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MECHANISM OF THE Mg^{2+}-FACILITATED SPECIFIC CLEAVAGE OF THE TERMINAL PHOSPHORYL GROUP OF ADENOSINE 5′-TRIPHOSPHATE

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The exchange rate constant between free Mg^{2+} and Mg^{2+} bound to adenosine 5′-triphosphate (ATP) was determined at various temperatures from the ^{31}P-NMR spectra of ATP in the absence and presence of Mg^{2+}. The activation free energy of this exchange reaction showed that Mg^{2+} binds asymmetrically to the β- and γ-phosphoryl groups and that it coordinates with the β-phosphoryl group more tightly than with the γ-phosphoryl group of ATP. On binding, Mg^{2+} becomes located closer to the β-phosphoryl group. This asymmetric location of Mg^{2+} weakens the chemical bond of the terminal bridged phosphoryl group, thus causing specific cleavage of this group. This mechanism was confirmed by an ab initio molecular orbital calculation, and by experiments on the stability of ATP in aqueous solution.

Most biological processes in which ATP participates, such as the release of bio-energy by ATPases and the transfer of a phosphoryl group by kinases, require Mg^{2+}. Thus the chemical and electronic structures of ATP-Mg complexes and of ternary complexes of ATP-Mg enzyme (or its model compounds) have been studied extensively to elucidate the mechanism of the cleavage of the terminal phosphoryl group from ATP. Much attention has been paid to why the bond between P_γ and O_8, not between P_β and O_9, of ATP (see Fig. 1) is specifically cleaved, and why Mg^{2+} is necessary for the cleavage of ATP, but so far these problems have not been solved [1–5].

To elucidate the molecular mechanism of the cleavage of the phosphoryl group in ATP and the role of Mg^{2+}, we measured the ^{31}P-NMR spectra of ATP under various conditions. The NMR spectra of 5 mM ATP in the presence and absence of Mg^{2+} at pH 7.4 and 4°C were recorded (Fig. 2A). The peaks of P_β and P_γ, but not that of P_α, shifted to lower fields in the presence of 5 or 10 mM Mg^{2+}, as observed previously [6]. These peaks correspond to those of a 1 : 1 complex of ATP with Mg^{2+} [7]. At Mg^{2+} concentrations of less than 5 mM, the shifts of the P_β and P_γ signals were less, but the signals were broader. These spectral changes reflect the exchange of free Mg^{2+} with Mg^{2+} chelated to the nonbridged β- and γ-phosphoryl groups (O_5 and O_6, respectively) of ATP [8]. The off-rate constants k of Mg^{2+} from ATP in the exchange reaction were determined from the simulated spectra [9,10] shown in Fig. 2B.

From the NMR spectra of 5 mM ATP in the presence of 2.5 mM Mg^{2+} at various temperatures

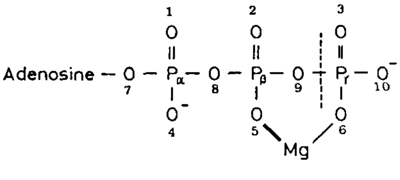

Fig. 1. Possible structure of ATP-Mg^{2+} complex. Mg^{2+} is supposed to be located closer to O_5 than to O_6 (see text).

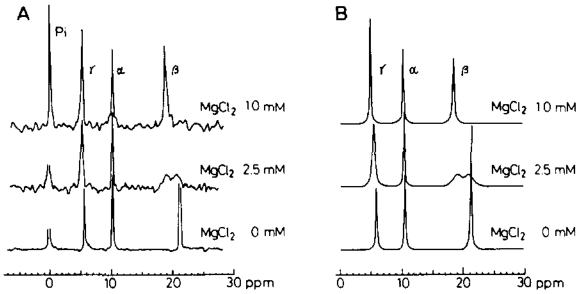

Fig. 2. ^{31}P-NMR spectra of ATP in the absence and presence of Mg^{2+} (A) and their simulated spectra (B). NMR spectra of 5 mM ATP in 2 mM Tris-HCl buffer (pH 7.4) containing 100 mM KCl were measured with a JEOL NMR spectrometer FX-200 at 80.76 MHz in the presence of 1 ml ^2H_2O with 85% orthophosphate (P_i) as an external standard at 4°C. Fourier-transformed NMR spectra were recorded by imposing 10 Hz line broadening. Simulated spectra of P_β and P_γ with 2.5 mM Mg^{2+} were determined with $k = 200$ and $k = 57$ s^{-1}, respectively, while that of P_α was drawn arbitrarily.

