An affinity resin for the F₁ sector of the *Escherichia coli* ATP synthase was prepared by coupling the b subunit to a solid support through a unique cysteine residue in the N-terminal leader: b<sub>24–156</sub> α form of b lacking the N-terminal transmembrane domain, was able to compete with the affinity resin for binding of F₁.-Truncated forms of b<sub>24–156</sub>, in which one or four residues from the C terminus were removed, competed poorly for F₁ binding, suggesting that these residues play an important role in b-F₁ interactions. Sedimentation velocity analytical ultracentrifugation revealed that removal of these C-terminal residues from b<sub>24–156</sub> resulted in a disruption of its association with the purified δ subunit of the enzyme. To determine whether these residues interact directly with δ, cysteine residues were introduced at various C-terminal positions of b and modified with the heterobifunctional cross-linker benzophenone-4-maleimide. Cross-links between b and δ were obtained when the reagent was incorporated at positions 155 and 158 (two residues beyond the normal C terminus) in both the reconstituted b<sub>24–156</sub> complex and the membrane-bound F₁-F₄ complex. CNBr digestion followed by peptide sequencing showed the site of cross-linking within the 177-residue δ subunit to be C-terminal to residue 148, possibly at Met-158. These results indicate that the b and δ subunits interact via their C-terminal regions and that this interaction is instrumental in the binding of the F₁ sector to the b subunit of F₀.

In the process of oxidative phosphorylation or photophosphorylation, the electron transport chain generates a transmembrane proton gradient. The ATP synthase, or F₁F₄-ATPase, allows protons to flow down this electrochemical gradient and uses the energy obtained to synthesize ATP (for reviews, see Refs. 1–4). Under appropriate conditions ATP synthase can be reconstituted on a membrane and houses the catalytic sites for ATP synthesis, the electron transport chain generates a transmembrane proton gradient across the membrane, and the enzyme uses the energy obtained to synthesize ATP by translocating protons across the membrane. The 156-residue b subunit is believed to span the membrane once at its hydrophilic N terminus, whereas the remainder of the protein is very hydrophilic. δ is thought to exist as a dimer in the complex (5–7), and proteolysis studies have shown that the hydrophilic region of δ is required for the association of F₁ with the membrane (7–9). Removal of two residues from the C terminus of b disrupts normal assembly of the complex (10), as does mutation of Gly-131 to aspartate (11). Thus, the b subunit is essential for linking the F₁ and F₄ sectors and likely plays a key role in the coupling of energy from proton translocation to ATP synthesis.

The crystal structure of the mitochondrial F₁ has shown that the α and β subunits alternate in a hexagonal ring structure, with two long α-helices from γ extending into a hole in the center of the ring (12). Recent evidence strongly suggests that γ and probably ε rotate relative to α and β during catalysis (13–16). Several studies have implied that the δ subunit is located near the top of the αβ cluster (17–20). Linking b, δ is required for the binding of F₄ to F₀. Membranes of mutant *E. coli* strains expressing truncated forms of δ showed little ATPase activity (21), suggesting that F₁ cannot bind to the membrane in the absence of δ. In truncation and mutagenesis studies using the mitochondrial (22, 23) and yeast (24) homologues of δ, called OSCP<sup>1</sup> for oligomycin sensitivity conferring protein, the C-terminal region of OSCP was implicated in F₀ binding.

The only subunit of F₀ able to span the distance from the membrane to the top of F₁ is b. The hydrophilic portion of b is dimeric, highly α-helical, has an elongated shape, and binds to F₁ (25). Although chemical cross-linking of *E. coli* ATP synthase has failed to reveal b-δ cross-links, such products have been obtained with the chloroplast (26) and mitochondrial (27) enzymes. In the chloroplast work, the cross-link produced by 1-ethyl-3,3-(dimethylaminopropyl)-carbodiimide was mapped to the C-terminal part of b. The site in δ was determined to be within the cyanoen bromide fragment encompassing residues Val-1 to Met-165 of the 187-residue polypeptide. Recent studies (28–31) have demonstrated the interaction of *E. coli* b and δ in the absence of other subunits.

