*Proc. Natl. Acad. Sci. USA*
Vol. 82, pp. 7550–7554, November 1985
Biochemistry

# Studies on the mechanism of oxidative phosphorylation: Effects of specific $F_0$ modifiers on ligand-induced conformation changes of $F_1$

AKEMI MATSUNO-YAGI, TAKAO YAGI, AND YOUSSEF HATEFI*

Division of Biochemistry, Department of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, CA 92037

*Communicated by Paul D. Boyer, July 19, 1985*

**ABSTRACT** **Aurovertin is a fluorescent antibiotic that binds to the catalytic $\beta$ subunits of the mitochondrial $F_1$-ATPase and inhibits ATP synthesis and hydrolysis. ATP, ADP, and membrane energization in submitochondrial particles (SMP) alter the fluorescence of $F_1$-bound aurovertin. These fluorescence changes are considered to be in response to the conformation changes of $F_1$-ATPase. This paper shows that the ATP-induced fluorescence change of aurovertin bound to SMP or complex V (purified ATP synthase complex $F_0$–$F_1$) is inhibited when these preparations are pretreated with oligomycin or *N,N'*-dicyclohexylcarbodiimide (DCCD). This inhibition is not seen with isolated $F_1$-ATPase. These and other results have suggested that modifications of the DCCD-binding protein in the membrane sector ($F_0$) of the ATP synthase complex are communicated to $F_1$, thereby altering the binding characteristics of ATP to the $\beta$ subunits. By analogy, it is proposed that modifications (e.g., protonation/deprotonation) of the DCCD-binding protein effected by protonic energy alter the conformation of $F_1$ and bring about the substrate/product binding changes that appear to be essential features of the mechanism and regulation of oxidative phosphorylation.**

The following recent findings have suggested that the mitochondrial $F_1$-ATPase undergoes catalytically significant conformation changes during oxidative phosphorylation. (*i*) In agreement with an early prediction of Boyer *et al.* (1, 2), it has been shown that $F_1$-bound ADP and $P_i$ can form $F_1$-bound ATP in an isoenergetic process involving negligible free energy change (3–6). The energy-requiring steps in oxidative phosphorylation are considered to be substrate (ADP and $P_i$)-binding (1, 7) and product (ATP)-releasing (1, 4, 8). (*ii*) The three catalytic sites on isolated $F_1$-ATPase interact, resulting in negative cooperativity with respect to substrate (MgATP) concentration and positive catalytic cooperativity in the sense that substrate binding to the second and third sites greatly enhances turnover by increasing the rate of product (ADP) removal from the first site (4, 8–11). Some of these cooperative effects have also been observed in the direction of ATP synthesis (ref. 12; unpublished data). (*iii*) In oxidative phosphorylation catalyzed by phosphorylating submitochondrial particles (SMP), partial uncoupling or attenuation of the rate of respiration not only diminishes the apparent $V_{max}$ ($V_{max}^{app}$) for ATP synthesis but also alters the apparent $K_m$ ($K_m^{app}$) for ADP and $P_i$. Partial uncoupling increases the $K_m^{app}$ for ADP and $P_i$, with $\ln(V_{max}/K_m)$ being a linear function of the uncoupler concentration (i.e., the free energy of the system) (ref. 7; see also ref. 13). Attenuation of the rate of respiration up to $\approx$60% by an electron-transfer inhibitor decreases these $K_m^{app}$ values; double-reciprocal plots of $V_{max}^{app}$ for ATP synthesis versus ADP or $P_i$ concentrations at several fixed concentrations of the electron-transfer inhibitor is a set of parallel lines for either ADP or $P_i$ as the variable substrate (14). Under these conditions, there is no change in $V_{max}/K_m$ and little or no change in the membrane potential $\Delta\psi$ (14). (*iv*) In SMP, the energy-induced fluorescence response of bound aurovertin, a reporter of $F_1$ conformation change (15, 16), decreases in parallel with decreases in $\Delta\psi$ and $V_{max}^{app}$ for ATP synthesis, as the respective assay systems are treated with incremental amounts of an uncoupler (14). Thus, it appears that energy-induced affinity changes of $F_1$ for ATP, ADP, and $P_i$ and the changes in $K_m^{app}$ for ADP and $P_i$ in response to alterations of the transmembrane electrochemical potential of protons, $\Delta\bar{\mu}_{H^+}$, and respiration rate are essential features of the mechanism and regulation of oxidative phosphorylation.

