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# Effect of the weakly acidic uncoupler 2,4-dinitrophenol and dimethyl sulfoxide on the coordination of $Mg^{2+}$ with ATP

## Possible mechanism of activation of the isolated $F_1$-ATPase by 2,4-dinitrophenol

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The exchange rate constants between $Mg^{2+}$-free and $Mg^{2+}$-bound ATP were determined under various conditions by line shape analysis of the $^{31}P$-NMR spectrum based on the exchange reaction, and the thermodynamic parameters of this exchange reaction were determined from the temperature dependence of its rate constants. Analysis of the activation enthalpy change $\Delta H^{\ddagger}$ showed that $Mg^{2+}$ is coordinated with the $\beta$- and $\gamma$-phosphoryl groups of ATP asymmetrically, being in closer proximity to the $\beta$-phosphoryl group. The weakly acidic uncoupler 2,4-dinitrophenol increased this asymmetric coordination of $Mg^{2+}$, and this effect was enhanced by the further addition of dimethyl sulfoxide. The hydrolysis of ATP in aqueous solution correlated well with the degree of asymmetry of $Mg^{2+}$ coordination. Thus, this asymmetric coordination specifically weakens the $O-P_\gamma$ bond at which specific cleavage of ATP catalyzed by most ATPases takes place in the presence of $Mg^{2+}$. In this paper, the mechanism of activation of isolated ATPase ($F_1$-ATPase) by 2,4-dinitrophenol, and that of ATP synthesis by isolated $F_1$-ATPase in the presence of dimethyl sulfoxide are considered on the basis of these results. The essential role of the OH group of Ser-174 of the $\beta$-subunit of $F_1$-ATPase in ATP hydrolysis is also discussed.

## 1. Introduction

ATP is the common high-energy compound in living organisms. Its synthesis and hydrolysis are catalyzed by ATP synthases in various energy-transducing membrane systems, such as mitochondria and chloroplasts. Energy-utilizing ATPases such as $Na^+,K^+$-ATPase, $H^+,K^+$-ATPase or $Ca^{2+}$-ATPase support transport of $Na^+$, $K^+$, $H^+$ and $Ca^{2+}$ through membranes against their chemical potentials. Recently, the primary structures of these ATPases were determined [1–5]. However, the molecular mechanisms of ATP synthesis and hydrolysis in these enzymic reactions are not yet fully understood. In general, $Mg^{2+}$ is necessary for the synthesis and hydrolysis of ATP, and ATP is split at the terminal phosphoryl bond, $O_3-P_\gamma$ (cf. scheme 1) in the hydrolysis of ATP. The reason for the requirement of $Mg^{2+}$ and the mechanism of specific cleavage of the ATP molecule cannot be clarified solely from the primary

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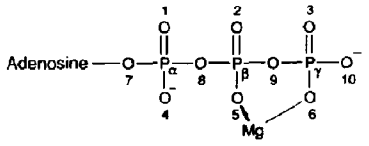
Abbreviations: ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; DNP, 2,4-dinitrophenol; DMSO, dimethylsulfoxide; HPLC, high-performance liquid chromatography.




Chart

Scheme 1. Structure of the ATP molecule coordinated with $Mg^{2+}$. The bold line for the coordination indicates the stronger bonding. The oxygen atoms in ATP are numbered as shown in the chemical structure.

structures of ATPases, since they should be directly related to the electronic structure of ATP in the presence of $Mg^{2+}$.

Recently, we found in $^{31}$P-NMR studies that in aqueous solution $Mg^{2+}$ is mainly coordinated to the $\beta$- and $\gamma$-phosphoryl groups of ATP in a manner such that it is located closer to the $\beta$-phosphoryl group than to the $\gamma$-phosphoryl moiety [6]. This asymmetric coordination of $Mg^{2+}$ results in weakening of the $O_9$–$P_\gamma$ bond at which most ATP hydrolyzing enzymes cleave the ATP molecule. This mechanism was confirmed by ab initio molecular orbital calculations [7].

The weakly acidic uncoupler DNP is known to stimulate the activities of both membrane-bound and isolated $F_1$-ATPase [8,9]. Furthermore, isolated $F_1$-ATPase is reported to synthesize ATP in medium containing the organic solvent DMSO [10,11]. Thus, as an extension of our previous studies [6,7], we examined the effects of DNP and DMSO on the stability of the ATP molecule in relation to its electronic structure.

