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Ab initio MO Calculations of Model Molecules for Ribozyme Reaction Including an Mg^{2+} Ion

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Ab initio molecular orbital (MO) calculations are performed for the model molecule of ribozyme. For the models of initial and transition states of splicing reaction, stable binding sites of the Mg^{2+} ion are investigated. The energetics of the reaction and the effect of hydration on the Mg^{2+} ion are discussed.

Self-splicing and ribozyme reactions by *Tetrahymena* RNA catalysis are interesting phenomena in science with the interest of the origin of life.1) Mg^{2+} ion is supposed to play an important role in it.2) However, details of the role of Mg^{2+} ion remain unknown. Toh, Imamura and Kanda3) performed ab initio molecular orbital calculations using model molecule, $[PO_4(CH_3)_2]^{2-}$-Mg^{2+}. They investigated energies and populations of some model molecules at several binding sites of Mg^{2+} ion in the initial and transition states of the transesterification reaction. Though their intension is novel, their calculations are not necessarily reliable because they used minimal basis on each atom and did not optimize the position of Mg^{2+} ion. The distance between oxygen and magnesium ion used in their work is fixed at 1.49 Å, which seems to be too short. They also neglected the hydration of Mg^{2+} ion. In this paper, we first perform geometry optimization of Mg^{2+} ion in the initial and transition states of the ribozyme reaction by ab initio MO calculations for the same model molecule $[PO_4(CH_3)_2]^{2-}$-Mg^{2+} as used by Toh et al.3) Next, at these geometries, we examine the energetics of this reaction by using hydrated Mg^{2+} ion and the charges of the compounds with and without the hydration.

Model molecules used in this calculation are shown in Fig. 1 for initial state and in Fig. 2 for transition state. This model assumes that this splicing reaction is S_N2 type in organic chemistry. Considering the symmetry of this model, we investigated several binding sites of Mg^{2+} ion. We perform ab initio MO calculations using the program HONDO7.4) Basis sets for the P and Mg atoms are the ECP ones proposed by Wadt snd Hay5) with splitting valence s functions. That for oxygen is Huzinaga-Dunning's [9s5p/4s2p]6) and that for carbon is taken from Huzinaga's table.7) STO-3G set8) is used for hydrogen. For Mg atom, the p functions which are derivatives of the valence s functions are added so that the Hellmann-Feynman theorem is satisfied.9) This is quite effective in searching the optimized position of the Mg^{2+} ions.10) Results of the geometry optimization for both initial and transition states are shown in Table 1.

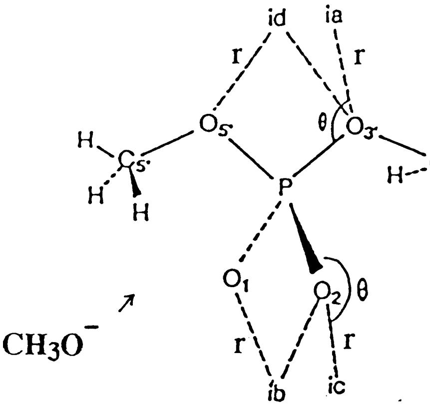

Fig. 1. Model molecule for the initial state of ribozyme reaction. The positions of Mg^{2+} ion are indicated by ia--id.

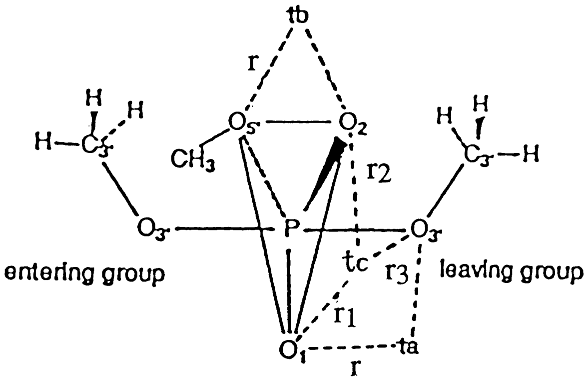

Fig. 2. Model molecule for the transition state of ribozyme reaction. The positions of Mg^{2+} ion are indicated by ta--tc.

