Evidence for a Nucleotide Binding Site on the Isolated β Subunit from *Escherichia coli* F_1-ATPase. Interaction between Nucleotide and Aurovertin D Binding Sites†

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ABSTRACT: The nucleotide binding capacity and affinity of the isolated β subunit from *Escherichia coli* F_1-ATPase[1] have been studied with radiolabeled ADP and ATP by an equilibrium dialysis technique. Each mole of β subunit in the presence of EDTA bound 1 mol of ADP or ATP with K_d values of 25 μM and 50–100 μM, respectively. At a saturating concentration, aurovertin enhanced the affinity of ADP or ATP for the isolated β subunit by 3–6-fold. The K_d values for the binding of ADP or ATP were also assessed through the enhancing effect of ADP on [^{14}C]aurovertin binding (Issartel, J.-P., Klein, G., Satre, M., & Vignais, P. V. (1983) *Biochemistry 22*, 3485–3492); the K_d values determined by this approach were several times lower than in the absence of aurovertin, in agreement with results obtained by direct titration with radiolabeled ADP or ATP.

Understanding of the molecular events controlled by the catalytic sector of the H^+-linked ATPases requires investigation of the function fulfilled by each subunit of this sector. Typically, in the case of *Escherichia coli*, reconstitution from isolated subunits and analysis of phenotypic defects in mutants have led to the conclusion that the two major subunits α and β play a central role in catalysis (Futai, 1977; Downie et al., 1979). The nucleotide binding sites in mitochondrial, bacterial, and chloroplast F_1 are restricted to the α and β subunits. This has been shown by exploring the binding of photoactivable nucleotide analogues (Wagenvoord et al., 1977, 1979, 1980; Lunardi et al., 1977, 1981; Scheurich et al., 1978; Cosson & Guillory, 1979; Carlier et al., 1979; Williams & Coleman, 1982; Czarnecki et al., 1982; Bar-Zvi et al., 1983) or alkylating nucleotide analogues (Budker et al., 1977; Esch & Allison, 1978; Hulla et al., 1978; Drutsa et al., 1979; Kumar et al., 1979; Lowe & Beechey, 1982; Bitar, 1982; Wakagi & Ohta, 1982) or by studying changes in the circular dichroism spectra of the α and β subunits isolated from F_1 of the thermophilic bacterium PS3 (Ohta et al., 1980). By measurement of the aurovertin fluorescence intensity at different concentrations of ADP, Verschoor (1982) concluded that a low-affinity ADP binding site was present on the β subunit of mitochondrial F_1. To our knowledge, direct nucleotide binding studies with isolated subunits had been reported only by Dunn & Futai (1980) in the case of *E. coli* F_1 and Gromet-Elhanan & Khananshvili (1984) in that of *Rhodospirillum rubrum*, respectively. Dunn & Futai (1980), using the equilibrium dialysis technique, found a high-affinity binding site for ADP or ATP on the isolated α subunit of *E. coli* F_1 but failed to detect any binding to the isolated β subunit. After completion of the work described in the present paper, Gromet-Elhanan & Khananshvili (1984) reported a high-affinity binding site for ADP or ATP (K_d = 4–6 μM) on the isolated β subunit of *R. rubrum* F_1 in an EDTA medium and a supplementary site of very low affinity ($K_d \simeq 200$ μM) when the binding assay was performed in a $MgCl_2$ medium. As described here, we have determined the binding parameters of ADP and ATP, with respect to the purified β subunit from *E. coli* F_1, by an equilibrium dialysis technique and by titration or [^{14}C]aurovertin binding. The results by both approaches are in good agreement, showing that the isolated β subunit from *E. coli* can bind either ADP or ATP with, however, a different affinity.

