# Detection of ATP-Dependent Conformational Change in the $F_1$ Portion and $\beta$ Subunit of *Escherichia coli* $H^+$-ATPase Using 8-Anilinonaphthalene-1-sulfonate†

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ABSTRACT: The bindings of ATP to *Escherichia coli* coupling factor ATPase ($F_1$) and its isolated $\beta$ subunit were studied with 8-anilinonaphthalene-1-sulfonate (ANS). The fluorescence of ANS increased upon addition of $F_1$ or the $\beta$ subunit, and this fluorescence was quenched on addition of ATP. Thus, ANS bound to the hydrophobic region of $F_1$ or the $\beta$ subunit, and the binding of ATP was detected as a quenching of ANS fluorescence. The quenching of the fluorescence suggested that $F_1$ or the $\beta$ subunit underwent a conformational change on binding to ATP. With and without ATP, 5.0 and 3.6 mol of ANS, respectively, bound to 1 mol of $F_1$. On the other hand, with or without ATP about 1 mol of ANS bound to $\beta$. The fluorescence quenching was specific for ATP and was not observed with GTP or CTP. It was dependent on pH, being higher at acidic pH, but it was not enhanced by $MgCl_2$. The dissociation constants of $F_1$ and the $\beta$ subunit for ATP were estimated to be $10^{-4}$–$10^{-5}$ M. The significance of these binding sites is discussed in relation to the mechanism of the ATPase.

The proton-translocating ATPase[1] ($H^+$-ATPase) complex is composed of two portions $F_1$ and $F_0$ [for reviews, see Fillingame (1981), Futai & Kanazawa (1980, 1983), and Senior & Wise (1983)]. The peripheral membrane portion of the complex, $F_1$, has five subunits ($\alpha$, $\beta$, $\gamma$, $\delta$, and $\epsilon$), and isolated $F_1$ has ATPase activity. $F_0$ is an integral part of the membrane and mediates proton translocation between the two compartments separated by the membrane. The entire $F_0F_1$ complex is found in membranes of mitochondria, chloroplasts, and bacteria.

The nucleotide binding sites of $F_1$ have been shown to be in the $\alpha$ and $\beta$ subunits by chemical modification experiments with general protein reagents and photoaffinity analogues of ATP [for reviews, see Futai & Kanazawa (1983) and Senior & Wise (1983)]. From these studies the catalytic site of ATPase has been suggested in the $\alpha$ or $\beta$ subunit or the interface between the two sununits. Studies on isolated subunits of *Escherichia coli* also indicated that the $\alpha$ subunit has a nucleotide binding site and undergoes an ATP-dependent conformational change. The isolated $\alpha$ subunit of *E. coli* has a tight binding site for ADP and ATP (1 mol of nucleotide/mol of $\alpha$) with $K_D$ values of 0.9 $\mu$M and 0.1 $\mu$M, respectively (Dunn & Futai, 1980). Physical studies by Paradies (1980, 1981), Paradies & Kagawa (1981), and Dunn (1980) showed that this subunit undergoes a large conformational change when it binds nucleotide. Senda et al. (1983) also detected an ATP-dependent conformational change of the $\alpha$ subunit as a change of trypsin sensitivity. This conformational change may be important for catalysis and assembly of the ATPase. Circular dichroism measurement indicated the presence of high- and low-affinity sites, respectively, in $\alpha$ and $\beta$ subunits from a thermophilic bacterium PS3 (Ohta et al., 1980). However, no nucleotide site has yet been demonstrated in the isolated $\beta$ subunit of *E. coli*.

In this study we detected binding of ATP to the isolated $\beta$ subunit or $F_1$ of *E. coli* using 8-anilinonaphthalene-1-sulfonate (ANS). ANS is known to show fluorescence with a high quantum yield upon binding to a hydrophobic region of a protein, although its emission is low in aqueous solution (Weber & Young, 1964). The flurescence of ANS increased upon addition of the purified $\beta$ subunit or $F_1$, and this fluorescence was quenched on addition of ATP ($10^{-3}$–$10^{-4}$ M). Thus, ATP bound to $F_1$ or the $\beta$ subunit and induced a conformational change which was detected by quenching of ANS fluorescence. The ATP-dependent conformational change may include change in the hydrophobic region of $F_1$ or $\beta$ subunit, because ANS is known to bind to hydrophobic regions of proteins.