In the present work, we examine the interaction between *E. coli* b and F₁. An F₁ affinity resin has been generated by linking the hydrophilic portion of b to a solid support. By using binding
interaction between residues at the C terminus of \( b \) and the C-terminal region of \( \delta \).

**EXPERIMENTAL PROCEDURES**

**Construction of Plasmids**—Molecular biological procedures were carried out as described by Sambrook et al. (32). Plasmid pMR2, which was used as an intermediate during the construction of pPB2, was generated from pSD80 (33) by elimination of an NdeI site outside of the entire cloning region, followed by insertion of the PCR-amplified uncF gene from pSD51 (25) into the EcoRI and HindIII sites. The PCR primer was designed such that the initiating ATG codon is part of an NdeI site.

Purified pPB2, encoded the \( b_{24–152} \) protein, was constructed as follows. pDM3 (34) was used as the template for PCR, using the mutagenic primer 5'-GGCCATATAGGACCGGTGATACCCCTCTAGC-3'. The 5' end contains an NdeI site and the 3' end is complementary to the beginning of the gene encoding \( b_{24–152} \). The initial ATG codon is underlined. The second primer for PCR was the M13 forward sequencing primer. The resulting PCR product was inserted into the NdeI and HindIII sites of pMR2 to encode a protein beginning with the amino acid sequence MERC bound to the resin. The mixture was incubated at room temperature overnight to allow for the formation of the desired DNA fragment. To confirm the formation of the desired DNA fragment, the PCR product was excised from agarose gel slices and was purified.

Plasmids expressing other variants of \( b_{24–156} \) were transformed into E. coli DH5\( \alpha \) and were purified as described previously (34). Expression and Purification of Proteins—\( b_{24–156} \) and \( b_{146–24} \) were expressed and purified as described (34). Cysteine-containing and truncated forms of \( b_{24–156} \) as well as the \( b_{24–156} \) gene were expressed and purified in the same manner as \( b_{24–156} \), except that proteins containing cysteine residues were purified in the presence of 1 mM dithiothreitol (DTT).

Plasmids encoding wild type or mutated full-length \( b \) protein, appropriate restriction endonucleases were used to cut the desired fragment from the pDM3-based plasmid and transfer it to pDM3 (34). The construction of plasmid pSD114 encoding \( b_{24–156} \) has been described previously (34). Production of the F, Affinity Resin—DTT was removed from \( b_{24–156} \) by passing it through a Sephadex G-25 size exclusion column equilibrated with 50 mM triethanolamine HCl (TEA-HCl), pH 7.5, 1 mM EDTA. Sulfo-link Coupling gel, obtained from Pierce, was washed with 8 volumes of 50 mM TEA-HCl, pH 7.5, 5 mM EDTA before addition of 2.5 mg of \( b_{24–156} \) per ml of resin. The mixture was incubated at room temperature for 1 h, and the resin was then washed in buffer containing cysteine residues were purified in the presence of 1 mM dithiothreitol (DTT).

Plasmids encoding wild type or mutated full-length \( b \) were transformed into the \( uncF \) E. coli strain KM2 (35). Expression of the proteins and purification of the membranes were performed as described (34). The \( \delta \) subunit was expressed and purified as described previously (31). \( F_1 \) was purified to homogeneity using standard SDS-PAGE methods (36).

Cyanogen Bromide Cleavage and Analysis of Cross-linked Products—Cross-linked \( b_{24–156} \) were incubated with 50 mM Tris-HCl, pH 8.0, and 100 mM NaCl, and 1 mM EDTA. After incubation, the mixture was centrifuged to sediment the resin, and 10 \( \mu \)l of the supernatant solution were analyzed by SDS-PAGE.