This paper is concerned with the role played by $F_0$ in the conformation changes of $F_1$. The results suggest that modifications at the *N,N'*-dicyclohexylcarbodiimide (DCCD)-binding protein influence the ligand-induced conformation changes of the catalytic $\beta$ subunits of $F_1$.

## MATERIALS AND METHODS

SMP (17), complex V (ATP synthase complex $F_0$–$F_1$) (18), and $F_1$-ATPase (19) were prepared from bovine heart mitochondria by published procedures. SMP were washed once with a buffer containing 0.25 M sucrose and 10 mM Tris acetate (pH 7.5) and was suspended in the same buffer at 40–60 mg of protein per ml. The heat-activation step in the $F_1$-ATPase preparation was omitted. Protein concentration was determined by the biuret method (20) in the presence of 0.1% deoxycholate or by the method of Lowry *et al.* (21). Inhibitors were added in ethanolic solution at the concentrations indicated to SMP (10 mg of protein per ml), complex V (5 mg of protein per ml), or $F_1$-ATPase (1 mg of protein per ml) in a buffer containing 0.25 M sucrose and 50 mM Tris acetate (pH 7.5). The mixture was then incubated at 0°C (SMP and complex V) or at room temperature ($F_1$-ATPase) and sampled for measurements of aurovertin fluorescence and ATPase activity.

Aurovertin fluorescence was monitored by an SLM photon-counting spectrofluorometer at 30°C in the above buffer. When SMP were used, the assay mixtures contained, in addition, 0.5 mM EDTA, 3 mM $MgCl_2$, and 20 mM potassium phosphate (pH 7.5) (15). The amounts of enzyme added per ml to the assay medium were: 0.2 mg of SMP, 0.094 mg of complex V, and 0.025 mg of $F_1$-ATPase. Aurovertin was added at 0.63 $\mu$M from a stock solution in absolute ethanol whose concentration had been determined by using an extinction coefficient of 42,700 $M^{-1} \cdot cm^{-1}$ (22). Additions of ATP, 2',3'-*O*-(2,4,6-trinitrophenyl)adenosine 5'-triphosphate (TNP-ATP), succinate, etc., were made as indicated in the figures. The wavelengths of excitation and emission were 366

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: SMP, phosphorylating submitochondrial particles; $F_1$, $F_1$-ATPase; $F_0$, membrane sector of the ATP synthase complex; TNP-ATP, 2',3'-*O*-(2,4,6-trinitrophenyl)adenosine 5'-triphosphate; DCCD, *N,N'*-dicyclohexylcarbodiimide; $Ph_3SnCl$, triphenyltin chloride; $Bu_3SnCl$, tributyltin chloride; $V_{max}^{app}$ and $K_m^{app}$, apparent $V_{max}$ and $K_m$.

*To whom reprint requests should be addressed.

and 470 nm, respectively. TNP-ATP showed no fluorescence with this setting of wavelengths within the concentration range used and had only a small filter effect on the aurovertin fluorescence (≤2% decrease by 0.4 μM TNP-ATP).

ATPase activity was measured at 30°C by the ATP regenerating system as described (10) in the presence of 50 μg of SMP, 25 μg of complex V, or 1 μg of $F_1$-ATPase per ml. In the assay of SMP for ATPase activity, 13 μM rotenone and 5 μM carbonylcyanide *m*-chlorophenylhydrazone were also present (23). Oligomycin sensitivity of ATPase activity was estimated by addition of 5 μg of oligomycin per ml to the assay mixtures. The assay system was not affected by the concentrations of inhibitors carried with the samples.

The sources of chemicals used were as follows: ATP and oligomycin were from Boehringer Mannheim; pyruvate kinase, lactic dehydrogenase, phospho*enol*pyruvate and *p*-(chloromercuri)benzoic acid, $ClHgC_6H_4COOH$, from Sigma; DCCD and tributyltin chloride ($Bu_3SnCl$) from Aldrich; triphenyltin chloride ($Ph_3SnCl$), from ICN; and TNP-ATP, from Molecular Probes, Junction City, OR. Other chemicals were reagent grade. Aurovertin and venturicidin were gifts, respectively, from R. B. Beechey (University College of Wales) and D. E. Griffiths (University of Warwick).

