The Escherichia coli uncA gene codes for the α subunit of the F1-sector of the membrane proton-ATPase. Mutations in this gene cause loss of ATPase and ATP synthesis activity and, in some instances, derangement of F1 structure. From three mutants (uncA401, uncA453, and uncA447), an F1 of normal size and subunit structure may be purified. In this work, purified soluble F1 from these three mutants has been compared with F1 from a normal strain in respect to (a) binding of ATP and ADP, both in the presence and absence of Mg, (b) effects of aurovertin on the binding of ATP and ADP, (c) binding of aurovertin itself, (d) effects of ADP on the fluorescence of bound aurovertin, (e) reactivity of essential residues using dicyclohexylcarbodiimide and 4-chloro-7-nitrobenzofurazan to modify carboxyl and tyrosyl residues, respectively. UncA F1 was normal or similar to normal in ATP- and ADP-binding capability. An increase in binding affinity for ADP and ATP was induced in normal F1 by aurovertin, but was not induced in uncA F1. Both normal F1 and uncA F1 bound ~2 mol of aurovertin/mol of F1, with Ka of approximately 3 μM, the only difference being that the bound aurovertin showed 70% higher fluorescence in uncA F1 as opposed to normal F1, whereas ADP induced marked enhancement of bound aurovertin fluorescence in normal F1 (Ka ADP = 1 to 2 μM), no such effect of ADP was seen in uncA F1. The reactivity of essential carboxyl and tyrosyl residues was similar in uncA and normal F1.

It is suggested that α ↔ β inter-subunit conformational interaction is abolished in F1 from these three uncA mutants and it is hypothesized that such interaction is required for normal F1 catalyzed ATP hydrolysis and synthesis.
was reconstituted by dialysis at 24 °C against 200 volumes of 50 mM sucinate-Tris, pH 6.0, 10% (v/v) glycerol, 0.1 mM diethylthreitol, 0.1 mM EDTA, 2.0 mM ATP; 2.0 mM MgCl₂. Full (96%) recovery of ATPase activity was reached at 24 h.

**Binding of ADP and ATP to F₁**—Prior to the incubation with nucleotide, F₁ samples were freed of unbound nucleotide and equilibrium was achieved. The following Tris/Na₂SO₄, 0.5 mM EDTA, pH 7.5, as follows. The F₁ sample was diluted 1:3 with 50 mM Tris/Na₃SO₄, 0.5 mM EDTA, pH 7.5, and 2 volumes of cold saturated (NH₄)₂SO₄ solution were added. The suspension stood 30 min on ice and was then centrifuged for 30 min at 2 °C (20,000 × g). The pellet was redissoled in a minimal amount of buffer and passed through Sephadex G-25 (0.5 × 24 cm).

Incubation with [2,8-³H]ADP or [2,8-³H]ATP was then carried out at room temperature for 60 min in the same buffer (± 2.5 mM MgSO₄). 120 µg of F₁ were added and the final volume was 0.3 ml. After 60 min, duplicate 0.1 ml samples were applied to centrifuge columns (Sephadex G-50F, Ref. 14) and eluted by centrifugation. Eluates were weighed, analyzed for protein content, and counted. Where Mg or aurovertin were present in the binding incubation, they were also present in the centrifuge columns at the same concentration. We found that recovery of F₁ protein in the eluates ranged from 60 to 70%, and was inconsistent enough to necessitate protein and volume analysis of every sample. Binding parameters were analyzed by linear least squares regression analyses of Scatchard plots.

**Reaction of F₁ with DCCD**—The reaction conditions followed those described by Satre et al. (17). Incubation of F₁ (0.2 mg/ml) with 10 µM DCCD at 30 °C in 50 mM morpholino propane sulfonic acid, pH 6.5, for 10 min was found to give 50 to 70% inhibition of normal ATPase activity. Radioactive [³²P]DCCD labeling was measured the reaction was stopped by passing the F₁ through centrifuge-columns. UncA F₁ samples were run in parallel with normal F₁, samples and treated identically. The amount of [³²P]DCCD bound was estimated from protein and radioactivity assays of the eluates. Sodium dodecyl sulfate (2%) and P-mercaptoethanol (1%) were then added to final concentrations noted and 5 to 10 µg samples were analyzed by sodium dodecyl sulfate gel electrophoresis.

