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Why is Mg^{2+} necessary for specific cleavage of the terminal phosphoryl group of ATP?

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The mechanism of specific cleavage of the terminal phosphoryl group in hydrolysis of ATP, and the role of Mg^{2+} in the hydrolysis were studied by ab initio molecular orbital calculations. The tetravalent anion of methyl triphosphate was used as a model of the ATP anion, and its electronic structures were determined as a function of the distance between Mg^{2+} and its β-phosphoryl group. We found that the closer location of Mg^{2+} to the β-phosphoryl group than to the α- or γ-phosphoryl group was effective in weakening the P–O bond at which the cleavage of ATP catalyzed by most enzymes takes place. Moreover, the orbital coefficient of the frontier electron of P_γ, which is related to the nucleophilic reaction, was shown to increase greatly with increasing interaction between Mg^{2+} and the β-phosphoryl group.

1. Introduction

The enzyme-catalyzed hydrolysis of ATP to ADP and P_i is the main source of energy for most biological processes, and Mg^{2+} is known to be essential for this phosphoryl transfer reaction. There have been many studies on the role of Mg^{2+} in this reaction [1–10], but its precise function, especially in the specific cleavage of the terminal phosphoryl group of the ATP molecule, is still unknown.

Recently, we carried out a ^{31}P-NMR spectroscopic study on the interaction of ATP with Mg^{2+} [11]. We measured the exchange rate between free ATP and Mg^{2+}-bound ATP at various temperatures. The free energy of activation of this exchange reaction showed that Mg^{2+} binds asymmetrically to the β- and γ-phosphoryl groups of ATP, binding to the β-phosphoryl group being tighter than that to the γ-phosphoryl group of ATP. In other words, on binding to ATP, Mg^{2+} becomes located closer to the β-phosphoryl group. We proposed that this asymmetric location of Mg^{2+} weakens the chemical bond of the terminal bridged phosphoryl group between O_9 and P_γ, and that this is why cleavage takes place between O_9 and P_γ, not between O_9 and P_β (cf. chemical structure of ATP in fig. 1). This proposal was supported by an ab initio molecular orbital (MO) calculation for the divalent anionic form of methyltriphosphate coordinated with Mg^{2+} as a model of the ATP-Mg complex [11].

As an extension of this study, we carried out ab initio MO calculations for the tetravalent anionic form of ATP. There have been many quantum-chemical studies on the ATP molecule [3,12–14]. However, the characteristic electron structures of ATP directly related to ATP hydrolysis at the P_γ-O_9 position do not seem to have been de-

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termined clearly. Furthermore, there has been no ab initio MO calculation of the tri- or tetravalent anionic form of ATP coordinated with Mg^{2+}.

In this study we performed MO calculations on the magnesium-bound tetravalent anionic form of methyl triphosphate as a realistic model of ATP in the physiological environment, and considered the role of Mg^{2+} in ATP hydrolysis. We found that the total energy of such a highly charged anion as tetravalent methyl triphosphate did not converge in the calculations by usual MO programs. However, we later succeeded in performing the MO calculations, and in determining the electronic structure of ATP relevant to specific cleavage of its terminal phosphoryl group.

2. Methods

Ab initio MO calculations were performed at the STO-3G level using an IMSPACK program: a version of the GAUSSIAN-70 program in the library program package of the Computer Center, Institute for Molecular Sciences, Okazaki, Japan, coded by K. Morokuma, S. Kato, K. Kitaura, I. Ohmine, S. Sasaki and S. Obara. As ATP is too large to allow MO calculation on the whole molecule, a model of ATP, methyl triphosphate, was chosen for the calculations. The values of bond lengths and angles deduced by Cini et al. [15] from X-ray analysis of a complex of ATP and Mg^{2+} were adopted as initial values in the three-dimensional structure.

First, we found that the total energy of negatively charged methyl triphosphate (tetravalent anionic form) did not converge after 20-times iterated SCF calculations of the first set of calculations in the usual ab initio programs (GAUSSIAN-70 and -80), but that the values tended to converge. Thus, we adopted the atomic densities, obtained in the above calculation (values at the 20th iteration), as the initial values for the next SCF calculations. The total energy of the molecule converged after 15–16 additional iterations. This technique should also be useful for ab initio MO calculations on other highly charged biological molecules.

