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THE β SUBUNIT OF THE ESCHERICHIA COLI ATP SYNTHASE EXHIBITS A TIGHT MEMBRANE BINDING PROPERTY

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SUMMARY: We have developed a chromatographic procedure to analyze the association of the subunits of the Escherichia coli F_1F_o-ATP synthase with the cytoplasmic membrane. Minicells containing [^{35}S]-labeled ATP synthase subunits are treated with lysozyme, solubilized, and chromatographed on a Sepharose CL-2B column in buffer containing urea and taurodeoxycholate. ATP synthase subunits are resolved into membrane intrinsic and membrane extrinsic subunits. Interestingly, a significant amount (36%) of the F_1 subunit β fractionates with the membrane intrinsic F_o subunits. About half of this amount (19%) of β is non-specifically bound to the membrane. Interaction of β with the membrane is not mediated by the amino terminal portion of β.

INTRODUCTION: In Escherichia coli, the proton translocating ATP synthase (EC 3.6.1.3) consists of two portions: the F_1, which is the membrane extrinsic catalytic domain and; the F_o, which is membrane intrinsic and conducts protons across the cytoplasmic membrane. The F_1 consists of the five subunits α, β, γ, δ, and ε, present in a stoichiometry of 3:3:1:1:1, respectively (1). The F_o consists of the a, b, and c subunits occuring in a 1:2:10 stoichiometry (1). The F_1 is thought to be organized into a core $\alpha_3\beta_3\gamma$ ATPase which is conected to the F_o via interactions mediated by the δ, ε, a and b subunits (2,3,4,5).

The F_1 sector is regarded as soluble because it may be dissociated ("stripped") from the F_o using low ionic strength buffer containing EDTA. Stripping removes virtually all of the F_1 activity from the membrane. On the other hand, there is evidence that stripping does not completely dissociate the F_1 from the F_o. Schneider and Altendorf found in their search for an F_o purification method that a column with bound ATP synthase

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washed with stripping buffer resulted in only partial separation of the F_1 and the F_o: the F_o was contaminated with large amounts of α and β (6). Friedl and Schairer reported that repeated washings of liposomes containing the ATP synthase with stripping buffer resulted in 90% loss of F_1 coupled function, but that greater than 50% of the F_1 subunits remained bound to the liposomes (7). Similarly, we have found that the δ and ε subunits are stripped from liposomes more efficiently than the α, β and γ subunits (9). Bragg and Hou have described non-specific binding of F_1 to phospholipid vesicles (8). Hermolin et al. have mentioned a similar phenominon with E. coli membrane vesicles, to which the F_1 exhibits non-specific or low affinity binding (5). The experiments presented here demonstrate an interaction between the β subunit and the F_o and the membrane which may explain the membrane binding property of the F_1 sector.