**Reaction of F₁ with Nbf⁻**—The reaction conditions followed those described by Lunardi et al. (18). Incubation of normal F₁ (0.5 mg/ml) with 100 µM Nbf⁻ at 30 °C in 0.2 M sucrose, 2 mM EDTA, 10 mM triethanolamine, pH 7.5, for 20 min in the dark was found to give around 65% inhibition of ATPase activity. Labeling of the F₁ with radioactive Nbf⁻ was measured as for [³²P]DCCD labeling above, except that β-mercaptoethanol was omitted during gel electrophoresis. To compare unca F₁ and normal F₁, samples were run in parallel and treated identically.

**Routine Procedures**—Protein was analyzed by Miller's method (19), ATPase activities were assayed as described by Senior and Brooks (19) using 5-min incubation period at 30 °C. Sodium dodecyl sulfate gel electrophoresis in 10% tube gels or in 10 to 25% slab gels, slicing of gels and counting of radioactivity in gel slices were done as described previously (12, 20).

**Chemicals**—[2,8-³H]ATP and [2,8-³H]ADP were obtained from New England Nuclear. [U-¹⁴C]Nbf and dicyclohexyl[¹⁴C]carboximidide were obtained from Research Products International.

**RESULTS**

**Assembly of F₁ in unca Mutant Strains**—Five unca mutations have been characterized by Gibson and co-workers using genetic complementation analyses. The properties of the soluble membranes and soluble F₁ from strains carrying these mutations are described in Table I. Strains carrying the uncaA401, uncaA447, and uncaA453 mutations have been described previously (11). These three strains have in common the fact that a soluble five-subunit F₁ aggregate of normal size may be purified from them using the same purification procedure as for F₁.

Strain AN1159 (uncaA462) appeared to form a normal F₁ aggregate on membranes as judged by biochemical characteristics and gel electrophoretic analyses (data not shown). The F₁ is released by low ionic strength washing in the normal way, but the F₁ then apparently dissociates in solution. During the S-300 gel filtration step, no F₁ aggregate peak was seen at the normal effluent position (260 ml). Instead two peaks of protein at M, approximately 110,000 (300 ml) and 50,000 (35 ml) were seen, and each contained a and β subunits of F₁.

Various procedures were tried to stabilize soluble uncaA462 F₁, such as purification at 23 °C versus 4 °C, use of 10% methanol instead of glycerol, inclusion of 2.5 mM MgCl₂, and solubilization with CHCl₃ instead of low ionic strength buffer. None improved the stability of the uncaA462 F₁.

The uncaA450 mutation has been described before (11). No F₁ aggregate forms here, even on the membranes.

Mutation in the unca gene therefore gives rise to a range of structural effects in F₁, ranging from inability to form an F₁ aggregate through formation of a readily dissociating F₁ to ability to form an F₁ aggregate with normal structural stability. In the remainder of this paper we used only the three strains which formed a stable F₁ aggregate so that we could assess the functional defects in soluble F₁, which are conferred by the unca mutation.

**Binding of Aurovertin to Normal and unca Mutant F₁**—The binding of aurovertin to normal membranes, normal soluble F₁, and unca mutant soluble F₁ is documented in Table II. Aurovertin binds to the β subunit of F₁ and the binding site is retained even in isolated β subunit (8, 21). The unca mutants have a normal F₁ β subunit, and aurovertin was found to bind to the unca F₁ preparations with normal Kᵢ. The stoichiometry of binding (2 mol of aurovertin/mol of F₁) was the same in normal and unca mutant F₁. A notable difference between the unca F₁ and normal F₁ was that the maximum fluorescence of the aurovertin-F₁ complex was significantly higher (by 63 to 73%) in unca F₁. This was a

