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Role of Mg^{2+} in the ribozyme system

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Recently, several kinds of catalytic activities have been identified from L-19 IVS RNA or 'ribozyme'. In the catalytic process of ribozyme, Mg^{2+} is required. We investigated the role of Mg^{2+} by molecular orbital calculation. Based on the results of the calculation, a model for the self-splicing reaction was constructed.

Ribozyme; Mg^{2+}; Ab initio molecular orbital calculation

1. INTRODUCTION

The *Tetrahymena* ribosomal RNA intervening sequence mediates self-splicing, removing itself from the precursor ribosomal RNA. The shortened form of the intervening sequence, L-19, has several kinds of enzymatic activities. Because of these enzymatic activities, the RNA has been termed 'ribozyme' [1–3].

The self-splicing reaction is essentially a sequence of transesterification reactions. However, the detailed mechanism has remained unknown. Firstly, the role of Mg^{2+} is not clear. Mg^{2+} is required in the enzymatic activities of ribozyme. On the other hand, it is known that metal cations are involved in many cases of nucleotidyl transfer and phosphoryl transfer reaction [4,5]. Therefore, Mg^{2+} is supposed to play an important role in the ribozyme system. Secondly, each transesterification step in the self-splicing produces a 3′-hydroxyl group. However, the cleavage of the phosphodiester bond catalyzed by heavy metal ions produces a 5′-hydroxyl group. Therefore, the attack of the 2′-hydroxyl group must be restricted in the ribozyme system.

Here, we constructed a model of transesterification in the ribozyme system which can explain both the above-mentioned points. The validity of the model was investigated by population analysis of molecular orbital (MO) calculation.

2. MATERIALS AND METHODS

The self-splicing reaction is a sequence of transesterification reactions. Each reaction is supposed to proceed via an S_N2 in-line mechanism. In this mechanism, entering nucleophile binds to the phosphorus, resulting in a trigonal bipyramid as a transition state or reactive intermediate [4].

In order to investigate the role of Mg^{2+} in this reaction, MO calculation was carried out. The entire structure of the nucleotide is too large to be subjected to the calculation. Therefore, we used only the region of the phosphodiester bond as a model molecule for calculation. These model molecules of the initial state are shown in fig.1a–c, and those of the trigonal bipyramidal state are depicted in fig.2e–g. Each model molecule in fig.1 is coordinated by Mg^{2+} in different ways. The bond lengths and angles of the phosphodiester

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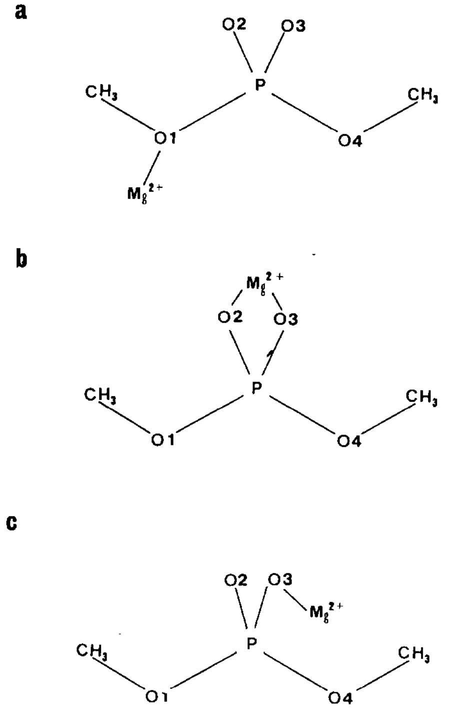

Fig.1. Model molecules of the phosphodiester bond with Mg^{2+}. Mg^{2+} was placed in the plane including O1, P and O5 for model (a), and O2, O3 and P for models (b) and (c). Bond angles ∠ Mg^{2+}-O1-CH_3 of (a) and ∠ Mg^{2+}-O3-P of (c) were taken to be 110°. For (b), Mg^{2+} was placed to be bound with both O2 and O3. The bond length between Mg^{2+} and oxygen was taken to be 1.49 Å.

bond in the initial state were taken from the geometry of $Na_5P_3O_{10} \cdot I(C_2)$ [6]. The bond lengths of the trigonal bipyramidal state were taken to be the same as those of the initial state. In the trigonal bipyramidal state, entering and leaving groups were placed in line, and the plane including the triply coordinated phosphate was set to be vertical to the line.