## Experimental Procedures

*Materials.* The sources of materials were as follows: ATP, Daiichi Pharmaceutical Co.; ANS, Sigma Chemical Co.; Sephadex G-25 (Fine) and DEAE-Sepharose CL-6B, Pharmacia; Bio-Gel HTP, Bio-Rad. Other materials were of the highest grade available commercially.

*Preparations. E. coli* $F_1$ (four-subunit form without the $\delta$ subunit) was prepared from strain KY7485 ($\lambda asn$-5) (Kanazawa et al., 1979) as described previously (Futai et al., 1974) and stored at −80 °C until use. For experiments $F_1$ was precipitated with ammonium sulfate (65% saturation), dissolved in buffer A (50 mM succinate–Tris, pH 6.3, 2.0 mM EDTA, and 3.0 mM $\beta$-mercaptoethanol) and dialyzed extensively against the same buffer. The $\alpha$, $\beta$, and $\gamma$ subunits of $F_1$ were isolated by hydroxyapatite (Bio-Gel HTP) and DEAE-Sepharose column chromatographies from $F_1$ dissociated by cold treatment (Dunn & Futai, 1980) and concentrated by ultrafiltration (Amicon Filter, PM10). The resulting subunits were essentially homogeneous and were stored at −80 °C until use. They were dialyzed extensively against buffer A at 4 °C before binding experiments.

*Assays.* ATPase activity (Futai et al., 1974) and protein (Lowry et al., 1951) were measured as described previously. All fluorescence measurements were carried out at 25 °C in a Hitachi spectrofluorometer, Model 650-10, equipped with

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[1] Abbreviations: ANS, 8-anilinonaphthalene-1-sulfonate; ATPase, adenosinetriphosphatase; $F_1$, catalytic portion of the proton-translocating ATPase ($H^+$-ATPase); $\alpha$, $\beta$, $\gamma$, $\delta$, and $\epsilon$, subunits of $F_1$; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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an incubator to maintain a constant temperature. Fluorescence of ANS was assayed in a cuvette (light path 1.0 cm) at 470 nm with excitation at 350 nm. For fluorometric titrations ANS dissolved in ethanol was added to protein solutions in buffer unless otherwise specified. The final concentration of ethanol was kept below 1%. The number of binding sites for ANS in $F_1$ or the $\beta$ subunit was determined by gel filtration (Hammel & Dreyer, 1962; Arai et al., 1975). The $F_1$ or $\beta$ subunit was dissolved in buffer A with or without 1.0 mM ATP and passed through a column of Sephadex G-25 (1.0 cm × 50 cm) equilibrated with the same buffer containing ANS at various concentrations. The concentration of ANS in the effluent was measured in terms of the absorbance at 350 nm, and the amount of ANS bound to $F_1$ or the $\beta$ subunit was calculated from the peak and trough of the chromatogram. Absorption was measured in a Hewlett-Packard 8450A UV/VIS spectrophotometer.

## Results

*Binding of ANS to E. coli $F_1$ or Its $\beta$ Subunit.* It is known that the fluorescence of ANS has a low quantum yield in aqueous solution with an emission maximum at 515 nm. On addition of *E. coli* $F_1$, ANS showed fluorescence with a high quantum yield, and its emission maximum shifted to 470 nm (Figure 1A, trace 1), suggesting that ANS bound to a hydrophobic domain of the $F_1$ molecule. ANS had essentially no effect on the ATPase activity of $F_1$ at concentrations of up to 0.5 mM (data not shown). Therefore, it could be used for analysis of possible conformational transition of $F_1$ or its subunit upon addition of substrates or cofactors such as ATP, ADP, phosphate, and $MgCl_2$. When ATP was added, the fluorescence decreased with a slight blue shift of the emission maximum (Figure 1A, trace 2). These results suggest that the hydrophobic domains of $F_1$ underwent an ATP-dependent conformational change that could be detected as quenching of the fluorescence. However, the fluorescence of ANS bound to $F_1$ did not change on addition of 2.0 mM potassium phosphate or 5.0 mM $MgCl_2$ (data not shown), suggesting that a conformation transition, if any, dependent on these compounds could not be detected when ANS was used. Thus, bound ANS responded only to the ATP-dependent conformational change.