## RESULTS

The antibiotic aurovertin is an inhibitor of $F_1$-ATPase (15, 16, 24–30). In bovine heart and *Escherichia coli* $F_1$, aurovertin binds to the β subunits (27, 29, 30). In the latter, the binding stoichiometry has been shown to be 1 mol of aurovertin per β subunit (29). Upon binding to isolated bovine heart $F_1$, the fluorescence of aurovertin increases 50- to 100-fold (15, 25). Addition of ADP enhances this fluorescence, whereas addition of ATP or $Mg^{2+}$ results in partial quenching (15, 16, 26, 28). According to Chang and Penefsky (15), the dissociation constants of the $F_1$–aurovertin complex were 0.52, 0.07, 0.013, and $0.04 \times 10^{-6}$ M in the presence of ATP, ADP, $Mg^{2+}$, or buffer, respectively. In SMP, $F_1$-bound aurovertin displays fluorescence enhancement upon membrane energization (15, 16, 25, 26). The extent of the energy-induced fluorescence increase correlates well with $\Delta\psi$ and $V_{max}^{app}$ for ATP synthesis when these parameters are altered by addition of incremental amounts of an uncoupler (14). The changes in the fluorescence of bound aurovertin are considered to be in response to the conformation changes of $F_1$-ATPase (15, 16).

As shown in Fig. 1*A*, the binding of aurovertin to a preparation of ATP synthase complex (complex V) resulted in an enhancement of aurovertin fluorescence, which was partially quenched by subsequent addition of 1.25 mM ATP. However, when complex V was pretreated with the $F_0$ inhibitor oligomycin, the ATP-induced quenching was greatly diminished (see also refs. 16 and 31). Essentially identical effects were observed when the concentration of added ATP was increased to 10 mM. Also, similar results were obtained when complex V was pretreated with DCCD or with $ClHgC_6H_4COOH$ at concentrations that inhibited the ATPase activity of the complex ≈90%. Addition of ATP to aurovertin-treated SMP caused a small enhancement in fluorescence, which was also prevented when the particles were pretreated with oligomycin or DCCD (Fig. 1*B*). In the case of oligomycin, it was shown that the extent of inhibition of ATP-induced quenching of aurovertin fluorescence was oligomycin concentration dependent and correlated well with inhibition of ATPase activity at various oligomycin concentrations (Fig. 2). Also, the order of additions of aurovertin and oligomycin or of ATP and oligomycin to either complex V or SMP did not alter the inhibition of ATP-induced fluorescence quenching. Fig. 1*C* shows that treatment of isolated $F_1$-ATPase with oligomycin did not affect the ATP-induced fluorescence quenching. Similar results were obtained when $F_1$ was pretreated with $ClHgC_6H_4COOH$. Pretreatment with DCCD was not attempted because DCCD is known to react with the β subunits of isolated $F_1$. The results of Figs. 1 and 2 indicate, therefore, that the ligand-induced change in the fluorescence of $F_1$-bound aurovertin is influenced by alterations in $F_0$. In the case of $ClHgC_6H_4COOH$, the inhibitory modification of $F_0$ could be reversed by treating the particles with dithiothreitol. This treatment also reversed the inhibition of ATP-induced fluorescence change of aurovertin.

