as double-bonded oxygen bases. From the rationale which we advance for the separation into families, we consider that Gramstad is probably correct and that our β values (which average β_i's from many diverse properties and from experimental information of variable precision) are insufficiently precise for the distinctions to become evident.

(27) Many of these correlations will be reported in future papers of this series.

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Conformation of 3′-Nucleotides. Crystal Structure of Uncharged 3′-Uridine Monophosphate Monohydrate and Hydrogen Bonding to the Furanose Ring Oxygen

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Abstract: An accurate X-ray crystallographic investigation of the structure of 3′-UMP in the free acid form has been carried out. 3′-UMP in the solid state exists as an uncharged molecule, unlike AMP, CMP, and GMP, which are zwitterions in the solid state. The uncharged phosphate group exhibits shorter P-O distances (P-O(ester), 1.567 Å; P-OH, 1.502, 1.521 Å) compared to anionic phosphate groups, indicating that a distinction between the individual $P-O^-$ and P-OH based purely on the basis of bond distances alone without considering the phosphate group as a whole is not valid. The ribose has the C(2′)-endo conformation; the pseudorotation parameters are $P = 165°$ and $\tau_m = -38.9°$. The anti conformation ($\chi_{CN} = 55.8°$) of the molecule and the g^+ orientation of O(5′) across C(4′)-C(5′) are stabilized by an intramolecular C(6)-H⋯O(5′) hydrogen bond. The phosphate group assumes the characteristic staggered conformation across the P-O bonds. The torsion angles ψ' (C(5′)-C(4′)-C(3′)-O(3′)) and ϕ' (C(4′)-C(3′)-O(3′)-P) are respectively 150.1 and 226.3°. The molecular conformation derived from NMR and energy calculations is in broad agreement with the X-ray results. The molecules related by the *a* translation are on top of each other at 6.3 Å apart; facing this stack is another related by the 2_1 screw. The two stacks interleave such that the O(2) from one and H(C5) from the other project into each other. Hydration is promoted by cation coordination; when the ions form an "inner-sphere" complex, as in most crystals of nucleotides with metal ions, the basic features of the "rigid nucleotide" are conserved. However, deviations from the "rigid nucleotide", though not favored, are possible under appropriate conditions such as the formation of "outer-sphere" complexes or coordination of metal ions through the phosphate groups. The furanose ring oxygen participates in a hydrogen bond (O(2′)-H⋯O(1′), 2.912 Å; H⋯O(1′), 1.80 Å; O(2′)-H⋯O(1′), 168°), an uncommon occurrence in the usual nucleosides, and the first example in a nucleotide. It is suggested that a similar, but an *intra*molecular hydrogen bond from O(2′)-H of one residue to the O(1′) of the next residue on the 5′ side is possible for RNAs and might indicate one of the important functions of 2′-OH in RNA compared to DNA. The crystals of 3′-UMP·H_2O are orthorhombic, $a = 6.133$ (1), $b = 7.465$ (2), $c = 30.256$ (4) Å, space group $P2_12_12_1$; final R value is 0.051 for 1512 reflections $> 2\sigma$.

The conformation analysis of nucleic acid polymers has been greatly facilitated by the availability of conformational data on mononucleotides and by the recognition of the preferred conformations of these monomers.[1] Stereochemical information on mononucleotides have been gathered mainly by using X-ray and NMR techniques. Though extensive studies by NMR on 3′-nucleotides have been carried out, very few X-ray diffraction studies have been carried out on 3′-nucleotides compared to 5′-nucleotides. It has been demonstrated that the 3′- and 5′-phosphate bonds in mononucleotides behave differently with respect to radiation-induced breakage[2] and radiation sensitization.[3] This paper describes the result of our crystallographic studies on 3′-UMP. All the mononucleotides AMP, CMP, and GMP (see discussion later) exist in the solid state as zwitterions, but UMP does not, thereby affording an opportunity to study the conformation of an uncharged nucleotide.

Experimental Section

Transparent, plate-like crystals of 3′-UMP (Nutritional Biochemical) were obtained by slow evaporation of an aqueous solution (crystals kindly supplied to us by Dr. H. C. Box of our department). The unit cell dimensions were obtained by a least-squares refinement of 45 high-angle reflections ($\theta \geq 45°$) and are given in Table I along with other pertinent crystallographic data. Complete intensity data were collected to the limit of $2\theta = 165°$ for Cu Kα radiation. The stationary crystal-stationary counter techniques[4] were employed for obtaining the intensities with a 5° takeoff angle on a GE XRD-6 diffractometer; 1743 reflections were measured out of which 231 had their intensities less than twice the background in that $\sin\theta/\lambda$ range, and were given zero weight during the refinement. The crystal was mounted with the b^* along the ϕ axis of the goniostat and the difference in absorption as a function of ϕ[5] was measured for the axial reflections and was used for correcting approximately the anisotropy of absorption. The data were processed in the usual way.