Each model molecule shown in figs 1 and 2 is subjected to MO calculation at the minimal basis set level [7], using the ab initio program GAMESS [8]. For magnesium and phosphorus, effective core potentials were introduced [9,10]. There was no geometry optimization procedure in the calculations.

3. RESULTS AND DISCUSSION

Naturally, calculations of biological systems should also consider the solvent. However, introducing solvent molecule(s) into the model would lead to inextricable complexity in the computation. Furthermore, as the geometry of the ribozyme system is unknown, little security would be added to our calculation by considering the solvent. With these limitations in mind, our computations have led to the following results.

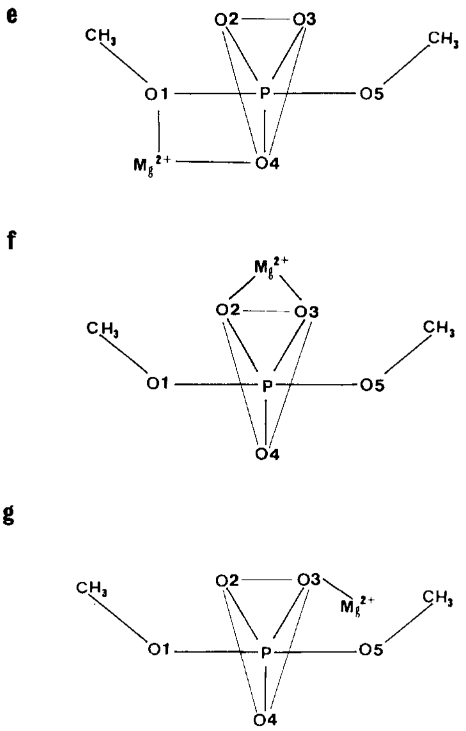

Fig.2. Model molecules of the trigonal bipyramidal state with Mg^{2+}. Mg^{2+} was placed in the plane including O1, P, O4 and O5 for model (e), and O2, O3, O4 and P for models (f) and (g). The bond angle ∠ Mg^{2+}-O3-P of model (g) was taken to be 110°. For (e) and (f), Mg^{2+} was placed to be bound with two oxygens, O1 and O4 for model (e), and O2 and O3 for model (f). The bond lengths of O2-P, O3-P and O4-P were taken to be the same as those of O2-P and O3-P in fig.1; and the bond lengths of O1-P and O5-P were taken to be the same as those of O1-P and O4-P in fig.1. The angles ∠ O-P-O on the plane including O2, O3, O4 and P were taken to be 120°. The methyl group corresponding to the 5′-carbon was placed to be bound to O2 for (e), O4 for (f) and O2 for (g).

Model molecules of the phosphodiester bond in the initial state are shown in fig.1a–c. The differences among the three models are only the patterns of coordination of Mg^{2+}. The total energies and charges on phosphorus of each model molecule were calculated, using the ab initio SCF program (see table 1). As shown in table 1, model c is the most stable of the three with respect to total energy. This result is consistent with the observation that Mg^{2+} preferentially coordinates with the region of the phosphate of nucleotides [11]. In such a state, Mg^{2+} is supposed to increase the electrophilicity of the phosphate by its electron-withdrawing effect, accelerating transesterification [1]. In order to evaluate the electron-withdrawing effect of Mg^{2+}, the charge on the phosphorus of the phosphodiester bond model molecule without Mg^{2+} was calculated (see table 1 d). As shown in table 1, the increase in electrophilicity of the phosphate by electron withdrawal with Mg^{2+} cannot be expected in the conformation of model c. Therefore, the requirement of Mg^{2+} in self-splicing indicates other roles for Mg^{2+} in the reaction.