Similar results were obtained when the isolated $\beta$ subunit (Figure 1B): after addition of the $\beta$ subunit, the fluorescence of ANS increased with a shift of the emission maximum, and this fluorescence was quenched by ATP, suggesting that the $\beta$ subunit bound ATP and that the ATP-dependent conformational change of the hydrophobic domain was detected as quenching of the fluorescence of ANS. It is noteworthy that this is the first indication of binding of ATP to the isolated $\beta$ subunit of *E. coli*. The fluorescence of ANS bound to the $\beta$ subunit was not affected by addition of $MgCl_2$ or potassium phosphate. Thus, the bound ANS responded only to the ATP-dependent conformational change of the $\beta$ subunit, as shown with $F_1$. The fluorescence of ANS (50 $\mu$M) increased linearly with the amount of $F_1$ (up to 2 nmol) or $\beta$ subunit (up to 5 nmol) added. Fluorescence of the ANS complex of $F_1$ was higher than that of the $\beta$ subunit when the emission was expressed per nanomole of protein (of $F_1$ or $\beta$): the values for fluorescence of the ANS–$F_1$ and ANS–$\beta$ complexes were 11.5 and 5.3 relative emission/nmol, respectively, and values for the fluorescence quenching of ANS-$F_1$ and ANS-$\beta$ on addition of ATP were 2.6 and 0.6 relative fluorescence/nmol, respectively.

The fluorescence of ANS also increased on addition of the isolated $\alpha$ subunit. However, only low quenching was observed