Solution and Refinement of the Structure. The position of the phosphorus atom (P) was found from a sharpened Patterson synthesis but the P-phased electron density maps did not reveal the structure completely. A model consisting of P and 10 atoms was used as a starting structure. The other nonhydrogen atoms were found by a successive least-squares refinement combined with difference electron density maps. The R factor $[(\sum||F_o| - |F_c||)/(\sum|F_o|)]$ at the end of the isotropic refinement was 0.11. Further refinements were continued with anisotropic temperature factors for the nonhydrogen atoms and the R value converged to 0.073. A difference electron density synthesis at this stage revealed all the hydrogen atoms. The final cycles of refinement were calculated using a full-matrix least-squares refinement[6] with individual isotropic and anisotropic temperature factors for the hydrogen and nonhydrogen atoms, respectively. The final R value was 0.051. The quantity minimized was $\Sigma w(|F_o|^2 - (1/k^2)|F_c|^2)^2$ where the weight $w = 1/\sigma^2(|F_o|^2)$ and k is the scale factor. Atomic scattering factors and the anomalous

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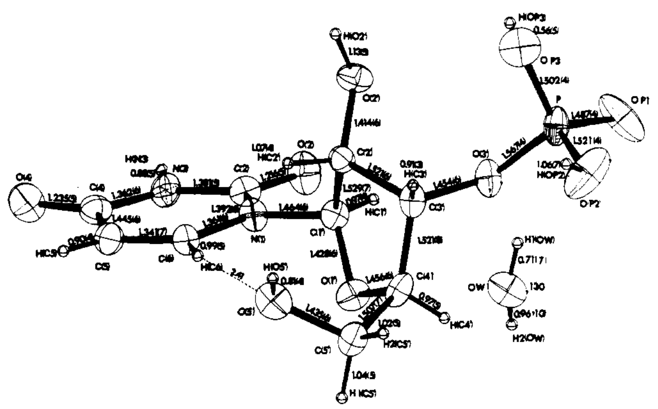

Figure 1. Bond distances (Å) in 3′-UMP. The esd's given in parentheses refer to the last digit. Note the intramolecular hydrogen bonding C(6)-H(C6)···O(5′). The two phosphate P—OH distances are only slightly longer than the P═O bond and are nearly equal to the P—O⁻ bond. (See text for explanation.)

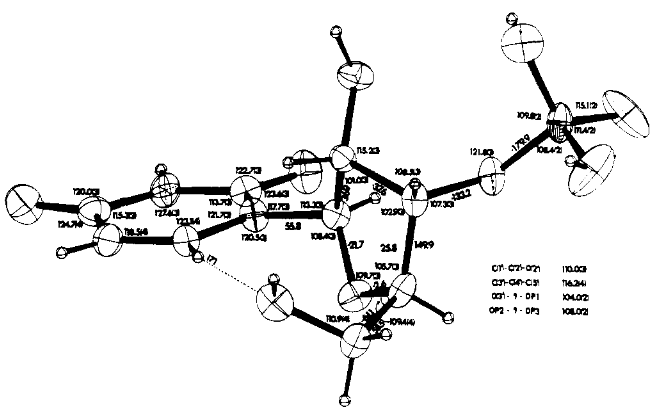

Figure 2. Bond angles (deg) and some relevant torsional angles in 3′-UMP. The anti conformation of the molecule and the g$^+$ orientation across C(4′)-C(5′) are stabilized by the internal C(6)-H(C6)···O(5′) hydrogen bonding. The ribose has the preferred C(2′)-endo conformation. The phosphate group assumes the characteristic staggered conformation with respect to the ester bond.

Table I. Crystallographic Data for 3′-UMP

stoichiometry	$C_9H_{13}N_2O_9P{\cdot}H_2O$
a, Å	6.133 (1)
b, Å	7.465 (2)
c, Å	30.256 (4)
vol, Å^3	1385 Å^3
space group	$P2_12_12_1$
μ, cm^{-1}	14.2
d(obsd), g cm^{-3}	1.64
d(calcd), g cm^{-3}	1.641
dimensions of crystal, mm	0.35 × 0.22 × 0.12
no. of reflections ($I \geqslant 2\sigma$)	1512
$R = \Sigma\|\|F_o\| - \|F_c\|\|/\Sigma\|F_o\|$	0.051
temp = 22 ± 3 °C	
Cu Kα = 1.54051 Å	

dispersion corrections are from International Tables.[7] For the hydrogens, the scattering factors given by Stewart, Davidson, and Simpson[8] were used.