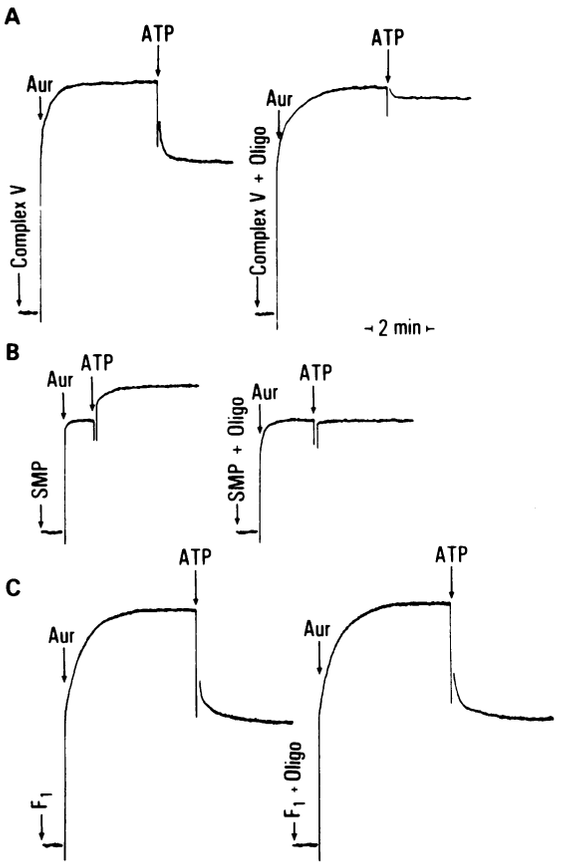


FIG. 1. Effect of oligomycin on the ATP-induced changes in the fluorescence of aurovertin (Aur) bound to complex V (*A*), SMP (*B*), and $F_1$-ATPase (*C*). Treatment of enzymes with oligomycin (oligo) and measurements of aurovertin fluorescence were carried out as described. The concentrations of oligomycin and the degrees of inhibition of ATPase activity were 11 μg/mg of protein and 98% inhibition for complex V, 3 μg/mg of protein and 97% inhibition for SMP, and 50 μg/mg of protein and no inhibition for $F_1$-ATPase. Where indicated, the final concentrations of added aurovertin and ATP were, respectively, 0.63 μM and 1.25 mM in this and subsequent figures. In the case of SMP, the experiments were repeated with SMP pretreated with 30 μM $Bu_3SnCl$ to ensure that, in the absence of oligomycin, the ATPase activity of the particles was inhibited. Under these conditions also, ATP induced an increase in the fluorescence of SMP-bound aurovertin in the absence of oligomycin but not in its presence (see text for the effect of organotin compounds).

It was of interest to see whether the effect of oligomycin, DCCD, and $ClHgC_6H_4COOH$ is brought about by inhibition of ATP binding to $F_1$ (32–34). However, this did not appear to be the case. When TNP-ATP was used instead of ATP, it was found that TNP-ATP at saturating concentrations resulted in a greater degree of quenching of the fluorescence of aurovertin bound to complex V (Fig. 3 A and B) and that treatment of complex V with oligomycin had no detectable effect on the extent of TNP-ATP-induced quenching. This may be related in part to the fact that TNP-ATP binds to $F_1$ more tightly than does ATP (35). However, when TNP-ATP

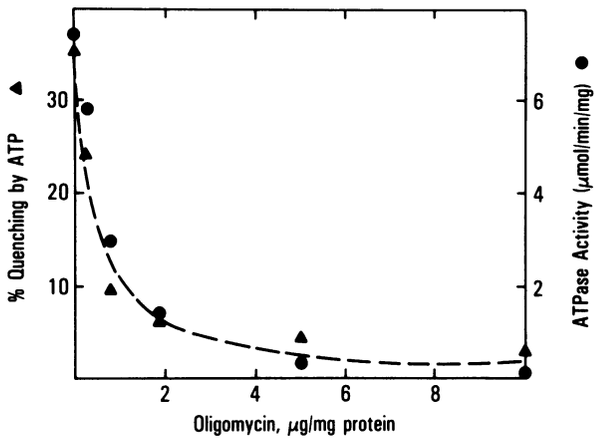


FIG. 2. Correlation between inhibitions of complex V ATPase activity and ATP-induced quenching of the fluorescence of bound aurovertin at different oligomycin concentrations. Complex V was incubated with the indicated concentrations of oligomycin and assayed for ATPase activity as described. ATP-induced fluorescence quenching was measured as in Fig. 1.

was added after ATP to oligomycin- and aurovertin-treated complex V (Fig. 4 *Top*, left trace), then TNP-ATP did not induce fluorescence quenching, suggesting prevention of TNP-ATP binding by the bound ATP. A similar experiment in the absence of oligomycin showed only the degree of quenching effected by ATP, which was added first, and