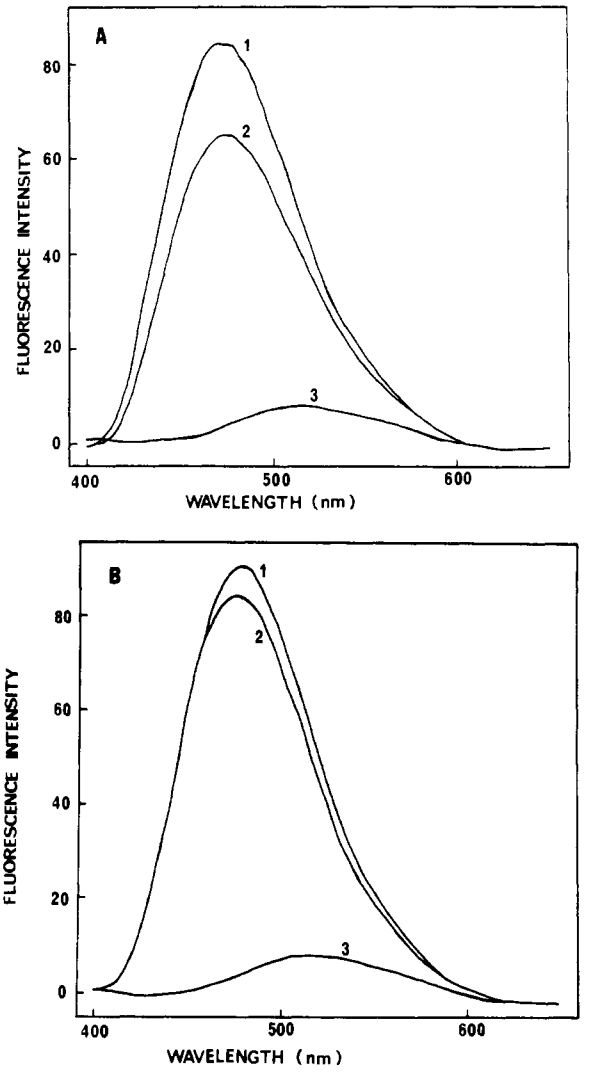


FIGURE 1: Fluorescence emission spectra of ANS bound to *E. coli* $F_1$ and its subunit. (A) Fluorescence emission spectra of $F_1$ (0.47 mg, 1.3 nmol) in buffer A [50 mM succinate–Tris (pH 6.3), 2.0 mM EDTA, 3.0 mM $\beta$-mercaptoethanol] was recorded 10 min after addition of 50 $\mu$M ANS (trace 1). The emission spectrum of the ANS–$F_1$ complex was also recorded in the presence of ATP (trace 2): $F_1$ was incubated with 50 $\mu$M ANS at 25 °C for 10 min. Then 1.0 mM ATP was added, and the fluorescence emission spectrum was recorded 5 min later. The emission spectrum of 50 $\mu$M ANS in buffer A is shown as a control (trace 3). All spectra were taken at 25 °C and are uncorrected and shown as relative fluorescence (excitation 350 nm; scan speed 60 nm/min). Other conditions were as described under Experimental Procedures. (B) Fluorescence emission spectra of the ANS–$\beta$ subunit complex (0.15 mg, 3 nmol) recorded at pH 6.3 in buffer A (traces 1 and 2). Spectra were recorded 10 min after addition of ANS (trace 1) or 5 min after addition of 1.0 mM ATP (after incubation with ANS) as described for (A) (trace 2). The emission spectrum of 50 $\mu$M ANS in buffer A is shown as a control (trace 3). Other conditions were as described for (A) or under Experimental Procedures.

when ATP was added, and $MgCl_2$ and potassium phosphate had no effect. The ATP-dependent quenching of ANS fluorescence with the $\alpha$ subunit was about 10% of that with the $\beta$ subunit in the presence of 1.0 mM ATP at the same concentrations of subunits. Because of this low quenching, we did not study the binding of ANS and ATP to the $\alpha$ subunit in detail.

*Number of Binding Sites for ANS in $F_1$ and the $\beta$ Subunit.* We determined how many molecules of ANS bound to $F_1$ or the $\beta$ subunit by gel filtration, since it was interesting to know whether the decrease in fluorescence was due to change in the number of ANS molecules bound to the proteins upon ATP

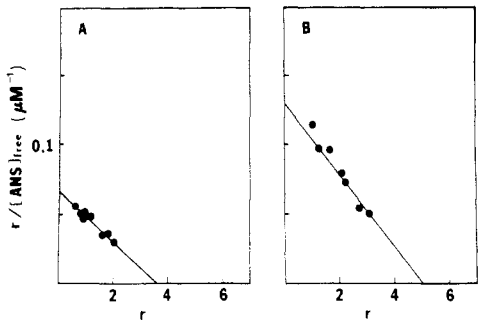


FIGURE 2: Scatchard plot of ANS binding to *E. coli* $F_1$. Binding of ANS to $F_1$ was measured in the presence (A) or absence (B) of 1.0 mM ATP. A series of gel filtrations were carried out with Sephadex G-25 equilibrated with buffer A (with or without ATP) containing various concentrations of ANS. $F_1$ (2.0 mg) in 150 $\mu$L of buffer A was applied each time. Results are plotted according to Scatchard (1949): $r$, number of ANS molecules per mole of $F_1$; ANS, free ANS concentration. The molecular weight (360 000) of $F_1$ was calculated assuming $\alpha_3\beta_3\gamma$ and used for calculation of $r$. Values for the molecular weights of subunits are from the primary structure (Futai & Kanazawa, 1983). It should be noted that the $F_1$ used had no $\delta$ subunit. Other conditions were as described under Experimental Procedures.

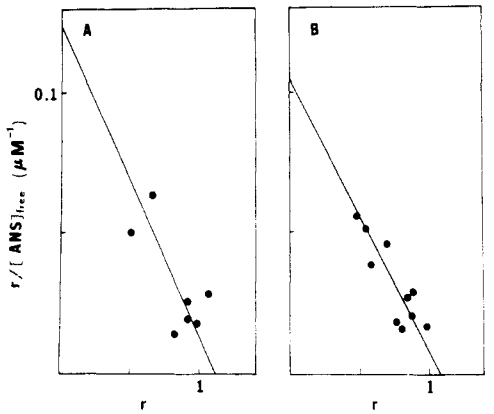


FIGURE 3: Scatchard plot of ANS binding to the *E. coli* $\beta$ subunit. Binding of ANS to the $\beta$ subunit was measured in the presence (A) or absence (B) of 1.0 mM ATP. The $\beta$ subunit (0.9 mg) in 80 $\mu$L of buffer A was applied to a Sephadex G-25 column equilibrated with buffer A (with or without ATP) containing various concentrations of ANS. Results are plotted according to Scatchard (1949) as described in the legend of Figure 2. The molecular weight of the $\beta$ subunit was taken as 50 117 (Futai & Kanazawa, 1983). Other conditions were as described under Experimental Procedures.