**Analytical Ultracentrifugation**—Analytical ultracentrifugation was carried out using a Beckman XL-A ultracentrifuge at 20 °C. Buffer containing 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 1 mM EDTA (centrifugation buffer) was used. The rotor speed was 60,000 rpm, and scans were taken at 10-min intervals. During analysis of \( \delta \), 1 mM DTT was added to the centrifugation buffer, the buffer used during experiments in which \( \delta \) and forms of \( b \) were mixed contained 0.5 mM DTT. The data were analyzed with the Beckman software using the time derivative method of Stafford (30). Sedimentation equilibrium experiments were performed at 20 °C and 20,000 rpm using \( b_{24–156} \) at concentrations of 0.5, 1.0, or 2.0 mg of protein per ml in centrifugation buffer. Three molecular weight determinations were made at each concentration.

**Cross-linking of Membranes**—Membranes containing \( F \), \( F_0 \), complexes including either wild type or mutated \( b \) were diluted to 0.25 mg of total protein per ml with buffer containing 50 mM Tris-HCl, pH 8.0, 5 mM MgCl\(_2\), and 10% glycerol. Benzenzophenone-4-maleimide (BPM) (Mo- lecular Probes, Eugene, OR) dissolved in dimethylformamide (DMF) was added to a final concentration of 1 mM, and the mixture was allowed to stand at room temperature for 30 min. Controls were performed in which only DMF was added to the membranes. The treated membranes were exposed to wave ultraviolet light from Ultra-Violet Products model TM-36 transilluminator for 5 min. As a control some BPM-modified samples were placed on the transilluminator but were removed before it was turned on. After illumination, SDS-PAGE sample buffer was added to the samples, which were then heated at 100 °C for 5 min and analyzed by SDS-PAGE followed by Western blotting.

**Assays for F1 Interaction with Affinity Resin**—To assay for 

**Other Methods**—SDS-PAGE was performed by the method of Laemmli (41) using 15% separating gels. The proteins were stained with Coomassie Brilliant Blue R-250. Protein blotting onto PVDF membrane. After blotting, the membrane was stained briefly with Coomassie Blue or treated with 2% CNBr in 70% formic acid. After 30 min the slices had swollen back to their original sizes. At this time the electrophoresis was performed and the gel sliced and incubated at 37 °C overnight. The pH of the slices was then adjusted by three successive 15-min incubations in 150 mM of 10 mM Tris-HCl, pH 8.0, followed by 15-min incubations in 150 mM of 1 mM Tris-HCl, pH 8.0, 150 mM of 67 mM Tris-HCl, pH 6.8, and 150 mM of SDS-PAGE sample buffer containing DTT. The slices were placed in the wells of a second SDS-polyacrylamide gel that had been pre-electro-phoresed for 1 h at 100 V in the presence of 50 mM Tris-HCl, pH 8.0, 0.1% SDS, containing 0.1 mM sodium thioglycolate to scavenge free radicals in the gel. Electrophoresis was carried out in the presence of 0.1 mM thioglycolate. The gel was either stained with Coomassie Blue or Western blotted onto a polyvinylidene difluoride (PVDF) membrane.

After blotting, the membrane was stained briefly with Coomassie Blue and destained with 30% methanol before the bands of interest were excised and analyzed by peptide sequencing.

**Acknowledgments**—We thank the late R. H. Atwood and W. W. Ing olden-Holstein of Universität Osnabrück, Germany. Anti-\( \delta \) polyclonal antibodies were raised against purified \( \delta \) subunit (43), and the anti-\( \delta \) antibodies were affinity purified on a column containing immobilized recombinant \( \delta \) (31). Antibodies were labeled with \( ^{125}I \) by the IODO-GEN method (44). Protein concentrations were determined by the method of Bradford (45) or Lowry et al. (46). Peptide sequencing was performed at the Laboratory for Macro.
The volume of trapped liquid, BSA was included in all incubations. As a control for trapping (see text). The resin was then sedimented by centrifugation, and the pellet was resuspended in SDS-PAGE sample buffer and analyzed by SDS-PAGE. The asterisks indicate experiments in which the control resin, bearing cysteine instead of bMERC, was used. A, a wide variety of b24–156 concentrations was used to determine the range in which substantial effects would be observed. B, b24–156, b24–155, and b24–152 were each tested at dimer concentrations of 1 and 10 μM to determine their relative strengths of binding.

