Journal of Bioenergetics and Biomembranes, Vol. 19, No. 3, 1987

On the Mechanism of the Reconstitution of F_1-Depleted ATPase Complex with Purified F_1: Possible Conformational Effects[1]

Shengguang Li,[2,3] Ying Zhang,[2] and Zhihuan Lin[2]

Received June 30, 1986; revised November 26, 1986

Abstract

The membrane sector (F_0) of H^+-ATPase was prepared by trypsin and urea treatment of F_1–F_0 and reconstituted with purified F_1. The oligomycin sensitivity of the reconstituted F_1–F_0 complex obtained by treating F_1 or F_0 with Mg^{2+} before binding is much higher than that obtained without Mg^{2+} treatment. The greater change in the intrinsic fluorescence of the reconstituted F_1–F_0 complex obtained by Mg^{2+} treatment suggests that conformational changes may occur during the reconstitution. We deduce that Mg^{2+} binds to membrane lipids, thus decreasing membrane fluidity and changing the physical state of the lipids to provide a suitable microenvironment for conformational changes in F_0. The data also suggest that the conformational change in the F_0 portion of the F_1–F_0 complex can be transmitted to the F_1 portion, the conformation of which is in turn altered, resulting in the formation of an F_1–F_0 complex with high oligomycin sensitivity. On the other hand, Mg^{2+} may act on F_1 directly to induce a suitable conformational change which is then trnsmitted to F_0, resulting in the formation of an H^+-ATPase with greater sensitivity to oligomycin.

Key Words: Conformational interaction; Mg^{2+}; H^+-ATPase; reconstitution; soluble F_1-ATPase; mitochondria.

[1]Abbreviations: STED, 0.25 M sucrose, 10 mM Tris-SO_4, 0.2 mM EDTA, and 1 mM dithiothreitol, pH 8.0; NADH, nicotinamide adenine dinucleotide, reduced form; olig., oligomycin; OSCP, oligomycin sensitivity conferring protein; F_6, coupling factor 6; F_1, coupling factor one (or F_1–ATPase); $F_1^{+Mg^{2+}}$ and $F_1^{-Mg^{2+}}$, the F_1 treated and untreated with 1 mM Mg^{2+} respectively; F_0, the membrane sector proteins of the H^+–ATPase; TUF_0, trypsin-urea $-F_0$; EUF_0, EDTA–urea $-F_0$; $F_0^{+Mg^{2+}}$ and $F_0^{-Mg^{2+}}$, the F_0 treated and untreated with 1 mM Mg^{2+} respectively; $(F_1 \cdot F_0)^{+Mg^{2+}}$ and $(F_1 \cdot F_0)^{-Mg^{2+}}$, the reconstituted $F_1 \cdot F_0$ complex containing Mg^{2+}-treated F_1 and F_0 and untreated F_1 and F_0 respectively; $F_1 \cdot F_0^{+Mg^{2+}}$ and $F_1 \cdot F_0^{-Mg^{2+}}$, the reconstituted H^+–ATPase complex derived from the binding of purified F_1 to the F_0 treated and untreated with Mg^{2+} respectively; $F_1^{+Mg^{2+}} \cdot F_0$ and $F_1^{-Mg^{2+}} \cdot F_0$, the reconstituted H^+–ATPase derived from the binding of F_0 to the purified F_1 treated and untreated with Mg^{2+} respectively.

[2]Institute of Biophysics, Academia Sinica, Beijing, China.

[3]To whom correspondence should be addressed.

0145-479X/87/0600-0273$05.00/0 © 1987 Plenum Publishing Corporation

Introduction

Since Boyer (1965) proposed the conformational coupling hypothesis to account for the mechanism of oxidative phosphorylation, considerable evidence was accumulated indicating the occurrence of energy-linked conformational changes in the F_1 sector of ATPase. Recently Penefsky (1985) observed that DCCD and oligomycin which bind to the F_0 sector impair binding of substrate to the F_1 catalytic sites. This is strong evidence that DCCD binding produces a conformational change in the F_0 portion of the complex that is transmitted to F_1. Our experiments also showed that free Mg^{2+} (0.67 mM) induced competitive inhibition of free F_1 but competitive activation of membrane-bound F_1 (Ye and Lin, 1985). The differing effects of Mg^{2+} may be explained if there is a conformational difference in the catalytic sites of the free and membrane-bound F_1. The conformation of the latter may be influenced by the F_0 embedded in the membrane lipids. In this paper we report evidence for a conformational interaction between F_1 and F_0 based on studies which are carried out by binding the F_1-depleted H^+–ATPase complex and purified F_1. These experiments indicate that conformational changes in F_0 or F_1 induced by Mg^{2+} can be transmitted to F_1 or F_0 respectively, which in turn affects the efficiency of the reconstitution.

