effect (${}^{\gamma}E \rightleftharpoons {}_{\gamma}E$) or only a minor one (${}^{\beta}_{\gamma}T \rightleftharpoons {}^{\beta}_{\gamma}T$) on the backbone torsion angle. Thus, *a transition between ${}^{\gamma}E$ and ${}_{\gamma}E$ is possible without major perturbation of the backbone.*

The frequent occurrence of proline in β turns and its importance in protein folding suggest that those conformations which allow ${}^{\gamma}E \rightleftharpoons {}_{\gamma}E$ interconversion might be entropically favored over those restricting the ring mobility.

Summary

The relative flexibilities of the two most widely occurring five-membered rings in biological polymers are compared here in the framework of the pseudorotation concept which provides an elegant way of gaining an insight into the inherent mobilities of these systems and their associated effects on their respective polymer backbone. The conformational analysis of these ring systems as presented here would eventually lead to a better molecular understanding of the relationships between internal motions and functions of the nucleic acids and proteins such as collagen. Although disorder of proline rings has been known for some time, disorder of furanose rings has been observed only recently and it has been characterized in only a few cases so far (e.g., see ref 6). Disorder in crystals of oligonucleotides seems also likely and may be the culprit (at least in part) behind the high crystallographic R values or anomolous thermal parameters in some of the reported oligonucleotide structures[48,49] and drug-nucleotide complexes.[50] We have presented a method for extracting well-defined geometries of the disordered static states of the furanose ring which can be restrained during crystallographic refinement and will be particularly useful in the structural investigation of the nucleic acids and their constituents.

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Registry No. Tetrahydrofuran, 109-99-9; pyrrolidine, 123-75-1.

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Ternary Complexes as Models for Protein-Metal-Nucleic Acid Interactions: Structure of Palladium(II) Complex with Glycyl-L-tyrosine and Cytidine

M. Sabat, K. A. Satyshur, and M. Sundaralingam*

Contribution from the Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin—Madison, Madison, Wisconsin 53706. *Received July 21, 1982*

Abstract: The structure of the palladium(II) complex with glycyl-L-tyrosine and cytidine, Pd(Gly-L-Tyr)(Cyd)·6.5H_2O ($C_{20}H_{25}N_5O_9Pd$·6.5H_2O), has been determined from X-ray diffraction data. The compound crystallizes in orthorhombic space group D_2^8-$I222$ with unit cell parameters a = 15.433 (1) Å, b = 21.088 (1) Å, c = 18.257 (4) Å, V = 5942 Å^3, Z = 8, D_c = 1.57 g cm^{-3}. The structure was solved by heavy-atom methods and refined to an R of 0.065 (R_w = 0.091) by using 3027 reflections with $F > 3\sigma(F)$. The Pd ion occupies the center of a square whose corners are formed by (1) the free carboxyl O(6) of the tyrosine, (2) the deprotonated nitrogen N(5) of the peptide bond, (3) the free amino N(6) of the glycine, and (4) the ring nitrogen N(3) of the cytidine. The plane of the cytidine ring is twisted 51.1° from the square plane. The tyrosine ring slants up at an angle of 41.0° over the square plane with Pd-C (ring) distances from 3.68 to 4.60 Å. The conformation of the peptide side chain is characterized by the torsion angle $\chi_{C\alpha-C\beta}$ of 49.4°. The cytidine molecule exhibits energetically favored conformational features: the anti conformation around the glycosyl bond (χ_{CN} = 47.4°), the ${}^2T_3$ (P = 160.9°, τ_m = 38.1°) sugar pucker, and the gauche$^+$ (Ψ = 54.9°) conformation about the exocyclic C(4′)-C(5′) bond. The intermolecular interactions are described by the sequence O(4′)···Cyd···Tyr with rather extensive stacking of the cytidine and tyrosine rings and an interaction between the sugar (ring oxygen) and the base. The tyrosine OH group is involved in hydrogen bonding to the carbonyl O(9) of the glycine moiety from an adjacent complex.

Protein-nucleic acid complexes occur as a result of electrostatic, stacking, and hydrogen-bonding interactions between amino acid side chains and peptide backbones of proteins and nucleic acid bases, phosphates, and sugar moieties. Interactions between proteins and nucleic acids may also be promoted by metal ions.[1,2] The significance of the divalent metal ions in formation of nucleic acid-enzyme complexes during DNA replication and RNA synthesis has been already recognized.[3,4] Metal ions were found to have important effects on packing of DNA molecules in DNA-polylysine complexes.[5] It has been shown[6] that stacked ATP-tryptophan adducts could be stabilized by ionic bridges in which metal ion (Mn^{2+}, Cu^{2+}, or Zn^{2+}) is bounded to the two components. Ions such as Zn^{2+} and Cu^{2+} can mediate interactions between polypeptides containing glutamic acid and tyrosine residues and polynucleotides.[7]

Detailed structural information about ternary metal-amino acid(peptide)-nucleoside(nucleotide) complexes is required for further elucidation of the metal ion role in protein-nucleic acid

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interactions. However, only a few studies have been devoted to the crystal structure of the ternary complexes. The structures of the glycyl(glycinato)copper(II) complexes with cytidine,[8] cytosine,[9] adenine,[10] and 9-methyladenine[11] have been reported. In all these cases, the simplest peptide, i.e., Gly—Gly, was used, and no information could be obtained about possible interactions of the amino acid side chains. The preparation and the determination of the crystal structure of a Pd(II) ternary complex with glycyl-L-tyrosine and cytidine were carried out in this study in order to provide such structural details.