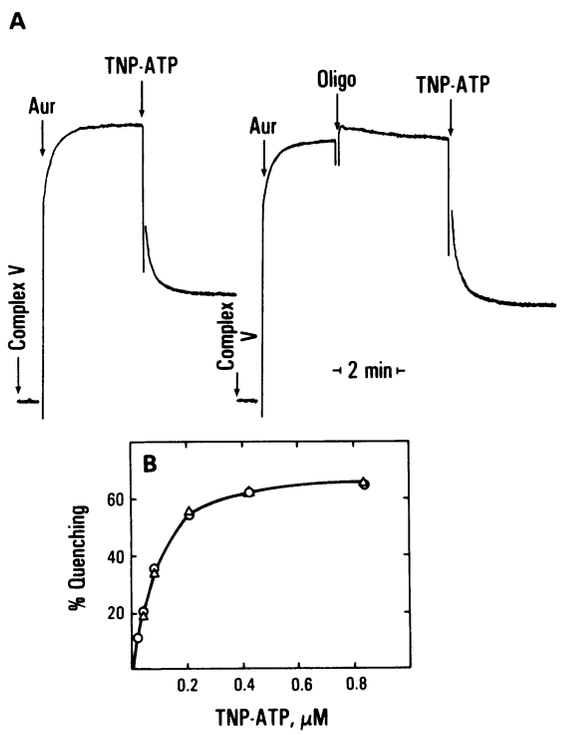


FIG. 3. Quenching of the fluorescence of complex V-bound aurovertin (Aur) induced by TNP-ATP. In *A*, the concentrations of added TNP-ATP and oligomycin (Oligo) were, respectively, 0.42 μM and 30 μg/mg of complex V. When oligomycin was added after TNP-ATP, no further change was observed. In *B*, complex V was preincubated with (△) or without (○) 5 μg of oligomycin per mg of protein. The ATPase activity of the oligomycin-treated samples was inhibited by 98%. Fluorescence quenching of aurovertin induced by the indicated concentrations of added TNP-ATP was measured as described. The degree of quenching was corrected for the filter effect of TNP-ATP, which, at the maximum TNP-ATP concentration used, accounted for 3.3% decrease of aurovertin fluorescence.

subsequent addition of TNP-ATP did not increase quenching to the level expected from TNP-ATP alone (Fig. 4 *Top*, right trace). Further, in the absence or presence of oligomycin (Fig. 4 *Middle* and *Bottom*, respectively), addition of ATP after TNP-ATP diminished the extent of quenching achieved with TNP-ATP to nearly the level expected from ATP alone (see Fig. 1*A*). The concentrations of ATP (1.25 mM) and TNP-ATP (0.42 μM) used in the experiments of Fig. 4 were so chosen to demonstrate maximal competitive effects. At lower concentrations of ATP as the first added ligand, subsequent addition of 0.42 μM TNP-ATP did cause a further quenching; and with TNP-ATP as the first added ligand at 0.42 μM and lower concentrations of ATP as the second added ligand, the rate and the extent of reversal of quenching were less. These results indicate, therefore, that pretreatment of complex V with oligomycin does not inhibit ATP binding but does inhibit the aurovertin fluorescence response to ATP, probably by a change in the ATP-induced conformation of the β subunit. Whatever the change, it is not detectable when the ligand is either TNP-ATP or ATP in the presence of added $Mg^{2+}$ (data not shown).

Among the $F_0$ inhibitors mentioned above, DCCD and oligomycin appeared to react with the same $F_0$ subunit, the DCCD-binding proteolipid (36–38). $ClHgC_6H_4COOH$ could also be reacting with the same subunit, possibly at the single cysteine, which is located six residues away from the DCCD-reactive Glu-58 (38). Hence, it was of interest to see whether other $F_0$ inhibitors would cause the same effect on the ATP-induced fluorescence change of bound aurovertin. The inhibitors used were the organotin compounds and venturicidin. They were selected because of their lack of effect on the ATPase activity of isolated $F_1$, lack of uncoupling effect in SMP, and strong inhibitory effect on the ATPase activities of SMP and complex V (39, 40). Another