## 2. Materials and methods

ATP was purchased from Oriental Yeast Co. Ltd (Tokyo), and was used as a concentrated aqueous solution (200 mM) at pH 7.4. The preparation was confirmed not to be contaminated with any metal ions that affect the $^{31}$P-NMR spectrum of ATP. Other reagents used were of the highest grade commercially available.

The $^{31}$P-NMR spectra of ATP solutions were recorded at 80.76 MHz in a JEOL FX-200 NMR spectrophotometer, by inserting a capillary containing 85% orthophosphoric acid as an external standard into the sample tube [6]. The standard sample solution contained a known amount of ATP, $MgCl_2$ and 10% $^2H_2O$ in 5 mM Tris-HCl buffer, pH 7.4. The effects of DNP and DMSO were assessed at final concentrations of 0.5 mM and 40%, respectively. Computer simulations of spectral data were carried out as described previously [6,12].

ATP hydrolysis was examined at 60 °C. The reaction mixture consisted of 8 mM ATP, 8 mM $MgCl_2$, 6.7 mM $Na_2SO_4$ and 10 mM Tris-$H_2SO_4$ buffer, pH 7.4, unless otherwise noted. At appropriate times, aliquots of the reaction mixture were removed for the determination of the amount of ATP hydrolyzed according to the method of Hashimoto et al. [13], by HPLC on a column of TSK gel, DEAE 2SW (4.6 mm × 25 cm), with a mobile phase consisting of 67 mM $Na_2SO_4$ in 100 mM Tris-$H_2SO_4$ buffer, pH 7.4.

## 3. Results

### 3.1. $^{31}$P-NMR spectra of ATP in the presence of $Mg^{2+}$

From analysis of the $^{31}$P-NMR spectra of ATP in the presence of $Mg^{2+}$, Misawa et al. [14] reported that there occur two exchange reactions between ATP and $Mg^{2+}$:

$$\text{ATP-Mg} \rightleftharpoons \text{ATP} + \text{Mg} \quad (1)$$

$$\text{ATP-Mg} + \text{ATP} \rightleftharpoons \text{ATP} + \text{ATP-Mg} \quad (2)$$

The exchange rate constants of the two reactions are referred to as $k_1$ and $k_2$, respectively.

In a previous study [6], we determined the exchange rate constant between $Mg^{2+}$-free and $Mg^{2+}$-coordinated ATP from the $^{31}$P-NMR spectra of ATP based on the apparent exchange reaction consisting of the reactions, eqs 1 and 2. For analysis of the interaction of $Mg^{2+}$ with the ATP molecule, exchange reaction 1 is of primary importance. Thus, in the present study, we determined $k_1$ as follows: (i) The $^{31}$P-NMR spectra of ATP at various concentrations were recorded in the presence of $Mg^{2+}$ while maintaining a fixed

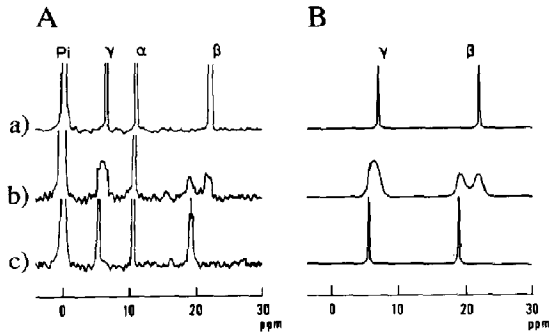



Fig. 1. Effect of $Mg^{2+}$ on the $^{31}P$-NMR spectrum of ATP (A) and its computer-simulated spectrum (B). $^{31}P$-NMR spectra of 4 mM ATP without $Mg^{2+}$ (a), with 2 mM $Mg^{2+}$ (b), and with 8 mM $Mg^{2+}$ (c), in a solution consisting of 5 mM Tris-HCl, pH 7.4, and 10% $^2H_2O$, at 4°C.

molar ratio of ATP/$Mg^{2+}$ = 2.0, and the apparent rate constant $k_{app}$ was determined by line-shape analysis. (ii) Then the value of $k_1$ was determined from the dependence of $k_{app}$ on ATP concentration.