Materials and Methods

Materials. ADP, ATP, and AMP were purchased from Boehringer. [U-^{14}C]ADP (514 Ci/mol) and [2,8-^{3}H]ATP (40–10^3 Ci/mol) were obtained from Amersham. They were diluted to a specific radioactivity of 19 and 75 Ci/mol for [^{14}C]ADP and [^{3}H]ATP, respectively. Luciferin–luciferase (Lumit PM) was from Lumac. Aurovertin D was extracted, purified, and radiolabeled as described by Issartel et al. (1983). BF_1 from *E. coli*, strain K12 PP12 genetically related to PP11 (Portier, 1980), and its β subunit were purified as previously described (Satre et al., 1982). BF_1 was stored at 0–4 °C in 50 mM Tris-HCl,[1] 20% methanol, 2 mM ATP, 2 mM EDTA, and 2.5 mM 2-mercaptoethanol, final pH 7.4, at the concentration of 5–10 mg/mL. The β subunit was either used directly after purification or stored in liquid N_2 in 25 mM sodium succinate, 4 mM EDTA, 5 mM ATP, 2.5 mM 2-mercaptoethanol, and 200 mM LiCl, final pH 6.5.

Estimation of Protein-Bound ADP and ATP. The nucleotide content of the β subunit was determined by the luciferin–luciferase assay. Fifty micrograms of β directly isolated from BF_1 was desalted by the technique of centrifugation filtration through a short column of Sephadex G-50 equilibrated in 10 mM Tris-HCl–0.5 mM EDTA, pH 7.5, as described by Penefsky (1977). The β subunit preparation was then treated by 0.75 N perchloric acid (final concentration) for 30 min at 0 °C. The denatured protein was discarded by centrifugation and the supernatant was neutralized by KOH. After sedimentaion of the potassium perchlorate precipitate by centrifugation, ADP and ATP were assayed in the supernatant by a procedure similar to that described by Lundin et al. (1976). No detectable amount of ADP or ATP was found, indicating that the isolated β subunit was virtually devoid of bound ADP or ATP.

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[1] Abbreviations: F_1-ATPase, water-soluble component of the ATPase complex; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate.

0006-2960/84/0423-6591$01.50/0

Binding Studies. The binding parameters of radiolabeled aurovertin and nucleotides to the β subunit were determined by equilibrium dialysis, after the β subunit preparation had been desalted by Sephadex G-50 chromatography by the method of Penefsky (1977) (cf. above). Equilibrium dialysis for the study of [^{14}C]aurovertin binding was performed at 20 °C for 90 min in a "Dianorm" apparatus with microcells (2 × 250 μL) and Spectrapor 2 cellulose membranes (cutoff 10000–12000). The media used were a sucrose medium composed of 0.25 M sucrose, 0.5 mM EDTA, and 10 mM Tris-HCl, final pH 7.5, and a saline medium consisting of 200 mM NaCl, 0.5 mM EDTA, 10 mM Tris-HCl, and Tween-20, 0.02% (v/v), final pH 7.5. The same saline medium was used for the study of binding parameters of radiolabeled nucleotides. In this case, equilibrium dialysis was performed for 3.5 h. Tween-20 was used to avoid protein aggregation that has a tendency to occur in the presence of cations. At the concentration of 0.02%, Tween 20 did not interfere with the binding of aurovertin or nucleotides to β. One of the compartments of the microcells contained the β subunit; the other compartment contained the radiolabeled aurovertin or the radiolabeled nucleotides. After dialysis, radioactivity in the compartment without protein corresponded to the free aurovertin or the free nucleotides; radioactivity in the other compartment corresponded to either bound plus free aurovertin or bound plus free nucleotides. In assays bearing on radiolabeled nucleotides, samples were spotted on silica gel plates (DC Alufolien Kieselgel 60 F 254, Merck) and chromatographed by using a solvent made of dioxane, 2-propanol, NH_4OH, 20%, and H_2O (40:20:36:34 v/v) supplemented with 7 mM EDTA (Bronnikov & Zakharov, 1983). The plates were then autoradiographed. The autoradiographs clearly showed that the radiolabeled ADP and ATP had not been altered during dialysis.

Binding of [^{14}C]ADP to the β subunit was also assayed by elution–centrifugation through Sephadex G-50 (Penefsky, 1977). The β subunit (50 μg) was incubated in 100 μL of the sucrose medium described above with increasing amounts of [^{14}C]ADP for a 30-min period; then the sample was applied to small columns of Sephadex G-50 and centrifuged.

