, 6 mg/liter dihydroxybenzoate, 340 mg/liter l-arginine, 45 mg/liter uracil, and either 0.4% glucose or 0.8% sodium succinate. Anaerobic incubations were carried out in BB1 GasPak pouches.

Enzyme Assays—E. coli membranes were isolated and assayed for

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Plasmid Constructions—The vector for all the plasmids used for these studies was pACYC184 (18), and all confer chloramphenicol resistance. The E. coli β plasmid pWSB39 has been described previously (13). The B. megaterium plasmid pWSB59 was constructed by cloning an SphI-PvuII fragment from the B. megaterium β plasmid pCAH1.3 (19) into pACYC184 that had been digested with SphI and NruI. An AvrII site was constructed in the E. coli β gene at the same location in which it exists in the B. megaterium β gene as follows. A 1168-base pair PstI fragment was cloned from the E. coli β plasmid pRPG31 (20) into M13mp18. Single-stranded DNA was isolated and used as a template for mutagenesis with the primer 5’-ATCCATACCTAGATGGCGAT-3’, using the Amersham mutagenesis kit. The presence of the resultant AvrII site, which did not affect the amino acid sequence of the E. coli β subunit, was verified by sequencing the entire insert, which was then cloned into pUC19, creating plasmid pCB2. An AvrII-Ndel fragment carrying the 3’ end of the E. coli β gene and the entire E. coli ε gene was cloned into the B. megaterium β plasmid pMAS112 (12) which had been digested with AvrII and Ndel to create pWSB55, a plasmid carrying a chimeric E. coli-B. megaterium β gene and the entire E. coli ε gene. An SphI-Mael fragment carrying just the chimeric β gene was cloned from pWSB55 into pACYC184, which had been digested with SphI and NruI, to create pWSB58. To construct pWSB61, the mutagenized fragment from pCB2 was first cloned into pACYC184 as a HindIII-BamHI fragment to create pWSB65. An EcoRI-AvrII fragment from pWSB65 was then ligated to an EcoRI-AvrII fragment from pWSB59 to create pWSB66, a plasmid carrying the C-terminal region of the B. megaterium β subunit joined to the upstream PstI-AvrII region of the E. coli β subunit. A HindIII-PstI fragment carrying the first 84% of the E. coli β gene was then cloned from pWSB39 into pWSB66, which had been digested with HindIII and PstI, to create pWSB61, coding for the E. coli β gene carrying a C-terminal substitution of the last 16% of the B. megaterium β gene.

RESULTS

Construction of Chimeric β Subunits—We have previously constructed a set of chimeric E. coli-B. megaterium β plasmids, the results with which indicated that the energy coupling defect seen in E. coli β mutants complemented with B. megaterium β subunits might lie in the C-terminal 39% of the gene (13). In order to better define the region associated with the phenotype, we constructed the additional chimeric β subunits, shown in Fig. 1 along with the other β subunits used in these studies. All these constructions used plasmid pACYC184 as the cloning vector, so all confer chloramphenicol resistance. Plasmid pWSB39 and pWSB59 encode the intact E. coli and B. megaterium β subunits, respectively. Plasmid pWSB58 encodes the B. megaterium β subunit containing a C-terminal substitution of 76 amino acid residues from the E. coli subunit. Plasmid pWSB61 encodes the E. coli β subunit containing a C-terminal substitution of 79 amino acid residues of the B. megaterium subunit. These substitutions represent the C-terminal 18% of the β subunit. The constructions are described under “Materials and Methods.” The intact E. coli β gene does not contain an AvrII site near the 3’ end, but the engineering of that site into the E. coli sequence, required for the construction of pWSB61 and pWSB58, did not change the resultant amino acid sequence.

Genetic Complementation of β Mutants—The E. coli β deletion strain JP17 was transformed with the plasmids described in Fig. 1. The transformants were replica-plate-onto minimal succinate or minimal glucose plates and incubated at 37 °C under aerobic conditions or anaerobic conditions. The results are shown in Table I. All four β plasmids restored aerobic growth on succinate to the β deletion strain JP17. Only the intact E. coli β plasmid, pWSB39, and the chimeric plasmid consisting of the B. megaterium β gene with the C-terminal E. coli substitution, pWSB58, restored anaerobic growth on minimal glucose medium to JP17. The E. coli β subunit carrying the C-terminal B. megaterium substitution, encoded by pWSB61, behaved genetically like the intact B. megaterium β subunit. Neither restored anaerobic growth to the β deletion strain. As expected, all transformed colonies grew aerobically on minimal glucose medium, even those transformed with the vector alone. These results showed that although the chimeric β subunits could restore functional ATP synthase activity to the β mutant, the “one-way uncoupled” phenotype (12) conferred to β mutants by the B. megaterium β subunit is localized to amino acid residues in the C-terminal 16% of the β subunit.