FIG. 1. Binding of F1 to affinity resin and competition by soluble forms of b, as analyzed by SDS-PAGE. The F1 affinity resin, prepared from bMERC as described under “Experimental Procedures,” was incubated for 1 h in the presence of purified F1 and the indicated soluble forms of b, expressed as concentration of the dimer. BSA was included as a control for trapping (see text). The resin was then sedimented by centrifugation, and the pellet was resuspended in SDS-PAGE sample buffer and analyzed by SDS-PAGE. The asterisks indicate experiments in which the control resin, bearing cysteine instead of bMERC, was used. A, a wide variety of b24–156 concentrations was used to determine the range in which substantial effects would be observed. B, b24–156, b24–155, and b24–152 were each tested at dimer concentrations of 1 and 10 μM to determine their relative strengths of binding.

molecular Structure at Purdue University (West Lafayette, IN) using an Applied Biosystems 470A sequencer.

RESULTS

F1 Affinity Resin—To characterize better the binding between the F1 sector and the hydrophilic portion of b, we coupled this region of b to a solid matrix to form an affinity resin for F1. The Sulfo-link coupling gel from Pierce, to which proteins can be coupled specifically via thiol groups, was chosen as a matrix. Since the hydrophilic region of b has no cysteine residues, the site of coupling to the resin can be specified by site-directed mutagenesis of b. Because the C-terminal region was thought most likely to be involved in b-F1 contacts, we introduced a cysteine residue near the N terminus of the hydrophilic region. A construct encoding the polypeptide sequence MERCN-SHY24–L156 (bMERC) was produced as described under “Experimental Procedures.” The first four amino acids of this sequence were taken from the E. coli enzyme 3-methyladenine-DNA glycosylase I, because of the polar nature of the sequence and the high expression of this enzyme in a recombinant system (47).

The purified bMERC was coupled to the Sulfo-link resin as described under “Experimental Procedures,” at a final concentration of 2.2 mg per ml of resin. Upon incubation of the modified resin with the F1 complex followed by centrifugation, a significant amount of F1 sedimented with the resin, as determined by SDS-PAGE (Fig. 1A, first lane). Only a small amount of F1 co-sedimented with the resin that had been modified with cysteine (Fig. 1A, last lane). The F1 present in this pellet was probably not bound specifically to the resin but rather was trapped between and within the resin particles. As a control for the volume of trapped liquid, BSA was included in all incubations; similar amounts of BSA were observed to co-sediment with both resins under all conditions used (Fig. 1). Thus the bMERC-modified resin is able to bind F1 specifically. The faint band migrating at the position of b24–156 in the first lane represents a trace of bMERC eluted from the resin during the incubation in SDS sample buffer. Most likely this arose from instances in which only one subunit of the dimer became covalently coupled to the resin.

Competition for F1 Binding by the SDS-PAGE Assay—To test the ability of the hydrophilic portion of b to compete with the resin for binding to F1, the resin was incubated with F1 in the presence of increasing amounts of b24–156 (formerly known as b24-L; Ref. 33). After centrifugation and analysis of the pellet by SDS-PAGE, the amount of F1 bound to the resin was observed to decrease as the concentration of b24–156 increased, until essentially no F1 was bound by the resin at 6.5 μM of b24–156 dimer (Fig. 1A). Note that the amounts of soluble b24–156 trapped within the pelleted resin provide an internal representation of the amount of competitor added. These experiments demonstrate that b24–156 is able to compete with the bMERC-modified resin for binding to F1, establishing a simple competition assay for determining the relative affinity of any mutant form of b for F1.