Materials and Methods

Preparation of Oligomycin-Sensitive ATPase

Oligomycin-sensitive ATPase complex was prepared according to the method of Yu and Yang (1985), which is a modification of the method of Hatefi *et al.* (1974). Briefly, submitochondrial particles from pig heart are solubilized with cholate (0.15 mg/mg protein; 10 mg protein/ml) in 0.25 M sucrose, 10 mM Tris-SO_4, pH 8.0, 100 mM $(NH_4)_2SO_4$, and 0.1 mM EDTA. After dialysis against 200 volumes of 0.25 M sucrose, containing 10 mM Tris-SO_4, pH 8.0, 0.5 mM EDTA, 0.1 mM 2-mercaptoethanol. 2% (v/v) methanol, 1 mM $MgCl_2$, and 0.1 mM ATP with four changes in 16 hours, the suspension was diluted with 1/2 volume of distilled water and precipitated at 30% ammonium sulfate saturation. The precipitate was removed and the supernatant was brought to 50% ammonium sulfate saturation. The precipitate was collected and dissolved in sucrose, 10 mM Tris, pH 7.4, 0.2 mM EDTA, and 1 mM dithiothreitol. This F_0–F_1 had an activity of 0.28 μmol $\times$ min^{-1} $\times$ mg^{-1} protein in P_i-ATP exchange and hydrolyzed ATP with an activity of about 10 μmol $\times$ min^{-1} $\times$ mg^{-1}. The oligomycin sensitivity of the ATPase was over 95%. Its lipid content was unusually high, 390 μg/mg protein.

Preparation of F_1

F_1 was prepared according to the method of Senior *et al.* (1970) with some modifications (Lin *et al.*, 1981). The specific activity of F_1 was 80–100 μmol ATP hydrolyzed $\times$ min^{-1} $\times$ mg^{-1} protein. It was kept at 4°C in 55% saturated $(NH_4)_2SO_4$. Before use, the suspension was spun down, and the F_1 pellet was redissolved at 1 mg protein/ml in a solution containing 40 mM Tris-SO_4 and 1 mM EDTA, pH 8.0.

Preparation of F_1-Depleted H^+-ATPase (F_0)

The depletion was carried out by two methods. (1) F_1-Depleted H^+-ATPase (trypsin–urea–F_0 or TUF_0) was prepared according to the method of Racker (1963) for submitochondrial particles. After incubating the oligomycin-sensitive ATPase complex and trypsin in a ratio of 10 : 1 (w/w) at 30°C for 45 min, trypsin inhibitor was added (five times the amount of trypsin). Urea was added to 2 M at 0°C for 45 min and centrifuged at 0°C for 1 h at 105,000 *g*. The supernatant was discarded and the F_0 pellets homogenized in a solution containing 0.25 M sucrose, 10 mM Tris-SO_4, 0.2 mM EDTA, and 1 mM DTT at pH 8.0 (STED). (2) F_1-Depleted H^+-ATPase (EDTA–urea $-F_0$ or EUF_0) was prepared according to the method of Lee and Ernster (1967) from EDTA particles. The H^+-ATPase complex (10 mg protein/ml) containing 2 mM EDTA at pH 8.5 was saturated with N_2 and subjected to sonic oscillation for 30 sec. An equal volume of 8 M urea was added at 0°C for 30 min. Then the suspension was diluted with 4 volumes of 0.25 M sucrose and centrifuged at 105,000 *g* for 40 min. The sediment was washed by homogenization with 10 volumes of 0.25 M sucrose and centrifuged at 105,000 *g* for 40 min. The particles were finally suspended in STED.