The effect of $Mg^{2+}$ on the $^{31}P$-NMR spectrum of ATP at 4°C is shown in fig. 1A. The peaks of the β- and γ-phosphoryl groups shifted to lower field with increase in $Mg^{2+}$ concentration, first becoming broad (at 2 mM $Mg^{2+}$), and then sharp again (at 8 mM $Mg^{2+}$). The splitting of the signal of the β-phosphoryl group into two peaks followed by their coalescence with increase in $Mg^{2+}$ concentration is especially noteworthy. These features are typical of exchange reactions [12]. The shape and position of the signal of the α-phosphoryl group was insensitive to $Mg^{2+}$. Thus, the β- and γ-phosphoryl groups, but not the α-phosphoryl group, participate in the exchange of free and $Mg^{2+}$-bound ATP, as observed previously [6,15,16].

For determination of the apparent exchange rate constant $k_{app}$, we simulated the NMR spectra of ATP in the presence of $Mg^{2+}$ by the method described previously [12]. Because $Mg^{2+}$ was coordinated with the β- and γ-phosphoryl groups, these peaks were simulated separately, and the simulated spectra are shown in fig. 1B. The values of $k_{app}$ thus determined were always greater with the β-phosphoryl group than with the γ-phosphoryl group, indicating that the affinity of $Mg^{2+}$ for the β-phosphoryl group is greater than that for the γ-phosphoryl group. In trace b in fig. 1B, the exchange rate constant appears to be greater with the γ-phosphoryl group than with the β-phosphoryl group. However, this is due to the smaller chemical shift of the γ-phosphoryl group on addition of $Mg^{2+}$, and the $k_{app}$ determined with the β-phosphoryl group was in fact greater than that with the γ-phosphoryl group. Differences in strength of coordinated bonds are commonly observed in the chelation of metal ions with chelating reagents.

With both phosphoryl groups, the values of $k_{app}$ increased linearly with increase in concentration of ATP, as shown in fig. 2. The value of the intercept on the $k_{app}$-axis obtained by extrapolation of the straight lines to [ATP] = 0 gives the value of $k_1$. It is noteworthy from fig. 2 that the increase in $k_{app}$ with [ATP] is not large and that at low [ATP], such as less than 2 mM, $k_1$ can be regarded as essentially the same as $k_{app}$.

For determination of the thermodynamic parameters of the exchange reaction, $k_1$ was determined at various temperatures between 5 and

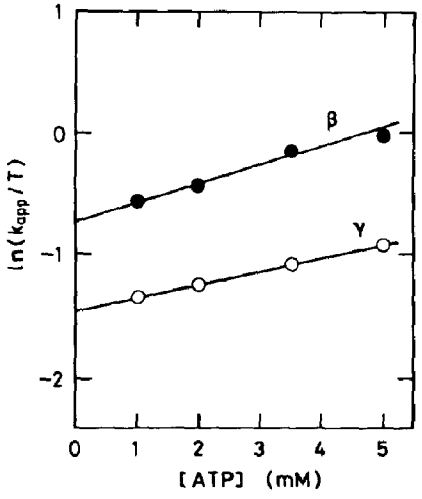



Fig. 2. Dependence of the apparent exchange rate $k_{app}$ on the concentration of ATP. Values of $k_{app}$ were determined by the simulation of spectra for the β- (●———●) and γ-phosphoryl (○———○) groups of ATP measured at 4°C with various concentrations of ATP keeping the molar ratio of ATP/$Mg^{2+}$ = 2.0. $T$, absolute temperature.

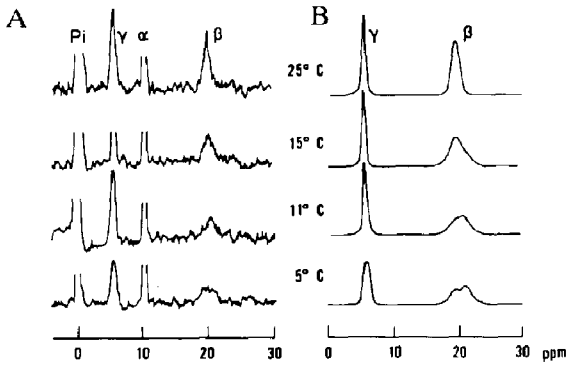



Fig. 3. Temperature dependence of the $^{31}$P-NMR spectrum of ATP in the presence of $Mg^{2+}$. The $^{31}$P-NMR spectra of a solution containing 7 mM ATP, 3.5 mM $Mg^{2+}$, 5 mM Tris-HCl, pH 7.4 and 10% $^{2}H_2O$ were measured at various temperatures (A), and the spectra of signals of the $\beta$- and $\gamma$-phosphoryl groups were simulated (B).