Such experiments were carried out using b24–155 and b24–152, which lack one and four residues from the C terminus, respectively. It was found that these forms of b competed very poorly, relative to b24–156, with the bMERC-modified resin (Fig. 1B). At a dimer concentration of 10 μM, b24–155 showed a small amount of competition, whereas at the same concentration b24–152 showed no detectable competition by the SDS-PAGE assay. It is evident, however, that very weak competition is difficult to detect in this manner, as it requires seeing a small difference in band intensity.

Competition for F1 Binding by the Soluble ATPase Activity Assay—To determine weak competition more reliably and in a quantifiable way, the assay was modified such that soluble ATPase activity, rather than bound ATPase protein, was measured. Under the conditions used in these assays, more than 90% of the added enzyme was bound by the resin. Competition for F1 binding by b24–156, b24–155, and b24–152 was determined by the increase in ATPase activity remaining in the supernatant solution when these forms of b were added to the incubations. As expected, the amount of F1 in the supernatant solution increased sharply with increasing concentration of b24–156, whereas the ability of b24–155 and b24–152 to compete with the bMERC-modified resin was far weaker, although still detectable (Fig. 2). These results show that the C-terminal residues of b are essential for its proper interaction with F1-ATPase. The preparations of soluble b were tested directly for ATP hydrolysis activity to make certain that trace contamination with an enzyme such as alkaline phosphatase could not account for the increase in soluble ATPase activity. In no case could such a contaminant account for more than 2% of the observed soluble activity.

Sedimentation Velocity Analysis—Recent ultracentrifugation results from this laboratory have provided evidence for the formation of an elongated complex by two molecules of the hydrophilic region of b and one molecule of the δ subunit (31). We performed further centrifugation experiments to determine whether the reduced binding of the C-terminal truncations of b to F1 could be due to loss of contacts with δ. The isolated b24–156 and δ subunits showed sedimentation coefficients (Table I) which were similar to those previously reported for b24–156 (25) and δ (31). The increase in the s20,w upon mixture of equimolar amounts of dimeric b24–156 and δ is confirmation that these proteins form a complex in solution. Mixtures of δ with b24–155 or with b24–152 resulted in s values only slightly above the weighted averages of the two components, indicating that bind-
Effect on its binding to residues near the N terminus of the complex formed when coefficient comparable with amounts of rpm as described under "Experimental Procedures." The abscissa represents concentration of the activity present in the supernatant solution when the control resin, bearing cysteine instead of D_47HCO, was used.

TABLE I

Sedimentation velocity analysis of δ with soluble b domains

| Protein       |  \\( s_{20, w}(S) \) ± S.D. | -δ | +δ |
|---------------|-----------------------------|----|----|
| δ             | 1.84 ± 0.01                 | 2.70 ± 0.01 |
| b_{24-156}    | 1.60 ± 0.03                 | 1.85 ± 0.01 |
| b_{24-155}    | 1.42 ± 0.04                 | 1.74 ± 0.03 |
| b_{34-152}    | 1.79 ± 0.03                 | 2.25 ± 0.02 |

*Mean of three determinations ± S.D.

Cross-linking of b to δ in the F_1F_0 complex. Membranes were prepared from uncF E. coli cells complemented by a plasmid expressing either b containing the E155C mutation (A), b containing the 158C mutation (B), or the wild type b subunit (both panels). The membranes were treated with BPM and/or UV light as shown and were then analyzed by Western blotting. The blots were probed with antibodies raised against either b or δ.

Cross-linking of b and δ—Individual cysteine residues were introduced into full-length b at positions 150, 151, and 155. A fourth construct was made that encoded a protein, referred to as b158C, having two residues, glycine and cysteine, attached to the C terminus of b. It was anticipated that the glycine would provide conformational flexibility to the C-terminal cysteine, increasing the likelihood of obtaining a cross-link. Plasmids bearing these mutated forms of b were all able to complement the uncF strain KM2 for growth on minimal media with succinate as the sole carbon/energy source, indicating that the b-F_1 interaction was not disrupted in the mutants.

