PHOSPHATE- N BASE HYDROGEN BONDS INVOLVING PROTON TRANSFER WITH REFERENCE TO THE NON-ENZYMIC HYDROLYSIS OF ATP

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SUMMARY:
IR spectra of aqueous solutions of 1:1 mixtures of $H_2PO_4^-$ and various N bases have been studied as models for (POH···N) $\rightleftharpoons$ ($P^-O···H^+N$) hydrogen bonds. 50% proton transfer is observed when the pKa of the protonated N base is 1.1 smaller than that of the phosphate group. The hydrogen bonds are easily polarizable near this equilibrium. These results strongly support the conclusion that such bonds contribute 1) to the self-association of ATP and ADP and 2) to the association of the hydrolysis products ADP and inorganic phosphate.

Hydrogen bonded complexes between phosphate groups and basic nitrogen atoms have been reported for many biological systems, for example histidine-nucleotide interactions in ribonuclease-inhibitor complex (1) (2), nucleohistones (3), DNA-spermine association (4), and self-association of ATP (5) (6). The interactions extend from OH···N hydrogen bonds to $N^+H···^-O$ ion-pair formation. In order to determine the proton transfer equilibria OH···N $\rightleftharpoons$ $O^-···H^+N$ and the symmetry of such hydrogen bonds in aqueous solutions, we studied 1:1 mixtures of $H_2PO_4^-$ with various N bases, dependent on the pK_a values* of the bases by infrared spectro-

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* pK_a values of the conjugate acids of the bases in water.

scopy. The IR bands of phosphate groups and of N bases are very sensitive to proton attachment (7).

The studies of these model systems should give us more information about hydrogen bonds in aqueous solution of hydrolysing ATP (8) (9). ATP and ADP have acidic and basic proton acceptor sites which may act as hydrogen bonding donors or acceptors, depending on pH. We assumed such hydrogen bonds 1) between the adenine ring and the phosphate chain which contribute to the self-association of the nucleotides at acidic pH (8) (6), and 2) between ADP and inorganic phosphate at non-enzymic hydrolysis of ATP (9).

MATERIAL AND METHODS

KH_2PO_4, pK_a = 6.93 and the N bases 2-methylpyrazine, pK_a = 1.45*, 4-hydroxypyridine, pK_a = 3.27*, pyridine, pK_a = 5.25*, 4-picoline, pK_a = 6.02*, 1-methylimidazole, pK_a = 6.95*, 2-methylimidazole, pK_a = 8.05*, 4-picolylamine, pK = 9.65* were commercial samples of reagent grades (pK_a values are taken from ref. (10) (11) (12)). The concentrations of the 1:1 mixtures were 1.0 M. IR spectra were recorded on a Perkin-Elmer 325 spectrometer at 25°C. Experimental procedure and evaluation of the spectra, especially with regard to the continous absorption, are the same as described in ref. (13) (20).

RESULTS AND DISCUSSION

In fig. 1 IR spectra of mixtures of $H_2PO_4^-$ with four different N bases are given. The following phosphate bands were used for evaluation (14)(15): at 1160 cm^{-1} ν_{as} PO_2^- of the $H_2PO_4^-$ grouping and at 985 cm^{-1} ν_s PO_3^{2-} of the PO_3^{2-} grouping. The band at 1160 cm^{-1} indicates the H_2PO_4 con-

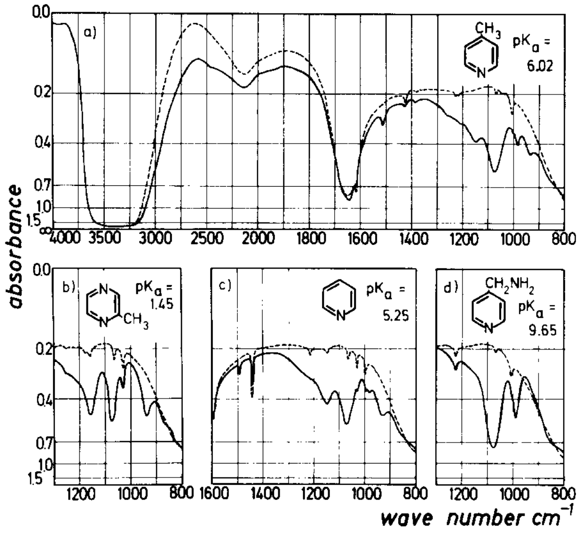

Fig. 1 IR spectra of 1.0 M aqueous solutions of N bases
------- and 1:1 mixtures of N bases with KH_2PO_4 ————
a) 4-picoline
b) 2-methylpyrazine
c) pyridine
d) 4-picolylamine

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centration, the band at 985 cm^{-1} the HPO_4^{2-} concentration. With increasing pK_a of the base the band at 1160 cm^{-1} decreases wheras the band at 985 cm^{-1} increases, indicating the dissociation of one proton of $H_2PO_4^-$. Simultaneously the bands of the N bases change due to the attachment of the protons at the N atoms of the bases. For example, in the case of the pyridine-phosphate mixtures (fig. 1c) the band at 1442 cm^{-1} is a ring vibration of pyridine, that at 1490 cm^{-1} of pyridinium. With addition of $H_2PO_4^-$ to the pyridine solution, the band at 1442 cm^{-1} decreases and that at 1490 cm^{-1} arises.