### Table I

<table>
<thead>
<tr>
<th>Bacterial stain</th>
<th>Genotype</th>
<th>Properties of membranes and F₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN 718&lt;sup&gt;a&lt;/sup&gt;</td>
<td>uncaA01</td>
<td>Zero ATPase activity. Washed membranes are proton-leaky. Stable F₁ aggregate present in wash. Normal F₁ reconstitutes washed membranes.</td>
</tr>
<tr>
<td>AN 1111&lt;sup&gt;b&lt;/sup&gt;</td>
<td>uncaA447</td>
<td>Zero ATPase activity. Washed membranes are proton-leaky. F₁ aggregate present in wash but it readily dissociates. Normal F₁ reconstitutes washed membranes.</td>
</tr>
<tr>
<td>AN 1137&lt;sup&gt;b&lt;/sup&gt;</td>
<td>uncaA453</td>
<td>Zero ATPase activity. Washed membranes are not proton-leaky. No F₁ aggregate present in wash. Normal F₁ does not reconstitute washed membranes. Abnormal α remains bound to washed membranes.</td>
</tr>
<tr>
<td>AN 1113&lt;sup&gt;b&lt;/sup&gt;</td>
<td>uncaA462</td>
<td>Zero ATPase activity. Washed membranes are not proton-leaky. No F₁ aggregate present in wash. Normal F₁ does not reconstitute washed membranes.</td>
</tr>
</tbody>
</table>

<sup>a</sup> Isogenic with AN 1111, AN 1113, and AN 1137. Properties of AN 718 purified F₁ are the same as those of AN 249 purified F₁ described in Ref. 11.

<sup>b</sup> As published by Dunn and Futai (8).

---

**Table II**

<table>
<thead>
<tr>
<th>Aurovertin binding to normal and unca mutant F₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kᵢ&lt;sup&gt;p&lt;/sup&gt;</td>
</tr>
<tr>
<td>µM</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Normal membranes&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal soluble F₁</td>
</tr>
<tr>
<td>Isolated normal β subunit&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>uncaA401 soluble F₁</td>
</tr>
<tr>
<td>uncaA447 soluble F₁</td>
</tr>
</tbody>
</table>

<sup>c</sup> Scatchard analysis of binding curves.

<sup>b</sup> Lineweaver-Burk analysis of binding curve.
PM. 

lized, ADP was added stepwise. Enhancement of fluorescence is shown. From a double reciprocal plot the calculated strains.

Aurovertin in soluble ADP-Addition of ADP to normal membranes or normal 10 preparations. It is interesting to note that the fluorescence of resulting from a somewhat changed conformation of with aurovertin and saturating ADP. 

consistent difference and presumably reflects a difference in environment of the aurovertin binding site in 

uncA mutants normally, in fact the titration curves for binding of ADP, no enhancement of aurovertin fluorescence was elicited in the uncA mutant FI preparations. It is interesting to note that the fluorescence of the uncA FI with aurovertin was equal to that of the unc FI with aurovertin and saturating ADP.

Satre et al. (21) suggested previously that isolated β subunit of normal E. coli F₁ did not show ADP-enhanced fluorescence of bound aurovertin although the results (which were not shown) were apparently not definitive. This would suggest that inter-subunit conformational communication is necessary to see the effect. To demonstrate this, we dissociated normal F₁ by a procedure modified from that of Dunn and Futai (8), as described under “Materials and Methods.” After 40 h of dialysis in “dissociating buffer” at 4 °C, ATPase activity was zero, and the dissociated F₁ showed no enhancement of fluorescence by ADP (Fig. 2). This dissociated F₁ did bind aurovertin normally, in fact the titration curves for binding of aurovertin to the original intact F₁ and dissociated F₁ were identical. The dissociated F₁ was then reassociated by dialysis against “reconstitution buffer” at 24 °C for 24 h, at which time 99% of the original ATPase activity was restored. The reassociated F₁ now showed ADP-induced enhancement of fluorescence to aurovertin (Fig. 2). 