In all cases the protein concentration was assayed by the Coomassie G250 blue method (Bradford, 1976), with bovine serum albumin as the standard. Radioactivity was measured by liquid scintillation counting (Patterson & Greene, 1965) in an Intertechnique SL 30 scintillation counter.

Results

Use of [^{14}C]Aurovertin Binding as a Probe To Determine the ADP Binding Capacity of the Isolated β Subunit from E. coli. By means of [^{14}C]aurovertin D, three aurovertin binding sites per *E. coli* F_1 have been demonstrated (Issartel et al., 1983). One of these sites has a high-affinity binding (K_d = 0.2 μM); the other two have lower affinities (K_d = 5–10 μM). The isolated β subunit binds [^{14}C]aurovertin D with a stoichiometry of 1 and a K_d value of about 6 μM. In the presence of ADP, the binding affinity of the [^{14}C]aurovertin binding site was markedly increased (K_d = 1.4 μM). We took advantage of this situation and used aurovertin as a specific probe to investigate the ADP binding parameters of the β subunit isolated from *E. coli* F_1. Equilibrium dialysis was performed with increasing concentrations of ADP at fixed concentrations of free [^{14}C]aurovertin D. The experiment illustrated in Figure 1A shows that the amount of [^{14}C]aurovertin D bound to the β subunit is enhanced upon increasing ADP concentration. On the basis of the fact that the [^{14}C]-aurovertin and ADP binding sites on the β subunit are distinct

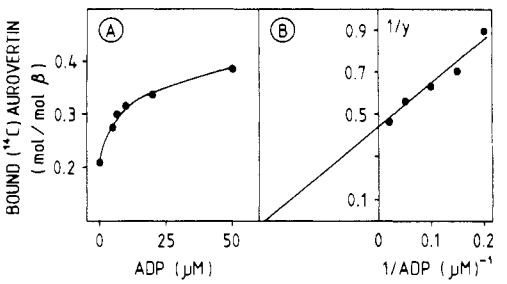
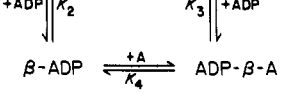


FIGURE 1: Effect of varying the concentration of ADP on the binding capacity of the isolated β subunit of *E. coli* for [^{14}C]aurovertin D. Binding of [^{14}C]aurovertin D was performed by equilibrium dialysis as described under Materials and Methods. The assay was carried out at a fixed concentration of β subunit (3.5 μM); the free [^{14}C]-aurovertin D concentration was adjusted to a nearly constant value (2.15–2.30 μM). (A) Direct plot. (B) Double-reciprocal plot of the data of (A). As explained under Results, the intercept on the base line is equal to $-1/K_3$; K_3 is the K_d value related to the ADP binding to β subunit in the presence of aurovertin D. The experimental data corresponding to the points were fitted by a straight line using eq 8 (see the text) with K_1 = 6 μM, K_4 = 1 μM, and N = 1.

Scheme I



and that the kinetics of inhibition of *E. coli* F_1 are consistent with the formation of a ternary complex (Issartel et al., 1983), the Scheme I is tentatively proposed, where A is aurovertin, K_1 is the equilibrium constant relative to aurovertin binding to the β subunit, K_2 is the equilibrium constant relative to ADP binding to the β subunit, K_3 is the equilibrium constant relative to ADP binding to the aurovertin–β complex, and K_4 is the equilibrium constant relative to aurovertin binding to the ADP–β complex. β–ADP and β–A correspond to the binary complexes of the β subunit with bound ADP and aurovertin, respectively, and ADP–β–A is the ternary ADP–β–aurovertin complex. Thus, bound aurovertin may exist either as β–A or as ADP–β–A, and

$$[\text{A bound}] = [\beta\text{–A}] + [\text{ADP–}\beta\text{–A}] \quad (1)$$

The total concentration of β subunit ($[\beta_T]$) is

$$[\beta_T] = [\beta\text{–ADP}] + [\beta\text{–A}] + [\text{ADP–}\beta\text{–A}] + [\beta]$$

