A QUANTITATIVE STUDY OF ORGANIC PHOSPHATE-AMINE INTERACTION BY ^{31}P NUCLEAR MAGNETIC RESONANCE

Robert Katz,† Herman J. C. Yeh* and David F. Johnson

Section on Microanalytical Services and Instrumentation
Laboratory of Chemistry, NIAMDD, NIH, Bethesda, Md. 20014

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Summary

Interactions between the phosphate group of 4-deoxypyridoxine 5'-phosphate and different protonated amines were quantitatively measured by means of $\{^{31}P\}-^{1}H$ nuclear magnetic double resonance technique combined with pD titration. An interaction of the phosphate group with added amine resulted in a measurable difference in the ^{31}P chemical shift of these phosphate-containing samples with and without amine [$\Delta\delta(^{31}P)$]. Basic amino acids and biogenic amines had significant measurable $\Delta\delta(^{31}P)$ values. No interactions were observed for acidic or neutral α, β and γ-amino acids.

A detailed and systematic knowledge of the interaction between the group of various coenzymes, among them vitamin B_6 phosphates, with apoenzymes[1-6] as well as interactions between biogenic amines and adenosine triphosphate[7-11] could provide a basis for the development of new drugs and enzyme inhibitors. It could also contribute to the elucidation of many enzymatic processes and nucleic acid synthesis. ^{1}H Nmr has been used to study the association between biogenic amines and organic phosphate-containing substrates.[12-14] Results of these studies implied the existence of phosphate-amine interactions.[12,13] ^{31}P Nmr is more suited for the study of these interactions than ^{1}H nmr due to the broader scale of chemical shift

†Visiting Fellow 1971-1973. Andrulis Research Corp., 4720 Montgomery Lane, Bethesda, Maryland 20014.
*To whom correspondence should be addressed.

obtained for this nucleus. Using a $\{^{31}P\}$-^{1}H nmdr technique[15] and pD titration[1] on a modified Varian HA-100 nmr spectrometer,[16] we wish to report here a quantitative evaluation of these interactions, thus indicating the greater usefulness of the ^{31}P over the ^{1}H nmr method for this purpose. 4-Deoxypyridoxine 5'-phosphate (4-dPNP)[17] was used as the phosphate containing model compound. Biogenic amines, amino acids and dipeptides were used as protonated amine-containing substrates.

Fig. 1 shows a typical ^{31}P nmr NaOD titration curve[18] of a 0.04M 4-dPNP solution in D_2O with and without L-histidine. In the titration curve obtained for ^{31}P, the irradiation frequency was taken such that a maximum decoupling