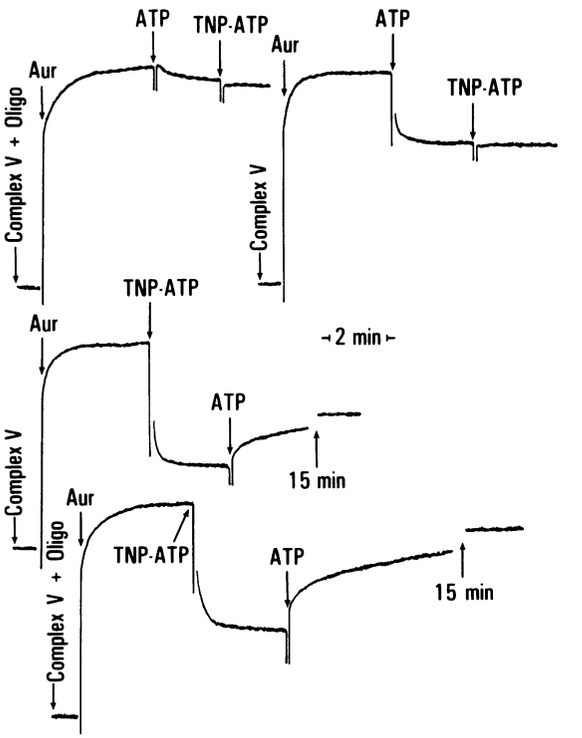


FIG. 4. Competition between ATP and TNP-ATP in quenching the fluorescence of complex V-bound aurovertin (Aur). Where indicated, complex V was pretreated with 5 μg of oligomycin (Oligo) per mg of protein, which resulted in 98% inhibition of ATPase activity. The small decrease in fluorescence upon addition of TNP-ATP after ATP was due to the filter effect of TNP-ATP (see the legend to Fig. 3).

reason for their selection was that genetic evidence has suggested that in yeast the sites of action of organotin compounds and venturicidin are different from that of oligomycin (41). Pretreatment of SMP or complex V with $Bu_3SnCl$ or $Ph_3SnCl$ at concentrations that caused ≈90% inhibition of ATPase activity altered neither the succinate-induced (or ATP-induced; data not shown) fluorescence enhancement of aurovertin bound to SMP nor the ATP-induced quenching of the fluorescence of aurovertin bound to complex V (Fig. 5). Essentially similar results were obtained when venturicidin was used as the ATPase inhibitor acting on $F_0$. Further, treatment of complex V with organotin compounds did not interfere with the inhibition by oligomycin of ATP-induced fluorescence quenching of bound aurovertin. Thus, when preparations of complex V treated with aurovertin and tributyltin chloride were further treated with oligomycin, the ATP-induced quenching of aurovertin fluorescence was inhibited in the same manner as was shown in Fig. 1 (data not shown).

## DISCUSSION

It has been shown in SMP and complex V that the ATP-induced fluorescence change of $F_1$-bound aurovertin can be inhibited by treating SMP or complex V with certain reagents that act on the $F_0$ segment of the ATP synthase complex. These reagents are oligomycin, DCCD, and $ClHgC_6H_4$-COOH, all of which inhibit ATP synthesis and hydrolysis at the level of $F_0$. That not all ATPase inhibitors acting on $F_0$ can inhibit the ATP-induced fluorescence change of bound aurovertin is clear from the results obtained with the use of organotin compounds and venturicidin. Genetic evidence suggests that in yeast the latter reagents act at $F_0$ sites other than oligomycin (41). By contrast, there is evidence that oligomycin and DCCD react with the same $F_0$ subunit, namely the DCCD-binding proteolipid (ATPase subunit 9 of yeast) (36–38). Thus, it appears that modifications at the DCCD-binding protein are communicated to the $\beta$ subunits of $F_1$, resulting in inhibition of the ATP-induced fluorescence change of aurovertin, which according to available evidence also binds to the $\beta$ subunits (27, 29, 30).