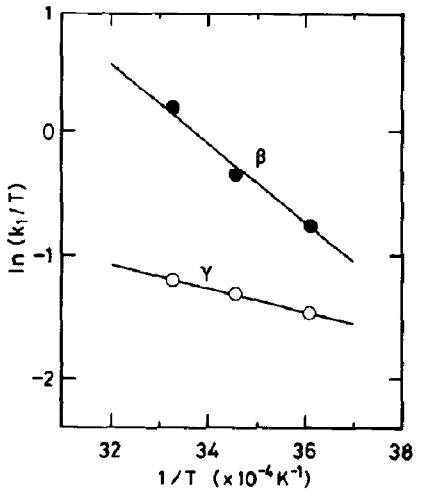



Fig. 4. Arrhenius plot of the exchange rate constant $k_1$. $T$, absolute temperature.

25 °C. As shown in fig. 3, the peaks of the $\beta$- and $\gamma$-phosphoryl groups became sharper with increasing temperature, indicating that the exchange reaction between free and $Mg^{2+}$-bound ATP was enhanced at higher temperatures. With the $\beta$-phosphoryl group the value of $k_1$ at 27 °C was about 400 $s^{-1}$, which was less than the corresponding value of 1500 $s^{-1}$ at 25 °C reported by Misawa et al. [14]. However, the latter value was determined at higher ionic strength, and we obtained a similar, higher value of $k_1$ under conditions comparable to theirs, i.e., in 100 mM KCl. The small discrepancy between our values and theirs is also ascribable to the fact that they determined the exchange rates by simulating all the phosphorus signals simultaneously on the assumption that the coordination of $Mg^{2+}$ with the $\beta$- and $\gamma$-phosphoryl groups of ATP is symmetric, whereas we performed line-shape analysis with each signal separately.

The values of the activation free energy change $\Delta G^{\ddagger}$, activation enthalpy change $\Delta H^{\ddagger}$ and activation entropy change $\Delta S^{\ddagger}$ were determined from the Arrhenius plot of $k_1$ shown in fig. 4. As summarized in table 1, at 25 °C, $\Delta G^{\ddagger}$ with the $\beta$-phosphoryl group is essentially the same as that with the $\gamma$-phosphoryl group, while the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ with the $\beta$- and $\gamma$-phosphoryl groups are different. In particular, the value of $\Delta H^{\ddagger}$ with the $\beta$-phosphoryl group is about 4-times that with the $\gamma$-phosphoryl group, indicating stronger coordination of $Mg^{2+}$ with the $\beta$-phosphoryl group than with the $\gamma$-phosphoryl group. A similar tendency was observed in a previous study [6], although the conclusion in the latter work was deduced from the temperature dependence of $k_{app}$.

### 3.2. Effects of DNP and DMSO on the $^{31}$P-NMR spectrum of ATP

The commonly used, weakly acidic uncoupler DNP activates both membrane-bound $H^+$-ATPase

Table 1

Thermodynamic parameters for the exchange reaction of $Mg^{2+}$-free and $Mg^{2+}$-bound ATP

Values of $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ of $k_1$ with the $\beta$- and $\gamma$-phosphoryl groups were determined from the temperature-dependent changes in $^{31}$P-NMR spectra of ATP.

| Additions | | $\Delta G^{\ddagger}$ (25 °C, kJ $mol^{-1}$) | $\Delta H^{\ddagger}$ (kJ $mol^{-1}$) | $\Delta S^{\ddagger}$ (J $mol^{-1}$ $K^{-1}$) |
|---|---|---|---|---|
| None | $\beta$ | 58.9 | 28.4 | −102 |
| | $\gamma$ | 61.8 | 7.6 | −182 |
| 0.5 mM DNP | $\beta$ | 59.8 | 49.8 | −34 |
| | $\gamma$ | 60.5 | 8.6 | −174 |
| 0.5 mM DNP and 40 (w/v)% DMSO | $\beta$ | 59.5 | 114.3 | 184 |
| | $\gamma$ | 61.5 | 39.5 | −74 |