UncA F₁-ATPase

Fig. 1. ADP-induced enhancement of fluorescence of bound aurovertin in solubelange F₁, from normal and uncA mutant E. coli strains. Aurovertin (10 μM) was added to soluble F₁ in 0.25 M sucrose, 10 mM Tris/Cl, 0.5 mM EDTA, pH 7.4. After the fluorescence stabilized, ADP was added stepwise. Enhancement of fluorescence is shown. From a double reciprocal plot the calculated Kₐ, ADP = 0.9 μM. 

Enhancement of Fluorescence of Bound Aurovertin by ADP—Addition of ADP to normal membranes or normal soluble F₁, which have been previously saturated with aurovertin brings about a further enhancement of fluorescence of the bound aurovertin (21). In a series of experiments, we deduced the Kₐ, ADP for this effect from Lineweaver-Burk analyses of data relating enhanced fluorescence to ADP added between 0 and 30 μM (Fig. 1). In 0.25 M sucrose, 50 mM Tris/Cl, 0.5 mM EDTA, pH 7.4, the Kₐ, ADP was 1.0 μM (membranes), and in 0.25 M sucrose, 10 mM Tris/Cl, 0.5 mM EDTA, the Kₐ, ADP was 0.9 μM (soluble F₁). In 50 mM Tris/So₄, 0.5 mM EDTA, pH 7.5, the Kₐ, ADP (soluble F₁) was 2.0 μM. This effect seems therefore to reflect binding of ADP at a relatively high affinity site. The effect was studied in F₁ from the uncA mutants (Fig. 1) and the result was dramatic, the effect was completely absent. Even at 150 μM ADP, no enhancement of aurovertin fluorescence was elicited in the uncA mutant FI preparations. It is interesting to note that the fluorescence of the uncA FI with aurovertin was equal to that of the unc F₁ with aurovertin and saturating ADP.

In a series of experiments, we dissociated normal F₁, and leave no doubt that neither the affinity nor the stoichiometry of ADP binding in the presence of Mg is altered drastically in the mutant F₁ preparations.

Binding of ADP to F₁ from Normal and uncA Mutant Strains—Two buffer systems were used. The first consisted of 50 mM Tris/So₄, 0.5 mM EDTA, pH 7.5. The second consisted of the same buffer with 2.5 mM MgSo₄ added. Concentrations of free ADP studied ranged from 0 to 100 μM. It was established in preliminary experiments that the binding of ADP at pH 8.0 and pH 7.5 was the same and that 60-min incubation at room temperature was required to achieve maximal binding.

Fig. 3 shows the binding of ADP to normal F₁ in the presence of Mg. Scatchard analysis gave values of Kₐ, ADP = 4.0 μM and n = 1.99 ADP/F₁ (mol/mol). Fig. 4 shows the binding of ADP to the three uncA F₁ preparations in the presence of Mg. The three curves are similar, especially at the lower ADP concentrations. Lack of sufficient enzyme prevented us from obtaining enough data to obtain good Scatchard plots. The curves are clearly similar to normal however and leave no doubt that neither the affinity nor the stoichiometry of ADP binding in the presence of Mg is altered drastically in the mutant F₁ preparations.

Fig. 5 shows the binding of ADP to normal F₁ in the absence of Mg. The Scatchard analysis (Fig. 5) gave values of Kₐ, ADP = 26 μM and n = 1.04 ADP/F₁ (mol/mol). The stoichiometry and tightness of binding of ADP was clearly different in the presence as compared with the absence of Mg. In the presence of 10 μM aurovertin the enzyme bound ADP more tightly and the Kₐ, ADP = 16 μM and n = 1.09 ADP/F₁ (mol/mol). The effect of aurovertin was therefore to decrease Kₐ, ADP. Fig. 6...
UncA F$_1$-ATPase

$\mu$m; uncA453, 10$\mu$m. Therefore, the major differences from the normal F$_1$ were the decreases in $K_d$ ADP in uncA401 and uncA453. We also tested the effects of 10$\mu$m aurovertin on ADP binding to the three uncA F$_1$ in the absence of Mg. The binding curves were identical with those shown in Fig. 6, i.e. in the three uncA mutant F$_1$ enzymes there was no effect of aurovertin on ADP binding. This contrasts with the effect of aurovertin in normal F$_1$ noted above.

