A Molecular Orbital Treatment of Phosphate Bonds of Biochemical Interest. I. Simple LCAO MO Treatment

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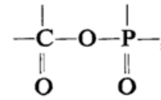
It is well known that adenosine triphosphate (ATP) is used as a catalyst in varions kinds of in vivo chemical processes. Kalckar[1] and Lipman[2] for the first time pointed out that the energy change of hydrolysis of phosphate P–O bond of ATP was much greater than that of ordinary phosphate bonds and that this energy liberated by hydrolysis might promote endergonic reactions. Thus P–O bonds of ATP were named "high energy phosphate bonds" characterized by the sign "P$\sim$O". Further, adenosine diphosphate (ADP), phosphoenol pyruvate (PEP) and acetyl phosphate (AcP) are some of the phosphates which were believed to have high energy phosphate bonds[1-4,5]; but they can hardly be used in in vivo reactions. In this they are different from ATP[5].

Recently it has been found in calorimetrical measurements by Podolsky and Morales[6] that the enthalpy change in hydrolysis of ATP is considerably smaller than the value hitherto reported and is parallel with those of simple polyphosphates. This finding has been supported by Benzinger and Kitzinger[7], and by Burton[8]. These results suggested that there might be a revision of our understanding with respect to the role of ATP[6-8].

Kalckar[1] and Lipman[2] studied the high energy phosphate bond on the basis of the mesomeric theory. Lately Ooshika[9] assumed in their quantum-chemical discussions that the phosphate bonds of ATP were of high energy. Grabe[10] calculated the charge density of the system  $-\underset{\|}{\overset{|}{\underset{O}{C}}}-O-\underset{\|}{\overset{|}{\underset{O}{P}}}-$, and discussed at which side of the bridge oxygen the bond dissociation occurred. Gillespie et al.[11] stressed the necessity of studies on this problem from the point of view of reaction kinetics.

In this paper the authors have carried out a systematic computation on the electronic energy changes, the reactivity indexes and charge distributions of four organic phosphates, ATP, ADP, PEP and AcP, with so-called "high energy" bonds and, for comparison's sake, two phosphates lacking such bonds, adenosine monophosphate (AMP) and glycerol 1-phosphate (GLP), by a simple molecular orbital approximation. From these calculated results they have concluded that, so far as the phosphate parts only are considered, the difference, if it might exist, in the energy of

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[1] H. M. Kalckar, *Chem. Revs.*, **28**, 71 (1941).
[2] F. Lipman, *Advances in Enzymol.*, **1**, 99 (1941).
[3] P. Oesper, *Arch. Biochem.*, **27**, 255 (1950).
[4] T. L. Hill and M. F. Morales, *J. Am. Chem. Soc.*, **73**, 1656 (1951).
[5] E. Baldwin, "Dynamic Aspects of Biochemistry", Cambridge Univ. Press, New York, 1951.
[6] R. L. Podolsky and M. F. Morales, *J. Biol. Chem.*, **218**, 945 (1956).
[7] T. B. Benzinger and C. Kitzinger, *Z. Naturforsch.*, **10b**, 375 (1955).
[8] K. Burton, *Nature*, **181**, 1594 (1958).
[9] Y. Ooshika, *Seitai-no-kagaku*, **7**, 322 (1955).
[10] B. Grabe, *Biochim. Biophys. Acta*, **30**, 560 (1958); *Archiv Fysik*, **15**, 207 (1959).
[11] R. J. Gillespie, G. Maw and C. A. Vernon, *Nature*, **171**, 1147 (1953).

hydrolysis between two kinds of phosphates can not be attributed to the difference of π electronic energy change, though their reactivity is somewhat different.

Method of Calculation

The calculation is carried out in the approximation of the simple LCAO MO (linear-combination-of-atomic-orbitals molecular orbital) treatment neglecting overlap. For AcP and PEP the conjugation is spreading throughout the molecule. For ATP, ADP and AMP, on the other hand, phosphate parts only are taken into account, i. e., the adenine part, which is separated from the phosphate part by the non-conjugating ribose, is assumed not to be interacting with the phosphate part. On this assumption a few essential discussions will be given in the following section. AMP, GLP and other low energy phosphates come to have the same conjugated system in this approximation. Phosphorus atoms in the phosphates have a nearly tetrahedral, sp^3-hybridized configuration, with one electron jumping up into one of $3d$ orbitals. Strictly speaking, $2p$ π electrons on the neighboring oxygen atoms can interact not only with these d orbitals but also, though presumably only slightly, with the sp^3-hybridized orbitals[12,13]. Taking into account all of these interactions, however, complicates the problems extremely and makes it practically impossible to treat large molecules of interest. We conveniently assume, therefore, that only one hybridized d orbital on a phosphorus atom, say e. g. d^3s hybridized orbital which can afford to give a directed valence of tetrahedral symmetry, conjugates with $2p\pi$ orbitals of the neighboring oxygens in this simple treatment. Accordingly ATP, for instance, is regarded as a system consisting of 13 orbitals and 20 electrons.

