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Diamagnetic Susceptibilities of Various Inorganic Phosphates and Organic Phosphate Esters

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The diamagnetic susceptibilities of various inorganic phosphates as well as various organic phosphate esters, such as ATP, ADP, and phosphocreatine, were determined by Gouy's method. The diamagnetic susceptibility of H_3PO_4 was estimated to be $-43.5\times10^{-6}\,cm^3 mol^{-1}$ from plots of the diamagnetic susceptibility against the total number of electrons in the cation by measuring various inorganic phosphates. The free-energy changes (ΔG's) for the hydrolysis of the phosphate ester were found to correlate with the differences in the diamagnetic susceptibilities between the phosphate esters and their products of hydrolysis. The diamagnetic susceptibility of $:PO_3H_2$ was obtained to be -28—$39\times10^{-6}\,cm^3\,mol^{-1}$, which decreased with increasing ΔG.

There are abundant results concerning the diamagnetic susceptibilities of various organic and inorganic compounds, as well as those of mixtures.[1—9] It is well known that it is possible to estimate the diamagnetic susceptibilities of organic compounds by using an additive property of the magnetic susceptibility (Pascal' additive law).[1] On the other hand, the diamagnetic susceptibilities of organic phosphate esters have been scarcely reported, except for that of phosphatidylcholine.[10]

One of the purposes of this paper was to determine every molar diamagnetic susceptibility (χ_M) of the phosphate esters, such as ATP, ADP, glucose 6-phosphate, and phosphocreatine. Another was to determine the difference between the χ_M of the phosphate ester and χ_M of its product of hydrolysis ($\Delta\chi$), which was obtained by the following equation:

$$\chi(RO:PO_3H_2) - \chi(RO:H) = \Delta\chi, \qquad (1)$$

where $\chi(RO:PO_3H_2)$ and $\chi(RO:H)$ denote the χ_M of the phosphate ester and the χ_M of the product of hydrolysis, respectively. Since the magnetic susceptibility exhibits an additive property, Eq. 1 can be replaced by

$$\Delta\chi = \chi(:PO_3H_2) - \chi(:H)$$

and

$$= \chi(PO_3H_2^+) - \chi(H^+), \qquad (2)$$

where $\chi(:PO_3H_2)$, $\chi(:H)$, $\chi(PO_3H_2^+)$, and $\chi(H^+)$ denote the χ_M of $:PO_3H_2$, the χ_M of :H, the χ_M of $PO_3H_2^+$ and the χ_M of H^+, respectively. The notation, ":" in $:PO_3H_2$ and :H denotes a non-bonding pair of electrons. $\chi(:PO_3H_2)$ can be expressed as

$$\chi(:PO_3H_2) = \Delta\chi + \chi(:H) \qquad (3)$$

from Eq. 2.

It is widely known that the ΔG's for the hydrolysis of ATP and phosphocreatine are higher than that for the other reaction, since the electronic configuration of the P–O bond for ester formation makes different from that of other bonds due to a resonance hybrid of the phosphate group. The diamagnetic susceptibilities of the substances reflect the electronic configuration, including the chemical bond. ΔG can be considered as being energy related to the electronic configuration. It was considered that $\Delta\chi$ in Eq. 1 could be determined by an extrapolation to $\Delta G=0$ in the presence of a nonbonding pair of electrons between P and O in the phosphate ester. The relationship between $\chi(:PO_3H_2)$ and ΔG for the hydrolysis of the phosphate ester was examined.

Experimental

Measuring Apparatus. The diamagnetic susceptibilities were measured by Gouy's method at 25±1°C in an air-conditioned room. An electronic balance (A & D, ER-182A) was used. The permanent magnet used had a maximum field strength of 7400 Oe ($1\,Oe=10^3 A\ m^{-1}$) with a 1.5 cm gap of the pole pieces, whose diameter was 5 cm across.