Reconstitution Experiments

Mg^{2+} treatment of F_0 particles was carried out in 1 mM Mg^{2+} at 0°C for 4 h, and dialyzed at 0–4°C against Mg^{2+}-free buffer, which was changed four times in 2 hours. The treatment with ammonia (30 mM NH_4^+) or Ca^{2+} (1 mM) was carried out similarly as the Mg^{2+} treatment.

Mg^{2+}-treated F_1 was prepared by incubating the F_1 in 1 mM Mg^{2+} at room temperature (20–25°C) for 30 min. When it was added to the reconstitution system, the concentration of Mg^{2+} was greatly diluted, by 50–100 fold. The reconstitution experiments were carried out by incubating F_0 (400–500 μg) and F_1 (about 0.1 activity unit) (Kagawa and Racker, 1966) in the STED solution for 5 min (except Fig. 1) at 30°C in a final volume of 210 μl. Other experimental conditions are indicated in the figures and tables.

Measurement of the Reconstituted ATPase Activity

ATPase activity was measured spectrophotometrically by coupling the reaction to pyruvate kinase and lactate dehydrogenase reactions, and monitoring the oxidation of NADH at 340 nm (Pullman *et al.*, 1960; Li and Sun, 1983) in 2 ml of reaction medium containing 42 μmol Tris-maleate, pH 8.0, 1.67 μmol $MgSO_4$, 1 μmol phosphoenol pyruvate, 0.15 μmol NADH, 1.73 unit pyruvate kinase, 1.0 unit LDH, and 1 μmol ATP-Na_2. The reaction was started by adding F_1-ATPase or H^+-ATPase complex. The specific activity of ATPase was expressed as μmol NADH $\times$ min^{-1} $\times$ mg^{-1}.

Measurement of Intrinsic Fluorescence

The measurement was carried out in 2 ml of the reaction mixture containing 0.25 M sucrose, 10 mM Tris-SO_4, 0.02 mM EDTA, 1 mM DTT, pH 8.0, and a certain amount of the F_1, F_0, or F_0–F_1 complex. The intrinsic fluorescence of F_0 or F_1 or reconstituted $F_0 \cdot F_1$ complex was traced with a Hitachi 650–60 spectrofluorimeter using 270 and 295 nm as the excitation wavelengths and the fluorescence emission of each as scanned at 280–410 nm and 305–410 nm. The scans were compensated for background fluorescence.

Determination of Mg^{2+} Bound to F_0

The Mg^{2+} was determined by atomic absorption spectrophotometric analysis.

Protein content was determined by Lowry's method (Lowry *et al.*, 1951) using bovine serum albumin as standard.

Results and Discussion[4]

The efficiency of reconstitution of F_0 with F_1 was determined by the extent of inhibition by oligomycin. The ATPase activity of F_1 was reduced by 80% in the presence of 0.67 mM Mg^{2+} and that of F_0–F_1 increased by 140%. Figure 1A shows that the reconstitution efficiency of EUF_0 and F_1 in the presence of Mg^{2+} is high and 95–100% oligomycin inhibition is seen within 5 min, while in the absence of Mg^{2+} only about 60% oligomycin sensitivity can be reached. In the latter case the inhibition by oligomycin increases gradually with increasing incubating time; after a 30-min incubation about 95% oligomycin sensitivity is reached as well. Obviously, Mg^{2+} does promote efficient reconstitution of EUF_0 and F_1. The results obtained here of the reconstitution in the absence of Mg^{2+} are similar to those obtained by reconstitution of F_1-depleted submitochondrial particles and F_1 reported by

