A Molecular Orbital Treatment of Phosphate Bonds of Biochemical Interest. II. Metal Chelates of Adenosine Triphosphate

By Kenichi FUKUI, Akira IMAMURA and Chikayoshi NAGATA

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In our previous paper[1] attempts were made by way of the simple LCAO MO method to correlate the energy-donor ability of adenosine triphosphate (ATP) with the π electronic energy of this compound. However, it was found that, so far as only the phosphate part is considered, the π electronic energy change of ATP in the course of hydrolysis, the reactivity index, and the π electron density at the phosphorus atom are not significantly different from those of such other high energy phosphate compounds as adenosine diphosphate (ADP), phosphoenol pyruvate (PEP) and acetyl phosphate (ACP), nor from those of such low energy phosphate compounds as adenosine monophosphate (AMP) and glycerol 1-phosphate (GLP).

These findings could not explain the efficiency of ATP as a biological specific. That is, among many so-called high energy phosphate compounds, ATP holds a unique position in the sense that ATP alone is, in general, used as an energy source in *in vivo* energy transfer reactions. Therefore, some other factors must be taken into consideration in order to explain the biochemical characteristic of ATP. This was pointed out by the present authors in the previous paper, where the possible importance of the metal chelate formation of ATP in the *in vivo* reaction was suggested. In fact, the participation of metal ions in the biological functions of ATP has been established in many experiments. In line with these experiments, various models of ATP-metal complex have been proposed. However, no detailed discussion and calculation have yet been carried out.

In the present paper, a comparison will be made of such theoretical indices as the stabilization energy due to the formation of metal chelate, the reactivity index and the π electron density for various models with the experimental facts, especially with the enzymatic hydrolysis of ATP. On the basis of this comparison, the most probable structure of the ATP-metal complex will be sought.

1) K. Fukui, K. Morokuma and C. Nagata, This Bulletin, 33, 1214 (1960).

The Method of Calculation and the Models Adopted

As in the previous paper, the calculations were carried out by means of the simple LCAO MO method. For the sake of comparison, the same values for the parameters of the Coulomb and the resonance integrals of phosphorus and oxygen atoms have been adopted as in the previous paper. However, in these calculations it was necessary to determine the parameters for the chelate-forming part of the ATP-metal complex. The resonance integrals between the metal and the oxygen atom or the nitrogen atom, $l_{M-O}\beta$, or $l_{M-N}\beta$, are both taken to be $0.4\,\beta$, where β is the resonance integral of two adjacent carbon atoms in benzene. This value was determined by considering the distance of the chelate bond and also the nature of the orbitals participating in the π conjugation.*

The value of Coulomb integral for the metal, α_M, is difficult to determine. Therefore, calculations have been carried out for model 2 for various values, for $\alpha+3\beta$, $\alpha+\beta$, $\alpha-\beta$ and $\alpha-3\beta$. The theoretical indices obtaiaed by utilizing these varying parameters are not very different from one another, and the qualitative conclusion is not changed with these varying parameters. Therefore, the calculations for some models have been carried out for two parameters, $\alpha+\beta$, and $\alpha-\beta$. For model 1, rather tedious calculations are required, therefore, the calculations have been carried out for the $\alpha+\beta$ parameter value.

By utilizing these parameters, such theoretical indices as the stabilization energy due to the chelate formation, the superdelocalizability and the total π electron density at the phosphorus atoms have been calculated for the following models (Fig. 1).

* The resonance integrals between oxygen and metal have been tentatively determined to be 0.4β, which may be an intermediate value between that of oxygen-phosphorus bond in ATP, 0.6β, and that of the usual hydrogen bond. For many kinds of metals, the values of resonance integral are probably different. But so far as the relative comparison on the stability of chelate compounds is concerned, the change in the resonance integral can be included in the change in the Coulomb integral, so that the approximate estimation of the resonance integral may not cause any serious errors in these calculations.

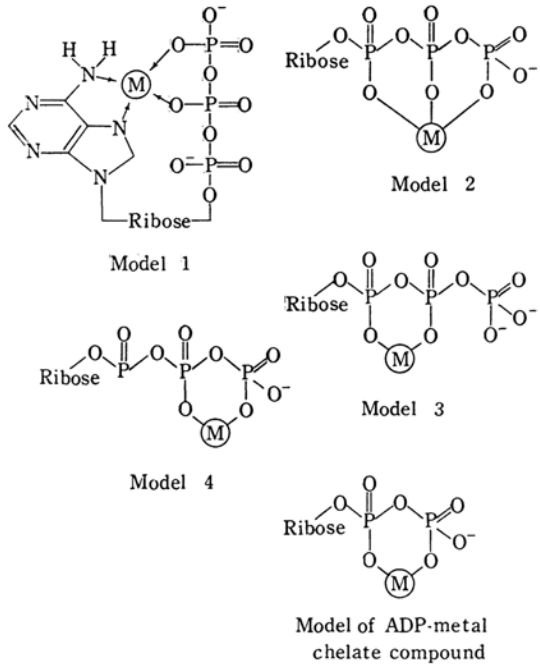

Fig. 1. The models of metal chelate compounds adopted in the calculation.