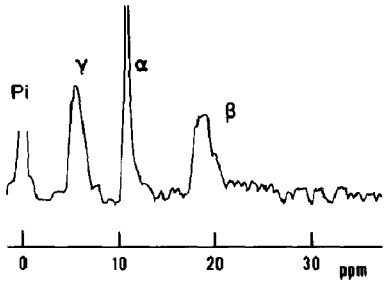



Fig. 5. Effect of DNP plus DMSO on the $^{31}$P-NMR spectrum of ATP in the presence of $Mg^{2+}$. The $^{31}$P-NMR spectrum of ATP was recorded in solution consisting of 4 mM ATP, 2 mM $Mg^{2+}$, 40% DMSO, 0.5 mM DNP, 5 mM Tris-HCl, pH 7.4, and 10% $^2H_2O$ at 20 °C.

($F_0F_1$-ATPase) in mitochondria and isolated $H^+$-ATPase ($F_1$-ATPase), although most uncouplers only activate the membrane-bound $H^+$-ATPase [8,9]. Thus, DNP is thought both to act as a protonophore and to interact directly with $F_1$-ATPase [9]. Furthermore, purified $F_1$-ATPase was reported to synthesize ATP under hydrophobic conditions, such as in the presence of DMSO [10,11]. Thus, it was of interest to examine the effects of these reagents on the electronic structure

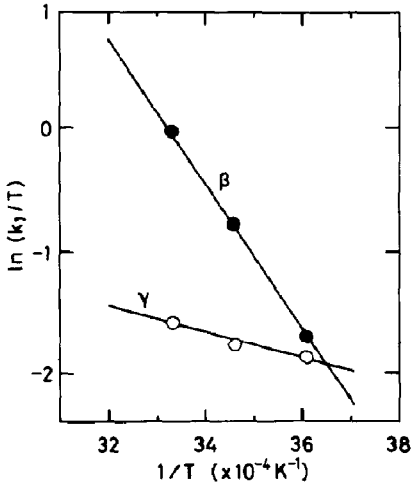



Fig. 6. Arrhenius plot of the exchange rate constant $k_1$ in the presence of 0.5 mM DNP. Values of $k_1$ were determined from the $^{31}$P-NMR spectra of ATP in the presence of 0.5 mM DNP under similar conditions to those in fig. 1. $T$, absolute temperature.

of ATP. We measured the $^{31}$P-NMR spectra of $Mg^{2+}$-coordinated ATP in the presence of 0.5 mM DNP or 40 (w/v)% DMSO or both under similar conditions to those described above. Fig. 5 shows an example of the composite effect of DNP and DMSO on the $^{31}$P-NMR spectrum of ATP in the presence of $Mg^{2+}$. These reagents apparently enhanced the exchange between free $Mg^{2+}$ and $Mg^{2+}$-bound ATP (cf. fig. 1). DNP and DMSO singly also affected the $^{31}$P-NMR spectrum of ATP. An Arrhenius plot of $k_1$ in the presence of 0.5 mM DNP is shown in fig. 6, and the thermodynamic parameters are summarized in table 1.

As seen in table 1, DNP increased $\Delta H^{\ddagger}$ with the $\beta$-phosphoryl group by about 21 kJ $mol^{-1}$, while no increase was observed with the $\gamma$-phosphoryl group, showing that DNP enhances the asymmetric coordination of $Mg^{2+}$ with ATP causing it to move closer to the $\beta$-phosphoryl group. The marked increase in $\Delta S^{\ddagger}$ in the exchange reaction for the $\beta$-phosphoryl group may be attributable to the release of water molecules bound to $Mg^{2+}$ and the $\beta$-phosphoryl group by the enhanced coordination of the $\beta$-phosphoryl group with $Mg^{2+}$ upon formation of the ATP-Mg-DNP ternary complex, as observed in ion-pair complex formation for methyl orange with alkali metal cations [17]. On the other hand, the values of $\Delta S^{\ddagger}$ for the $\gamma$-phosphoryl group in the absence and presence of DNP were very similar. The reason why $\Delta S^{\ddagger}$ with the $\gamma$-phosphoryl group was not affected appreciably by formation of a complex with DNP was probably as follows: As the interaction of the terminal $\gamma$-phosphoryl group with $Mg^{2+}$ is relatively weak, the entropic change caused by formation of a complex of DNP with $Mg^{2+}$-coordinated ATP would be very small compared with that of hydration of the ionized oxygen atom in the $\gamma$-phosphoryl group.