Binding of ATP to F$_1$ from Normal and uncA Mutant Strains—Fig. 7 shows the binding of ATP to normal soluble F$_1$ in the absence of Mg, with and without aurovertin present. Control tests showed that under the conditions of these experiments, where ATP was 100$\mu$m, 3% of the ATP was hydrolyzed (limit of detection by colorimetric analysis) at the end of the 60-min binding incubation. This showed that it was ATP and not ADP binding that was being measured in Fig. 7.
**UncA F₁-ATPase**

1.0 -

2.0 -

5.0 -

8.0 -

10.0 -

FIG. 6. Binding of ADP to uncA mutant F₁, in the absence of Mg. Binding of ADP was measured in 50 mM Tris/SO₄, 0.5 mM EDTA, pH 7.5, as described under "Materials and Methods."

1.8 -

2.2 -

2.8 -

3.6 -

4.2 -

ATP BOUND (mol/mol)

[ADP] μM

Fig. 6. Binding of ADP to uncA mutant F₁, in the absence of Mg. Binding of ADP was measured in 50 mM Tris/SO₄, 0.5 mM EDTA, pH 7.5, as described under "Materials and Methods."

1.8 -

2.2 -

2.8 -

3.6 -

4.2 -

ATP BOUND (mol/mol)

[ATP] μM

FIG. 7. Binding of ATP to normal F₁, in the absence of Mg. Binding of ATP was carried out in 50 mM Tris/SO₄, 0.5 mM EDTA, pH 7.5. Top, binding curve. Aurovertin (10 μM) was added to F₁, 5 min before the addition of ATP. Incubation with ATP was for 60 min. Bottom, Scatchard plot.

(Refer to Fig. 5 and the text below where competitive effects of ATP and ADP are noted.) In the absence of aurovertin, K₀ for ATP was 42 μM and saturation apparently occurred at 0.62 ATP/F₁ (mol/mol). With aurovertin present, K₀ for ATP was 25 μM and n = 0.64 ATP/F₁ (mol/mol). The results are similar to those of Fig. 5, where ADP binding was assessed under similar conditions. The ATP is bound with lower affinity than the ADP, but aurovertin had the same effect of decreasing K₀ in both experiments. It is not clear why the maximum ATP binding (0.62 to 0.64 mol/mol) is lower than the ADP binding (1.04 to 1.09 mol/mol). One explanation could be that ATP dissociates more during the centrifuge-column procedure (which is not a true equilibrium binding procedure) because of its higher K₀. It was shown in competitive binding experiments that where 25 μM ADP (radioactive) and 0.5 mM ATP (nonradioactive) or where 40 μM ATP (radioactive) and 0.5 mM ADP (nonradioactive) were incubated with F₁, binding of the radioactive ligand was reduced to very low levels, suggesting that ATP and ADP are competing for the same site at these concentrations.

Fig. 8 shows the binding of ATP to uncA mutant F₁, in the absence of Mg. Comparison with Fig. 7 shows that the uncA F₁ enzymes are not markedly dissimilar to normal. Consistent with the idea that ATP and ADP are binding at the same site under these conditions, a comparison of Fig. 8 with Fig. 6 shows the same pattern of changed affinity for both the ligands ATP and ADP (cf. normal F₁ in Figs. 5 and 7). Aurovertin had no effect on the binding of ATP by the three uncA mutant F₁ enzymes under the conditions in Fig. 8, contrasting with its effect on normal F₁ (Fig. 7).