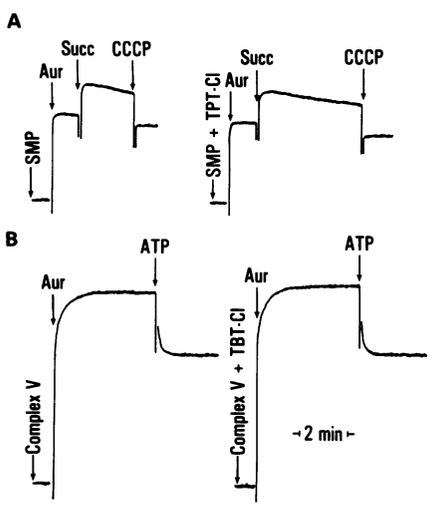


FIG. 5. Effect of organotin compounds on respiration-induced fluorescence enhancement of SMP-bound aurovertin (Aur) (*A*), and ATP-induced fluorescence quenching of complex V-bound aurovertin (*B*). Experimental conditions were as described. In *A*, SMP was treated with 60 $\mu$M $Ph_3SnCl$ (TPT·Cl). The inhibition of ATPase activity was 99%. In *B*, complex V was treated with 50 $\mu$M $Bu_3SnCl$ (TBT·Cl). The inhibition of ATPase activity was 88%. Where indicated, the concentrations of added sodium succinate (Succ), carbonylcyanide *m*-chlorophenylhydrazone (CCCP), and ATP were, respectively, 6 mM, 2.5 $\mu$M, and 1.25 mM. CCCP had a small filter effect amounting at 2.5 $\mu$M CCCP to 6.5% of the fluorescence decreases shown.

The suppression of the ATP-induced fluorescence change of aurovertin suggests an alteration in the conformation of the $\beta$ subunits. While this work was being prepared for publication, a paper appeared by Penefsky indicating that treatment of SMP with oligomycin or DCCD inhibits ATP binding to $F_1$ (42). These experiments were done at ATP concentrations ($\leq$0.1 $\mu$M) substoichiometric with respect to $F_1$. By contrast, however, the data of Fig. 4 show that ATP at physiological concentrations binds to oligomycin (or DCCD)-treated SMP or complex V, competes with the bound TNP-ATP, and alters the TNP-ATP-induced fluorescence change of aurovertin. Therefore, although we do not disagree with the results of Penefsky obtained at substoichiometric ATP concentrations relative to $F_1$ $\beta$ subunits, it is clear that treatment of complex V or SMP with inhibitory amounts of oligomycin or DCCD does not abolish the interaction of $F_1$ with ATP when the latter is added at physiological concentrations. Perhaps modification of the DCCD-binding protein by DCCD or oligomycin alters the characteristics of ATP binding to the $\beta$ subunits in such a manner that, at the extremely low ATP concentrations (molar ratio of $F_1$/ATP = 6) used by Penefsky (42), the effect is seen as inhibition of ATP binding.

Should it prove correct that modifications of the DCCD-binding protein affect ligand binding by $F_1$ (see also refs. 32–34, 42), then an important role of the DCCD-binding protein may be as follows. Energy-induced modifications (e.g., protonation/deprotonation) of the DCCD-binding protein would be communicated to the $\beta$ subunits of $F_1$ resulting in substrate/product binding changes which appear to be essential features of the mechanism of oxidative phosphorylation.

We thank Calixto Munoz for preparation of mitochondria and complex V. This work was supported by United States Public Health Service Grant AM08126. This is publication number 3913-BCR from the Research Institute of Scripps Clinic, La Jolla, CA 92037.

1. Boyer, P. D., Cross, R. L. & Momsen, W. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2837–2839.
2. Boyer, P. D. (1979) in *Membrane Bioenergetics*, eds. Lee, C. P., Schatz, G. & Ernster, L. (Addison-Wesley, Reading, MA), pp. 461–479.
3. Feldman, R. I. & Sigman, D. S. (1982) *J. Biol. Chem.* **257**, 1676–1683.
4. Grubmeyer, C., Cross, R. L. & Penefsky, H. S. (1982) *J. Biol. Chem.* **257**, 12092–12100.
5. Feldman, R. I. & Sigman, D. S. (1983) *J. Biol. Chem.* **258**, 12178–12183.
6. Yoshida, M. (1983) *Biochem. Biophys. Res. Commun.* **114**, 907–912.
7. Hatefi, Y., Yagi, T., Phelps, D. C., Wong, S.-Y., Vik, S. B. & Galante, Y. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1756–1760.
8. Gresser, M. J., Myers, J. A. & Boyer, P. D. (1982) *J. Biol. Chem.* **257**, 12030–12038.
9. Cross, R. L., Grubmeyer, C. & Penefsky, H. S. (1982) *J. Biol. Chem.* **257**, 12101–12105.
10. Wong, S.-Y., Matsuno-Yagi, A. & Hatefi, Y. (1984) *Biochemistry* **23**, 5004–5009.
11. O'Neal, C. C. & Boyer, P. D. (1984) *J. Biol. Chem.* **259**, 5761–5767.
12. Stroop, S. D. & Boyer, P. D. (1985) *Biochemistry* **24**, 2304–2310.
13. Kayalar, C., Rosing, J. & Boyer, P. D. (1976) *Biochem. Biophys. Res. Commun.* **72**, 1153–1159.
14. Yagi, T., Matsuno-Yagi, A., Vik, S. B. & Hatefi, Y. (1984) *Biochemistry* **23**, 1029–1036.
15. Chang, T. & Penefsky, H. S. (1973) *J. Biol. Chem.* **248**, 2746–2754.
16. Chang, T. & Penefsky, H. S. (1974) *J. Biol. Chem.* **249**, 1090–1098.
17. Hansen, M. & Smith, A. L. (1964) *Biochim. Biophys. Acta* **81**, 214–222.