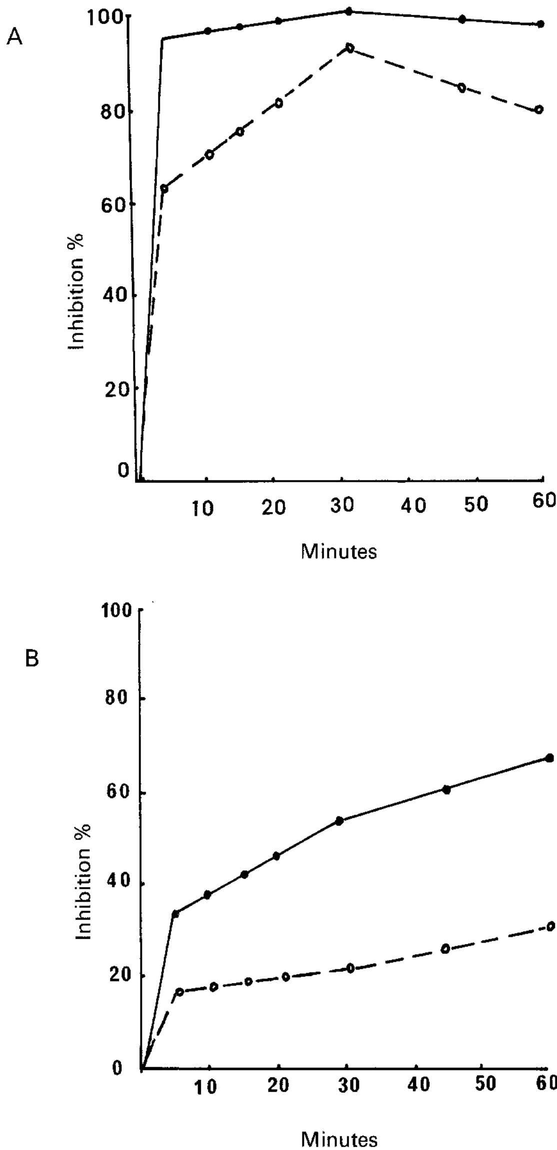

Fig. 1. Comparison of the reconstitution efficiency of binding purified F_1 with EUF_0 or TUF_0 in the presence and absence of Mg^{2+}. ATPase activity was measured by the spectrophotometric method as described in Materials and Methods. The reaction was started by adding 60 μl of the reconstituted $F_0 \cdot F_1$ complex to 2 ml of the reaction medium as described in the text. For the assay of oligomycin sensitivity, 2 μg oligomycin was introduced into the assay medium. ———, Reconstitution in the presence of Mg^{2+}; – – – – –, reconstitution in the absence of Mg^{2+}. (A), Reconstitution of EUF_0 with F_1; (B), reconstitution of TUF_0 with F_1.

Slater's laboratory (Vadineanu *et al.*, 1976). However, the improved efficiency of the reconstitution produced by Mg^{2+} can likely be interpreted in terms of cationic screening of negative membrane charges, thus increasing the affinity to F_1, as suggested by Ernster's laboratory (Sandri *et al.*, 1983). In Fig. 1B it may be seen that the reconstitution efficiency of TUF_0 and F_1 following a 5-min incubation in the presence of Mg^{2+} is about 100% higher than in the absence of Mg^{2+}. Both in the presence and absence of Mg^{2+}, the reconstitution efficiency measured by the extent of inhibition by oligomycin increases with increasing incubation time. Even after a 60-min incubation, the reconstitution efficiency in the presence of Mg^{2+} remains double that in the absence of Mg^{2+}. The difference in reconstitution efficiency between TUF_0 and EUF_0 may be related to their different preparative methods. The trypsin used in the preparation of TUF_0 may damage the membrane and retard some of the conformational changes in F_0, especially OSCP, and F_6 may be degraded.

Table I compares the oligomycin sensitivity of reconstituted F_0–F_1 complex, when the TUF_0 is pretreated with 1 mM Mg^{2+} or untreated TUF_0 is used. It is seen that the efficiency with this Mg^{2+}-treated F_0 was over 20 times higher than that with untreated F_0.

Table II compares the efficiency of different cations in the reconstitution of $F_1 \cdot F_0$ containing TUF_0 treated with different cations. The efficiency of reconstitution containing 1 mM Mg^{2+}-treated F_0 was higher than that containing the same concentration of Ca^{2+}-treated F_0 and much higher than that containing 30 mM NH_4^+-treated F_0. According to Ernster's laboratory (Sandri *et al.*, 1983) the amount of negative membrane charges neutralized

Table I. Comparison of the Oligomycin Sensitivity of $F_1 \cdot F_0^{+Mg^{2+}}$ and $F_1 \cdot F_0^{-Mg^{2+}}$

Samples	ATPase activity[a] (ΔA_{340}/min) – Oligomycin	ATPase activity[a] (ΔA_{340}/min) + Oligomycin	Sensitivity to oligomycin (%)
$F_1 \cdot F_0^{+Mg^{2+}}$	0.096	0.028	70.8
$F_1 \cdot F_0^{-Mg^{2+}}$	0.069	0.067	2.9