Model 1 represents the ATP-metal complex making a chelate between the adenine and the phosphate parts. Taking the metal as the magnesium cation, Szent-Györgyi[2] postulated a quadridentate chelate. Epp[3] et al. suggested, on the basis of their infrared data of ATP in the presence of and in the absence of Mg^{2+}, that such a complex formation could be recognized, although no direct evidence was obtained as to whether the complex formation was intramolecular or intermolecular.

In addition, James,[4] on the basis of the data on the rotatory dispersion of ATP, ADP and AMP, suggested the existence of some interaction between adenine and phosphate parts. Model 2 was proposed by Melchior[5] in view of the higher stability of the ATP-metal complex than those of ADP and AMP.

Theoretically there exist two other possible chelate complex structures between the polyphosphate chain and the metal. They are designated as models 3 and 4; the theoretical indices have been calculated with respect to these structures, and also for the ADP-chelate complex for the sake of comparison.

2) A. Szent-Györgyi, "Biogenetics," Academic Press, New York (1957).
3) A. Epp, T. Ramasarma and I. R. Wetter, *J. Am. Chem. Soc.*, **80**, 724 (1958).
4) B. H. Levendahl and T. W. James, *Biochim. Biophys. Acta*, **21**, 298 (1956).
5) N. Melchior, *J. Biol. Chem.*, **206**, 615 (1954).

In ATP there are two sites at which chelation can take place, that is, the amino group of the purine ring or the polyphosphate chain. In order to decide the site where the metal ion combines with the ATP molecule, Khan and Martell[6] measured the equilibrium constant of the ATP complex with various metal ions and concluded that the amino group of the purine ring is probably not involved in the chelate formation of ATP, because little or no interaction was observed between metal ions and adenosine. In view of these experiments, there is no need to consider the ATP-metal chelation on the purine ring only. Needless to say, however, model 1 can not be eliminated by this experiment, because in that model the participation of both the purine ring and the polyphosphate parts are considered.

Results and Discussion

Stabilization Energy Due to the Metal Chelate Formation.—In Table I, the stabilization energies calculated with regard to various models for ATP-metal complexes are indicated. For the sake of comparison, the calculated results for the ADP-metal complex are also included. As may clearly be seen in this table, the stabilization energies for models 3 and 4 are considerably smaller than those for models 1 and 2. Therefore, the latter two models are more plausible than the former two. The propriety of models 3 and 4 is, moreover, threatened conclusively by the fact that the stabilization energies for models 3 and 4 are smaller than that of the ADP-metal complex; this finding contradicts the fact that the ATP

Table. I. Stabilization energy due to metal chelate formation

Chelate compound	The value of the Coulomb integral of the metal, α_M	Stabilization energy (in units of β)
ADP-metal chelate compound	$\alpha+\beta$	3.609
	$\alpha-\beta$	3.800
ATP-metal chelate compound model 1	$\alpha+\beta$	7.890
ATP-metal chelate compound model 2	$\alpha+3\beta$	9.326
	$\alpha+\beta$	9.330
	$\alpha-\beta$	9.614
	$\alpha-3\beta$	9.503
ATP-metal chelate compound model 3	$\alpha+\beta$	2.442
	$\alpha-\beta$	2.634
ATP-metal chelate compound model 4	$\alpha+\beta$	1.109
	$\alpha-\beta$	1.301

6) M. M. T. Khan and A. E. Martell, *J. Phys. Chem.*, **66**, 10 (1962).

complex has a higher stability than those of ADP or AMP. Hence, from the standpoint of the stabilization energy, model 1 or 2 remains the most favorable.

Reactivity Indices and Enzymatic Hydrolysis. —As reactivity indices such as the superdelocalizability for nucleophilic attack, $S_r^{(N)}$, and the π electron density at the phosphorus atoms of ATP- and ADP-metal complexes have been calculated; they are indicated in Tables II and III. Superdelocalizability, S_r, is a good index representing the reactivity of atom r in conjugated molecules, the greater the value of a position, the more reactive that position. Accordingly, from Table II, a prediction for the most susceptible position in ATP for the nucleophilic attack is possible. That is, for models 1 and 4, the innermost and the terminal phosphorus atoms (they are referred to as P_1 and P_3, respectively) are expected to be most susceptible to nucleophilic attack, whereas model 2 predicts the following order of reactivity: $P_3 > P_2 > P_1$, where the middle phosphorus atom is designated as P_2. For model 3 and also for free ATP, P_2 is predicted as the most reactive.

In comparing these theoretical predictions with the experimental positions of enzymatic hydrolysis, we can choose the model 1 as the most probable chelate complex of ATP, because the theoretical indices for this model explain well the experimental position of enzymatic hydrolysis. That is, the most commonly encountered type of reaction of ATP is the cleavage of the terminal phosphate group, P_3; moreover, not a few reactions involving the cleavage of the innermost group, P_1, have also been recognized. On the other hand, the middle phosphate group is very resistant to such enzymatic attacks. These situations are schematically shown in Fig. 2, in which the position of cleavage is shown by an arrow. The theoretical values for model 4 explain well the experimental position of enzymatic hydrolysis, but this model is not suitable in view of the stabilization energy, as has been stated in the previous section.