with $K_1 = [\beta][A]/[\beta\text{–A}]$, $K_2 = [\beta][\text{ADP}]/[\beta\text{–ADP}]$, $K_3 = [\beta\text{–A}][\text{ADP}]/[\text{ADP–}\beta\text{–A}]$, and $K_4 = [\beta\text{–ADP}][A]/[\text{ADP-}\beta\text{-A}]$

$$K_1K_3 = K_2K_4 \quad (2)$$

Equation 1 can be written

$$[\text{A bound}] = \frac{[\beta_T][A](K_3 + [\text{ADP}])}{K_1K_3 + K_3[A] + [A][\text{ADP}] + K_4[\text{ADP}]} \quad (3)$$

In the absence of ADP, the concentration of bound aurovertin, $[\text{A bound}]_0$, is

$$[\text{A bound}]_0 = [\beta_T][A]/K_1 + [A] \quad (4)$$

Equation 4 can be rearranged into the form

$$\frac{[\beta_T][A](K_3 + [\text{ADP}])}{(K_1 + [A])(K_3 + [\text{ADP}])} = \frac{[\beta_T][A](K_3 + [\text{ADP}])}{K_1K_3 + K_1[\text{ADP}] + [A]K_3 + [A][\text{ADP}]} \quad (5)$$

Table I: Compared Effect of ADP and ATP Added at a Saturating Concentration on the Binding of [^{14}C]Aurovertin D to *E. coli* β Subunit[a]

ligand	concn (μM)	[^{14}C]aurovertin D bound (mol/mol of β)	free [^{14}C]aurovertin D (μM)
none		0.26	1.98
ADP	500	0.51	1.80
ATP	500	0.41	1.88

[a] Binding of [^{14}C]aurovertin D was performed by equilibrium dialysis as described under Materials and Methods. The β subunit concentration was 2 μM. The medium consisted of 0.25 M sucrose, 0.5 mM EDTA, and 10 mM Tris-HCl, final pH 7.5.

The difference between the reciprocals of [A bound]$_0$ and [A bound] can be written as

$$1/[\text{A bound}]_0 - 1/[\text{A bound}] = \frac{(K_1 - K_4)[\text{ADP}]}{[\beta_T][\text{A}](K_3 + [\text{ADP}])} \quad (6)$$

It follows that

$$(1/[\text{A bound}]_0 - 1/[\text{A bound}])^{-1} = 1/y = \frac{[\beta_T][\text{A}]K_3}{(K_1 - K_4)[\text{ADP}]} + \frac{[\beta_T][\text{A}]}{K_1 - K_4} \quad (7)$$

Equation 7 can be rewritten as

$$\frac{1}{y} = \frac{N[\text{A}]K_3}{(K_1 - K_4)[\text{ADP}]} + \frac{N[\text{A}]}{K_1 - K_4} \quad (8)$$

where N is the total number of aurovertin binding sites per β subunit when [A bound]$_0$ and [A bound] are expressed in moles of bound aurovertin per mole of β subunit.

When $1/y$ was plotted vs. $1/[\text{ADP}]$, a straight line was obtained; the intercept on the base line was equal to $-1/K_3$. The above equation is valid provided that the concentration of free aurovertin ([A]) remains constant. In the experiment illustrated in Figure 1, the aurovertin D concentration ([A]) was adjusted to maintain the concentration of free [^{14}C]aurovertin D at a constant value, between 2.15 and 2.30 μM. In Figure 1B the plot of $1/y$ vs. $1/[\text{ADP}]$ gave a straight line. In eq 8, the value given to N was 1; values of 6 μM and 1 μM were given to K_1 and K_4, respectively (Issartel et al., 1983). On the basis of these data, the calculated value for K_3 was 4.7 μM (Figure 1). From eq 2, a K_2 value of 28 μM could then be deduced.

From the data in Table I it is clear that ATP also is able to enhance aurovertin binding to the β subunit.