3. Results and discussion

Since NMR studies showed that Mg^{2+} coordinates with P_β and P_γ (O_5 and O_6, respectively), but not with P_α (O_4) of the ATP molecule [1,11,16], we carried out an MO calculation of the tetravalent anion of methyl triphosphate, a model of the ATP anion, as a function of the distances between Mg^{2+} and P_β. Fig. 1 shows the profiles of change in total energy, and overlap populations of P_β-O_9 and P_γ-O_9 as a function of the distance between O_5 and Mg^{2+}. The overlap populations between atoms X and Y, N(X, Y), were calculated using the following definition.

$$N(\mathrm{X}, \mathrm{Y}) = \sum_i N_i(\mathrm{XY})$$

$$N_i(\mathrm{XY}) = 2\sum_p \sum_r C_{ip}^{\mathrm{X}} C_{ir}^{\mathrm{Y}} S_{pr}^{\mathrm{XY}}$$

where i is the number of molecular orbital, p and r the numbers of atomic orbitals in atoms X and Y, respectively, and S_{pr}^{XY} the overlap integral be-

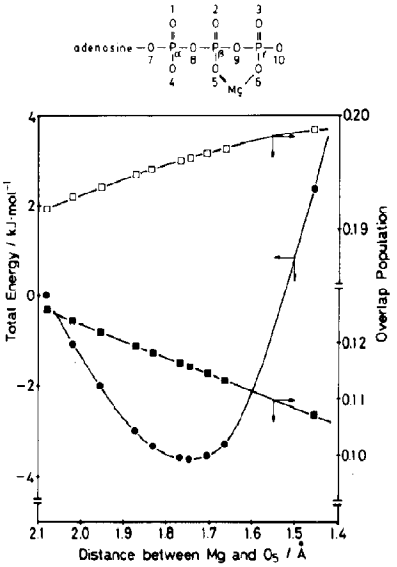

Fig. 1. Total energy and overlap population of methyl triphosphate anion coordinated with Mg^{2+} as a function of the distance between O_5 and Mg^{2+}. The total energy (●) is shown relative to the length of O_5-Mg determined by X-ray analysis (= 2.0815 Å). Changes in the overlap populations of P_β-O_9 (□) and P_γ-O_9 (■) are also shown.

tween the p-th and r-th atomic orbitals. The overlap population thus obtained serves as a good index for predicting the ease of breaking a bond between X and Y. The length of the bond between O_5 and Mg^{2+} was successively shortened along the initial geometric direction of O_5-Mg from the value of 2.08 Å deduced from X-ray data [15]. The total energy was minimal at an O_5–Mg distance of about 1.75 Å. The overlap population of P_γ-O_9 decreased almost linearly with decrease in length of O_5-Mg. In contrast, the overlap population of P_β-O_9 increased with decrease in length of O_5-Mg.

Fig. 2 shows the net atomic charge densities and overlap populations in the structure determined from X-ray data (panel A) and in the most stable structure when the distance between O_5 and Mg^{2+} is 1.75 Å (panel B). The results in fig. 2 clearly indicate that with stronger interaction between O_5 and Mg^{2+} (fig. 2B), the overlap population of P_γ-O_9 decreases, but that of P_β-O_9 increases. These results show that the stronger coodination of Mg^{2+} to O_5 specifically weakens the bond between P_γ and O_9. This is apparently the reason why specific cleavage of ATP takes place at this position when Mg^{2+} coordinates to ATP and is consistent with our previous conclusion based on a ^{31}P-NMR study [11]. It is interesting that the length of O_6-Mg is shorter than that of O_5-Mg in the solid crystal of the ATP-Mg complex [15], indicating that the stable structure of ATP-Mg in the solid state is different from the structure that facilitates cleavage of the P_γ–O_9 bond in aqueous solution.