TABLE II. SUPERDELOCALIZABILITY, S_r, OF THE PHOSPHORUS ATOM FOR NUCLEOPHILIC ATTACK

Compound	The value of the Coulomb integral of the metal α_M	$S_{P_1}^{(N)}$	$S_{P_2}^{(N)}$	$S_{P_3}^{(N)}$
ADP (phosphate part only)		1.182	1.099	
ADP-metal chelate compound	$\alpha+\beta$	1.094	1.180	
	$\alpha-\beta$	1.100	1.188	
ATP (phosphate part only)		1.183	1.191	1.099
ATP-metal chelate compound model 1	$\alpha+\beta$	1.182	1.101	1.180
ATP-metal chelate compound model 2	$\alpha+3\beta$	1.094	1.102	1.181
	$\alpha+\beta$	1.093	1.101	1.180
	$\alpha-\beta$	1.100	1.106	1.188
	$\alpha-3\beta$	1.097	1.104	1.184
ATP-metal chelate compound model 3	$\alpha+\beta$	1.093	1.100	1.098
	$\alpha-\beta$	1.100	1.107	1.099
ATP-metal chelate compound model 4	$\alpha+\beta$	1.182	1.101	1.180
	$\alpha-\beta$	1.182	1.108	1.188

TABLE III. TOTAL π ELECTRON DENSITY, q_r, OF THE PHOSPHORUS ATOM

Compound	The value of the Coulomb integral of the metal α_M	q_{P_1}	q_{P_2}	q_{P_3}
ADP (phosphate part only)		0.270	0.329	
ADP-metal chelate compound	$\alpha+\beta$	0.279	0.272	
	$\alpha-\beta$	0.276	0.268	
ATP (phosphate part only)		0.269	0.268	0.329
ATP-metal chelate compound model 1	$\alpha+\beta$	0.270	0.277	0.272
ATP-metal chelate compound model 2	$\alpha+3\beta$	0.278	0.276	0.270
	$\alpha+\beta$	0.279	0.277	0.272
	$\alpha-\beta$	0.276	0.275	0.268
	$\alpha-3\beta$	0.277	0.275	0.269
ATP-metal chelate compound model 3	$\alpha+\beta$	0.279	0.278	0.329
	$\alpha-\beta$	0.276	0.275	0.329
ATP-metal chelate compound model 4	$\alpha+\beta$	0.269	0.277	0.272
	$\alpha-\beta$	0.269	0.274	0.268

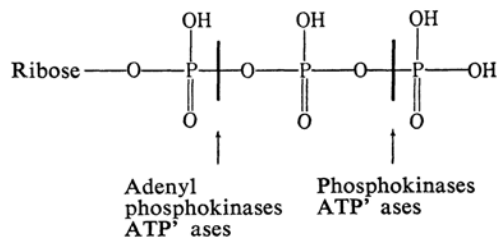

Fig. 2. Positions of the enzymatic cleavage of ATP (After Ref. 7).

In Table III the total π electron densities for various models are indicated. The smaller the value in the table, the more reactive the position for the nucleophilic attack. Also, in this case an identical conclusion is obtained as in the case of the superdelocalizability. That is, model 1 remains the most probable structure in *in vivo* reactions.

A Comparison of the Theoretical Indices with the Nuclear Magnetic Resonance Spectra of ATP.—Recently an ever-increasing number of data have been accumulated regarding the nuclear magnetic resonance (NMR) spectra of ATP, but no quantitative data for the theoretical analysis is available. Therefore, in this paper some qualitative discussions will be undertaken in comparison with the results of Cohn's experiment.[7] Cohn measured the shift in resonance frequency of the three phosphorus atoms in ATP relative to that of H_3PO_4. The shift for P_2 was largest and appeared at higher magnetic fieldes, while the spectra of P_1 and P_3 were superimposed upon each other and appeared at lower magnetic fields than those of P_2. If the chemical shift is exclusively influenced by the electron density, we can expect, on the basis of the above experiment, the following order of electron density at the phosphorus atoms in ATP: $P_2 > P_1 \sim P_3$. However, as may be seen in Table III, the degree of the electron densities in free ATP is $P_3 > P_2 \sim P_1$. This discrepancy means that the chemical shift of phosphorus atoms is influenced not only by the electron density around them, but also by other factors. That is, the contribution from the terms of the diamagnetism as well as the paramagnetism should be taken into consideration in order to explain correctly the shift of the phosphorus atom in ATP.

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Faculty of Engineering
Kyoto University
Sakyo-ku, Kyoto (*K. F.*)

Electrical Communication Laboratory
Nippon Telegraph and Telephone
Musashino, Tokyo (*A. I.*)

National Cancer Center Research Institute
Chuo-ku, Tokyo (*C. N.*)

7) M. Cohn, "Mechanisms of Enzymic Cleavage of of Some Organic Phosphates" presented at the Symposium on Enzymatic Reaction Mechanisms, held at Gatlinburg, Tennessee, April, 1959.