Further addition of DMSO to this solution resulted in greater increases in both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ with the $\gamma$-phosphoryl groups and more particularly with the $\beta$-phosphoryl group. These results indicate that DMSO (i) enhances the interaction energy between $Mg^{2+}$ and the $\beta$-phosphoryl group, and (ii) stabilizes the ATP-Mg-DNP ternary complex with liberation of hydrated water. Thus, it is expected that DNP facilitate the hydrolysis of

Table 2

Effects of $Mg^{2+}$, DNP and DMSO on the rate of ATP hydrolysis

The rate of ATP hydrolysis, $k_{hydro}$, was calculated as the amount of ATP hydrolyzed divided by the incubation time (A–C, 16 h; D–G, 12 h).

| Addition | $k_{hydro}$ (% $h^{-1}$) |
|---|---|
| (A) 8 mM ATP | 0.090 |
| (B) 8 mM ATP + 4 mM $Mg^{2+}$ | 0.108 |
| (C) 8 mM ATP + 8 mM $Mg^{2+}$ | 0.119 |
| (D) 8 mM ATP + 16 mM $Mg^{2+}$ | 0.229 |
| (E) 8 mM ATP + 8 mM $Mg^{2+}$ + 4 mM DNP | 0.155 |
| (F) 8 mM ATP + 8 mM $Mg^{2+}$ + 35 (w/v)% DMSO | 0.221 |
| (G) 8 mM ATP + 8 mM $Mg^{2+}$ + 4 mM DNP + 35 (w/v)% DMSO | 0.262 |

$Mg^{2+}$-coordinated ATP in aqueous solution, and the hydrolysis is greater in the presence of DMSO. We could not determine thermodynamic parameters in the presence of DMSO without DNP, since the exchange rate was too rapid to analyze the NMR spectra.

### *3.3. Effects of $Mg^{2+}$, DNP and DMSO on the hydrolysis of ATP in aqueous solution*

We next examined the effects of DNP and DMSO on the hydrolysis of ATP in aqueous solution at pH 7.4 and 60°C. In all cases, ATP hydrolysis was linear during the incubation time. As shown in table 2, the ATP molecule itself was stable, undergoing only about 2% hydrolysis in 24 h. Its rate of hydrolysis was increased in the presence of $Mg^{2+}$, the increase being proportional to the $Mg^{2+}$ concentration. The hydrolysis at a molar ratio of $Mg^{2+}$ to ATP of 1 (C in table 2) corresponds closely to that of a 1:1 complex between the two due to the high affinity of $Mg^{2+}$ for ATP [18], and the hydrolysis at a ratio of more than 1:1 (D in table 2) corresponded to that of a 2:1 complex between the two in which the coordination of each $Mg^{2+}$ with ATP was bidentate [18]. Thus, the latter hydrolysis should be complex and involve a mechanism other than that for a 1:1 complex. DNP at 4 mM and DMSO at 35 (w/v)% greatly accelerated the hydrolysis of ATP, increasing the rate of hydrolysis to 1.3- and 1.9-fold, respectively, of that in the presence of 8 mM $Mg^{2+}$ alone. In the presence of both DNP and DMSO (plus $Mg^{2+}$), the rate of ATP hydrolysis was about 3-fold that of ATP alone. These results are in good accordance with the stability of the ATP molecule deduced from its $^{31}$P-NMR spectra.

## 4. Discussion

In this study, we determined the exchange rate constant $k_1$ between $Mg^{2+}$-free and $Mg^{2+}$-bound ATP under various conditions from the $^{31}$P-NMR spectra of ATP. By analysis of the thermodynamic parameter $\Delta H^{\ddagger}$, we confirmed our previous results obtained by analysis of $k_{app}$ [6] that $Mg^{2+}$ is coordinated asymmetrically to the $\beta$- and $\gamma$-phosphoryl groups, being in closer proximity to the former. We also determined thermodynamic parameters from the temperature dependence of $k_1$ in the presence of DNP and DMSO. DNP greatly increased the asymmetric coordination of $Mg^{2+}$, and this effect was greatly enhanced by the further addition of DMSO. As results on the $^{31}$P-NMR of ATP are in good accordance with those on ATP hydrolysis, the asymmetric coordination of $Mg^{2+}$ is concluded to be of primary importance for specific hydrolysis of the ATP molecule. According to our molecular orbital calculations, the closer proximity of $Mg^{2+}$ to the $\beta$-phosphoryl group of the ATP molecule specifically weakens the $O_9$–$P_\gamma$ bond, which is the site of cleavage of ATP catalyzed by ATPases, and this also increases the susceptibility of the $P_\gamma$ atom to nucleophiles [7].