Binding of [^{14}C]ADP to the Isolated β Subunit. The proposed model in Scheme I postulates interaction between ADP and aurovertin D binding sites on the β subunit. As ADP or ATP increases the binding affinity for [^{14}C]aurovertin, one may wonder whether the converse is true, i.e., whether aurovertin increases the binding affinity of [^{14}C]ADP for β. The effect of aurovertin on the binding parameters of [^{14}C]ADP with respect to the isolated β subunit is described now.

In preliminary assays, direct binding of [^{14}C]ADP to the β subunit was assayed by the elution–centrifugation method (see Materials and Methods). This method, however, did not allow detection of any significant binding of [^{14}C]ADP to the β subunit, probably due either to the low affinity of the ADP binding site or to the rapid dissociation of the ADP–β subunit complex. With a gel filtration technique, Ohta et al. (1980) also failed to detect an ADP binding site on the β subunit of F_1 from PS3.

Equilibrium dialysis was then performed as described under Materials and Methods with [^{14}C]ADP concentration up to 70 μM. Since equilibrium is reached after a few hours, the stability of the isolated β subunit could be affected. The integrity of the β subunit was checked after dialysis by the ability to form a fluorescent complex with aurovertin D. The enhancement of the fluorescence intensity of a given amount of aurovertin D was identical in the presence of equal amounts of freshly prepared β subunit or dialyzed β subunit. Proteolysis also did not occur, as checked by NaDodSO$_4$–polyacrylamide gel electrophoresis.

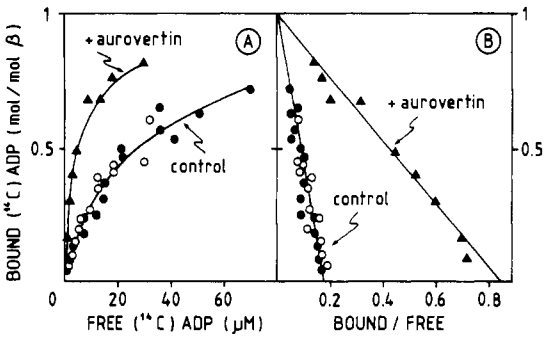


FIGURE 2: Determination by equilibrium dialysis of the binding parameters of [^{14}C]ADP with respect to the isolated β subunit from *E. coli*. Experiments were carried out as described under Materials and Methods in 200 mM NaCl, 0.5 mM EDTA, and 10 mM Tris-HCl, final pH 7.5 (●), or in the same medium plus 0.02% Tween-20 (○). The β subunit concentration was 4 μM. In (▲), aurovertin D was added to a final free concentration of 26 μM; Tween-20 was present at the final concentration of 0.02% (v/v). (A) Direct binding curves. (B) Scatchard plots.

Table II: Effect of ATP and AMP on the Binding Efficiency of Isolated *E. coli* β for [^{14}C]ADP[a]

ligand	concentration (μM)	[^{14}C]ADP bound (mol/mol of β)	free [^{14}C]ADP (μM)
none		0.49	25
AMP	143	0.43	22.9
ATP	57	0.27	19.6

[a] Binding was performed by equilibrium dialysis technique as described under Materials and Methods in 200 mM NaCl, 0.5 mM EDTA, and 10 mM Tris-HCl, final pH 7.5. The concentration of β subunit was 2.7 μM.

The direct [^{14}C]ADP binding curves and the corresponding Scatchard plot are illustrated in Figure 2. The data clearly show that the isolated β subunit of *E. coli* F_1 contains an ADP binding site with a K_d value of about 23 μM. As shown also in Figure 2, ADP binding was not changed in the presence of Tween-20 added at the final concentration of 0.02% (v/v). The presence of Tween-20 prevented aggregation of β subunits during the course of dialysis in the presence of Mg^{2+}.

At a concentration of 26 μM, aurovertin D did not modify the amount of bound [^{14}C]ADP, but it enhanced the affinity of β for [^{14}C]ADP, the K_d value being shifted from 23 μM to 4.8 μM. The K_d values obtained by direct assay of [^{14}C]ADP binding in the presence or absence of aurovertin were very close to those obtained by the indirect method based on binding enhancement of [^{14}C]auvovertin D in the presence of ADP, namely, 28 μM and 4.7 μM, respectively. These results confirmed the validity of the model illustrated in Scheme I.