The Coulomb integral of the atom or group X, that of the carbon atom attached to X and the resonance integral between that carbon atom and X are written as $\alpha+a_X\beta$, $\alpha+a_r\beta$ and $l\beta$, respectively, where α and β are the Coulomb and the resonance integrals of a carbon atom and a C–C bond in benzene, respectively. Since the precise value of a_{-O-} seems unsettled, two parallel computations are carried out for two a_{-O-} values. In Table I, together with a_X's of oxygen atoms in the phosphate part, are listed the numerical values of a_X, a_r and l adopted by us, which do not differ so much from those which were previously used by the present authors[21,22] and others[15,16]. If some oxygen atoms assigned to different a_X's should be considered to be equivalent, the mean value of their a_X's is employed; for instance, a_X of each oxygen atom in the $-PO_3^{2-}$ group is regarded as 1.0 and that in the $-PO_2^-$ group as 1.25.

TABLE I. PARAMETERS USED IN THE CALCULATION

1. Phosphate part

X	a_X
$=O$	2.0
$-O^-$	0.5
$-O-$	0.5, 2.0

2. Others

X	a_X	a_r	l
$=O$	2.0	0.2	$\sqrt{2}$
$-OH$	0.5	0	0.6
$-CH_3$	3.0	−0.1	1

The resonance integral between a phosphorus atom and an oxygen atom, β_{P-O}, is tentatively taken 0.6 β, regardless of the bonding states of the oxygen atom (–O– type, O=type or $-O^-$ type) from a rather simple theoretical consideration on the overlap integral[13], but the numerical value itself is not essential to our results of calculation, as will be stated below. Furthermore, it is hardly possible to decide semiempirically the Coulomb integral of the phosphorus atom in the valence state in question, $\alpha_P=\alpha+a_P\beta$, because of the lack of sufficient experimental data and knowledge on the d electron phenomena. Therefore in order to avoid the unreliability of the results caused by a rather arbitrary choice of a_P, we vary the a_P value as widely as possible, as 0, −0.2, −0.4, −0.6, −1.0, −1.5, −2.0, −3.0 and $-\infty$. It should be stressed concerning this point that by means of this sliding of a_P values we can practically afford to evade not only the ambiguity of the a_P value but also the arbitrariness of the β_{P-O} value and moreover, partly, the error due to the above-stated conventional choice of the orbitals on a phosphorus atom, at least for ATP, ADP and AMP where conjugated systems are consisting of only phosphorus and oxygen atoms. In this sense the present treatment has a very general character. Judging from the ionization potential of a $3p$ electron ($I=10.98$ eV.)[10] and from Pauling's electronegativity scale ($\chi_P=2.1$), −0.6 or smaller would be appropriate for the real a_P value of a hybridized d electron.

The present authors have previously presented the frontier electron theory and have succeeded in interpreting theoretically the experience on

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[12] R. S. Mulliken, C. A. Rieke, D. Orloff and H. Orloff, *J. Chem. Phys.*, **17**, 1248 (1949).

[13] H. H. Jaffé, *J. Phys. Chem.*, **58**, 185 (1954).

[14] C. Nagata, K. Fukui, T. Yonezawa and Y. Tagashira, *Cancer Resarch*, **15**, 233 (1955).

[15] H. H. Jaffé, *J. Chem. Phys.*, **20**, 279 (1952).

[16] L. E. Orgel et al., *Trans. Faraday Soc.*, **47**, 113 (1951).

the chemical reactivity of π electron system[17-21]. Here we do not repeat the details of the theory but we mention only the fact that the superdelocalizability, S_r, has been known as a good intermolecular reactivity index for the substitution and the addition reactions. Also for hydrolysis of phosphates S_r is expected to remain a good measure of reactivity and is used in the present paper for discussing the ease of hydrolysis of phosphates.