METHODS: Materials: [^{35}S]methionine (1000 Ci/mmol) was purchased from New England Nuclear. SDS was from BDM Chemicals Ltd. Sepharose CL-2B was from Pharmacia Fine Chemicals. Sodium tauro-deoxycholic acid was obtained from Sigma. Ultra pure urea was from Schwarz/Mann. All other chemicals and media were of a commercially available high purity.
Bacterial strain, plasmids and minicell purification: DK3 is used for minicell preparations and is a derivative of DS410 which lacks the uncBEFHAGD genes (3). DK3 codes for the ε and i polypeptides. Plasmids, constructed as described (10), code for the polypeptides indicated in the parentheses following the plasmid designation. Plasmids were maintained in DK3 cultured in Luria broth with 30 mg/L chloramphenicol. Minicells were prepared as described from cultures harvested at OD_{600}=1.0 (11).
Minicell labeling, lysis and chromatography: Minicells ($2x10^9$), in 200 µl of minicell medium (11), were preincubated for 15 min at 37°, labeled with 50 µCi of [^{35}S]methionine for 60 min, and chased with 1 µM unlabeled methionine for 15 min. Minicell synthesis was stopped by cooling and all subsequent steps were performed at 0-4°C. Labeled minicells were washed once and resuspended in 100 mM HEPES pH 7.3, 5 mM $MgSO_4$, 100 µM PMSF, 40 µM spermine and frozen/thawed in buffer four times. T4 lysozyme was added to a final concentration of 3 µg/ml and the mixture kept at 0°C for 30 min. T4 lysozyme treated minicells were harvested in an Eppendorf microfuge (12,800xg) for 5 min, followed by solubilization with 40 µl of UTG buffer (6 M urea, 10 mM taurodeoxycholate, 10% glycerol, 50 mM HEPES pH 8.0, 2 mM EDTA, 150 mM NaCl, 2 mM DTT). The suspension was frozen/thawed three times, kept on ice 30 min, adjusted to 0.01% bromophenol blue dye, and applied to the top of a 14 cm (3.8 ml) Sepharose CL-2B column previously equilibrated with UTG buffer. Chromatography was performed at a flow rate of 1.0 ml per hour. A small aliquot of each 100 µl fraction was analyzed using a liquid scintillation counter (Beckman LS 7500).

Electrophoretic analysis and subunit quantitation: Chromatography fractions, precipitated with acetone, were analyzed on 20 cm 13% SDS-polyacrylamide slab gel as described (9). Fluorographic enhancement was performed and the film exposed for a length of time that produced autoradiogram band densities within the linear detection range of a Quick Scan R & D densitometer. A Numonics Corp Electronic Graphics Calculator was used with the densitometer integrator to compare the quantities of subunits.

Additional methods: Determination of the CL-2B column void volume was performed with exhaustively crosslinked E. coli cells. One milliliter of an OD_{600}=3.5 culture was adjusted to 5% formaldehyde and incubated at room temperature 30 min. The cells were centrifuged in an Eppendorf microfuged for 2 min, washed with UTG buffer, resuspended in UTG buffer and subjected to chromatography as described above. Fractions were brought to 1 ml with H_2O and the OD_{600} was measured (Gilford 240).

RESULTS: Characterization of the column chromatography technique: The elution profiles for the experiments reported below show two peaks of radioactivity (Fig. 1). The first peak centered at fraction 13 elutes just behind the column void volume and represents radiolabeled protein(s) bound to a very high molecular weight (> $40x10^6$) cellular fraction. Considering the apparent molecular weight of this peak, we suggest that the first peak consists of minicell membranes which have been extracted by

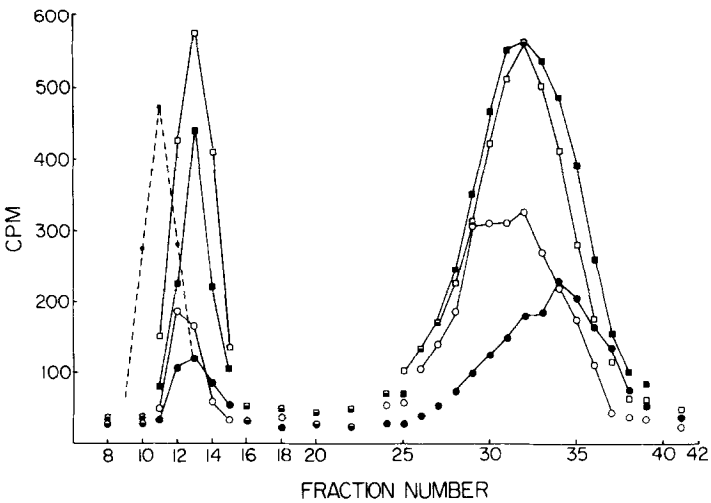

Figure 1. Separation of membrane intrinsic and membrane bound ATP synthase polypeptides. Radioactively labeled minicells were solubilized and chromatographed in UTG buffer as described in Methods. The elution profiles are from: DK3/pRPG54 (ΔuncB-D/acbδαγβε), ● ; DK3/pRPG32 (ΔuncB-D/βε), ○ ; DK3/pWSB1 (ΔuncB-D/β', M_r=42000, ε), □ ; DK3/pWSB2 (ΔuncB-D/β'' M_r=10000), ■ . The dashed line indicates the column void volume as measured by the elution position of E. coli cells.