Binding of [^{14}C]ADP was decreased by addition of ATP but not of AMP (Table II). Since ATP is a substrate, but AMP is not, these data point to the nucleotide specificity of the β subunit. Bindings of ATP and ADP were mutually exclusive, indicating that there is a single nucleotide binding site on the β subunit.

By equilibrium dialysis, [^{3}H]ATP was found to bind with low affinity. If one ATP binding site per β subunit is assumed,

Table III: Effect of Aurovertin Added at a Saturating Concentration on the Binding Affinity of the Isolated β Subunit for ADP or ATP[a]

radiolabeled ligand	additional ligand	K_d value (μM)
[^{14}C]ADP	none	23
[^{14}C]ADP	aurovertin D	4.8
[^{14}C]ADP[c]	$MgCl_2$	50[b]
[^{14}C]ADP[c]	$MgCl_2$ + aurovertin D	7.5
[^{3}H]ATP	none	50–100[b]
[^{3}H]ATP	aurovertin D	21

[a] Binding of [^{14}C]ADP or [^{3}H]ATP was performed by equilibrium dialysis as described under Materials and Methods in 200 mM NaCl, 0.5 mM EDTA, 10 mM Tris-HCl, and 0.02% (v/v) Tween-20, final pH 7.5. The β subunit concentration used ranged from 3.5 to 4 μM. When added, $MgCl_2$ and aurovertin were at the final concentrations of 200 and 25 μM, respectively. [b] For calculation of K_d values, the binding capacity of the isolated β was normalized to a value of 1 mol of nucleotide/mol of β. [c] EDTA was omitted.

the approximate K_d value was in the range of 50–100 μM (Table III). Likewise, in the presence of 200 μM Mg^{2+}, the ADP binding affinity was very low; if a binding stoichiometry of 1 is assumed, a K_d value of about 50 μM could be derived. When the same experiments were carried out in the presence of 25 μM aurovertin D, the nucleotide binding affinity of ATP and ADP for the β subunit was increased by 3–6-fold (Table III). The K_d value for [^{14}C]ADP, which is 2 times lower in the absence than in the presence of $MgCl_2$, was also decreased by about 6-fold upon addition of aurovertin.

Discussion

The β subunit of F_1-ATPase is thought to enclose the catalytic site, since it is the target for a number of inhibitors, for example, aurovertin in the case of mitochondrial and bacterial F_1 and the natural ATPase inhibitor in that of mitochondrial F_1; the β subunit also possesses a P_i binding site; furthermore, the β subunit in mitochondrial, bacterial, and chloroplast F_1 shares with the α subunit the ability to be covalently photolabeled by azido derivatives of ADP and ATP [for a review, see Vignais & Satre (1984)]. The fact that ADP or ATP added to the isolated β subunit of TF_1 from the thermophilic bacterium P3 induces changes in the circular dichroism properties of the protein was interpreted as the result of nucleotide binding to the β subunit (Ohta et al., 1980). However, these data do not provide direct evidence for an ADP/ATP binding site on β. Direct evidence for an ADP/ATP binding site on the β subunit requires the demonstration that the isolated β subunit has the ability to bind ADP or ATP. In the present paper, the presence of a nucleotide binding site on isolated β from *E. coli* F_1 is demonstrated through two different experimental approaches, namely, the direct measurement of the binding parameters of radiolabeled ADP or ATP by equilibrium dialysis and the effect of added ADP or ATP on the binding capacity and affinity for [^{14}C]aurovertin.