Materials. All of the inorganic phosphates used were of guaranteed grade, or the best commercially grade available. Disodium salts of various phosphate esters were used, except for monosodium salt of FMN. The contents of water and sodium were determined by measuring the dry weight at 110—120°C, and by using a sodium ion-selective electrode. The organic components, such as glucose and adenosine, were determined by various enzyme methods, liquid chromatography and spectrophotometry. The results are summarized in Table 1. The liquid chromatograms showed one peak for both AMP and ATP, and two peaks for ADP. In the case of ADP, it was estimated from the retention time and peak area that one can be assigned as 97.7% ADP, and the other as 2.3% AMP. By using Wiedemann's additive law,[1] the χ_M of ADP was revised to be $-196\times10^{-6}\,cm^3\,mol^{-1}$. The empirical formula given in Table 1 was derived within 4% error by taking into consideration of all of the analytical results. The already known diamagnetic susceptibilities were used to calculate the χ_M values of various compounds.[11] The χ_M values of H_2O, Na^+, H_2, and H^+ were known to be -12.96, -5, -3.96, and $0\times10^{-6}\,cm^3\,mol^{-1}$. From a calculation based on the additive property, the $\chi(:H)=\chi(H_2)-\chi(H^+)=-4.0\times10^{-6}\,cm^3\,mol^{-1}$ was obtained. Various χ_M of the phosphate esters were determined by excluding the diamagnetic susceptibilities of both crystal water and sodium ion.

Table 1. Analytical Results on Organic Phosphate Esters

	Water	Na	Enzyme method	Total	Spectro-photometry	Experimental formula	Enzyme used[a]
	%	%	%	%	%		
AMP 2Na	7.34	10.2	82.1	99.6	93.6	$C_{10}H_{12}N_5O_7P$ $Na_{1.86}$ $1.72H_2O$	MK, PK, LDH
ADP 2Na	6.76	8.38	81.8	96.9	96.1	$C_{10}H_{13}N_5O_{10}P_2$ $Na_{1.89}$ $1.95H_2O$	PK, LDH
ATP 2Na	6.44	6.97	82.5	95.9	98.9	$C_{10}H_{14}N_5O_{13}P_3$ $Na_{1.86}$ $2.19H_2O$	HK, G6P-DH
Glucose 6-phosphate 2Na	11.9	11.0	73.4	96.3		$C_6H_{11}O_9P$ $Na_{1.68}$ $2.32H_2O$	G6P-DH
Glucose 1-phosphate 2Na	5.08	12.9	72.5	90.5		$C_6H_{11}O_9P$ $Na_{2.00}$ $1.01H_2O$	G6P-DH, PGM
Phosphocreatine 2Na	21.2	14.1	63.4	98.7		$C_4H_8N_3O_5P$ $Na_{2.02}$ $3.88H_2O$	CK, HK, G6P-DH
FMN Na	15.9	5.26				$C_{17}H_{20}N_4O_9P$ $Na_{1.09}$ $4.21H_2O$	

a) MK: Adenylate kinase, PK: Pyruvate kinase, LDH: Lactose dehydrogenase, HK: Hexokinase, G6P-DH: Glucose 6-phosphate dehydrogenase, CK: Creatine kinase, PGM: Phosphoglucomutase. Molar absorption coefficient of AMP, ADP, and ATP: 15.4×10^3 at 260 nm.

Results and Discussion

The diamagnetic susceptibilities of various inorganic phosphates were determined, as shown in Table 2. The values of the molar susceptibilities were plotted against the total number of electrons in the cation of each salt in the usual way (Fig. 1).[1] From the intercept of Fig. 1, the χ_M of H_3PO_4 was obtained to be -43.5×10^{-6} $cm^3\,mol^{-1}$. In a similar way, the χ_M of pyrophosphoric acid was obtained to be -79.0×10^{-6} $cm^3\,mol^{-1}$ by subtracting the diamagnetic susceptibility of sodium $(4\times(-5\times10^{-6})$ $cm^3\,mol^{-1})$ from the χ_M of $Na_4P_2O_7$. Since pyrophoshphoric acid hydrolyzes to form phosphoric acid, Eq. 1 is modified to