[a]The assay of ATPase activity is described in the text. The reaction was started by adding 60 μl of the reconstituted $F_1 \cdot F_0$ to 2 ml reaction medium as described in Materials and Methods. 2 μg oligomycin was added just prior to the start of the reaction for the assay of oligomycin sensitivity. A_{340}/min represents the activity of ATPase under the same condition. $F_1 \cdot F_0^{+Mg^{2+}}$ and $F_1 \cdot F_0^{-Mg^{2+}}$ denote the reconstituted H^+-ATPase complex derived from the binding of purified F_1 to F_0, treated and untreated with Mg^{2+} respectively.

[4]All experiments in the figures and tables except Fig. 1A were carried out with trypsin–urea H^+-ATPase particles.

Table II. The Efficiency of the Reconstitution of Cation-Treated TUF_0 with Purified F_1: Effect of Various Cations[a]

Cations	Oligomycin sensitivity (%)
—	4.0
NH_4^+ (30 mM)	25.0
Ca^{2+} (1 mM)	52.0
Mg^{2+} (1 mM)	72.0

[a]The reconstitutions of purified F_1 and TUF_0 pretreated with 1 mM Mg^{2+} or 1 mM Ca^{2+} or 30 mM NH_4^+ respectively were carried out at 30°C for 5 min. For the detailed description, refer to the text.

by 30 mM NH_4^+ was greater than that neutralized by 1 mM Ca^{2+}. So, from our results the effect of cations promoting the reconstitution of $F_1 \cdot F_0$ can be interpreted not only as the cationic neutralization of the negative charges on the membrane, but also as the cationic effect on the conformation of F_0.

We further determined the Mg^{2+} content of Mg^{2+}-treated F_0 and untreated F_0 by atomic absorption spectrophotometric analysis. It was found that the Mg^{2+} content in the former was 20- to 50-fold higher than that in the latter. Yang *et al.* (1983) reported that when Mg^{2+} was bound to the lipid part of the reconstituted H^+-ATPase in the proteoliposomes, it reduced the fluidity of the lipid matrix. In our laboratory also we found that the fluidity of membrane lipids of H^+-ATPase complex decreased in the presence of Mg^{2+}, and Mg^{2+} exhibited an activating effect on the hydrolytic activity of H^+-ATPase (Ye and Lin, 1985). So we deduced that in the reconstitution of F_1-depleted particles with purified F_1, Mg^{2+} decreased the fluidity of the lipid environment of F_0 to provide a suitable physical state of the lipid for altering the conformation of F_0. This conformational change of F_0 can be transmitted to F_1, regulating its conformation and promoting the formation of $F_1 \cdot F_0$ complex with maximal oligomycin sensitivity. Recently Penefsky (1985) also proposed that the conformational change of F_0 can be transmitted to F_1. Furthermore, when F_1 was treated with 1 mM Mg^{2+} and reconstituted with untreated F_0 particles, we found that the reconstitution efficiency of Mg^{2+}-treated F_1 was about three times higher than that of untreated F_1 (Table III). This indicates that the reconstitution of $F_1 \cdot F_0$ can be promoted not only by treating F_0 with Mg^{2+} but also by treating F_1 with Mg^{2+}. We prefer the explanation that the effect of Mg^{2+} in promoting the reconstitution with higher efficiency was derived from the direct effect F_1, resulting in a conformation change, and after being bound, this conformation change of F_1 is transmitted to F_0 regulating the conformation of F_0 so as to better promote reconstitution with conferral of greater oligomycin sensitivity.

Table III. Comparison of the Oligomycin Sensitivity between $F_1^{+Mg^{2+}} \cdot F_0$ and $F_1^{-Mg^{2+}} \cdot F_0$

Samples	ATPase activity[a] (ΔA_{340}/min)		Sensitivity to oligomycin (%)
	− Oligomycin	+ Oligomycin	
$F_1^{+Mg^{2+}} \cdot F_0$	0.095	0.078	17.9
$F_1^{-Mg^{2+}} \cdot F_0$	0.143	0.136	4.9

[a]The assay of ATPase activity and its sensitivity to oligomycin is described under Materials and Methods. The $F_1^{+Mg^{2+}} \cdot F_0$ and $F_1^{-Mg^{2+}} \cdot F_0$ denote the reconstituted H^+-ATPase complex derived from the binding of F_0 to the purified, Mg^{2+}-treated and untreated F_1 respectively. The same amount of Mg^{2+} was added in control ($F_1^{-Mg^{2+}} \cdot F_0$) as in $F_1^{+Mg^{2+}} \cdot F_0$.