Results and Discussion

The π Electronic Energy Change of Hydrolysis.—The results of calculation of the π electronic energy change of hydrolysis for two a_{-O^-} values and various a_P values are listed in Table II. The listed value, $-\Delta E$, is expressed as the difference of π electronic energy between the hydrolysis products and the original reactant excepting water. For ATP, for instance, it becomes

$$-\Delta E=E(\mathrm{ADP})^{3-}+E(\mathrm{PO_4}^{3-})-E(\mathrm{ATP})^{4-}$$

The electronic energy of water is not included in this expression because it is a constant and can not precisely be evaluated. Therefore, not the absolute value of ΔE but its difference between the two kinds of phosphate or between ATP and other phosphates has practical meaning in further discussions.

As clearly seen in Table II, ΔE of each compound does not vary so much according to a_P values. Furthermore it should be noticed that its difference between the two kinds of phosphate bonds does not exceed -0.2β for any pair of a_P and a_{-O^-} values. This maximum value corresponds to ATP when a_P is zero and $a_{-O^-}=2.0$. The difference for $a_P=-1.0$, a most probable value of a_P, is about $-0.12\ \beta$. Putting β as -20 kcal./mol., it corresponds to ca. 2.5 kcal./mol. If β_{P-O} is taken to be $0.8\ \beta$, it becomes ca. 3 kcal./mol. by changing the energy value of β. Thus it might be said that in the difference of the free energy change of hydrolysis between the two kinds of phosphate only 2.5 to 3 kcal./mol. at most can be ascribed to the π electronic energy.

Consequently it is concluded that, so far as phosphate parts only are taken into account, the peculiarity of ATP in several in vivo reactions cannot be attributed to the electronic energy.

Superdelocalizability for a Nucleophilic Attack and the Reactivity of Phosphate Bond.—By this time most contributions to the problem of these organic phosphate bonds of biochemical interest have been carried out from the

TABLE II. π-ELECTRONIC ENERGY CHANGE OF HYDROLYSIS

1) $a_{-O^-}=0.5$.

Class	Compound	$-\Delta E$								
	$a_P=$	0	-0.2	-0.4	-0.6	-1.0	-1.5	-2.0	-3.0	$-\infty$
Low energy	AMP, GLP etc.	$2\alpha+1.143\beta$	$2\alpha+1.168\beta$	$2\alpha+1.173\beta$	$2\alpha+1.176\beta$	$2\alpha+1.182\beta$	$2\alpha+1.187\beta$	$2\alpha+1.191\beta$	$2\alpha+1.195\beta$	$2\alpha+1.200\beta$
High energy	ATP (I)[a]	1.176	1.198	1.199	1.199	1.199	1.199	1.199	1.199	1.200
	ATP (II)[b]	0.976	0.999	1.000	1.001	1.002	1.002	1.001	1.001	1.000
	ADP	1.174	1.196	1.197	1.198	1.199	1.199	1.199	1.199	1.200
	AcP	1.181	1.201	1.201	1.201	1.200	1.197	1.195	1.192	1.175
	PEP	1.163	—	1.193	—	1.201	—	1.206	1.207	1.200

2) $a_{-O^-}=2.0$.

Class	Compound	$-\Delta E$		
	$a_P=$	0	-1.0	-2.0
Low energy	AMP, GLP etc.	$2\alpha-1.654\beta$	$2\alpha-1.697\beta$	$2\alpha-1.735\beta$
High energy	ATP (I)[a]	-1.446	-1.575	-1.659
	ATP (II)[b]	-1.614	-1.763	-1.854
	ADP	-1.449	-1.576	-1.659

[a] The end "high energy" phosphate bond.

[b] The central "high energy" phosphate bond.

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17) K. Fukui, T. Yonezawa and H. Shingu, *J. Chem. Phys.*, **20**, 722 (1952).

18) K. Fukui, T. Yonezawa, C. Nagata and H. Shingu, ibid., **22**, 1433 (1954).

19) K. Fukui, T. Yonezawa and C. Nagata, This Bulletin, **27**, 423 (1954).

20) K. Fukui, T. Yonezawa and C. Nagata, *J. Chem. Phys.*, **26**, 831 (1957).

21) K. Fukui, T. Yonezawa and C. Nagata, *J. Am. Chem. Soc.*, **80**, 2268 (1958).

TABLE III. SUPERDELOCALIZABILITY OF THE PHOSPHORUS ATOM FOR NUCLEOPHILIC ATTACK

1) $a_{-O-}=0.5$.