$$\chi(H_2PO_3O : PO_3H_2) - \chi(H_2PO_3O : H)$$
$$= \chi(:PO_3H_2) - \chi(:H) = \Delta\chi, \qquad (4)$$

where $\chi(H_2PO_3 : O : PO_3H_2)$ and $\chi(H_2PO_3O : H)$ are expressed as the diamagnetic susceptibilities of pyrophosphoric acid and phosphoric acid, respectively. From Eq. 4 and the intercept in Fig. 1, we obtained $\chi(:PO_3H_2) = -39\times10^{-6}$ $cm^3\,mol^{-1}$.

Table 3 shows the χ_M of various phosphate esters (excluding the diamagnetic susceptibilities of both crystal water and sodium ion). The relationship between the $\Delta\chi$ and ΔG for the hydrolysis is given in Fig. 2. Well-known values of ΔG are quoted from a textbook of biochemistry. The χ_M of the phosphoric ester group decreased with increasing ΔG. By extrapolating the diamagnetic susceptibility at $\Delta G = 0$ in Fig. 2, $\Delta\chi$ became -34.5×10^{-6} $cm^3\,mol^{-1}$, which, in turn, yielded $\chi(:PO_3H_2) = -38.5\times10^{-6}$ $cm^3\,mol^{-1}$ by Eq. 3. The P–O bonding electrons were considered to be in the same situation as a non-bonding pair of the electrons at $\Delta G = 0$. Since $\chi(:PO_3H_2) + \chi(O) = \chi(H_2PO_4^-)$, the χ_M of $H_2PO_4^-$ was obtained to be -44×10^{-6} $cm^3\,mol^{-1}$ by adding the χ_M of oxygen atom ($\chi(O) = -5.3\times10^{-6}$ $cm^3\,mol^{-1}$) to $\chi(:PO_3H_2)$. This is in accord with the χ_M of $H_2PO_4^-$, obtained from inorganic phosphates.

Table 2. Diamagnetic Susceptibility of Inorganic Phoshphates

Phosphate	$-\chi_M$	$-\chi_M/10^{-6}$ $cm^3\,mol^{-1}$ for PO_4^{-3}, HPO_4^{-2}, $H_2PO_4^-$
	10^{-6} $cm^3\,mol^{-1}$	obtained by calculation [a]
Li_3PO_4	44.4	42.6
Na_3PO_4	60.3	45.3
Na_2HPO_4	54.7	44.7
NaH_2PO_4	49.1	44.1
K_3PO_4	84.3	45.3
K_2HPO_4	71.1	43.7
KH_2PO_4	57.0	44.0
$(NH_4)_2HPO_4$	67.5	44.5
$(NH_4)H_2PO_4$	56.2	44.7
$Na_4P_2O_7$	99.0	—

a) The values, 0, −0.6, −5, −13, -11.5×10^{-6} $cm^3\,mol^{-1}$, respectively were used as χ_M of H^+, Li^+, Na^+, K^+, NH_4^+.[11]

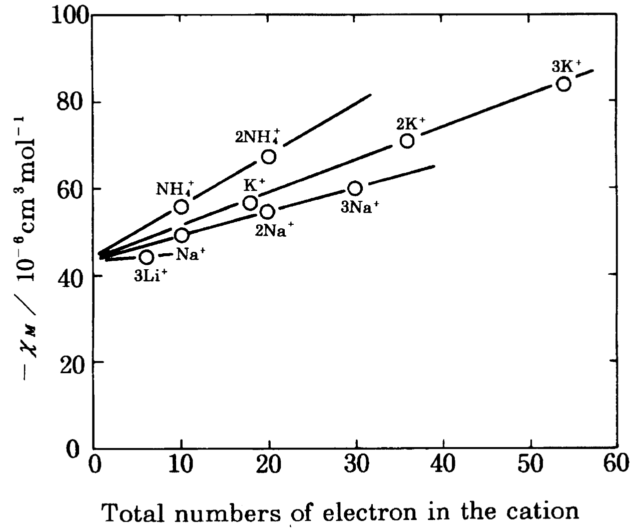

Fig. 1. Relationship between the molar diamagnetic susceptibilities of various salts and the total number of electrons in the cation.