In fig. 2a, the relative integral absorbance of the phosphate bands at 1160 and 985 cm^{-1} is plotted dependent on the pK_a of the N bases and the ΔpK_a, respectively, i.e., the pK_a of the protonated base minus the

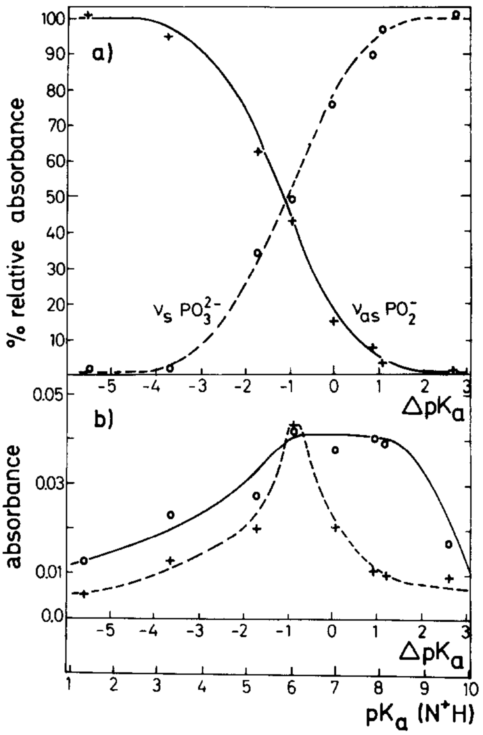

Fig. 2 a) Relative integral absorbance of ν_s PO_3^{2} at 988 cm^{-1}------ and ν_{as} PO_2 at 1160 cm^{-1} ———, dependent on the Δ pK$_a$ and the pK$_a$ of the N base, respectively.
b) Absorbance of the continuum at about 1900 cm^{-1} ——— and at about 1400 cm^{-1}------, dependent on the ΔpK$_a$ and the pK$_a$ of the base, respectively.

pK$_a$ of the acid. The curves show directly the removal of the protons from the phosphate groups dependent on the ΔpK$_a$. The inflection points of the sigmoid curves indicate that 50% proton transfer between $H_2PO_4^-$ and N bases is obtained at a pK$_a$ value of 3.5, i.e., at ΔpK$_a$ = -1.1.

Besides the changes with the bands a continous absorption is observed. In fig. 1a, the IR spectra of 4-picoline and the $H_2PO_4^-$+ 4-picoline mixture are plotted. The ΔpK$_a$ of the latter system is near the symmetry point of the proton transfer equilibrium. In the regions in which no or only weak bands are present a general increase of the absorbance is observed which begins at the band of the OH stretching vibration and extends towards smaller wave numbers. Continua in IR spectra have already been observed in many different systems ((16) pp. 696-703). They indicate the presence of easily polarizable hydrogen bonds (13)(16)-(18). The energy surfaces of such H bonds are double minima or very broad wells. They may be "symmetrical"*, for instance $(N^+H\cdots N) \rightleftharpoons (N\cdots H^+N)$ bonds in semiprotonated imidazole (19) or slightly asymmetrical, as for instance carboxylic acid-N base bonds (20). With more asymmetrical polarizable H bonds the continua do not extend as far towards small wave numbers. Hence one can distinguish between various types of polarizable hydrogen bonds when studying the continua in different wave number regions. (For more details on easily polarizable hydrogen bonds and the very strong interaction effects between these bonds and their environment see ref. (16)).

The continuous absorption is most intense with the mixtures of which the ΔpK_a value is near the inflection point of the proton transfer curve. This is shown in fig. 2b where the absorbance of the continuum is plotted at two different wave number values as a function of ΔpK_a. At smaller wave numbers only at 50% proton transfer (i.e. $\Delta pK_a \simeq 1.0$) a remarkable continuum can be observed wheras at greater wave numbers a plateau extends from about $\Delta pK_a = -3.0$ to $+2.5$. This can only be understood when the continuum is preferentially caused by $(POH\cdots N) \rightleftharpoons (P^-O\cdots H^+N)$ bonds. If only $(N^+H\cdots N) \rightleftharpoons (N\cdots H^+N)$ and $(POH\cdots O^-P) \rightleftharpoons (P^-O\cdots HOP)$ bonds are formed one would except an almost equal dependence at smaller and greater wave numbers (13)(19). Similar results