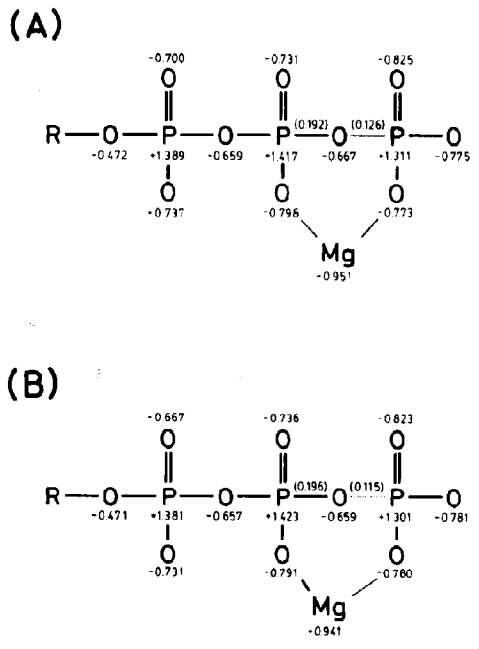

Fig. 2. Net atomic densities of ATP. Values in parentheses denote overlap populations between atoms. (A) Structure determined by X-ray analysis. (B) The most stable structure when the bond length between O5 and Mg^{2+} is 1.75 Å. R, methyl group.

The reactivity of ATP can be explained by the distribution of 'frontier electrons' related to a 'nucleophilic reaction'. The index of frontier electron density is usually used to compare the reactivity within a molecule. In MO calculations carried out in the present study, the numbers of atomic orbitals and total electrons were kept constant. Therefore, the LUMO coefficients are regarded as being a good index of reactivity between the different configurations. The changes in LUMO (lowest unoccupied molecular orbital) coefficient are shown in fig. 3. It is interesting that the coefficient of the 3p atomic orbital in the LUMO of the P_γ atom changes markedly from 0.0087 in the structure determined by X-ray analysis [15] shown in fig. 3A to 0.0184 in the most stable structure, where the distance between Mg^{2+} and O_5 is 1.75 Å, as shown in fig. 3B. This indicates that the reactivity of the P_γ atom increases significantly with increase in the interaction between Mg^{2+} and O_5. Recently, hydrolysis of ATP was shown to proceed through the attack of nucleophiles such as H_2O and OH^- on the P_γ atom [16]. The present results confirm this experimental trend.

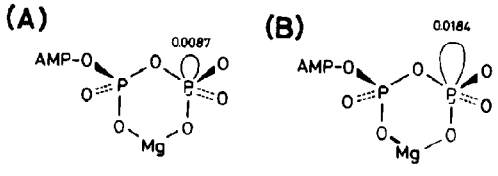

Fig. 3. Coefficient of the 3p atomic orbital of P_γ in the LUMO (lowest unoccupied molecular orbital), frontier orbital in ATP. Panels A and B: as for fig. 2.

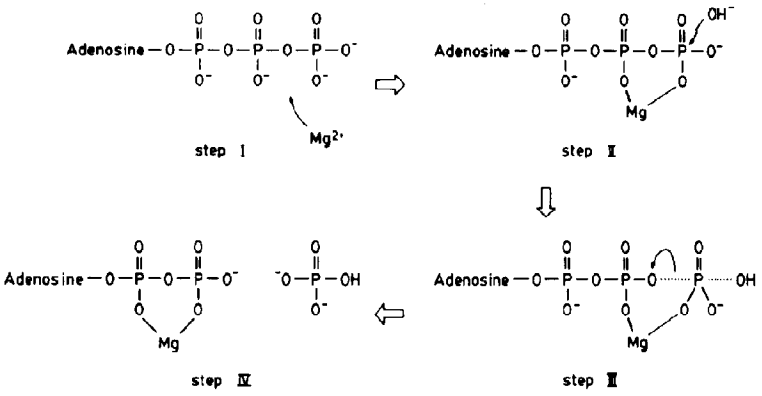

Fig. 4. Proposed mechanism of hydrolysis of ATP.

Based on the above results, the mechanism of hydrolysis of ATP can be depicted schematically as shown in fig. 4. We conclude that Mg^{2+} coordinated asymmetrically with O_5 and O_6 of the ATP molecule weakens the bond between P_γ and O_9, and that it also increases the frontier density of the P_γ atom. The former effect is directly related to the specificity of the position of hydrolysis of ATP, and the latter effect to the catalytic action of Mg^{2+} in accelerating the hydrolysis. This mechanism is probably involved in the hydrolysis of ATP by ATPases. An important role of ATPases is presumably to make Mg^{2+} coordinate more tightly with the O_5 atom than with the O_6 atom. This asymmetric coordination of Mg^{2+} is concluded to be essential for the catalytic action of ATPases. Further study on the detailed structure of the ATP-Mg complex in relation to the action of ATPases, such as the effect of hydration, is in progress by way of in vitro experiments and also by using MO calculations.

Acknowledgements

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