We have measured the intrinsic fluorescence of F_1, F_0, and $F_1 \cdot F_0$ complex both in the presence and absence of Mg^{2+}. When using 270 nm as the excitation wavelength (Fig. 2), the tyrosine and tryptophan residues absorb the energy to emit fluorescence, while using 295 nm as the excitation wavelength (Fig. 3) only the tryptophan residues emit fluorescence. Since only a small amount of F_1 (0.1 activity unit $= 2\,\mu$g, generally) was added to the reconstitution system, 210 μl in volume, and only 30 μl was taken to measure the intrinsic fluorescence, the emission fluorescence of F_1 was very weak at the two excitation wavelengths. The emission fluorescence spectra follow roughly a straight line (Figs. 2 and 3). However, the F_0, which consists mostly of hydrophobic proteins, contained more nonpolar amino acids such as tryptophan. Because of the high quantum yield of tryptophan, the fluorescence emitted from tyrosine may be masked by that emitted from tryptophan. Therefore, with the two excitation wavelengths stated above, only one emission from tryptophan with a maximum at 330 nm was observed. Although the intrinsic fluorescence spectrum with emission maximum at 330 nm for the reconstituted $F_1 \cdot F_0$ complex appeared similar to that of the F_0 at any of the two excitation wavelengths, the intensity of fluorescence of the $F_1 \cdot F_0$ complex was weaker than that of the F_0. Even in the absence of Mg^{2+} a difference of fluorescence intensity did exist between $F_1 \cdot F_0$ complex and F_0. *Although the differences in fluorescence intensity are small in Figs. 2 and 3, they have been reproduced in several experiments.* The decrease of fluorescence intensity exhibited by the reconstituted $F_1 \cdot F_0$ complex in the absence of Mg^{2+} was greater than that in the presence of Mg^{2+}. This showed that after binding of F_1 with F_0, a conformation change of F_0 resulted no matter whether the Mg^{2+} was present or not. It may be interpreted that the conformational change of F_0 altered the microenvironment of some tryptophan residue in F_0 and resulted in a significant decrease in the intensity of the fluorescence. But in the absence of Mg^{2+}, the ATPase activity had low oligomycin sensitivity in the reconstituted $F_1 \cdot F_0$ complex. F_1 and F_0 hence