the urea and detergent but not completely dissolved. We refer to this membrane fraction as "membrane remnants". The second broader peak centered at fraction 32 emerges from the column at the total volume of the column and represents radiolabeled proteins solubilized from the membrane by the chromatography buffer. Solubilization and chromatography of purified F_1F_o ATP synthase in UTG buffer results in dissociation of the complex into individual subunits which elute at the low molecular weight position (peak 2) (data not shown). The low molecular weight dye bromophenol blue elutes in fractions 28-36.

The β subunit fractionates with the F_o subunits a, b and c: Chromatography of labeled and solubilized DK3/pRPG54 (ΔuncB-D/acbδαγβε) minicells shows the presence of the majority of the F_o subunits a and b and all of the c subunit in peak one (Fig. 2). Significantly, a large portion of the F_1 subunit β fractionates in peak one. From densitometry we calculate that 36% of the β radioactivity is present in peak one. The α:β:γ:δ:ε stoichiometry in DK3/pRPG54 minicell membranes is 3:3:0.9:1.4:1.0, which compares favorably with the in vivo ratio of 3:3:1:1:1 (data not shown). This suggests that the β subunits bound to the membrane are not β subunits which have failed to assemble into an F_1 complex.

The F_1 subunits γ, δ, and ε are present in peak two, along with most of the α and β subunits. The majority of the vector encoded chloramphenicol acetyl transferase is also present in peak two, although a small amount occurs in peak one. A small amount of α also partitions in peak one. The absence of the very hydrophobic c subunit from peak two indicates that minicell membranes are not solubilized by this procedure, and that essentially all of the membrane remnants are present in peak one. The β subunit exhibits a tight association with the membrane: Minicells from DK3/pRPG32 (ΔuncB-D/βε) synthesize the β and ε subunits more efficiently than is observed with DK3/pRPG54 (ΔuncB-D/acbδαγβε) minicells. The analysis of minicells from DK3/pRPG32 shows that the β subunit binds to membranes (Fig. 3).