binding or to a conformational change. When $F_1$ was passed through a gel filtration column equilibrated with buffer containing ANS, ANS bound to $F_1$ was eluted as a single peak in the void volume, and the peak was followed by trough fractions containing low concentrations of ANS. Thus, the amount of ANS bound to $F_1$ could be calculated from the absorbances of the peak and trough. A Scatchard plot (Scatchard, 1949) was obtained from a series of such gel filtration experiments in the presence of various concentrations of ANS. As shown in Figure 2, $F_1$ bound 5.0 and 3.6 mol of ANS/mol in the presence and absence of ATP, respectively. The dissociation constants of ANS were 32 $\mu$M in the absence of ATP and 56 $\mu$M in its presence. These results suggest that the fluorescence change of the ANS–$F_1$ complex observed was mainly due to change in the number of molecules of ANS bound to $F_1$.

Similar experiments were carried out with the $\beta$ subunit. As shown in Figure 3, $\beta$ bound about 1 mol of ANS/mol regardless of the presence of ATP, and the dissociation constants in the presence and absence of ATP were similar: the $K_D$ values for ANS in the presence and absence of ATP were

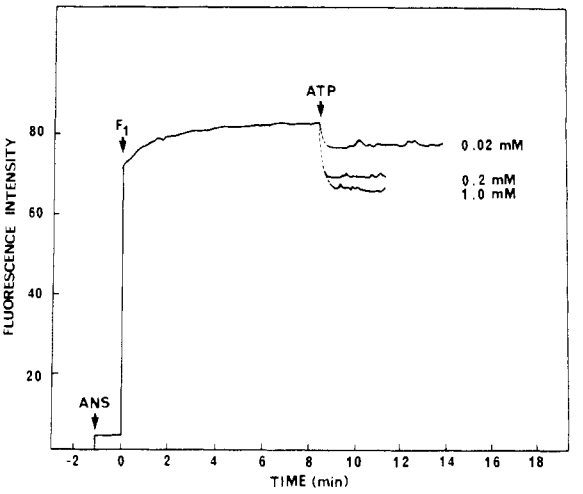


FIGURE 4: Kinetics of ANS binding to *E. coli* $F_1$. $F_1$ (0.90 mg) was incubated at pH 6.3 in 1.0 mL of buffer A. At the times indicated, concentrated solutions were added to give the following final concentrations: ANS, 50 $\mu$M; ATP, 0.02–1.0 mM.

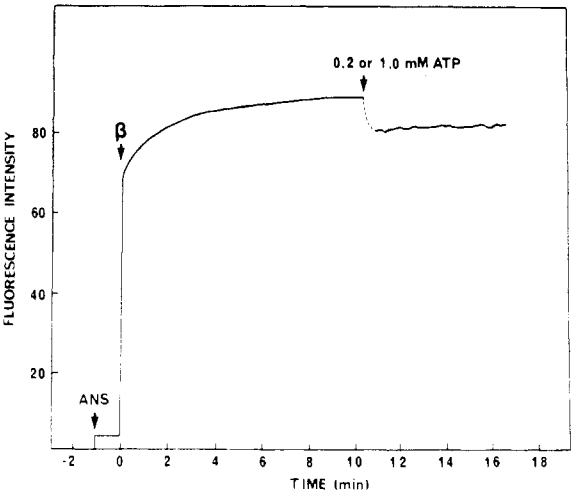


FIGURE 5: Kinetics of ANS binding to *E. coli* $\beta$. The $\beta$ subunit (1.0 mg) was incubated at pH 6.3 in 1.0 mL of buffer A. At the times indicated, concentrated solutions were added to give the following final concentrations: ANS, 50 $\mu$M; ATP, 0.2 or 1.0 mM.