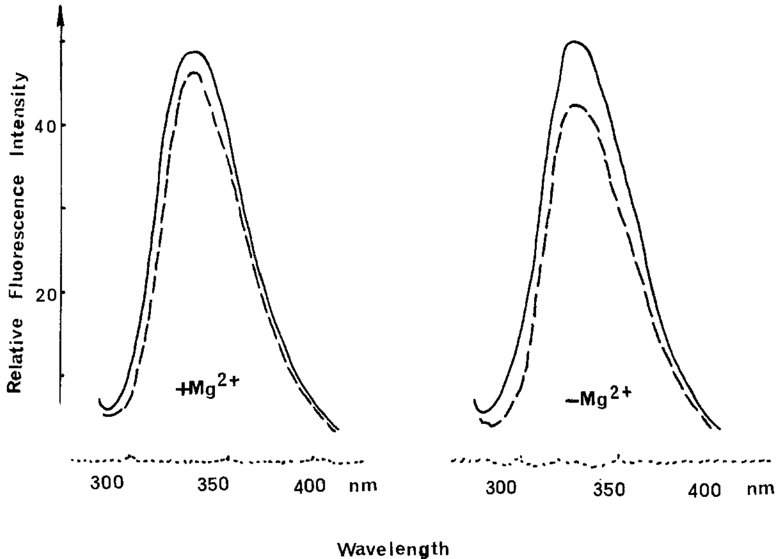

Fig. 2. The intrinsic fluorescence of $(F_1 \cdot F_0)^{+Mg^{2+}}$ and $(F_1 \cdot F_0)^{-Mg^{2+}}$ using 270 nm as excitation wavelength. 30 μl of $F_1 \cdot F_0$ complex was added to 2 ml of the reaction medium as described in Materials and Methods. F_1 and F_0 were added in the controls in the same amount as the F_1 and F_0 in the $F_1 \cdot F_0$ complex. The change in the intrinsic fluorescence was monitored on a Hitachi 650–60 spectrofluorimeter. $(F_1 \cdot F_0)^{+Mg^{2+}}$ and $(F_1 \cdot F_0)^{-Mg^{2+}}$ denote respectively the reconstituted $F_1 \cdot F_0$ complex containing Mg^{2+}-treated F_1 and F_0 and untreated F_1 and F_0. The fluorescence emissions of F_1 (···), F_0 (——), and $F_1 \cdot F_0$ complex (---) were monitored from 280 to 410 nm.

appear to be bound together to form a functionally "incomplete" complex. By means of ultracentrifugation, F_1 and F_0 particles can be separated again. When F_1-ATPase is centrifuged alone or with Mg^{2+} added, the total activity can be recovered in the supernatant. After reconstitution in the absence of Mg^{2+}, again full activity remains in the supernatant (Table IV). However, reconstitution with Mg^{2+}-treated F_0 and subsequent centrifugation yields practically no F_1 in the supernatant. We interpret the phenomenon as resulting from a sequence of interactions, each with its consequence. First, Mg^{2+} is bound to membrane lipids, decreasing the fluidity of the microenvironment and leading to the induction of a suitable conformation for F_0. This conformational change in F_0 is transmitted to F_1 which in turn undergoes conformational alteration. Alternatively, the first step in the sequence could also be a direct interaction between Mg^{2+} and F_1 with consequent change in its conformation which is then transmitted to F_0, with the regulation of F_0 conformation. Thus, the $F_0 \cdot F_1$ complex with high oligomycin sensitivity and better structural integrity is formed. *Thirdly, it should also be noted that the interaction between F_1 and F_0 appears quite weak in the absence*

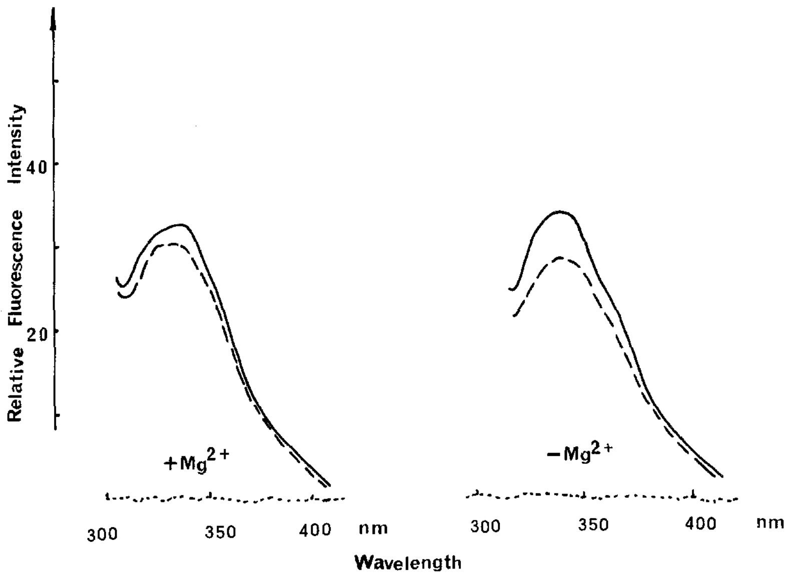

Fig. 3. The intrinsic fluorescence of $(F_1 \cdot F_0)^{+Mg^{2+}}$ and $(F_1 \cdot F_0)^{-Mg^{2+}}$ using 295 nm as excitation wavelength. $(F_1 \cdot F_0)^{+Mg^{2+}}$ and $(F_1 \cdot F_0)^{-Mg^{2+}}$ are as shown in Fig. 2. Other experimental conditions are the same as in Fig. 2. The fluorescence emissions of F_1 (······), F_0 (———), and $F_1 \cdot F_0$ complex (- - - - -) were recorded from 305 to 410 nm.