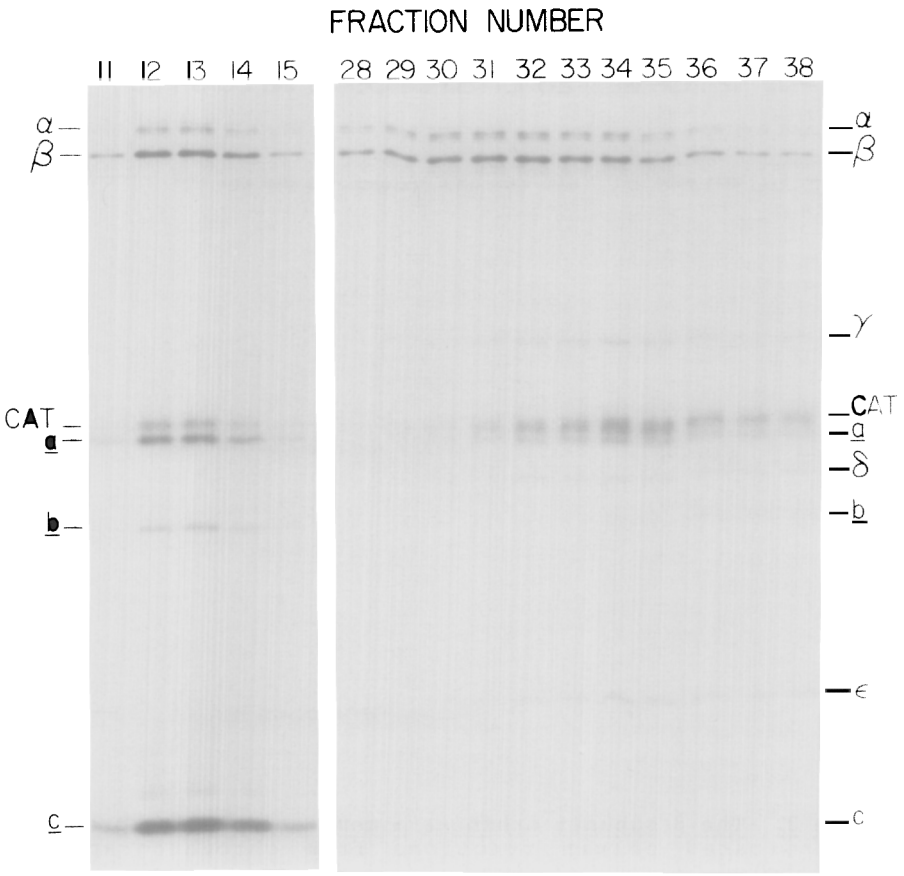

Figure 2. The β subunit fractionates with the F_o subunits. The indicated column fractions from chromatography of radiolabeled DK3/pRPG54 (ΔuncB-D/acbδαγβε) minicells were analyzed electrophoretically. The fractions correspond to the fractions designated ● in Fig. 1.

Densitometric analysis places the amount of β in peak one at 19%, approximately one half of the amount which is bound to membranes containing the F_o polypeptides. The ε subunit fractionates completely in peak two.

Using partial β polypeptides we have eliminated the possibility that the amino terminus of β is responsible for membrane binding. The plasmid pWSB1 codes for a carboxy-terminal β fragment 39989 daltons in size, consisting of residues 98-460, with an apparent molecular weight of 42000. Plasmid pWSB2 codes for an amino-terminal β fragment 10149 daltons in size, consisting of residues 1-95, with an apparent molecular weight of 10000. Chromatographic analysis of pWSB1 minicell products shows