Biochemical Complementation of β Mutants—Membranes were isolated from the β deletion mutant complemented with each plasmid and were assayed for ATPase and ATP synthase activity. Table I shows the results. Despite the fact that all of these cells were capable of growing on minimal succinate medium, isolated membranes displayed significant differences in specific ATP synthase activity. The highest activities were seen in membranes isolated from cells carrying either the intact E. coli β gene, pWSB39, or the B. megaterium β gene carrying the C-terminal E. coli replacement, pWSB58. Membranes isolated from cells carrying either the intact B. megaterium β gene, pWSB59, or the E. coli β gene carrying the B. megaterium C-terminal replacement, pWSB61, displayed very poor synthase activity, only marginally better than the control. However, the ATPase activities of membranes isolated from all these cells were essentially the same, despite the fact that they

| Plasmids | Aerobic | Anaerobic | ATPase | ATP Synthase |
|----------|---------|----------|--------|-------------|
| pACYC184 | −−      | <0.05    | 3 ± 2  |             |
| pWSB39   | ++      | 0.29 ± 0.05 | 20 ± 7 |             |
| pWSB61   | −−      | 0.20 ± 0.05 | 6 ± 3  |             |
| pWSB59   | −−      | 0.25 ± 0.03 | 9 ± 3  |             |
| pWSB58   | −+      | 0.21 ± 0.07 | 25 ± 1 |             |

Table I

Complementation of a β deletion strain with different β plasmids

The E. coli deletion strain JP17 was transformed with each of the plasmids described in Fig. 1. Transformants were patched on LB plates containing chloramphenicol and replica-plated onto minimal succinate and minimal glucose media. Succinate plates were incubated aerobically at 37 °C. Glucose plates were incubated anaerobically in BBL GasPak pouches at 37 °C. After 2 days growth was scored (+, good growth), + (growth), or − (no growth). Aerobically, all cultures grew on minimal glucose (not shown). Membranes were isolated from cultures of each transformant and assayed for ATPase and ATP synthase activities. The results are averages (±S.D.) from four different membrane preparations. For comparison, four membrane preparations from Unc- LE392 averaged 0.52 ± 0.07 μmol/min/mg of ATPase activity and 90 ± 15 nmol/min/mg of ATP synthase activity.

Fig. 1. Chimeric β subunits used in these studies. The bars represent a 5’ to 3’ schematic of the β genes present on each plasmid. The vertical line under the AvrII label represents the location of the AvrII site dividing the β gene in pWSB61, pWSB59, and pWSB58 into two sections: the 5’ 82% of the gene and the 3’ 18% of the gene. Sections derived from the E. coli β gene are labeled EC. Sections derived from the B. megaterium β gene are labeled BM. Details of the constructions are described under “Materials and Methods,” and details of the sequences are shown in Fig. 3.
isolated from cells carrying the E. coli with the 33392
the intact protons through the F₀ have not been defined. In
of the membrane protein. replacement coded for by pWSB58. All assays were done on 2 mg of
exhibiting this coupling defect are able to grow aerobically on a
ATPase. Fig. 2 shows that membranes isolated from cells car-
is an approximate measure of the rate of proton pumping and
fluorescence. The rate and extent of quenching described in Table I were assayed for their ability to carry out
the uncoupled phenotype is not related to differences in gross
the subunit, specifically of exchanging this small section between
effects on energy coupling of mutations in this section of the
subunit (5). The studies described in this paper examine the
B. megaterium plasmids. b
b
b
membrane preparations.

The amino acid sequences of the C-terminal 18% of the
E. coli and bovine mitochondria. The loop in which interacts with γ, the DEL-
loop, shows a single difference between E. coli and B. megaterium: the presence of an aspartate residue in the B.
subunit is indicated (Avr2). The amino acid differences between E. coli and B. megaterium β subunits are boxed. Digestion with Avr2 would cut the DNA at the point immediately preceding the codon for the leucine in the DIAIL sequence.

DISCUSSION

The catalytic cycle of the ATPase appears to involve rotation of the γ subunit relative to the αβ3 hexamer (10, 11), and the x-ray structure of the bovine mitochondrial ATPase shows that the γ subunit interacts with the β subunit in several places, one of which involves a large loop near the C terminus of the β subunit (5). The studies described in this paper examine the effects on energy coupling of mutations in this section of the β subunit, specifically of exchanging this small section between the β subunits of E. coli and B. megaterium.

The conformational changes in the F₁-ATPase that connect the energy of ATP hydrolysis or synthesis to the movement of protons through the F₀ have not been defined. In E. coli β mutants, the presence of a β subunit carrying the B. megaterium C terminus produces a defect in energy coupling. Cells exhibiting this coupling defect are able to grow aerobically on a

nonfermentable carbon source even though they exhibit significantly reduced ATP synthase activity. These cells are defective in their ability to grow anaerobically, although they have the same membrane-bound ATPase activity as cells that do not exhibit this defect. The C-terminal substitution appears to affect the forward and reverse coupling reactions differently: an indication that the defect is in the energy-coupling pathway and not catalysis. ATPases carrying this section on an otherwise E. coli β are still capable of ATP synthesis, ATP hydrolysis, and ATP-driven proton pumping. The coupling pathway has been impaired so that proton pumping ability is reduced, accounting for the uncoupled phenotype under anaerobic conditions. The C-terminal 18% of the B. megaterium β subunit therefore contains one or more amino acid substitutions, which directly affect the conformational coupling pathway: either the interaction of the β subunit with the γ subunit or the transmission of energy between the β-γ interaction and the catalytic site through the polypeptide backbone of the β subunit.

Fig. 3 shows the amino acid sequences of the C-terminal regions of the β subunits of E. coli, B. megaterium, and bovine mitochondria. The loop in which interacts with γ, the DEL-SEED loop, shows a single difference between E. coli and B. megaterium: the presence of an aspartate residue in the B. megaterium β subunit in place of a glutamate. Counting from this residue to the C terminus, E. coli has 76 residues, B. megaterium has 79 residues, and cow has 81 residues. In addition to the four C-terminal residues of the B. megaterium β subunit, there are 29 differences between E. coli and B. megaterium in this region, about half of which are very conservative changes. The coupling defect is therefore caused by one or more of a relatively small number of amino acid changes. Further analysis of these differences will start to define the conformational coupling pathway through the polypeptide backbone of the β subunit which physically links ATP synthesis or hydrolysis to the energy of proton translocation.

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