Class	Compound		$S_P^{(N)}$							
		$a_P=$ 0	-0.2	-0.4	-0.6	-1.0	-1.5	-2.0	-3.0	$-\infty$
Low energy	AMP, GLP etc.	1.555	1.412	1.290	1.185	1.012	0.848	0.724	0.553	0.000
High energy	ATP (I)[a]	1.733	1.525	1.362	1.236	1.037	0.860	0.730	0.555	0.000
	ATP (II)[b]	1.810	1.576	1.401	1.259	1.049	0.865	0.732	0.556	0.000
	ADP	1.708	1.513	1.359	1.233	1.036	0.858	0.730	0.555	0.000
	AcP	1.696	—	1.371	—	1.054	—	0.728	0.561	0.000
	PEP	1.600	—	1.322	—	1.031	—	0.734	0.559	0.000

2) $a_{-O-}=2.0$.

Class	Compound	$S_P^{(N)}$		
		$a_P=$ 0	-1.0	-2.0
Low energy	AMP, GLP etc.	1.907	1.092	0.749
High energy	ATP (I)[a]	1.956	1.099	0.751
	ATP (II)[b]	2.511	1.191	0.778
	ADP	1.954	1.099	0.751

[a] The end "high energy" phosphate bond.

[b] The central "high energy" phosphate bond.

energetical point of view. But it seems important and interesting at this juncture for us to discuss the reactivity or the ease of hydrolysis of phosphate bonds. Hydrolysis of phosphates would be initiated by the attack of a hydroxyl anion to the phosphorus atom in vivo or of some nucleophilic group or site of tissue in vivo. So the superdelocalizability, $S_P^{(N)}$, of the phosphorus atom for a nucleophilic attack is expected to represent a theoretical index to the ease of hydrolysis. In Table III are collected the results of the calculation of superdelocalizability for a nucleophilic attack for various a_P values. The larger the $S_P^{(N)}$ is, the more reactive for a nucleophilic attack is the phosphorus atom.

As is seen in Table III, the value of $S_P^{(N)}$ of each compound is reduced markedly with the decrease of a_P values; but it is noticed that comparison of $S_P^{(N)}$ of various compounds at the same a_P value shows us some difference between the two kinds of phosphate bond, which decreases with the decrease of a_P, as 0.05 to 0.2 when $a_P=0$, 0.04 to 0.1 when $a_P=-0.4$, 0.02 to 0.04 when $a_P=-1.0$, and less than 0.01 when $a_P=-2$ and $a_P=-3$. Even larger differences of the superdelocalizability, which are realized for larger values of a_P (smaller in the absolute value), would not be sufficient to cause a casual difference of actual ease of hydrolysis. Nor could the conspicuous role of ATP be attributed to this difference, for the difference for ATP is not at all markedly greater than that for other "high energy" phosphates*. So far as phosphate parts only are considered, it would be concluded that, though some distinction can be seen in the reactivity for hydrolysis between the "high energy" bond and the ordinary one, it does not seem great enough to cause a serious difference in the role in biochemical processes.

Total π Electron Density of Oxygen Atoms.—In some biochemical problems of reactivity of conjugated molecules, effects of subsidiary atoms and bonds in the compounds were often noticed to play an essential part as well as some steric factors. A few examples of this can be seen in the carcinogenic activity of condensed aromatic hydrocarbons[14)] and dimethylaminoazobenzene derivatives[22)] and also in auxin activity of benzoic acid derivatives[21)]. In this sense the total π electron density of oxygen atoms (q_{-O-} and q_{-O^-}) attached to a phosphorus atom may be expected to prevent electrostatically a nucleophilic reagent from approaching the phosphorus atom, where $-O-$ and $-O^-$ stand for an oxygen atom on the phosphate bond to be broken and an oxygen anion attached to the phosphorus atom on that bond, respectively. The calculation of them has been carried out only when a_P is

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* Of the two so-called high energy bonds in an ATP molecule, the inner one seems to be of very low energy (about 4 kcal. lower than the outer) from an energetic point of view, whereas the value of $S_P^{(N)}$ shows that the inner is the more reactive. It would not be possible to decide from this calculation, therefore, which bond is the easier to break.

22) C. Nagata, K. Fukui, T. Yonezawa, H. Kitano, Y. Inamoto, K. Kanai and Y. Tagashira, *Gann*, **46**, 346 (1955).

TABLE IV. TOTAL π ELECTRON DENSITY AND SUPERDELOCALIZABILITY OF OXYGEN ATOMS FOR ELECTROPHILIC ATTACK[a]

1) $a_{-O-}=0.5$.