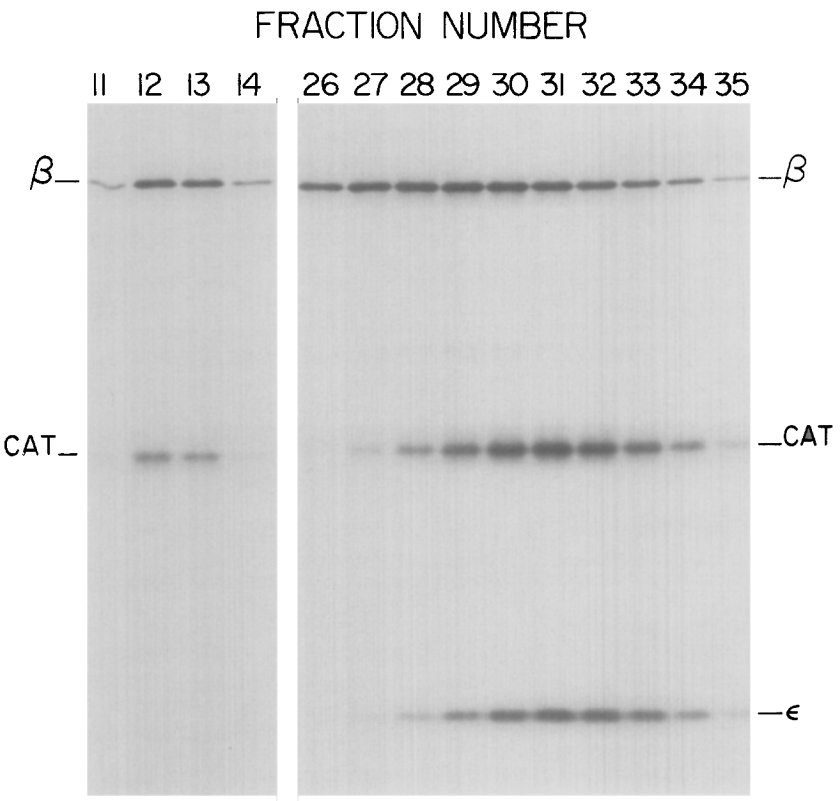

Figure 3. The β subunit exhibits a membrane binding property. The indicated column fractions from chromatography of radiolabeled DK3/pRPG32 (ΔuncB-D/βε) minicells were analyzed electrophoretically. The fractions correspond to the fractions designated O in Fig. 1.

that the 42000 dalton β, denoted β', elutes in peak one (Fig. 4). The 10000 dalton β, denoted β'', however, appears in peak two (Fig. 4).

DISCUSSION: The β subunit exhibits the property of binding to the F_o sector and appears to interact with E. coli membranes vesicles to an equal extent. Both of these β interactions offer an explanation for the difficulty in removing the F_1 subunits in general, and the β subunit in particular, from F_o preparations, liposomes, and E. coli membrane vesicles (5-9). Close proximity of a β subunit, or a portion of β, with the membrane is suggested by labeling of the ATP synthase in liposomes with a cross-linking phospholipid analogue, which results in significant labeling of β (12). We can not rule out the possibility that the β subunit

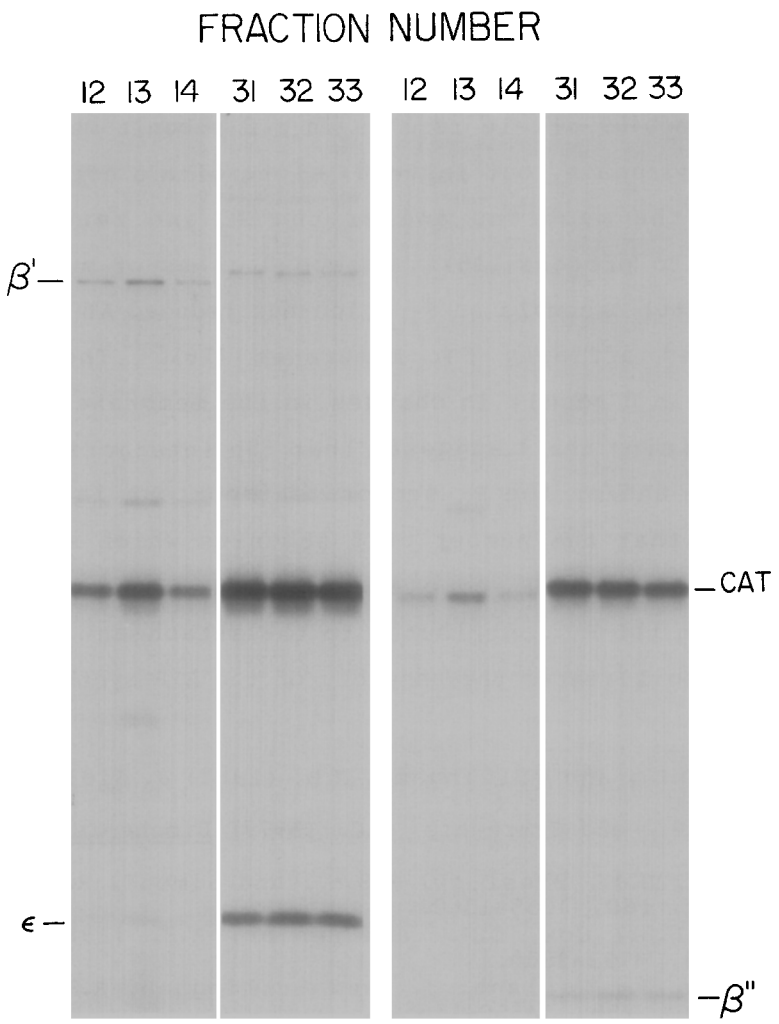

Figure 4. The carboxy terminus of β interacts with the membrane. The two panels on the left: the indicated column fractions from chromatography of radiolabeled DK3/pWSB1 (β', carboxy-terminal fragment, M_r=42000, ε) were analyzed electrophoretically. The two panels on the right: DK3/pWSB2 (β'', amino terminal fragment, M_r=10000) minicells were analyzed electrophoretically. The fractions correspond to the fractions designated □ (pWSB1) and ■ (pWSB2) in Fig. 1.