9.6 and 8.1 $\mu$M, respectively. These results suggest that ATP-dependent flurescence changes of the ANS–$\beta$ complex observed (Figure 1B) were not due to change in the number of ANS molecules bound to the $\beta$ subunit on binding ATP but to change in the conformation of the hydrophobic domain that bound ANS without release of ANS. On the other hand, the number of ANS molecules bound to $F_1$ decreased upon addition of ATP, as described above. Thus, the mechanism of the fluorescence quenching with the $\beta$ subunit may differ from that with the $F_1$. We have repeated the experiment for the Scatchard plot several times because the deviations, especially for the $\beta$ subunit, were relatively high but have obtained essentially the same results each time.

*Kinetics of ATP-Dependent Quenching of ANS Fluorescence with the $F_1$ and $\beta$ Subunit.* The fluorescence of ANS increased immediately after addition of $F_1$ or the $\beta$ subunit, but about 3 min was required to reach a plateau level. On the other hand, ATP-dependent quenching was a faster process, and the fluorescence reached a steady level within 1 min after addition of ATP (Figures 4 and 5). The quenching was dependent on the concentration of ATP. $MgCl_2$ had no effect, regardless of the time of its addition, suggesting that the conformational change of the $\beta$ subunit was induced by the binding of ATP, not MgATP. ATP-dependent quenching with

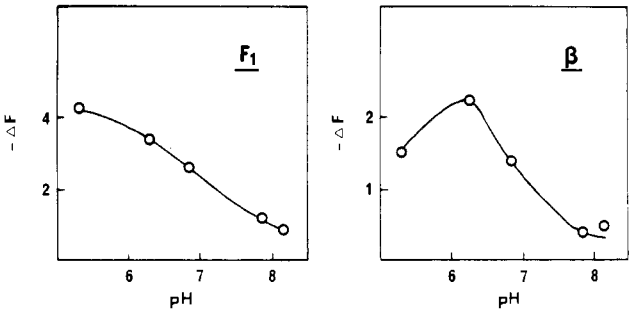


FIGURE 6: Effect of pH on ATP-dependent quenching of ANS fluorescence. $F_1$(18 $\mu$g) or its $\beta$ subunit (5 $\mu$g) was incubated with 50 $\mu$M ANS for 5 min in 1.0 mL of 50 mM buffers of the indicated pH values, and 1.0 mM ATP was added. Succinate–Tris (below pH 6.3) or Tris-HCl (above pH 6.8) was used. Fluorescence (excitation 350 nm; emission 470 nm) was measured, and plateau values of fluorescence quenching ($-\Delta F$) induced with ATP are plotted. Other conditions were as described in the text.

both $F_1$ and the $\beta$ subunit was also dependent on pH and was high at acidic pH (Figure 6). The fluorescence emissions of the ANS–$F_1$ and ANS–$\beta$ complexes were lower at alkaline pH: the ratios of the emission without ATP (at 470 nm) at pH 6.3 to that at 8.0 were 0.59 and 0.69, respectively, for the ANS–$F_1$ and ANS–$\beta$ complexes. Essentially the same results were obtained with ADP, but GTP and CTP of the same concentration as ATP had no effect. It was difficult to plot the reciprocal of the quenching at the plateau level ($-\Delta F$) to determine the $K_D$ value for ATP, because the extent of quenching was relatively low. However, the $K_D$ values of $F_1$ and the $\beta$ subunit for ATP was estimated to be about $10^{-4}$–$10^{-5}$ M from values obtained in repeated titrations of the quenching with different concentrations of ATP.