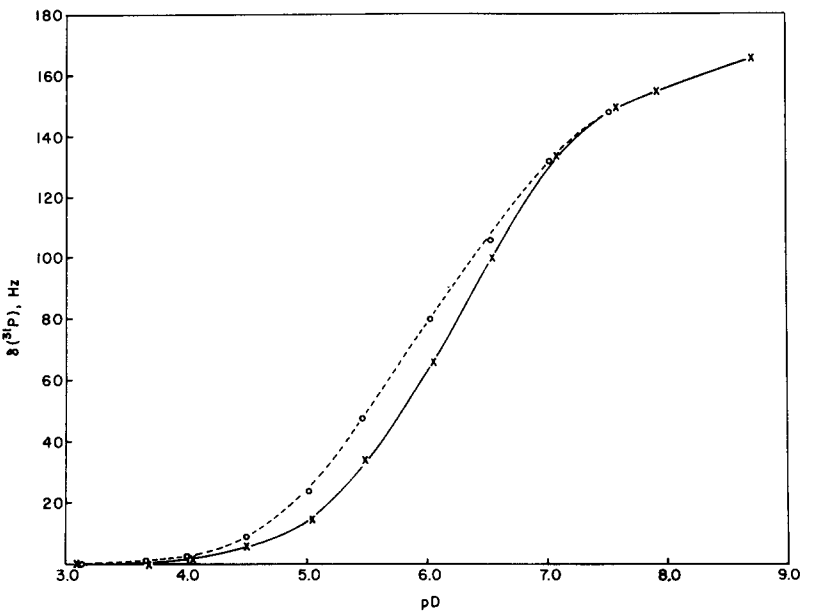

Figure 1. pD dependence of ^{31}P shift in Hz (measured at 23,487 Gauss) for a 0.04 M solution of 4-deoxypyridoxine 5'-phosphate (4-dPNP) in D_2O without (—X—X—) and with (—O—O—) L-histidine (4-dPNP:L-histidine = 1:3). A 5% TMS in CCl_4 solution was used as external ^{1}H lock.

1. pD values are direct readings from a Radiometer Copenhagen pH meter equipped with a Scale Expander.

effect [i.e. collapse of the 5-CH_2 doublet[19]] was observed. The frequency difference between a 4-dPNP reference sample (kept at pD 3.00) and a titrated 4-dPNP sample, at various pD values was referred to as $\delta(^{31}P)$. An increase in $\delta(^{31}P)$ indicates a downfield shift. These titration curves follow the change in the equilibrium between the monobasic and the dibasic phosphate ions. As long as this equilibrium is affected by the presence of the amine, the difference in $\delta(^{31}P)$ between the two curves becomes measurable. The difference between $\delta(^{31}P)$ of 4-dPNP with and without various protonated amine-containing substrates at constant pD (6.00-6.20) was defined as $\Delta\delta(^{31}P)$.

The value of $\Delta\delta(^{31}P)$[2] was affected by the nature of the amine, its concentration, and the ionic strength of additional cations present (Na^+ was added for pD adjustment[3]). Typical results of $\Delta\delta(^{31}P)$ vs. amine concentrations are shown in Fig. 2. The apparent association constants of L-norepinephrine·HCl, L-arginine·HCl and L-lysine·HCL (or L-histidine) to 4-dPNP calculated from these curves were found to be about 8.0, 5.0 and 3.0 M^{-1}, respectively. Thus, L-norepinephrine·HCl gave stronger interaction than L-lysine·HCl or L-arginine·HCl due to, among other factors, aromatic ring interactions.

Monomethyl phosphate (MMP) was used in a second set of measurements in order to characterize these possible intermolecular aromatic ring interactions. Some of the $\Delta\delta(^{31}P)$ values determined for both 4-dPNP and MMP with biogenic amines and amino acids are listed in Table I. The following general conclusions can be drawn from these data:

a) Biogenic amines and basic amino acids show significant interaction with the phosphate group.

b) The $\Delta\delta(^{31}P)$ value for an amine can be dependent on the nature of the phosphate containing substrates.

c) Substrates with 2 or more interacting amino groups show greater

2. A 0.2 pD unit lowering of the pK_{a2} value corresponding to a $\Delta\delta(^{31}P)$ of about 12.0 Hz (at 23,487 Gauss).