**Binding of Radioactive DCCD and Nbf to uncA Mutant F₁**—DCCD binds to the β subunit of E. coli F₁ (16) and is known to interact at the active site of PS3 F₁ (22) by reacting with a specific, highly reactive glutamyl group. To find out whether this glutamyl group was present and available for reaction with DCCD in uncA mutant F₁, we reacted the enzymes with DCCD under conditions where partial inactivation of normal F₁ was achieved (see Table III). Under these conditions essentially all the labeling of F₁ was in the β subunit of uncA F₁. The results of Table III also show that DCCD labeled predominantly the β subunit of uncA mutant F₁. The reaction between DCCD and F₁ seemed less complete with uncA F₁ than with unc⁺ F₁ as judged by incorporation of DCCD, suggesting a slightly slower rate of reaction between the reactive side chain and DCCD. This difference could by no means account for the total loss of ATPase activity conferred by the mutation.

Nbf is believed to interact with an essential uniquely reactive tyrosine residue in the β subunit of E. coli F₁ (17, 23). Reaction of 1 mol of Nbf/mol of F₁ eliminated ATPase activity...
TABLE III
Reaction of [14C]DCCD with soluble F1
A, B, and C are separate experiments in which normal F1 and uncA F1 were run in parallel and treated identically.

<table>
<thead>
<tr>
<th>F1, species</th>
<th>Inhibition of ATPase</th>
<th>[14C]DCCD bound to F1, pmol/mol</th>
<th>Counts in β-subunit, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.* Normal (unc+)</td>
<td>73</td>
<td>0.59</td>
<td>86</td>
</tr>
<tr>
<td>uncA401</td>
<td>0.42</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>uncA453</td>
<td>0.43</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>uncA447</td>
<td>0.61</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>B.* Normal (unc+)</td>
<td>82</td>
<td>0.61</td>
<td>76</td>
</tr>
<tr>
<td>uncA401</td>
<td>0.47</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>C.* Normal (unc+)</td>
<td>76</td>
<td>0.81</td>
<td>Not determined</td>
</tr>
<tr>
<td>uncA401</td>
<td>0.50</td>
<td>Not determined</td>
<td></td>
</tr>
</tbody>
</table>

* Means of quadruplicate determinations.
- Means of duplicate determinations.

(17). Previously Bragg and Hou (24) showed, using a spectrophotometric assay, that Nbf reacted with F1 from strain AN120 (uncA401), apparently with the same essential tyrosine residue which is present in normal F1. Using radioactive Nbf we have measured and compared the labeling of uncA, uncA401, uncA453, and uncA447 F1 preparations. Under conditions where about 65% of inactivation of ATPase activity was achieved, binding of Nbf was predominantly to the β subunit of uncA F1, with a small fraction of the label in the α subunit (15 to 30%). Essentially all of the Nbf was removed by incubation with β-mercaptoethanol. In previous reports of labeling of normal E. coli F1, by Nbf, Lunardi et al. (17) saw very little Nbf binding to α subunit, whereas Verheijen et al. (25) saw significant labeling of α subunit after short incubation periods.

The uncA401, uncA453, and uncA447 F1 bound the same amount of Nbf as the uncA F1, under the identical incubation conditions as above. Analyses of the gels of labeled uncA F1 showed that the labeling pattern in all three mutant F1 samples was the same as with uncA F1, i.e. β subunit contained most of the label with 14 to 25% in α. β-Mercaptoethanol removed essentially all the Nbf from all the mutant F1 preparations. Thus, our conclusion was that the essential uniquely reactive tyrosine on the β subunit which reacts with Nbf seems to be present in the uncA mutant F1 preparations.

DISCUSSION
Assembly of F1 in uncA Mutants—It is important to stress that mutation in the uncA gene can prevent normal assembly of F1, as described here and previously (11), and experiments aimed at deducing functional defects in the F1 must therefore be done on carefully characterized preparations. The three F1 preparations studied here appear to form structurally stable aggregates with normal subunit composition, molecular weight, and membrane-binding properties.