Table IV. Tightness of F_1 and F_0 Binding in $(F_1 \cdot F_0)^{+Mg^{2+}}$ and $(F_1 \cdot F_0)^{-Mg^{2+}}$

Samples[a]	Activity of free F_1 in the supernatant (ΔA_{340}/min)
$F_1^{-Mg^{2+}}$	0.086
$F^{+Mg^{2+}}$	0.077
$(F_1 \cdot F_0)^{-Mg^{2+}}$	0.082
$(F_1 \cdot F_0)^{+Mg^{2+}}$	0.003

[a]The symbols $(F_1 \cdot F_0)^{+Mg^{2+}}$ and $(F_1 \cdot F_0)^{-Mg^{2+}}$ are described in Fig. 2. $F_1^{+Mg^{2+}}$ and $F_1^{-Mg^{2+}}$ denote the F_1 treated and untreated with Mg^{2+} respectively. The amount of F_1 used in $F_1^{+Mg^{2+}}$ and $F_1^{-Mg^{2+}}$ was the same as in the reconstitution of $(F_1 \cdot F_0)^{+Mg^{2+}}$ and $(F_1 \cdot F_0)^{-Mg^{2+}}$. Each sample was centrifuged at 100,000 *g* for 20 min at room temperature in an AU-110 type air-driven ultracentrifuge (Chinese made). The hydrolytic activity of free F_1 in the supernatant was measured as stated in the text.

of Mg^{2+} (Table IV), which raises the question whether F_1 may be going on or off the membrane. If so, the ability of Mg^{2+} to increase oligomycin sensitivity of the ATPase reaction may be simply related to keeping F_1 bound to F_0. We regard the first sequence as more important and likely.

Acknowledgments

We thank Shan Sun and Guizhen Xu for their help with some experimental techniques and Changzhi Qu for her help with Mg^{2+} determination by atomic absorption spectrophotometric analysis. We also thank Prof. Fuyu Yang for his valuable advice. The authors would like to thank Dr. D. Rao Sanadi (Boston, USA) for critically reading and commenting on this manuscript.

References

Boyer, P. D. (1965). In *Oxidases and Related Oxidase Systems* (King, T. E., *et al.*, Eds), Wiley, New York, Vol. 2, p. 994.

Hatefi, Y., Stiggall, D. L., Galante, Y., and Hanstein, W. G. (1974). *Biochim. Biophys. Res. Commun.* **61**, 313–321.

Kagawa, Y., and Racker, E. (1966). *J. Biol. Chem.* **241**, 2461–2466.

Lee, C., and Ernster, L. (1967). *Methods Enzymol.* **10**, 543–548.

Li, S. G., and Sun, S. (1983). *Biochem. Biophys.* (in Chinese), **6**, 70–71.

Lin, Z. H., Wu, E., Sun, S., Xu, G. Z., and Zhang, K. (1981). *Biochem. Biophys.* (in Chinese), **5**, 53–56.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). *J. Biol. Chem.* **193**, 265–275.

Pullman, M., Penefsky, H., Datta, A., and Racker, E. (1960). *J. Biol. Chem.* **235**, 3322–3329.

Penefsky, H. S. (1985). *Proc. Natl. Acad. Sci.* **82**, 1589–1593.

Racker, E. (1963). *Biochem. Biophys. Res. Commun.* **10**, 435–439.

Sandri, G., Suranyi, E., Eriksson, L. E. G., Westman, J., and Ernster, L. (1983) *Biochim. Biophys. Acta* **723**, 1–6.

Senior, A. E., and Brooks, J. C. (1970). *Arch. Biochem. Biophys.* **140**, 257–266.

Vadineanu, A., Berden, J. A., and Slater, E. C. (1976). *Biochim. Biophys. Acta.* **449**, 468–479.

Yang, F. Y., Guo, B. Q., and Huang, Y. G. (1983). *Biochim. Biophys. Acta* **724**, 104–110.

Ye, J. J., and Lin, Z. H. (1985). In *The China–Japan Bilateral Symposium on Biophysics* (The Biophysical Society of China, ed.), Wuxi, China, pp. 278–281.

Yu, M. L., and Yang, F. Y. (1985). *Acta Biochim. Biophys. Sin.* **17**, 69–74.