Membrane preparations bearing F_1F_0 complexes containing the mutated b subunits were incubated with the photoreactive cross-linker benzophenone-4-maleimide (BPM) and then exposed to ultraviolet light. The samples were analyzed by Western blotting, using ¹²⁵I-radiolabeled monoclonal antibodies raised against b as probes. No cross-linking was observed with either bD150C or bK151C (data not shown). However, cross-linked products of about the same size were observed with both bE155C and b158C (Fig. 3). The new bands were approximately the size expected for a b-δ cross-link and showed reac-
tivity with anti-δ polyclonal antibodies (Fig. 3), indicating that cross-links had been formed between b and δ. Membranes containing cross-linked F₁F₀ showed no apparent loss of activity compared with control membranes treated with either BPM or UV light (data not shown).

To characterize further the cross-linked products, the E155C and 158C mutations were incorporated into b24–156. After modification of each of these proteins with BPM, reconstitution with F₁, and exposure to ultraviolet light, new bands of an appropriate size were observed on SDS-PAGE (Figs. 4A and 5A). The new bands were recognized by antibodies directed against b and δ (Figs. 4B and 5B), confirming the identity of a b24–156-δ cross-link. Exposure of the BPM-modified E155C and 158C proteins to UV light in the absence of F₁ caused an apparent reduction in the total amount of protein on the stained gels (Figs. 4A and 5A). Western blotting of similar samples revealed a series of dimeric and higher order aggregates (not shown), which were probably not visible on the stained gels because of their heterogeneous nature.

Cross-linking of the soluble forms of b gave rise to a second cross-link in each case that had a slightly greater mobility than b24–156 on SDS-PAGE (Figs. 4A and 5A). These cross-links were recognized by antibodies directed against b and δ (Figs. 4B and 5B), suggesting that in each case an internal cross-link had been formed in b24–156. This internal cross-link was not formed to the same extent in the absence of F₁.

Some cross-linked products of higher apparent molecular weight were observed with both the E155C and the 158C mutations (Figs. 4A and 5A). In each case, one of these cross-links was recognized by the anti-δ antibodies, whereas the other was not (Figs. 4B and 5B). The difference between b24–156 and its largest CNBr fragment is readily apparent on SDS-PAGE (Fig. 6). The arrow indicates the position of the bands that were cut from a subsequent blot and analyzed by protein sequencing.

**Fig. 4.** Cross-linking of b24–156 via position 155 to purified F₁. b24–156 containing the E155C mutation, as well as wild type b24–156 was modified with BPM and reconstituted with purified F₁. After exposure to UV light, the samples were analyzed by SDS-PAGE (A) and Western blotting (B), probing with ¹²⁵I-labeled antibodies raised against either b or δ. Controls were performed in which the reconstituted b24–156-F₁ complex was exposed only to BPM or to UV light, and in which the b24–156, with or without the E155C mutation, was treated in the absence of F₁.

**Fig. 5.** Cross-linking of b24–158 via position 158 to purified F₁. An experiment identical to that described in the legend to Fig. 4 was performed, except that the 158C mutation was used instead of the E155C mutation.

**Fig. 6.** Cyanogen bromide digestion of b24–156-δ cross-linked products. The bands corresponding to the b24–156-δ cross-links obtained with the E155C and the 158C proteins were excised from an SDS-polyacrylamide gel, treated with cyanogen bromide as described under “Experimental Procedures,” and analyzed by SDS-PAGE. The wt lane shows the position of unmodified b24–156. The arrow indicates the position of the bands that were cut from a subsequent blot and analyzed by protein sequencing.