Results and Discussion

The final positional parameters for nonhydrogen atoms are given in Table II and for hydrogen atoms in Table III. The observed and calculated structure factors are provided in the supplementary material (see paragraph at end of paper regarding supplementary material). The bond lengths and angles are illustrated in Figures 1 and 2. Bond angles involving the hydrogen atoms fall in the usual range for X-ray determinations.

Geometry and Ionization of the Phosphate Group. The bond distances and angles of the uridine moiety are similar to what has been observed in several other structures but the uncharged phosphate group exhibits shorter P-O (ester) and P-OH distances compared to anionic phosphates. 3′-UMP, not unexpectedly, exists as an uncharged molecule in the solid state; mononucleotides of all other bases exist as zwitterions in the crystalline state (Table IV). The pH of the concentrated solution of 3′-UMP (from which crystals were grown) was 1.35. The ionization constant of the primary phosphate group is <1, so that in solution less than 50% of the molecules are un-ionized. The keto oxygen O(4) of uracil can be protonated apparently only near pH ~0, as in the case of 1-methyluracil hydrobromide;[9] 3′-UMP is uncharged in the solid state. The structure of 5′-IMP[10] did not refine well enough to yield the locations of hydrogens; however, the increase in the C(5)-N(7)-C(8) angle[11-13] from the usual value of around 103° to 107°, we feel, indicates protonation of N(7), and consequently an ionized phosphate group. From similar considerations, we feel that 5′-GMP[14] is also a zwitterion in the solid state. The structure of another uncharged nucleotide, 5′-azaUMP, has been determined,[15] but the molecular dimensions have not been published. In this connection, it is interesting to note that hemiprotonation is possible in the solid state, as in polyribocytidylic acid and cytosine-5-acetic acid.[16]

The evidence for the un-ionized state of the phosphate group of 3′-UMP from the P═O and P—OH distances alone is not conclusive. But the actual location of the hydrogens on OP2 and OP3 and the appropriate hydrogen bonds they make clearly reflect the un-ionized state of the phosphate. A comparison of the bond distances of 3′-UMP with other uncharged phosphate groups (Table V) and charged phosphate groups (Table IV) indicates that it will be unwise to make a distinction between P—OH and P—O⁻ (just like the distinction between P═O and P—O⁻[17]) purely on the basis of bond distances alone without considering the phosphate group as a whole. As pointed out by one of the referees, the O⁻⩦P⩦O angle is usually the largest in anionic phosphates; this angle might serve as an additional criterion for distinguishing charged and uncharged phosphates. However, the geometry of the uncharged phosphate is still not well established owing to lack of a number of examples.

Conformation of 3′-UMP. The molecular conformation is shown in Figure 1 with the important conformation angles indicated in Figure 2. The ribose has the C(2′)-endo conformation. The four-atom least-squares plane with the least root mean square deviation of 0.011 Å is through C(1′), C(3′), C(4′) and O(1′); C(2′) and C(5′) are −0.60 and −1.19 Å from this plane. The pseudorotation parameters[18] are P = 165° and τ_m = −38.9°. The conformation across C(4′)-C(5′) is gauche-gauche or g$^+$. The anti conformation of the molecule (χ_{CN} = 55.8°) and the g$^+$ orientation of O(5′) are stabilized by an intramolecular C-H···O hydrogen bond. Such stabilizations have been pointed out in a number of nucleosides and nucleotides.[19-22]

The phosphate group assumes the characteristic staggered conformation with respect to the ester bond. The torsional angles across P-O(3′) for OP1, OP2, and OP3 with respect to C(3′) are respectively −179.9, 62.4, and −55.8°. The values for ψ'(C(5′)-C(4′)-C(3′)-O(3′)) and ϕ(C(4′)-C(3′)-O(3′)-P) are respectively 150.1 and 226.3°.