between 5 and 25°C, the k values in the exchange reactions with P_β and P_γ were determined. Then, the values of thermodynamic parameters were determined to be as shown in Table I. Both $\Delta H^{\neq}$ and $\Delta S^{\neq}$ affect the exchange reaction, and $\Delta G^{\neq}$ (P_β) at 25°C is about 7 kJ · mol^{-1} greater than $\Delta G^{\neq}$ (P_γ), indicating that Mg^{2+} binds more tightly to O_5 than to O_6. Namely, coordination of Mg^{2+} to the β- and γ-phosphoryl groups is not equivalent, but is asymmetric. Probably the chelated Mg^{2+} is located closer to O_5 than O_6 as shown diagramatically in Fig. 1. A certain configuration of the P_β and P_γ should be directly related to the asymmetric coordination of Mg^{2+}. This asymmetric location of Mg^{2+} should weaken the bond between P_γ and O_9 by its strong electron withdrawing ability through polarization of the bonding electrons, thus causing specific cleavage of the P_γ-O_9 bond in the ATP molecule.

TABLE I

THERMODYNAMIC PARAMETERS OF EXCHANGE REACTION OF ATP WITH Mg^{2+}.

	$\Delta G^{\neq}$ (25°C) (kJ·mol^{-1})	$\Delta H^{\neq}$ (kJ·mol^{-1})	$\Delta S^{\neq}$ (J·mol^{-1}·K^{-1})
P_β	26.4	16.2	−34
P_γ	19.0	5.3	−46

This idea was supported by an ab initio molecular orbital calculation (STO-3G) with a model compound of ATP, methyl triphosphate(bivalent anionic form). The molecular geometry of the triphosphate group was taken from that of ATP [11]. Since the total energy did not converge in usual ab initio programmes (Gaussian 70 and 80), the self-consistent field calculation was performed with selected atomic densities as initial values. When Mg^{2+} was assumed to be situated symmetrically at the same distance of 0.205 nm from both O_5 and O_6, the calculated overlap population between P_β and O_9 was 0.1710, and that between P_γ and O_9 was 0.1489 (Fig. 3A). The difference between these overlap populations became greater as the position of Mg^{2+} became closer to O_5: when the distance of Mg-O_5 was 0.1906 nm while that of Mg-O_6 remained at 0.205 nm, the overlap population of P_β-O_9 was increased to 0.1717, but that of P_γ-O_9 was decreased to 0.1417 (Fig. 3B).

Thus the stronger coordination of Mg^{2+} to O_5 is concluded to weaken the bond between P_γ and O_9. This explains why specific cleavage of ATP takes place at this position. This mechanism was supported by experiments on the stability of ATP in aqueous solution, which showed that Mg^{2+} enhanced the rate of ATP hydrolysis to ADP, as will be reported elesewhere. This mechanism should apply in enzymatic processes in which ATP is converted to ADP, since ATP is involved in these processes in the form of a complex with Mg^{2+}.

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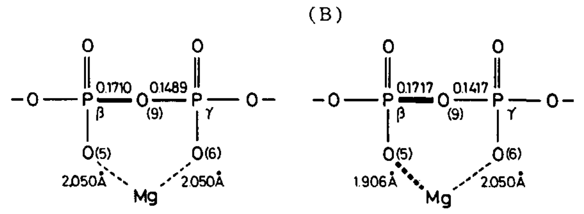

Fig. 3. Effect of symmetric(A) and asymmetric(B) locations of Mg^{2+} bound to O(5) and O(6) on the bond orders between P_β and O(9) and between P_γ and O(9) of a model compound of ATP, methyltriphosphate, calculated by the ab initio method.

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