**Peptide Analysis of b24–156-δ Cross-links**—To identify the region of δ involved in the cross-links to b24–156, a slice containing the cross-linked product from each cysteine mutation was cut from an SDS-polyacrylamide gel and treated with cyanogen bromide as outlined under “Experimental Procedures.” CNBr cleavage of b24–156 should give rise to fragments of 2, 12, and 126 residues, with the large C-terminal fragment (residues Ala-31 to Leu-156) containing the site of the cross-link. The δ subunit should give rise to fragments between 10 and 49 residues in length. The difference between b24–156 and its largest CNBr fragment is readily apparent on SDS-PAGE (Fig. 6). CNBr cleavage of each cross-linked product gave rise to a predominant fragment that is markedly larger than the 126-residue fragment derived from b24–156 alone (marked by an arrow in Fig. 6).
of the second subunit is in contact with another region of $b$. Removal of one or more hydrophobic residues might disrupt this interaction, causing a significant conformational change in the soluble $b$ protein. Such a conformational change to a more asymmetric shape would explain why the sedimentation coefficients of $b_{24–155}$ and $b_{24–152}$ were markedly lower than that observed for $b_{24–156}$ (Table I). In the absence of $\delta$, the hydrophobic face of the first $b$ subunit would not have its normal partner and might interact nonspecifically with the similarly exposed hydrophobic face of another $b_{24–156}$ dimer. This would explain why the slight aggregation of $b_{24–156}$ observed during sedimentation equilibrium centrifugation at high concentrations (34) is not seen with $b_{24–152}$.

Hydrophobic residues are conserved at positions corresponding to E. coli residues 153 and 156 in the $b$ subunit of many organisms (51). It is tempting to suggest that the importance of these hydrophobic residues in the $b-\delta$ interaction may provide the explanation for the observation that low ionic strength disrupts the binding of $F_1$ to $F_0$. However, other regions of $b$ must also be involved in $b-F_1$ interactions, since $b_{24–152}$ was able to compete to a minor extent with the affinity resin for binding to $F_1$ (Fig. 2). In this regard it is also noteworthy that some bacteria have $b$ subunits that lack the hydrophobic residues at the C terminus. For example, the $b$ subunit of the thermophile FS3 ends at the residue corresponding to position 148 in E. coli $b$ (52).

The difference in sedimentation coefficients observed between the $(b_{24–156})_2\delta$ and the $(b_{24–156})_2\delta$ complexes (Table I) was surprising. One possible explanation is that flexibility in the N-terminal region of the soluble $b$ construct allows the hydrophobic residues $Y^{24}VWPLMAAF^{33}$, present in $b_{24–156}$ but absent in $b_{34–156}$, to loop back and interact with $\delta$. Such an arrangement would be more compact, and the complex would therefore sediment faster than if the N-terminal helices were in a more extended conformation. Because the N termini of the $b$ subunits are anchored in the membrane in ATP synthase, it seems unlikely that residues 24–33 of $b$ normally interact with $\delta$ in the intact complex.

Takeyama and co-workers (10) showed that one aspect of the defective ATP synthase assembly caused by deletion of residues from the C terminus of $b$ was the failure of $F_0$ to form a functional proton pore in vivo. In subsequent work, Brusilow and co-workers (53, 54) demonstrated that the $\delta$ subunit was required for the formation of the proton pore from the cloned $F_0$ subunits. Here we have shown that the same region of $b$ implicated in the $F_0$ assembly process is essential for binding to $\delta$, strengthening the argument that the $b-\delta$ interaction is critical for the assembly of functional $F_0$. At present, however, we have
no evidence of how a signal arising from the interaction may be transmitted from the C-terminal end of $b$ to the membrane, where interaction with the other $F_o$ subunits would occur.