binds to the i polypeptide in the membrane, since DK3 contains the uncI gene.

Although the amino-terminus of β does not appear to be involved in membrane binding, it is difficult to infer which portion of β is responsible for binding. The only hydrophobic amino acid stretch is located in the carboxy terminal half of β, from residue 240 to 330 (β contains 460 residues) and probably forms the nucleotide binding site, or Rossmann fold (13,14).

The phenotypes of a number of uncD (coding for β) mutants suggest that the membrane binding property of β is important in vivo. The uncD409 allele results in a β subunit which does not form an F_1 aggregate, but instead, along with a normal α, binds tightly to the membrane and/or the F_o and renders the F_o impermeable to protons (15). A second class of mutants (e.g., uncD404 and 460) assemble an F_1 which has reduced ATPase activity but increased affinity for membranes (16). The fact that alterations in β result in changes in the membrane affinity of the F_1 containing the β suggests that the interaction of β with the membrane and/or the F_o sector is important in vivo. It is interesting that the number of β subunits which appear to be membrane bound in the F_1 complex is 1 in 3. Perhaps one of the β subunits in the F_1 contributes to the attachment of the F_1 to the F_o, or facillitates the assembly of the ATP synthase complex.

REFERENCES:

1. Foster, D.L., and Fillingame, R.H. (1982) J. Biol. Chem. **257**, 2009-2015.
2. Smith, J.B., and Sternweis, P.C. (1977) Biochemistry **16**, 306-311.
3. Klionsky, D.J., Brusilow, W.S.A., and Simoni, R.D. (1984) J. Bacteriol. **160**, 1055-1060.
4. Perlin, D.S., Cox, D.N., and Senior, A.E. (1983) J. Biol. Chem. **258**, 9793-9800.
5. Hermolin, J., Gallant, J., and Fillingame, R.H. (1983) J. Biol. Chem. **258**, 14550-14555.
6. Schneider, E., and Altendorf, K. (1982) Eur. J. Biochem. **126**, 149-153.
7. Friedl, P., and Schairer, H.U. (1981) FEBS LETTERS **128**, 261-264.
8. Bragg, P.D., and Hou, C. (1978) Can. J. Biochem. **56**, 559-564.
9. Aris, J.P. and Simoni, R.D. (1983) J. Biol. Chem. **258**, 14599-14609.
10. Gunsalus, R.P., Brusilow, W.S.A., and Simoni, R.D. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 320-324.
11. Klionsky, D.J., Brusilow, W.S.A., and Simoni, R.D. (1983) J. Biol. Chem. **258**, 10136-10143.
12. Hoppe, J., Montecucco, C., and Friedl, P. (1980) J. Biol. Chem. **258**, 2882-2885.
13. Kanazawa, H., Kayano, T., Kiyasu, T., and Futai, M. (1982) Biochem. Biophys. Res. Commun. **105**, 1257-1264
14. Kanazawa, H., and Futai, M. (1982) Ann. N. Y. Acad. Sci. **402**, 45-64.
15. Cox, G.B., Downie, J.A., Langman, L., Senior, A.E., Ash, G., Fayle, D.R.H., and Gibson, F. (1981) J. Bacteriol. **148**, 30-42.
16. Senior, A. E., Langman, L., Cox, G. B., and Gibson, F. (1983) Biochem. J. **210**, 395-403