## Discussion

This work provides the first evidence for an ATP binding site in the isolated $\beta$ subunit of *E. coli*. It is noteworthy that the $K_D$ value for ATP of the $\beta$ subunit is more than 100 times that of the $\alpha$ subunit: the $K_D$ value of the $\alpha$ subunit for ATP was reported to be 0.1 $\mu$M (Dunn & Fatai, 1980), whereas that of the $\beta$ subunit was estimated to be $10^{-4}$–$10^{-5}$ M in this study. This finding is consistent with the report of Lunardi et al. (1981) of preferential labeling of the $\alpha$ subunit of *E. coli* $F_1$ with a photoaffinity analogue at low concentration but almost equal labeling of the $\alpha$ and $\beta$ subunits at higher concentration. Nucleotide binding sites were also located in the $\alpha$ and $\beta$ subunits from $F_1$ of the thermophilic bacterium (Ohta et al., 1980), when binding was estimated from change in the circular dichroism spectrum, assuming that the same tyrosyl residue in isolated $\beta$ as that in $\beta$ assembled in $F_1$ responded to ATP binding. The isolated $\alpha$ and $\beta$ subunits of this organism had essentially the same $K_D$ values for ATP and ADP. However, ATP at low concentration bound to the $\alpha$ subunit assembled in $F_1$, while ATP at high concentration bound to both $\alpha$ and $\beta$ assembled in $F_1$, as judged by following binding by change of a specific tyrosyl residue in the $\beta$ subunit. Thus, it was concluded that a tight site is located in $\alpha$ and a loose site in $\beta$. We tried to detect binding to the $\beta$ subunit more directly with a centrifuge column procedure but were not successful (M. Senda, H. Kanazawa, and M. Futai, unpublished observation), although we detected binding to the $\alpha$ subunit by this procedure. As described previously (Senda et al., 1983) $\alpha$ subunit changed in sensitivity to trypsin upon binding of ATP, but no similar change in the $\beta$ subunit on binding of ATP could be detected. Recent studies using covalently bound analogues have suggested that the catalytic site may be at the interface between $\alpha$ and $\beta$ subunits (Bragg et al., 1982; Bruist & Hammes, 1981; Williams & Coleman, 1982). Our observation could be compatible with this suggestion if the major portion of the site is in the $\beta$ subunit and is detectable in the isolated $\beta$ subunit. Binding studies using nucleotide-free $F_1$ have shown that $F_1$ has six nucleotide binding sites (Cross & Nalin, 1982), a value which is consistent with the ideas of binding sites in both the $\alpha$ and $\beta$ subunits and $\alpha_3\beta_3\gamma$-type stoichiometry.

The ATP binding site observed in the $\beta$ subunit may be the same as that observed in intact $F_1$, because the two had similar $K_D$ values ($10^{-4}$–$10^{-5}$ M) of fluorescene quenching for ATP and pH curves of ATP-dependent conformational change. However, ATP had different effects on the numbers of ANS molecules bound to $F_1$ and $\beta$, as discussed above: ATP reduced the number of ANS molecules bound to $F_1$ but not $\beta$. This may be due to the binding of ANS molecules to different domains in $F_1$ and the isolated $\beta$ subunit, although $F_1$ and the $\beta$ subunit seemed to undergo similar conformational change on ATP binding. However, it seems likely that a domain(s) was (were) exposed in the isolated subunit that was inaccessible in the assembled complex. This possibility is supported by the fact that the $K_D$ of $F_1$ for ANS was about 4 times that of the $\beta$ subunit.

There is accumulating evidence of site–site cooperatively in hydrolysis of ATP by the $F_1$ molecule (Boyer et al., 1982; Senior & Wise, 1983). ATP (Cross et al., 1982) or its analogue (Cross & Nalin, 1982; Grubmeyer & Penefsky, 1981; Matsuoka et al., 1982) bound to the first site tightly and was hydrolyzed very slowly, but loose binding of ATP to the second and third sites accelerated the hydrolysis at the first site. Because the $K_D$ for ATP was relatively higher in the fluorescence quenching of ANS, the quenching observed here may be due to the conformational change of $F_1$ after binding of ATP to the second and third sites. Such sites may be in the $\beta$ subunit of $F_1$, because the isolated $\beta$ subunit responded similary to ANS and ATP.