Table 1. Energies of the initial and transition state models for the ribozyme reaction at the optimal geometries of Mg^{2+} ion and the effect of hydration on Mg^{2+} ion

			Without hydration		With hydration	
Mg site	Geometry r/Å	θ/degree	Total energy/au	Energy difference kcal mol^{-1}	Total energy/au	Energy difference kcal mol^{-1}
Initial state						
ia	1.95	104.60	-499.158523	94.3		
ib	2.14		-499.308839	0.0	-803.451729	0.0
ic	1.80	197.10	-499.295332	8.5		
id	2.14		-499.182177	79.5		
Transition state						
ta	1.90		-499.414277	-66.2	-803.465636	-8.7
tb	2.23		-499.328678	-12.5		
tc	r_1 2.04		-499.432033	-77.3	-803.324401	79.9
	r_2 2.27					
	r_3 2.20					

In our results without hydration, the site ib of Mg^{2+} is most stable for the initial state and the site tc is most stable for the transition state. These are different from Toh's results that ic and ta were most stable, respectively. We found that the Mg-O distance is elongated from 1.49 Å to 2.14 Å for the ib site and to 2.04 Å for the tc site during our geometry optimization. The Toh's fixed distance of 1.49 Å is certainly too short in comparison with these optimized distances.

Next we employed hydrated Mg^{2+} ion. Since Mg^{2+} ion is ordinarily six-coordinated by water, the ions at ib and ta sites are hydrated by four waters and that at tc site is hydrated by three waters. The distance between Mg and oxygen of water is 2.14 Å and the basis sets for oxygen and hydrogen in water are minimal. 7,8) The energies are also summarized in Table 1. In the case of the hydrated Mg^{2+} ion, the ta site is more stable than the tc site by 88.6 kcal mol^{-1}. To examine the hydration effect on this reaction, we take the ib site as the initial state and the ta site as the transition state. In Fig. 3 the energy profile of the initial and transition states are shown.

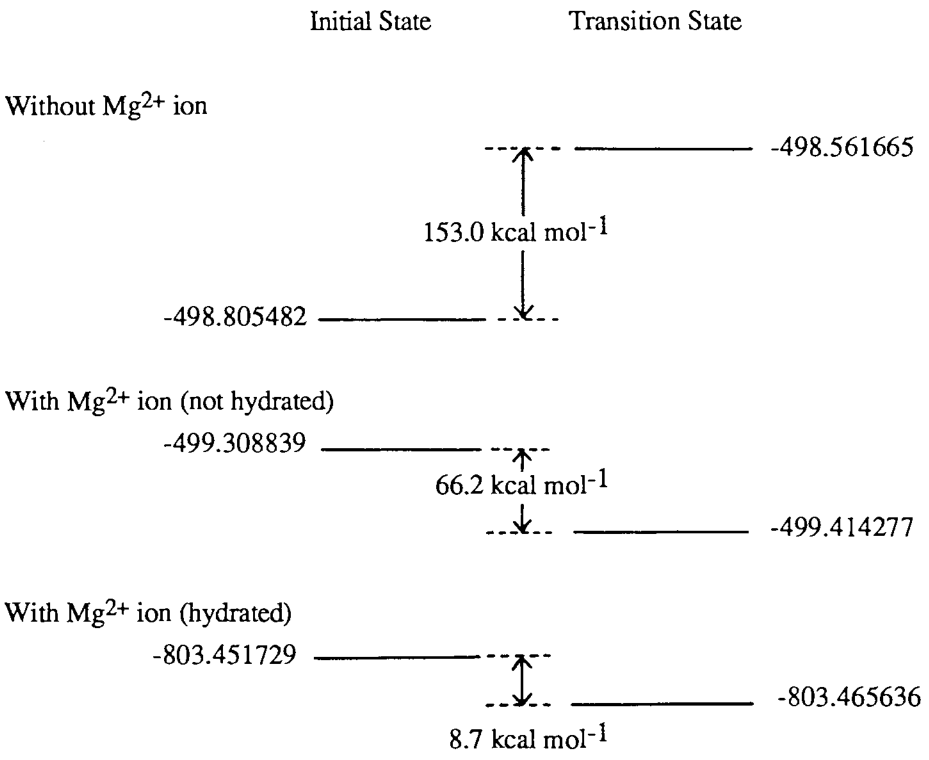

Fig. 3. Energy profile of the initial and transition states of the reaction with or without Mg^{2+} ion.