To elucidate the role of Mg^{2+}, ab initio SCF calculations for the transition or intermediate reactive state were carried out. In the S_N2 in-line reaction of the system that we considered, the transition state or reactive intermediate has a pentacoordinated trigonal bipyramidal conformation [4]. Mg^{2+} was coordinated with the model molecules of the trigonal bipyramidal state in different ways (see fig.2e–g). Total energies and overlap populations of each model molecule were calculated. The results are listed in table 2. In view of total energy, model (e) is the most stable of the three. In model (e), the overlap population between O1 and P is decreased and that between P and O5 increased by Mg^{2+} coordination. For comparison, overlap populations of O1-P and P-O5 of the trigonal bipyramidal state without Mg^{2+} were calculated (see table 2h). If O1 is taken to be the 3′-oxygen of the leaving group and O5 to be the 3′-oxygen of the entering group, this model corresponds to the self-splicing reaction. These results indicate that the role of Mg^{2+} is not the increasing of the electrophilicity of the phosphorus at the initial stage, but the weakening of the bond between the phosphorus and the leaving group, and strengthening of the bond between the phosphorus and the entering group in the trigonal bipyramidal stage.

Table 1

Total energies and charges on the phosphorus of model molecules (a)–(c) shown in fig.1

	Total energy (A.U.)	Charge on phosphorus
(a)	−385.950	+1.885
(b)	−385.798	+1.376
(c)	−386.012	+1.769
(d)	−	+1.786

The charge on the phosphorus of model molecules without Mg^{2+} is shown in row d. Total energies are expressed in atomic units (A.U.). 1 A.U. is 6.2751×10^2 kcal/mol

Table 2

Total energies, overlap population and its ratio for model molecules (e)–(g) shown in fig.2

	Total energy (A.U.)	Overlap population		Ratio O1-P/P-O5
		O1-P	P-O5	
(e)	−500.054	0.009	0.207	0.04
(f)	−499.465	0.168	0.161	1.04
(g)	−500.018	0.191	0.191	1.00
(h)	−	0.085	0.085	1.00

Overlap population and its ratio for model molecules without Mg^{2+} are shown in row h. O1, P and O5 correspond to those of the model molecules (see fig.2)

Based on these calculations, we constructed a model for the transesterification in the self-splicing reaction (see fig.3). At the initial stage, Mg^{2+} binds to one of the oxygens of the phosphate and the 2′-oxygen of the leaving group (fig.3a). Then, Mg^{2+} binds to the phosphate and 3′- and 2′-oxygens in the transition state (fig.3b). The significance of the coordination of Mg^{2+} with the 2′-oxygen is as follows. (i) Such a bond in the initial stage will ensure the coordination of Mg^{2+} not with the 5′-oxygen but with the 3′-oxygen during the process of the reaction. (ii) Even if the 2′-oxygen attacks the phosphate, the coordination of Mg^{2+} with the 2′-oxygen restricts the formation of a bond between the 2′-oxygen and phosphorus by the mechanism described above, i.e. the overlap

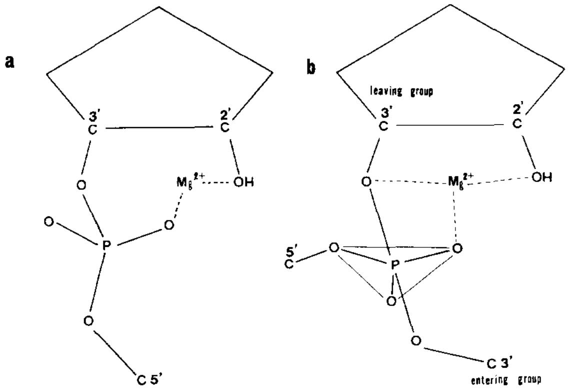

Fig.3. Model of Mg^{2+}-mediated transesterification in the self-splicing reaction. (a) For the initial stage and (b) for the trigonal bipyramidal stage. The numbers 2', 3' and 5' indicate the position of the carbon in the ribose of RNA.

population between the 2'-oxygen and phosphorus is decreased by Mg^{2+}. Recently, a molecular model for mRNA splicing, in which the splicing reaction proceeds via a 2',3'-cyclic state, was proposed [12]. In this model, the 5'-hydroxyl group attacks the 2',3'-cyclic phosphate, and the ligation between two exons is completed. However, a sequence of attacks of the 3'-hydroxyl group on the phosphodiester bond is considered to accelerate the self-splicing reaction [1], and we constructed the model following the latter idea. The validity of the two models in a self-splicing system will be evaluated by experimental studies.

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