**Registry No.** ATPase, 9000-83-3; ATP, 56-65-5; ANS, 82-76-8.

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# Phospholipids Chiral at Phosphorus. Synthesis of Chiral Phosphatidylcholine and Stereochemistry of Phospholipase D†

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ABSTRACT: Chirally labeled 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholines (DPPC) with known configuration were synthesized by N-methylation of chirally labeled 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE). Transphosphatidylation of ($R_P$)- and ($S_P$)-[$^{18}$O]DPPC catalyzed by phospholipase D from cabbage gave ($R_P$)- and ($S_P$)-[$^{18}$O]DPPE, respectively, as indicated by $^{31}$P nuclear magnetic resonance (NMR) analysis of [$^{18}$O]DPPE. Therefore, phospholipase D catalyzes transphosphatidylation with overall retention of configuration at phosphorus. The steric course of hydrolysis of DPPC catalyzed by the same enzyme was elucidated by the following procedures. Hydrolysis of ($R_P$)-[$^{17}$O,$^{18}$O]DPPC by phospholipase D gave 1,2-dipalmitoyl-*sn*-glycero-3-[$^{16}$O,$^{17}$O,$^{18}$O]phosphate ([$^{16}$O,$^{17}$O,$^{18}$O]DPPA) with unknown configuration. The latter compound was then converted to 1-[$^{16}$O,$^{17}$O,$^{18}$O]phospho-(*R*)-propane-1,2-diol by a procedure involving no P–O bond cleavage [Bruzik, K., & Tsai, M.-D. (1984) *J. Am. Chem. Soc. 106*, 747–754]. The configuration of the phosphopropane-1,2-diol was determined as $R_P$ by $^{31}$P NMR analysis following ring closure and methylation [Buchwald, S. L., & Knowles, J. R. (1980) *J. Am. Chem. Soc. 102*, 6601–6603]. The results indicated that hydrolysis of DPPC catalyzed by phospholipase D also proceeds with retention of configuration at phosphorus. Our results therefore support a two-step mechanism involving a phosphatidyl-enzyme intermediate in the reactions catalyzed by phospholipase D from cabbage.

The enzyme-catalyzed reactions involving a P–O bond cleavage can be categorized into the following types on the basis of the stereochemistry involved:

$$ROPO_2OR' \text{ (prochiral)} \rightleftarrows ROPO_2OR'' \text{ (prochiral)} \quad (1)$$

$$ROPO_2OR' \text{ (prochiral)} \rightleftarrows ROPO_3 \text{ (pro-prochiral)} \quad (2)$$

$$ROPO_3 \text{ (pro-prochiral)} \rightleftarrows R'OPO_3 \text{ (pro-prochiral)} \quad (3)$$

$$ROPO_3 \text{ (pro-prochiral)} \rightleftarrows PO_4 \text{ (pro-pro-prochiral)} \quad (4)$$

In the past 5 years, the steric course of a large number of enzymes has been elucidated by use of chirally labeled phosphates or phosphorothioates (Buchwald et al., 1982; Eckstein, 1983; Eckstein et al., 1982; Floss et al., 1984; Frey, 1982; Frey et al., 1982; Gerlt et al., 1983; Knowles, 1980; Lowe et al., 1981; Tsai, 1982; Webb, 1982). However, most of the reactions investigated previously involved nucleotides or sugar phosphates.

Recently, we have reported synthesis and configurational analysis of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE)[1] chirally labeled with $^{17}$O and/or $^{18}$O at phosphorus (Bruzik & Tsai, 1984). These compounds and the methodology developed are useful in studying the stereochemistry of phospholipases. In this paper, we report the syntheses of chirally labeled 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholines (DPPC), as well as their application to elucidate the steric courses of transphosphatidylation (reaction in eq 1) and hydrolysis (reaction in eq 2) catalyzed by phospholipase D (phosphatidylcholine phosphatidohydrolase, EC 3.1.4.4) from cabbage. Part of the work has been reported in a preliminary paper (Bruzik & Tsai, 1982) before the absolute configuration is known.

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[1] Abbreviations: DPPA, 1,2-dipalmitoyl-*sn*-glycero-3-phosphate; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; EDTA, ethylenediaminetetraacetate; HMDSA, 1,1,1,3,3,3-hexamethyldisilazane; HMPA, hexamethylphosphoramide; TLC, thin-layer chromatography; NMR, nuclear magnetic resonance.

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