It was supposed that both ΔG and the diamagnetic susceptibility might be influenced by the electronic configuration of the P–O bond, which, in turn, might be influenced by the magnetic field. The maximum $\Delta\chi$ at $\Delta G = 0$ in Fig. 2 was assigned to be the diamag-

Table 3. Diamagnetic Susceptibility of Phosphate Esters and Their Product of Hydrolysis

Phosphate ester (A)	$-\chi_M$ / 10^{-6} cm^3 mol^{-1}	Product of hydrolysis(B)	ΔG for A→B$^{a)}$ (kJ mol^{-1})
Adenosine	139		
AMP	171	Adenosine	9.21
ADP	196	AMP	29.3
ATP	226	ADP	29.3
Glucose	99.3		
Glucose 1-phosphate	135	Glucose	20.9
Glucose 6-phosphate	131	Glucose	13.8
Creatine	69.9		
Phosphocreatine	93.4	Creatine	43.1
Riboflavin	196		
FMN	226	Riboflavin	—

a) Cited from the textbook of biochemistry.

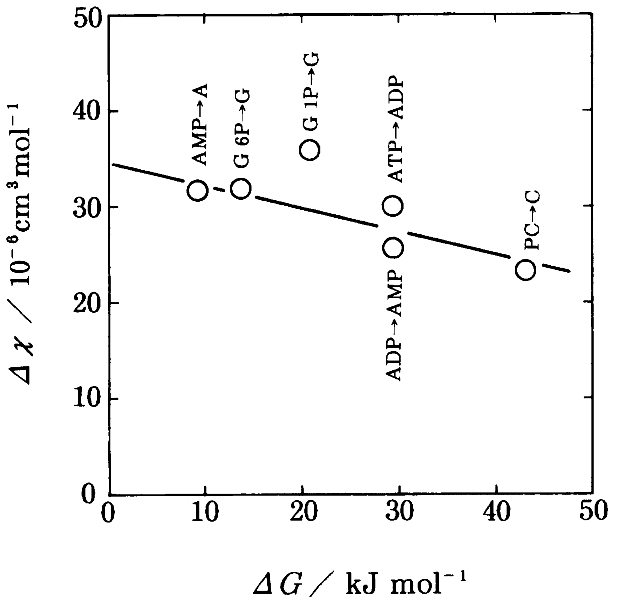

Fig. 2. Relationship between $\Delta\chi$ and ΔG. A: Adenosine, G: Glucose, G 6-P: Glucose 6-phosphate, G 1-P: Glucose 1-phosphate, C: Creatine, PC: Phosphocreatine.

netic susceptibility including a non-bonding pair of electrons in :PO_3H_2. On the other hand, the minimum $\Delta\chi$ should be involved in the highest energy-reserved chemical bond for a pair of electrons in :PO_3H_2. The difference of $\Delta\chi$ between the maximum and minimum values in Fig. 2 is about 12×10^{-6} cm^3 mol^{-1}. This difference must be in accord with the diamagnetic susceptibility of the bonding pair of electrons.

The difference of χ_M between FMN and riboflavin is 30×10^{-6} cm^3 mol^{-1} from Table 3. By inserting this difference in Fig. 2, ΔG for the hydrolysis of FMN to produce riboflavin was estimated to be about 19 kJ mol^{-1}. If the correlation given in Fig. 2 were to be generally accepted, the values of ΔG, or the bond energy, could be estimated by measuring the diamagnetic susceptibility.

There has been no paper concerning the relationship between ΔG and $\Delta\chi$ to date. Further investigation is necessary regarding the above-mentioned speculation.

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