It is interesting to compare the conformational features of 3′-mononucleotides in the solid state with those obtained in solution by NMR techniques.[23-25] From NMR studies it is found that the population of C(3′)-endo sugar pucker changes from 0.57 to 0.49 on going from a pH of 8.3 to 4.2 for 3′-UMP

Table II. Final Positional and Thermal Parameters of Atoms in 3′-UMP[a] with Estimated Standard Deviations in Parentheses

atom	x	y	z	u_{11} or u	u_{22}	u_{33}	u_{12}	u_{13}	u_{23}
P	581 (0)	1188 (2)	2927 (0)	38 (1)	26 (1)	18 (1)	10 (1)	−8 (1)	−2 (1)
O(2)	4231 (7)	1770 (4)	4847 (1)	54 (3)	19 (2)	30 (2)	0 (2)	−5 (2)	5 (1)
O(4)	4298 (7)	6699 (5)	5720 (1)	51 (3)	39 (2)	22 (2)	−7 (2)	1 (2)	5 (2)
O(1′)	5395 (6)	4325 (5)	3796 (1)	25 (2)	34 (2)	23 (2)	2 (2)	−2 (2)	4 (2)
O(2′)	−176 (6)	3165 (5)	3991 (1)	27 (2)	31 (2)	22 (2)	−8 (2)	−2 (1)	7 (2)
O(3′)	2201 (6)	2039 (5)	3267 (1)	41 (3)	21 (2)	25 (2)	3 (2)	−6 (2)	5 (1)
O(5′)	3832 (6)	7706 (4)	3487 (1)	40 (3)	23 (2)	37 (2)	2 (2)	−6 (2)	5 (2)
OP1	1119 (7)	−753 (4)	2937 (1)	27 (2)	31 (2)	22 (2)	−8 (2)	−2 (1)	7 (2)
OP2	1036 (7)	1997 (6)	2494 (1)	60 (3)	80 (3)	19 (2)	35 (3)	5 (2)	13 (2)
OP3	−1724 (7)	1644 (5)	3052 (1)	37 (2)	39 (2)	37 (2)	10 (2)	−7 (2)	11 (2)
OW	6771 (7)	148 (5)	3713 (1)	37 (2)	40 (2)	42 (2)	−8 (2)	−6 (2)	3 (2)
N(1)	3934 (7)	4523 (5)	4511 (1)	30 (3)	20 (2)	18 (2)	2 (2)	−3 (2)	4 (2)
N(3)	4297 (7)	4291 (5)	5272 (1)	37 (3)	29 (2)	13 (2)	2 (2)	−1 (2)	3 (2)
C(2)	4171 (7)	3394 (6)	4874 (1)	23 (3)	27 (2)	22 (2)	2 (2)	−3 (2)	4 (2)
C(4)	4256 (8)	6095 (6)	5340 (1)	25 (3)	28 (2)	26 (2)	−3 (3)	−2 (2)	−1 (2)
C(5)	4154 (9)	7173 (6)	4944 (2)	27 (3)	25 (3)	25 (2)	−3 (3)	−1 (2)	3 (2)
C(6)	4010 (9)	6338 (6)	4552 (2)	27 (3)	19 (2)	26 (2)	1 (2)	−2 (2)	2 (2)
C(1′)	3683 (8)	3693 (7)	4075 (1)	23 (3)	27 (3)	16 (2)	−1 (2)	−3 (2)	−1 (2)
C(2′)	1576 (9)	4234 (6)	3840 (1)	23 (3)	22 (2)	17 (2)	−1 (2)	−1 (2)	−1 (2)
C(3′)	2212 (9)	3951 (6)	3359 (1)	30 (3)	13 (2)	20 (2)	6 (2)	−6 (2)	6 (2)
C(4′)	4574 (9)	4567 (6)	3348 (1)	34 (3)	25 (3)	17 (2)	7 (3)	4 (2)	1 (2)
C(5′)	4962 (9)	6473 (7)	3204 (2)	33 (4)	34 (3)	31 (3)	−3 (3)	6 (2)	8 (2)

[a] T.F. = $\exp[-(u_{11}h^2 + u_{22}k^2 + u_{33}l^2 + u_{12}hk + u_{13}hl + u_{23}kl)]$. The entries in the tables are values ×10^4 for coordinates and ×10^3 for thermal parameters.

Table III. Final Positional Parameters for the Hydrogen Atoms with Standard Deviations in Parentheses[a]

atom	x	y	z
H(N3)	442 (8)	355 (7)	550 (1)
H(C5)	411 (8)	835 (6)	500 (1)
H(C6)	393 (8)	687 (6)	425 (2)
H(C1′)	391 (7)	242 (5)	412 (1)
H(C2′)	129 (6)	562 (5)	392 (1)
H(C3′)	127 (5)	454 (4)	318 (1)
H(C4′)	554 (9)	377 (6)	319 (1)
H1(C5′)	445 (9)	662 (6)	288 (1)
H2(C5′)	660 (8)	671 (6)	321 (1)
H(O2′)	−181 (8)	380 (6)	392 (1)
H(OP2)	24 (13)	315 (8)	235 (2)
H(OP3)	−227 (9)	148 (8)	319 (2)
H(O5′)	277 (7)	811 (6)	337 (1)
H1(OW)	571 (12)	−20 (8)	369 (2)
H2(OW)	806 (16)	−41 (11)	382 (2)