Conformation of the β Subunit in the uncA F1 Preparations—The aurovertin fluorescence measurements showed that the aurovertin site is somewhat abnormal in environment in the uncA mutants, although the Kd for aurovertin binding and the number of sites (two) was unchanged. The reaction DCCD and Nbf with the F1 preparations showed that the essential and uniquely reactive glutamyl and tyrosyl groups which are present on the β subunit of normal F1 are also available for reaction in uncA F1. Our evidence suggests, therefore, that the β subunit conformation in uncA F1 is not fully normal, but the changes we saw were of a minor nature, whereas the loss of ATPase activity is total.

Subunit-subunit-mediated Conformational Change in the uncA F1—Aurovertin was used to register conformational change on the β subunit of F1. Binding of ADP to the normal F1 induced an enhancement of fluorescence of bound aurovertin and this effect was abolished by dissociation of the F1 aggregate, but regained upon reassociation of the subunits into an active F1. Taken together with the data suggesting that the effect of ADP is not seen in isolated β subunit (21), it seems clear that subunit-subunit interaction is required for this effect. A very clear-cut defect of the uncA F1, was the complete absence of the ADP-induced enhancement of fluorescence of bound aurovertin. This could reflect loss of an ADP binding site, or loss of the subunit-subunit interaction necessary for the effect. As is discussed below, ADP binding appeared to be normal in uncA F1.

Other evidence of subunit-subunit interaction in normal F1 (and its elimination in uncA F1), came from the observation that aurovertin induced a change in the Kd for ADP and ATP binding in normal F1, but not in uncA F1.

Nucleotide Binding to Normal and uncA Mutant F1—All of our experiments were aimed at characterizing tight exchangeable sites, and we used ADP and ATP concentrations of 100 μM and less throughout. We found that normal F1 bound two ADP (mol/mol) in the presence of Mg and one ADP in the presence of EDTA alone. These values are consistent with previous studies (24, 27).

We found that all the ADP sites which were present on normal F1, were also present on each of the three uncA F1 preparations tested. With Mg present, two ADP sites were seen with Kd close to normal as judged from the binding curves. With EDTA present (Mg absent) the uncA401 F1 and uncA453 F1, both had decreased Kd ADP as compared to normal (this was also seen previously by Bragg and Hou with uncA401 F1, Ref. 24) and uncA447 F1, had Kd ADP close to normal. Whereas aurovertin decreased the Kd ADP in EDTA of normal F1, it has no effect on uncA F1. We should note that there was a discrepancy between the Kd ADP of 16 μM calculated in Fig. 5 for binding of ADP in the presence of aurovertin by the centrifuge-column direct binding approach (Mg absent) and the Kd ADP of 2 μM as measured in the same buffer by enhancement of bound aurovertin fluorescence.

Lunardi et al. found Kd ADP was 1 μM in direct binding experiments where Mg was absent (27) yet from aurovertin fluorescence enhancement measurements in a similar buffer the same group saw one site for ADP with Kd ADP = 10 to 20 μM (Statre et al., 21). Thus, differences in time of incubation, protein concentration, and buffer conditions appear to modulate this ADP site to change its apparent affinity. Also, the experimental techniques used may introduce some variation in the measured Kd ADP values.

Binding of ADP to normal F1 in EDTA buffer (Fig. 7) showed that there was one site present, which competed with ADP, and had higher Kd ATP than Kd ADP. This site was also changed by aurovertin as shown by decrease in Kd ATP. This site is clearly the same as the single ADP site seen in EDTA alone. Each of the mutant uncA F1 preparations had this site, and aurovertin did not change the Kd ATP in any of the uncA F1 preparations. On the basis of labeling studies with 8-azido-ATP in EDTA buffer Verheijen et al. (28) have suggested that a nucleotide binding site is changed in the uncA401 F1, α subunit. Our data are not in accord with this conclusion.