3. For details see legend to Table I.

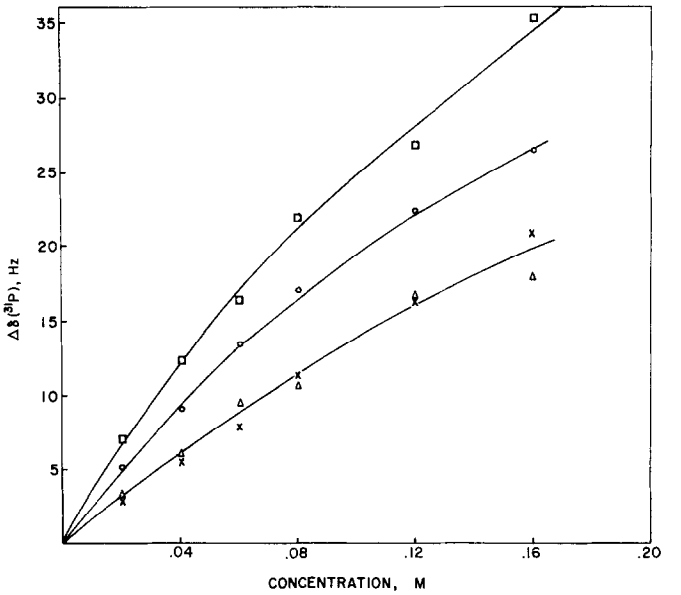

Figure 2. Dependence of $\Delta\delta(^{31}P)$ in Hz (measured at 23,487 Gauss) on the amine concentration at pD 6.00. $\Delta\delta(^{31}P) = \delta(^{31}P)_{4\text{-dPNP + amine}} - \delta(^{31}P)_{4\text{-dPNP}}$. Concentration of 4-deoxypyridoxine 5'-phosphate (4-dPNP) solution in D_2O was constant at 0.04M. L-Histidine (—X—X—), L-lysine·HCl (—Δ—Δ—), L-arginine·HCl (—O—O—) and L-norepinephrine·HCl (—□—□—).

$\Delta\delta(^{31}P)$ values than the corresponding monoamine substrates at the same concentration.

d) Other types of intermolecular interactions (e.g. interactions between aromatic rings) can enhance the $\Delta\delta(^{31}P)$ value.

e) At the concentrations studied, the α, β and γ-amino group of neutral or acidic amino acids does not significantly participate in the phosphate-amine interaction.

Titration curves of all protons of 4-dPNP with and without a non-aromatic amine substrate were practically identical and, thus, ^{1}H nmr offered little information about the phosphate-amine interaction.

4-dPNP and MMP are best suited for evaluation of phosphate-amine inter-

Table I Interaction Between Organic Phosphates and Protonated Amines

Amine Substrate	$(^{31}P)^a$ (in Hz)^b	
	4-Deoxypyridoxine 5'-phosphate[c] (4-dPNP)	Monomethyl phosphate[c] (MMP)
Tyramine·HCl	16.5	15.0
Histamine·2HCl	27.0	29.0
Dopamine·HCl	21.5	17.5
L-Norepinephrine·HCl	27.0	22.0
L-Alanine	2.0	1.0
L-Aspartic acid	6.5[d]	--
β-Alanine	0.5	0.0
γ-Aminobutyric acid	1.0	0.5
L-Lysine·HCl	16.5	15.0
L-Ornithine·HCl	18.0	19.5
L-Arginine·HCl	22.5	19.5
L-Histidine	18.5[e]	17.5[e]
L-Lysyl-L-lysine·2HCl	22.0	23.0

a) For definition, see caption of Fig. 2. b) Measured at 23,487 G. c) All values, determined at phosphate (0.04 M) to amine ratio of 1:3, are averages of 3 or more measurements at pD 6.00 for 4-dPNP and 6.15 for MMP. Possible standard error is ± 1.0 Hz. The salt effect due to Na^+ was found negligible and was not accounted for when Na^+ concentrations in the phosphate samples both with and without amine were equal (0.04M). d) This value is due entirely to salt effect. It is comparable to $\Delta\delta(^{31}P)$ obtained for a 0.04 M sample of 4-dPNP in D_2O, 0.16 M in NaCl. An equivalent concentration of Na^+ ions are present in the L-aspartic acid sample with 4-dPNP, after adjustment to pD 6.00, following the titration of the second carboxyl group with NaOD (pK_a=3.86). e) Corrected for salt effect because of differences in Na^+ content of the phosphate samples with and without amine.

actions between pD 5.5-6.5. Benzyl phosphonic acid allowed us to obtain $\Delta\delta(^{31}P)$ values for biogenic amines at biological pH's (at pD 7.00 - 8.00).

Preliminary studies of interaction between adenosine monophosphate and basic amino acid or biogenic amine show similar measurable $\Delta\delta(^{31}P)$ values.

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