[a] The entries in this table are values ×10^3.

but changes very little for 3′-AMP (from 0.38 to 0.37) for a change in pH from 7 to 6. But in the crystalline state all the 3′-mononucleotides, free acid or salt (see Table IV), with the exception of 3′-AMP, are in the C(2′) endo conformation; 3′-AMP has C(3′)-endo as the major pucker. The degree of pucker and the phase angle of pseudorotation for the S-type (C(2′)-endo) pucker, τ_s and P_s, are 42 and 153°, respectively, in good agreement with the X-ray values for 3′-UMP. The populations of 3′-mononucleotides range from 0.68 to 0.80 for g$^+$, 0.16 to 0.27 for t, and 0.06 to 0.08 for g$^-$. In the crystalline state, all the 3′-mononucleotides, with the exception of 3′-AMP, have the g$^+$ orientation for O(5′); for 3′-AMP, this orientation is g$^-$.

If the rotamer distribution of bond angle ϕ'(C(4′)-C(3′)-O(3′)-P) is analyzed using the three-rotamer model $\phi' = 60$ (g$^+$), 180 (t), and 300° (g$^-$), NMR data indicate significant population for $\phi' = 60°$, but X-ray results do not support such a conclusion[26] (see Table IV). The two rotamer models used by Alderfer and Ts'o[25] show that the population of t decreases from 0.69 to 0.52 on changing the pH from 8.4 to 4.2. For 3′-UMP, in the crystal, the ϕ' angle is 226.7°, between g$^-$ and t (and eclipsing the hydrogen H(C3′), the usual range found for 3′-nucleotides (Table IV). Theoretical calculations[27-34] show a broad minimum in this range for ϕ, agreeing with the X-ray results.

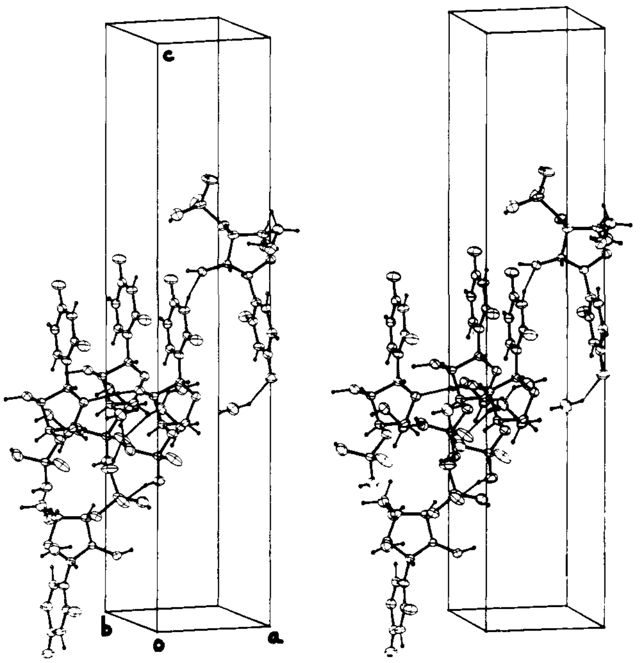

Figure 3. A stereoscopic view of the hydrogen bonding and packing in the crystal structure. Note that the two phosphate oxygens OP2 and OP3 take part in hydrogen bonding, and the ring oxygen takes part in hydrogen bonding from O(2′).

Hydrogen Bonding and Stacking. The stereochemistry of hydrogen bonding is summarized in Table VI and illustrated in Figure 3. The most important features of hydrogen bonding are (1) there is no self-association of the uracil moiety, (2) the ring oxygen O(1′) of ribose accepts a hydrogen bond, an uncommon occurrence in the usual nucleosides and the first example in nucleotides, (3) both the P-OH groups take part in very strong but nonlinear hydrogen bonding, and (4) there is a wealth of C-H⋯O interactions. The hydrogen atoms on the

Table IV. Conformation Details of Some 3′-Nucleotides, Their Salts, and 5′-Nucleotides and the Geometry of Their Phosphate Groups