Current Views on Subunit Location of Nucleotide Binding Sites in E. coli F1—The activo (ATP hydrolysis) site is thought to reside on β (7, 27). The Kd ATP is 0.2 to 0.4 μM (29, 30) or 0.6 μM (31) at pH 8 and Kd ADP for competitive inhibition is 0.3 μM (Ref. 31). Thus, none of the nucleotide-binding phenomena reported in this paper are likely to be due to interaction at the active site on β. Rather, they are at considerably tighter, yet exchangeable sites.

Dunn and Putai (8), Dunn (26), and Paradies (32) have
demonstrated directly that ATP binds to one site on purified α subunit. The $K_a$ ATP was 0.1 μM and ADP competes with ATP ($K_a$ ADP = 0.9 μM). This binding occurs in the absence of added Mg (5), apparently in the presence of EDTA (26, 32) and brings about a large conformational change in α. Dissociation of nucleotide from purified α is very slow (26). It appears that this binding site may be the site at which nonexchangeable "tightly bound" nucleotide is bound in intact $F_1$ (26, 32) in which case "α-nucleotide" complex would be the natural form of α subunit in intact $F_1$.

"Th αnuc" $F_1$ used in these studies contained 1.24 ADP and 0.68 ATP (mol/mol) of nonexchangeable tightly bound nucleotide when assayed as described by Leimgruber and Senior (33). Bragg and Hou previously saw no difference between unca401F1 and normal $F_1$ in tightly bound nucleotide content (24). The properties of the single nucleotide binding site seen by Dunn and Futai on isolated α subunit (above) differ markedly from the nucleotide-binding properties of intact $F_1$ as documented here. These considerations imply therefore that the binding of ADP and ATP to intact $F_1$ from normal or unca strains seen here was not to the site seen previously by Dunn and Futai on isolated α subunit. However, one could speculate that on incorporation into intact $F_1$, one or more of the α subunits might change in respect to the properties of its nucleotide site, so that this site becomes exchangeable under some conditions and changed both its relative affinity for ATP versus ADP, and its Mg responsiveness. Alternatively, one may propose that additional noncatalytic, tight, exchangeable sites are present in intact $F_1$, which account for the binding of ADP and ATP to intact $F_1$; as seen here and by others (24, 27).

Whichever of these alternatives is correct, there is growing evidence that the tight noncatalytic but exchangeable ADP/ATP sites are on α subunit in E. coli $F_1$. The recent paper of Lunardi et al. (27) seemed persuasive in this regard. There have also been recent reports detailing similar conclusions for beef heart mitochondrial $F_1$ (35–37) and for PS3 $F_1$ (38).

Availability for Reaction of Essential, Highly Reactive Residues in the β Subunit—Lack of catalytic activity in unca $F_1$ could be due to the fact that the β subunit conformation is altered sufficiently so that essential, highly reactive residues are shielded or rendered unreactive and no catalysis occurs. Here we have used DCCD to test whether the glutamyl residue at the active site (16, 22) is present in the unca $F_1$, and the data showed that it was reactive with DCCD. Similarly, the essential, highly reactive tyrosine on the β subunit with is labeled by Nbf, was also present and reactive in all three unca $F_1$ preparations (Bragg and Hou (24) previously demonstrated this in unca402 $F_1$). While these tests are not exhaustive, they are suggestive of the idea that the active site is normal in the unca $F_1$.

Hypothesis to Explain Lack of ATPase Activity in unca Mutants—We suggest that in unca mutants, subunit-subunit interaction between α and β subunits of $F_1$ is impaired such that conformational changes which are normally communicated from α to β and vice versa cannot occur. We hypothesize that it is the inability to carry out α ↔ β inter-subunit conformational coupling which is the cause of the loss of catalytic activity in unca $F_1$. It follows that such intersubunit conformational coupling is required for ATPase and ATP synthesis activity in normal $F_1$.

Detailed chemical analysis of the unca $F_1$ α subunits is now in progress here to locate the amino acid replacement sites.

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