18. Stiggall, D. L., Galante, Y. M. & Hatefi, Y. (1979) *Methods Enzymol.* **55,** 308–315.
19. Senior, A. E. & Brooks, J. C. (1970) *Arch. Biochem. Biophys.* **140,** 257–266.
20. Gornall, A. G., Bardawill, C. J. & David, M. M. (1949) *J. Biol. Chem.* **177,** 751–766.
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
22. Baldwin, C. L., Weaver, L. C., Brooker, R. M., Jacobsen, T. N., Osborne, C. E., Jr., & Nash, H. A. (1964) *Lloydia* **27,** 88–95.
23. Matsuno-Yagi, A. & Hatefi, Y. (1984) *Biochemistry* **23,** 3508–3514.
24. Lardy, H. A., Connelly, J. L. & Johnson, D. (1964) *Biochemistry* **3,** 1961–1968.
25. Bertina, R. M., Schrier, P. I. & Slater, E. C. (1973) *Biochim. Biophys. Acta* **305,** 503–518.
26. Van De Stadt, R. J. & Van Dam, K. (1974) *Biochim. Biophys. Acta* **347,** 253–263.
27. Verschoor, G. J., Van Der Sluis, P. R. & Slater, E. C. (1977) *Biochim. Biophys. Acta* **462,** 438–449.
28. Aleksandrowicz, Z. & Schuster, S. M. (1979) *Life Sci.* **24,** 1407–1418.
29. Issartel, J. P., Klein, G., Satre, M. & Vagnais, P. V. (1983) *Biochemistry* **22,** 3485–3492.
30. Issartel, J. P. & Vignais, P. V. (1984) *Biochemistry* **23,** 6591–6595.
31. Van De Stadt, R. J., Van Dam, K. & Slater, E. C. (1974) *Biochim. Biophys. Acta* **347,** 224–239.
32. Hoppe, J., Schairer, H. U., Friedl, P. & Sebald, W. (1982) *FEBS Lett.* **145,** 21–24.
33. Fillingame, R. H., Peters, L. K., White, L. K., Mosher, M. E. & Paule, C. P. (1984) *J. Bacteriol.* **158,** 1078–1083.
34. Fillingame, R. H. (1984) in *$H^+$-ATPase (ATP Synthase): Structure, Function, Biogenesis of the $F_0F_1$ Complex of Coupling Membranes*, eds. Papa, S., Altendorf, A., Ernster, L. & Packer, L. (Adriatica Editrice, Bari, Italy), pp. 109–118.
35. Grubmeyer, C. & Penefsky, H. S. (1981) *J. Biol. Chem.* **256,** 3718–3727.
36. Enns, R. K. & Criddle, R. S. (1977) *Arch. Biochem. Biophys.* **182,** 587–600.
37. Sebald, W. & Wachter, E. (1978) in *Energy Conservation in Biological Membranes*, eds. Schafer, G. & Klingenberg, M. (Springer-Verlag, Berlin), pp. 228–236.
38. Hoppe, J. & Sebald, W. (1984) *Biochim. Biophys. Acta* **768,** 1–27.
39. Stiggall, D. L., Galante, Y. M. & Hatefi, Y. (1978) *J. Biol. Chem.* **253,** 956–964.
40. Emanuel, E. L., Carver, M. A., Solani, G. C. & Griffiths, D. E. (1984) *Biochim. Biophys. Acta* **766,** 209–214.
41. Griffiths, D. E. (1976) in *The Structural Basis of Membrane Function* eds. Hatefi, Y. & Djavadi-Ohaniance, L. (Academic, New York), pp. 205–214.
42. Penefsky, H. S. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1589–1593.