A. 3′-Nucleotides and Their Salts

						bond distances, Å				
name	charge[d]	χ_{CN}, deg	sugar pucker	ψ	ϕ, deg	P=O	P—O (ester)	P—OH	P—O$^-$	ref
1. 3′-UMP·H_2O	0	55.8	C(2′)-endo	g$^+$	−133.3	1.487 (4)	1.567 (4)	1.502 (4)	1.521 (4)[b]	this work
2. 3′-CMP(mono)	−1	39.3	C(2′)-endo	g$^+$	−91.5	1.450 (5)	1.610 (5)	1.588 (5)	1.498 (5)	55
3. 3′-CMP(ortho)	−1	44	C(2′)-endo	g$^+$	−108.8	1.483 (3)	1.611 (3)	1.551 (3)	1.504 (4)	13
4. 3′-AMP·$2H_2O$	−1	3.8	C(3′)-endo	t	−122.8	1.486 (3)	1.612 (3)	1.579 (3)	1.477 (3)	56
5. 3′-thio-UMP[c]	−1	51	C(2′)-endo	g$^+$	−90.9	1.96 (5)[a]	1.62 (5)	1.61 (5)	1.42 (5)	58
methyl ester NH_4 salt		43	C(3′)-endo		−85.7	2.00 (5)[a]	1.67 (5)	1.68 (5)	1.48 (5)	
6. adenosine 3′-phosphonate ethanol solvate ammonium salt	−1	28.1	C(2′)-exo		−164.9					59
7. 3′-UMP·Na_2·$4H_2O$	−2	45.1	C(2′)-endo	g$^+$	−105.9	1.507 (5)	1.616 (5)	1.512 (5)	1.508 (5)	57a

B. 5′-Nucleotides

				bond distances, Å				
name	χ_{CN}, deg	sugar pucker	ψ	P=O	P—O (ester)	P—OH	P—O$^-$	ref
1. 5′-GMP·$3H_2O$	12.4	C(3′)-endo	g$^+$	1.499 (8)	1.608 (8)	1.569 (8)	1.508 (8)	14
2. 5′-AMP(mono)	25.7[e]	C(3′)-endo	g$^+$	1.495 (8)	1.610 (7)	1.566 (8)	1.514 (8)	60
3. 5′-AMP(ortho)	72.5	C(2′)-endo	g$^+$	1.493 (8)	1.602 (6)	1.571 (6)	1.507 (8)	61
4. 5′-dCMP	−6.0	C(3′)-exo C(2′)-exo	g$^+$	1.490 (2)	1.597 (2)	1.572 (2)	1.508 (2)	57b
5. 5′-IMP[f]	−20.2	C(3′)-endo	g$^+$	1.45 (2)	1.59 (2)	1.58 (2)	1.51 (2)	10

[a] These are P=S bonds. [b] This is a P—OH distance in this structure. [c] There are two independent molecules in this structure. [d] Charge on the phosphate. [e] Values taken from the compilation of Sundaralingam.[62] [f] This structure has a disorder around the phosphate group.

Table V. Phosphorus-Oxygen Distances (Å) in Structures with Uncharged Phosphate Groups

name	P—O1(H)	P—O2(H)	P=O	P—O	ref
3′-UMP·H_2O	1.521 (4)	1.502 (4)	1.487 (4)	1.567 (4)	this work
myoinositol phosphate	1.548 (2)	1.553 (2)	1.468 (2)	1.586 (2)	63
pyridoxal phosphate hemiacetal	1.519 (9)	1.529 (9)	1.487 (9)	1.600 (9)	64
phosphoric acid	1.57 1.57	1.58	1.52		Furberg[65]

Table VI. Hydrogen Bond Distances and Angles

				distance, Å			
donor D	hydrogen H	acceptor A	D-H	H···A	D···A	angle, deg D-H···A	data set
I. Uracil Base							
N(3)	H(N3)	O(2′)	0.88	2.03	2.904 (3)	178	$\frac{1}{2}+x,\frac{1}{2}-y,1-z$
II. Ribose Ring							
O(2′)	H(O2′)	O(1′)	1.13	1.80	2.912 (4)	168	$1-x,y,z$
O(5′)	H(O5′)	OP1	0.81	1.85	2.620 (4)	159	$x,1+y,z$
III. Phosphate Group							
OP2	H(OP2)	OP1	1.06	1.45	2.473 (4)	139	$\bar{x},\frac{1}{2}+y,\frac{1}{2}-z$
OP3	H(OP3)	OW	0.55	1.96	2.471 (4)	155	$-1+x,y,z$
IV. Water Molecule							
OW	H1(OW)	O(5′)	0.71	2.03	2.654 (3)	147	$x,y-1,z$
OW	H2(OW)	O(4)	0.96	1.85	2.694 (3)	146	$\frac{1}{2}+x,\frac{1}{2}-y,1-z$
V. C-H···O Interactions							
C(5)	H(C5)	O(2)	0.90	2.60[b]	3.445 (3)	158	$x,1+y,z$
C(6)	H(C6)	O(5′)	0.99	2.41	3.384 (3)	171	x,y,z
C(2′)[a]	H(C2′)	O(4)	1.07	2.59[b]	3.599 (4)	156	$x-\frac{1}{2},y+\frac{1}{2},1-z$
		(O5′)		2.56[b]	3.127 (3)	112	x,y,z
C(4′)	H(C4′)	OP3	0.97	2.35	3.275 (4)	160	$1+x,y,z$
C(5′)	H2(C5′)	OP2	1.02	2.54[b]	3.224 (4)	138	$1-x,\frac{1}{2}+y,\frac{1}{2}-z$