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[*] "Symmetrical" means that these hydrogen bonds have symmetrical energy surfaces when considered in isolated state.

were obtained with carboxylic acid-N base interactions (20). There are probably two reasons for this different behavior of the continuum at smaller and greater wave numbers:

1) The absorbance of the continuum is at smaller wave number - in agreement with the theory - much more sensitive to pertubations of the symmetry of the energy surface than the absorbance at higher wave numbers. This may be explained as follows: With increasing ΔpK_a, the weight of the polar proton boundary structure ($O^- \cdots H^+N$) increases. The polar environment (i.e. water molecules) traps the proton at the N atom more or less strongly, since the induced dipole interaction of this structure with the environment is stronger ((16) pp. 754 ff.). The energy surfaces become more asymmetrical and therefore the intensity is shifted towards higher wave numbers.

2) Usually the absorbance of polar structures, i.e. ($O^- \cdots H^+N$), is higher than of less polar ones.

The pK_a values of ATP and ADP are 6.53 and 6.44 for the terminal phosphate groups and 4.06 and 3.95 for the adenine residues (21)(22). Fig. 2 shows that hydrogen bonds between $H_2PO_4^-$ and $ADPH^{2-}$ can still be polarizable ($\Delta pK_a = 4.06 - 6.93 = -2.87$). Therefore we conclude that the continuum observed in aqueous solution of hydrolysed $ATPH^{3-}$ is caused by such polarizable hydrogen bonds. Furthermore, the results support our conclusion that hydrogen bonds between terminal phosphate groups and adenine residues contribute to the self-association of ATP and ADP at low acidic pH ($\Delta pK_a = -2.47$ and -2.49) (8).

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REFERENCES

1. Gorenstein, D.G., Wyrwicz, A.M., and Bode, J. (1976) J. Am. Chem. Soc., 98, 2308-2314.
2. Richards, R.F.M., and Wyckoff, H.W. (1971) The Enzymes, Vol. IV, P.D. Boyer, Ed., Chapter 24, Academic Press, New York.
3. Phillips, D.M.P. (1971) Histones and Nucleohistones, Plenum Press, London and New York.
4. Suwalsky, M., Traub, W., Shumueli, U., and Subirana, J.A. (1969) J. Mol. Biol., 42, 363-373.
5. Kennard, O., Isaacs, N.W., Motherwell, W.D.S., and Watson, G.D. in Jerusalem Symp. on Quantum Chem. and Biochem., E.D. Bergman and B. Pullman, Eds., Vol. IV, Academic Press, New York, 1972 pp. 114-121.
6. Gilligan III, T.J., and Schwarz, G. (1976) Biophys. Chem. 4, 55-63.
7. Zundel, G. (1969) Hydration and Intermolecular Interaction, Academic Press, New York, and Mir, Moscow 1972.
8. Matthies, M., and Zundel, G. (1976) submitted for publication.
9. Matthies, M., and Zundel, G. (1976) in preparation.
10. Phillips, R.S.J., Eisenberg, P., George, P., and Rutman, R.J. (1965) J. Biol. Chem., 240, 4393-4397.
11. Weast, R.C. (1974/75) Ed. Handbook of Chemistry and Physics, 55th Ed., Chem. Rubber Co., Cleveland, Ohio.
12. Perrin, D.D. (1965) Dissociation Constants of Org. Bases in Aqueous Solution, Butterworth, London.
13. Schiöberg, D., Hofmann, K.P., and Zundel, G. (1974) Z. Phys. Chem. (Frankfurt), 90, 181-188.
14. Steger, E., and Herzog, K. (1964) Anorg. Allgem. Chem., 331, 169-174.
15. Chapman, A.C., and Thirlwell, L.E. (1964) Spectrochim. Acta, 20, 937-947.
16. Zundel, G. (1976) in: The Hydrogen Bond, P. Schuster, G. Zundel, and C. Sandorfy Eds., North Holland Publ. Co., Amsterdam, ch. 15, pp. 683-766.
17. Zundel, G. (1970) Allgem. Prakt. Chem., (Wien) 21, 329-337.
18. Janoschek, R., Weidemann, E.G., Pfeiffer, H., and Zundel, G. (1972) J. Am. Chem. Soc., 94, 2387-2396.
19. Zundel, G., and Mühlinghaus, J. (1971) Z. Naturforschg. 26b, 546-555.
20. Lindemann, R., and Zundel, G. (1976) in preparation.
21. Taqui Khan, M.M., and Martell, A.E. (1966) J. Am. Chem. Soc. 88, 668-621.
22. Taqui Khan, M.M., and Martell, A.E. (1967) J. Am. Chem. Soc. 89, 5585-5590.