Weakly acidic uncouplers activate $H^+$-ATPase in mitochondria and submitochondrial particles, but in general do not have any effect on the activity of isolated ATPase ($F_1$-ATPase) [8]. However, DNP was reported to activate isolated $F_1$-ATPase in aqueous solution [9]. In the present study, we found that DNP increases the asymmetric coordination of $Mg^{2+}$ to ATP, probably by interaction with ATP-bound $Mg^{2+}$, and thus facilitates the hydrolysis of ATP. In this case, DNP could also act as a nucleophile to attack the $P_\gamma$ atom of the ATP molecule. Under more hydrophobic conditions than aqueous solution as in

enzymes, these two effects could be greater. The enhancing effect of DNP in the presence of DMSO supports this possibility. These findings explain the mechanism of activation of isolated ATPase by DNP.

As most weakly acidic uncouplers are phenols containing an OH group [8], they should activate isolated $F_1$-ATPase, but this has not been reported. One reason for this is the difference in their effective concentrations for uncoupling. DNP uncouples oxidative phosphorylation in mitochondria at about 50 $\mu$M, while other phenolic uncouplers such as SF 6847 and S-13 cause uncoupling at concentrations far below 1 $\mu$M [8,19]. The activation of isolated $F_1$-ATPase by uncouplers acting as nucleophiles requires rather high concentrations of uncouplers. Thus, their effects would not have been observed when activation of isolated ATPase was examined at their effective concentrations for uncoupling in mitochondria.

Isolated $F_1$-ATPase can synthesize ATP in aqueous solution, when DMSO is present [10,11]. On the other hand, the hydrolysis of ATP by isolated $F_1$-ATPase is stimulated in the presence of DMSO [20]. These contrasting results suggest that the 'labile' structure of ATP is important in both hydrolysis and synthesis of ATP by isolated $F_1$-ATPase. Actually, we found that DMSO caused an increase in the extent of ATP hydrolysis due to the induction of more pronounced asymmetric coordination of $Mg^{2+}$ with the $\beta$- and $\gamma$-phosphoryl groups in the ATP molecule. Further studies are required on details of this mechanism taking into consideration the effects of other factors, such as ionization of the terminal phosphoryl group, hydration of ATP, and 'water activity' [21].

Recently, mutation of the $\beta$-subunit of $F_1$-ATPase in *E. coli* was reported to result in about 90% loss of ATP hydrolysis activity [22], and this mutation was shown to be due to replacement of Ser-174 by Phe in this subunit [23,24]. Thus, this Ser residue is very important for the regulation of ATP hydrolysis activity. The role of this Ser residue can be explained from the present results. As shown in fig. 7, Ser is located at the catalytic site of ATPase, and regulates the interaction of $Mg^{2+}$ with ATP in such a way that coordination of $Mg^{2+}$ with the $\beta$- and $\gamma$-phosphoryl groups results

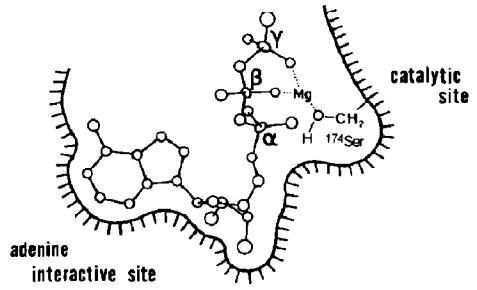



Fig. 7. Possible mechanism of the role of the Ser residue in the active site of $H^+$-ATPase. The conformation of ATP-Mg was drawn according to the crystal structure [25].

in a relatively labile or very labile electronic structure of ATP depending on the environment. The remaining weak ATP hydrolysis activity observed in the mutant (about 10% of the wild-type activity) is probably due to the coordination of $Mg^{2+}$ with a residue(s) other than Ser-174. However, this coordination would not be important in the regulation of ATP hydrolysis activity. In our study, the phenolic OH of DNP can be regarded as a model of the OH group in the Ser residue of ATPase. Further studies are in progress on the mechanism of ATP synthesis and hydrolysis catalyzed by ATPases.

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