[a] Bifurcated interaction. [b] H···O distances are longer for a hydrogen bond. [c] Intramolecular.

P-OH's are well located (the electron densities for H(OP2) and H(OP3) are respectively 0.4 and 0.3 e/Å^3), and they refined well with *B* values less than 5 Å^2. Consequently, we feel that, though these OP2-H···OP1 and OP3-H···OW are quite short, they do not appear to be symmetric. It is not clear what effect these short hydrogen bonds might have on the P-OH distances (a question raised by one of the referees); since the observed P-O and P-OH distances in 3′-UMP are *shorter* than what is usually found, it is unlikely that these strong hydrogen bonds provide an explanation for these shortenings.

The acceptance of a hydrogen bond by the ribose ring oxygen of nucleosides and nucleotides is quite unusual. In a few cases in which O(1′) participates,[35-42] the hydrogen bonding is weak and does not occur in normal nucleosides. Some examples follow: HN(6)···O(1′), 2.26 Å in formycin;[35] HC(8)···O(1′), 2.13 Å in virazole;[36] O(water)-H···O(1′), 2.40 Å in α-D-2′-amino-2′-deoxyadenosine monohydrate;[37] H···O(1) of 1.57 Å in ethyl-1-thio-α-D-glucofuranoside;[38] H(C5′)···O(1′) of 2.40 Å in 6-azacytidine;[39] H(OW)···O(1′) of 2.19 Å in toyocamycin monohydrate;[40] H(N4)···O(1′) of 2.06 Å in 3-deazacytidine;[41] H(N6)···O(1′) of 2.38 Å in 8-azatubercidin[42] (see also ref 43 for a short compilation of structures involving a hydrogen bond to O(1′)).

The acceptance of a hydrogen bond by O(1′) from 2′-OH and its stereochemistry as seen in this structure suggested that differences between the conformations of nucleic acid polymers with deoxy and ribose sugars might be due to hydrogen bonding from the O(2′)-H of one residue to O(1′) of the next residue on the 5′ side. Several workers have proposed earlier the involvement of 2′-OH hydrogen bonding to explain the higher stability of synthetic RNA compared to DNA of the same sequence (see ref 45 for a summary and other references), but these models differ regarding the acceptor of this hydrogen bond. The four proposed acceptors have been (a) the oxygen of the 3′-phosphate,[46] (b) the carbonyl oxygen of uracil,[47] (c) O(1′) of the adjacent residue,[48] and (d) an intramolecular water bridge.[49] The models (a) and (b) appear to be unfavorable stereochemically;[45] models (c) and (d) are stereochemically feasible. Also, in the crystal structure of tRNA, it has been noted[50] that a number of intrachain hydrogen bonds involving 2′-OH to O(1′) of adjacent residues are possible in view of the appropriate O(2′)···O(1′) distances. These observations in tRNA and the occurrence of a O(2′)-H···O(1′) hydrogen bond in 3′-UMP indicate that one of the roles of 2′-OH in RNA is through its intrastrand vertical hydrogen bonding to the O(1′) of the adjacent residue.

The wealth of the C-H···O interactions is also quite unusual and occurs also in 5-nitro-1-(β-D-ribosyluronic acid)uracil monohydrate[22,44] which also carries an **un**-ionized acid as an exocyclic substituent on the ribose.

The nucleotides are packed in the crystal in an unusual manner (Figure 3). The bases of nucleotides that are related by translation along the *a* axis are on top of each other at 6.3 Å apart. Facing this stack are the bases related by the 2_1 screw. These two stacks interleave such that the O(2) from one and H(C5) from the other project into each other. This type of base stacking is somewhat similar to that observed in 5-nitro-1-(β-D-ribosyluronic acid)uracil monohydrate.[44]