If there is no Mg^{2+} ion during the reaction, the transition state is higher than the initial state with energy barrier 153.0 kcal mol^{-1}. However, the coordination of Mg^{2+} ion makes the transition state more stable than the initial state by 66.2 kcal mol^{-1}. This suggests that the coordination of the Mg^{2+} ion enhances the S$_N$2 reaction by stabilizing the transition state. After the hydration for Mg^{2+} ion, the energy levels of the initial and transition states become almost the same. The hydration is effective for making the initial state stable. Nevertheless the transition state is slightly more stable than the initial state by 8.7 kcal mol^{-1}. Thus, we think that the effect of the Mg^{2+} coordination is more important than the effect of the hydration.

In Table 2 we show the overlap populations of the P-O$_3$' bond in the transition state with and without the Mg^{2+} ion. Without the Mg^{2+} ion, the overlap population of the P-O$_3$' bond of entering group and that of leaving group are equal. However, after the coordination of the Mg^{2+} ion, the overlap of the P-O$_3$' bond in the entering group increases and that of the leaving group decreases. The results with the hydrated Mg^{2+} ion also show the same tendencies. Thus, the existence of Mg^{2+} ion would facilitate the ribozyme reaction. Gross charges of P and Mg atoms are also listed in Table 2. The coordination of Mg^{2+} ion and the hydration thereof do not affect the gross charge of P, so that Mg^{2+} ion would not enhance the nucleophilicity of the reaction. The gross charge of Mg atom is close to the formal charge, +2. Hydration does not affec the charge of the Mg ion.

Table 2. Overlap population of P-O3' bonds and gross charges of P and Mg atoms in the transition state with or without Mg^{2+} ion

	Overlap population		Gross charge	
	P-O3' bond (entering group)	P-O3' bond (leaving group)	P	Mg
without Mg^{2+}	0.435	0.435	+2.35	
with Mg^{2+} ion(not hydrated)	0.538	0.224	+2.36	+1.99
with Mg^{2+} ion(hydrated)	0.515	0.247	+2.36	+1.97

In conclusion we have performed the geometry optimization of the Mg^{2+} ion in the model compounds of the initial and transition states of the ribozyme reaction by ab initio MO calculations. The optimal Mg-O distance is larger than the non-optmized one used by Toh et al. Further we examined the effect of hydration on Mg^{2+} ion for the reaction. The coordination of the magnesium and the hydration are found to be effective for stabilizing the transition state, and thereby for accelerating the reaction.

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References

1) T. R. Cech, *Science*, **236**, 1532 (1987).
2) N. Sugimoto, R. Kierzek, and D. H. Turner, *Biochemistry*, **27**, 638 (1988); *Chem. Lett.*, **1990**, 747.
3) H. Toh, A. Imamura, and K. Kanda, *FEBS Lett.*, **219**, 279 (1987).
4) M. Dupuis, J. D. Watts, H. O. Villar, and G. J. B. Hurst, Program Library HONDO7 (No. 544), Computer Center of the Institute for Molecular Science, Okazaki, Japan.
5) W. R. Wadt and P. J. Hay, *J. Chem. Phys.*, **82**, 284 (1985).
6) S. Huzinaga, *J. Chem. Phys.*, **42**, 1293 (1965); T. H. Dunning, Jr., *J. Chem. Phys.*, **53**, 2823 (1970).
7) S. Huzinaga, J. Andzelm, M. Klobukowski, E. Radzio-Andzelm, Y. Sakai, and H. Tatewaki, "Gaussian Basis Sets for Molecular Calculations," Elsevier, New York (1984).
8) W. J. Hehre, R. F. Stewart, and J. A. Pople, *J.Chem. Phys.*, **51**, 2657 (1969).
9) H. Nakatsuji, K. Kanda, and T. Yonezawa, *Chem. Phys. Lett.*, **75**, 340 (1980).
10) H. Nakatsuji, M. Hada, and T. Yonezawa, *Chem. Phys. Lett.*, **95**, 573 (1983).

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