Binding Affinity and Capacity of ADP and ATP for the Isolated β Subunit: Effect of Aurovertin. Two groups of authors (Dunn & Futai, 1980; Matsuoka et al., 1982) failed to detect nucleotide binding to isolated β from *E. coli*. A direct binding assay was used by Dunn & Futai (1980), but the added ADP concentrations were less than 2 μM. Matsuoka et al. (1982) used a fluorescent ATP analogue that had possibly no recognition site on the β subunit. The hydroxylapatite chromatography used to purify the β subunit by the two groups had possibly some deleterious effects on the structure of β. Here we demonstrate by equilibrium dialysis that the isolated β subunit of *E. coli* F_1 possesses a binding site for ADP and ATP with K_d values of 25 μM and 50–100 μM, respectively. After the present paper had been submitted for publication, Hirano et al. (1984) reported ATP-dependent conformational changes in the *E. coli* β subunit using as probe 8-anilinonaphthalene-1-sulfonate and concluded to the presence of an ATP binding site on the β subunit; the K_d value deduced from their results was in the range of 10–100 μM, a value in good agreement with that of 50–100 μM found in the present paper.

Another finding of interest is the marked influence of aurovertin on the binding affinity of radiolabeled ADP or ATP to the isolated β subunit. The affinity was increased by 3–6-fold; however the binding capacity of 1 mol of ADP or ATP per mol of β subunit remained unchanged. The inhibition of *E. coli* F_1-ATPase activity caused by aurovertin at low Mg^{2+} concentration is a mixed-type inhibition, i.e., inhibition is associated with an increased affinity of the substrate (Issartel et al., 1983). This again is consistent with the aurovertin-dependent increase in reactivity of isolated β with respect to ADP or ATP reported in this paper. The possibility that the inhibition of *E. coli* F_1 caused by aurovertin was due to retention of ADP at the catalytic site is not likely, however, since the ATPase activity was assayed in an ATP-regenerating system in which the ADP concentration is maintained at very low level.

Effect of ADP and ATP on the Binding Affinity and Capacity of [^{14}C]Aurovertin for the Isolated β Subunit. The fact that ADP enhances the binding affinity of the β subunit for [^{14}C]aurovertin and that the converse effect is also true is consistent with Scheme I, which implies the formation of a ternary complex aurovertin–β–ADP. It fits also with previous data from this laboratory on the effect of ADP on the binding of aurovertin to *E. coli* F_1 (Satre et al., 1980). Three aurovertin sites per F_1, one of high affinity (K_d = 0.2 μM) and the other two of low affinity (K_d = 3–5 μM and 7–10 μM), were revealed by addition of ADP or ATP; in the absence of nucleotide, only a fraction of the high-affinity binding site for aurovertin could be demonstrated (Issartel et al., 1983). Furthermore, a K_d value of 10–20 μM for ADP binding to *E. coli* F_1 was determined on the basis of the ADP-dependent enhancement of aurovertin fluorescence (Satre et al., 1980).

The stimulatory effect of ADP on the fluorescence of the aurovertin–F_1 complex has been repeatedly reported (Satre et al., 1980; Wise et al., 1981; Bragg et al., 1982); this is not the case for the isolated β subunit. To our knowledge, enhancement of the fluorescence of the aurovertin–β complex has been reported only in the case of isolated β from beef heart F_1 (Verschoor, 1982) and *E. coli* F_1 (Satre et al., 1980; Issartel et al., 1983). The weak fluorescence enhancement of the aurovertin β complex found by Verschoor (1982) allowed him to find a K_d value of 420 μM for ADP binding, a value 2 orders of magnitude higher than that found in the present work for the isolated β subunit of *E. coli*.

In conclusion, the definite demonstration of a high-affinity nucleotide binding site on the isolated β subunit reinforces the idea that the β subunit contains the catalytic site. Long-distance interactions between the aurovertin binding site on β and a nucleotide binding site, involving an $\alpha\beta$ contact, have been proposed by Wise et al. (1981). The presence on the isolated β subunit of both a nucleotide site and an aurovertin site and the fact that each ligand controls the binding parameters of the other one suggest on the contrary that the effects of added ADP or ATP on the fluorescence of the *E. coli* F_1–aurovertin complex are due to the binding of ADP or ATP and aurovertin to the same β subunit of F_1.

Registry No. ATPase, 9000-83-3; ADP, 58-64-0; ATP, 56-65-5; aurovertin D, 65256-31-7.

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