It is very interesting to note that the basic salts (Na^+, Ca^{2+}, Li^+) of mononucleotides have a tendency to contain more molecules of water of crystallization than the free acids, indicating that the cation coordination promotes hydration. Comparing the conformations of nucleotides as free acids and as salts of cations, it is found that, when the ions form an "inner-sphere" complex[51] as in most crystals of nucleotide complexes with metal ions, the basic features of the "rigid nucleotide"[26] are conserved. However, in the one example when an "outer-sphere" complex[51] is formed (5′-AMP·Ba·$7H_2O$ crystals[52]), the ribose displays a C(4′)-exo conformation. Also, when metal ion coordination takes place exclusively through the phosphate group, as in [Cu(5′-UMP)2,2′-dipyryidylamine)(H_2O)]$_2$·$5H_2O$,[53] the ribose ring in one UMP molecule has an unusual O(1′)-endo pucker. More remarkably, one of the 5′-UMP molecules has the sy**n** conformation, and further, as appears from the figure in the paper of Fischer and Bau,[53] both the nucleotides exhibit, not the usual g^+ conformation across C(4′)-C(5′), but the t (or gt) conformation. Also, in the structure of [Pt(en)(5′-CMP)]$_2$·$2H_2O$[54] the coordination of the phosphate group with platinum is probably the cause for the t conformation across C(4′)-C(5′) in one of the 5′-CMP molecules. It appears that the basic features of the "rigid nucleotide" hold good for a majority of nucleotides;[66] in a few cases, some of these features can be altered under appropriate conditions, such as "outer-sphere" formation of water molecules, metal ion coordination, or destacking (as indicated by Evans and Sarma).[24]

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Supplementary Material Available: Listing of structure factor amplitudes for the compound (11 pages). Ordering information is given on any current masthead page.

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Dynamics of Nonadiabatic Atom Transfer in Biological Systems. Carbon Monoxide Binding to Hemoglobin

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Abstract: In this paper we advance a theory of nonadiabatic molecular group transfer processes in biological systems, which can be described in terms of a radiationless transition between vibronic levels corresponding to two distinct electronic configurations. The resulting multiphonon rate expression exhibits a continuous variation from a temperature-independent nuclear tunnelling rate at low temperatures to an activated rate at high temperatures. The theory is applied for the study of the recombination reaction between CO and hemoglobin (CO/Hb) in the temperature range 2–100 K. This process is accompanied by an electronic spin change of the system, is induced by weak second-order spin-orbit coupling, and involves large nuclear changes, whereupon the nonadiabatic multiphonon treatment is applicable. The CO/Hb recombination rate is expressed in terms of a product of a second-order spin-orbit electronic coupling term and a thermally averaged nuclear Franck-Condon vibrational overlap term. The experimental temperature dependence of the CO/Hb recombination can adequately be accounted for in terms of our theory, provided that the shift in the iron equilibrium configuration between the "free" and "bound" states is 0.4–0.5 Å, the characteristic frequency of the motion of the iron, coupled to the deformation mode of the heme group is $\sim$100 cm^{-1}, the upper limit for the energy change involved in the exoergic process is ~ -0.05 to -0.1 eV, and the second-order spin-orbit coupling term is 0.1–1 cm^{-1}. These nuclear and electronic parameters concur with the available information concerning structural and spectroscopic data for hemoglobin and related compounds.

I. Introduction

Electron transfer (ET) and atom or molecule transfer (AT) reactions play a central role in a variety of biological systems. Consecutive ET reactions represent the key steps in the primary events of photosynthesis[2,3] and in the respiratory chain.[4] Proton transfer is one of the most important elementary steps in the action of hydrolytic enzymes[5,6] and in the primary photochemical processes in some visual pigments.[7] Ligand substitution, i.e., the transfer of heavy molecular groups, is important in the binding of substrate molecules to the metal centers in hydrolytic metalloenzymes.[8,9] The transfer of heavy molecular groups is also the dominant elementary process in the reversible uptake of dioxygen, carbon monoxide, and other small molecules by myoglobin (Mb), hemoglobin (Hb), and related compounds.[10] In terms of general methodology several of these processes (e.g., ET in bacterial photosynthesis,[2a] proton translocation in excited rhodopsin,[2b] and the recombination of CO with Mb or Hb[10]) may be viewed as unimolecular processes in a biological supermolecule, which are expected to exhibit an Arrhenius-type temperature dependence. However, as originally pointed out by Longuet-Higgins and Higgs,[11] in contrast to the predictions of this law, the rate constant is not expected to vanish for $T \rightarrow 0$ owing to zero-point energy effects. In modern terminology this would be restated as tunnelling of molecular groups prevailing for $T \rightarrow 0$ and resulting in a temperature-independent unimolecular rate constant at low temperatures.

During the last decade the dynamics of some elementary biological processes were investigated over a broad temperature range from cryogenic temperatures around 2 K up to room

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