The solution structure of residues 1–105 of $\delta$, a proteolytic fragment consisting of residues 1–134 of $\delta$, has been solved by NMR spectroscopy, but the structure of the entire 177-residue subunit could not be determined (49). Although the C-terminal regions of both proteins are predicted to be largely $\alpha$-helical, in the absence of concrete structural information from either region it is difficult to propose specific interactions between amino acid residues in $b$ and $\delta$. The proposed site of cross-linking, Met-158, lies before a predicted C-terminal helix encompassing residues $\delta$167–175 (55).

Our current results demonstrate that $b$ and $\delta$ interact via their C-terminal regions, and a recent study from our laboratory has shown that the $b_2\delta$ complex is extended enough to span the distance from the membrane to the N-terminal domain of $\alpha$ (31). Thus our work is consistent with the hypothesis that the $b$ and $\delta$ subunits form a stator to prevent $\alpha_2\delta_2$ from moving relative to $\gamma$. Since the stator must resist an appreciable torque (15), and since the $K_d$ of the $b$–$\delta$ interaction is relatively high (5–10 $\mu$M; Ref. 31), it is likely that the interaction of these subunits is stabilized by the presence of the other parts of the ATP synthase complex. A further possibility is that $\delta$ makes direct contact with other subunits, such as $\alpha$ or $\beta$, to stabilize the $b$-$F_1$ interaction. The newly developed competition assay for $b$-$F_1$ binding provides a simpler, more quantitative, and more sensitive way of detecting minor changes in $b$-$F_1$ affinity compared with earlier methods. We are currently using this assay to define other residues of $b$ that affect $b$-$F_1$ interactions.

Acknowledgments—We thank Drs. Gabriele Deckers-Hebestreit and Karlheinz Altendorf for providing the 10-IA4 and 10-6D1 monoclonal antibodies; Drs. Kimberly McCormick and Brian Cain for providing the 10-1A4 and 10-6D1 monoclonal antibodies; Hanna Abou Alfa for helpful discussions; Karlheinz Altendorf for providing the 10-1A4 and 10-6D1 monoclonal antibodies; and John West for helpful discussions and construction of plasmid pMR2; Dragon Biologics for providing $E$. coli antibodies; Drs. Kimberly McCormick and Brian Cain for providing the 10-1A4 and 10-6D1 monoclonal antibodies; and John West for helpful discussions and construction of plasmid pMR2; Hanna Abou Alfa for helpful discussions; and Faye Males for technical assistance.

REFERENCES
1. Boyer, P. D. (1997) Annu. Rev. Biochem. 66, 717–749
2. Junge, W., Lili, H., and Engelbrecht, S. (1997) Trends Biochem. Sci. 22, 420–423
3. Deckers-Hebestreit, G., and Altendorf, K. (1996) Annu. Rev. Microbiol. 50, 791–824
4. Weber, J., and Senior, A. E. (1997) Biochim. Biophys. Acta 1319, 19–58
5. Schairer, H. U., Hoppe, J., Sebald, W., and Friedl, P. (1997) Biochim. Biophys. Acta 1319, 19–58
6. Weber, J., and Senior, A. E. (1997) Biochim. Biophys. Acta 1319, 19–58
7. Schairer, H. U., Hoppe, J., Sebald, W., and Friedl, P. (1997) Biochim. Biophys. Acta 1319, 19–58
8. Weber, J., and Senior, A. E. (1997) Biochim. Biophys. Acta 1319, 19–58
9. Schairer, H. U., Hoppe, J., Sebald, W., and Friedl, P. (1997) Biochim. Biophys. Acta 1319, 19–58
10. Weber, J., and Senior, A. E. (1997) Biochim. Biophys. Acta 1319, 19–58
11. Schairer, H. U., Hoppe, J., Sebald, W., and Friedl, P. (1997) Biochim. Biophys. Acta 1319, 19–58
12. Weber, J., and Senior, A. E. (1997) Biochim. Biophys. Acta 1319, 19–58
13. Schairer, H. U., Hoppe, J., Sebald, W., and Friedl, P. (1997) Biochim. Biophys. Acta 1319, 19–58