Class	Compound		$S_{-O-}^{(E)}$	q_{-O-}	$S_{-O^-}^{(E)}$	q_{-O^-}
Low energy	AMP, GLP etc.		3.055	1.868	1.797	1.914
High energy	ATP	(I)[b]	2.508	1.773	1.783	1.912
		(II)[c]	2.550	1.773	1.475	1.925
	ADP		2.546	1.783	1.818	1.963

2) $a_{-O-}=2.0$.

Class	Compound		$S_{-O-}^{(E)}$	q_{-O-}	$S_{-O^-}^{(E)}$	q_{-O^-}
Low energy	AMP, GLP etc.		0.952	1.952	1.743	1.991
High energy	ATP	(I)[b]	0.900	1.906	1.742	1.990
		(II)[c]	0.878	1.903	1.417	1.991
	ADP		0.900	1.906	1.742	1.991

[a] O^- stands for the three or the two equivalent oxygens in $-PO_3{}^{2-}$ or $-PO_2{}^-$; $-O-$ stands for the bridge oxygen in phosphate bonds.

[b] The end "high energy" phosphate bond.

[c] The central "high energy" phosphate bond.

-1, since the choice of the value of a_P was proved in the previous paragraph not to affect the results so seriously.

On inspection of the fourth and the sixth columns of Table IV, ATP and ADP would be understood to be to a small extent preferential in hydrolysis, since the interference by the electron density in it must be smaller than in AMP.

Superdelocalizability of the Oxygen Atom for Electrophilic Attack.—In this case assuming, as was not done in the preceding paragraphs, that the initial stage of hydrolysis is the attack of proton or an electrophilic group upon the bridge oxygen atom of the phosphate bond, the superdelocalizability of the oxygen atom, $S_O^{(E)}$, for an electrophilic attack was calculated and listed in the third column of Table IV. The order of $S_{-O-}^{(E)}$ cannot explain the singularity of ATP in biochemical reactions. Nor can the order of the superdelocalizability of oxygen, $S_{-O^-}^{(E)}$, do so as clearly seen in the fifth column of Table IV.

In the foregoing discussion we found that the "high energy" phosphate bonds are not of electronically high energy, though to some extent they are more reactive, and that the conspicuous role which ATP exclusively plays in in vivo chemical reactions cannot be explained by these simple models of phosphates.

Two things must be investigated prior to the ascertainment of this conclusion. One of them is that the calculations in the present paper have been performed in the simple LCAO MO approximation: that is to say, no electron interaction has been explicitly taken into account. Interactions to be considered would occur also among $2p\pi$ electrons on a phosphorus atom. As stated in the previous section, however, since the wide sliding of a_P values from zero to minus infinity would considerably diminish the unreliability of this approximation, no serious alteration of results could be expected even in a more precise treatment.

The other is more essential. In the present paper AMP, ADP and ATP have been treated as if they were composed of only polyphosphate parts. No attention has been paid to the other conjugated part of the molecule, the adenine part, which is intervened and separated by the non-conjugated ribose group from the polyphosphate parts. This large conjugated system, however, would have some interaction with the polyphosphate part. Levendahl and James[23] found in their study on the rotatory dispersion of AMP, ADP and ATP that in ATP the last two phosphate groups and the amino group of adenine part were interacting, whereas no such interaction was noticed in the others, and suggested that this stabilized structure would an important significance in the peculiarity of the function of ATP. Szent-Györgyi[24] surmised that, as for ATP, among the 6-amino group and the 7-nitrogen of adenine parts and the two oxygens of the last two phosphate parts, the magnesium cation might intervene, through which the conjugation would spread over the molecule.

From the above calculations and discussions

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[23] B. H. Levendahl and J. W. James, *Biochim. Biophys. Acta*, 21, 298 (1956).

[24] A. Szent-Gyorgyi, "Biogenetics", Academic Press, New York, 1957.

we might say that the peculiar in vivo behavior of ATP must not be attributed to the polyphosphate part itself, but to the whole system including the polyphosphate part and the adenine part, interacting with each other directly or through a metal cation.

In the latter case the interaction of the polyphosphate part with a metal cation would considerably enhance the reactivity of its phosphorus atoms for a nucleophilic attack. Forming a weak bonding, metal cation will help them to interact. Calculation under the explicit consideration of these interactions is now going to be carried out with respect to several models for different interacting positions, extents of interaction and roles of metal cations. This will be published elsewhere.

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