Tyrosine side chains are of particular interest because they can give rise to both stacking and hydrogen-bonding interactions with nucleic acid constituents.[12] Furthermore, in some metal complexes, the tyrosine ring can be involved in a weak interaction with the metal ion.[13-17] The main aim of the study was, therefore, to establish the influence of these interactions on the conformation of the peptide and nucleoside moieties complexed to metal ion.

Experimental Section

Preparation of the Compound. A suspension of 0.1 g (0.4 mmol) of glycyl-L-tyrosine in 5 mL of distilled water was added to an aqueous solution of 0.14 g (0.4 mmol) of K_2PdCl_4 in 5 mL of distilled water. The mixture was heated gently to ~60 °C, and then an aqueous solution of 0.1 g (0.4 mmol) of cytidine also heated to ~60 °C was added to it with stirring. The pH of the resulting solution was raised to ~7 with 0.1 N KOH. The yellow well-formed crystals of the Pd(Gly-L-Tyr)(Cyd) complex precipitated during the subsequent slow cooling of the mother solution.

Crystallographic Procedures. A crystal of dimensions 0.10 × 0.15 × 0.25 mm was sealed into a capillary in the presence of a small droplet of the mother liquid to avoid any eventual decomposition. Preliminary oscillation and Weissenberg photographs showed symmetry of the orthorhombic system. The cell parameters obtained from least-squares refinement of setting angles for 18 reflections centered on the diffractometer were a = 15.433 (1), b = 21.088 (1), and c = 18.257 (4) Å, V = 5942 Å^3, and Z = 8. Systematic absences of the type $h + k + l = 2n + 1$ indicated one of the following space groups: $I222$, $I2_12_12_1$, Imm, or $Immm$. However, only the enantiomorphic space groups $I222$ and $I2_12_12_1$ should be considered, and the former was proved to be correct on the basis of the successful structure solution and refinement. Intensities were collected on an Enraf-Nonius CAD 4 diffractometer up to 2θ = 154° by the θ–2θ scan mode by using nickel-filtered Cu radiation (λ = 1.5418 Å). Lorentz-polarization and empirical absorption corrections based on the Eulerian angle ϕ were applied during data reduction.

The structure was solved by heavy-atom techniques (Patterson and Fourier syntheses) and refined by full-matrix least-squares procedures with anisotropic temperature factors for all atoms of the complex molecule. A difference Fourier synthesis revealed that some water molecules were disordered (see also below). Isotropic refinement of alternative positions of the water oxygen atoms with variable occupancy factors was carried out. All other oxygen atoms of molecules of hydration were refined with anisotropic temperature factors. Sixteen of the twenty-five hydrogen atoms in the complex molecule were also located from the difference Fourier synthesis. The remaining H atoms were fixed by standard geometry. The hydrogen atoms were given temperature factors one unit larger than the isotropic temperature factors of the atoms connected with the H atoms, and the contributions from the H atoms were kept fixed during the refinement. Of 3363 reflections collected, 324 reflections with $F_o < 3\sigma(F_o)$ and 12 low-angle reflections affected by strong secondary extinction were given zero weights in the final cycles of the refinement.

Table I. Fractional Positional Parameters for Non-Hydrogen Atoms of Pd(Gly-L-Tyr)(Cyd)·6.5H_2O[a]

atom	occupancy factor	x	y	z
Pd		−709.8 (4)	−2075.4 (3)	−874.1 (3)
O(2)		383 (4)	−834 (3)	−877 (3)
O(2′)		2693 (6)	−530 (4)	−1398 (5)
O(3′)		2648 (6)	776 (4)	−1418 (5)
O(4′)		1097 (4)	343 (3)	−2371 (4)
O(5′)		2167 (7)	161 (4)	−3675 (4)
O(6)		−242 (4)	−2945 (3)	−1105 (4)
O(7)		−428 (5)	−3953 (3)	−848 (5)
O(8)		−3322 (6)	−2115 (4)	−2953 (4)
O(9)		−2337 (4)	−2490 (4)	788 (4)
N(1)		820 (5)	−713 (3)	−2068 (4)
N(3)		−48 (5)	1584 (3)	−1693 (4)
N(4)		−512 (5)	−2299 (4)	−2558 (4)
N(5)		−1356 (4)	−2535 (4)	−165 (4)
N(6)		−1327 (6)	−1322 (4)	−448 (5)
C(1′)		1328 (6)	−146 (4)	−1882 (5)
C(2)		384 (6)	−1031 (4)	−1520 (5)
C(2′)		2288 (6)	−215 (4)	−1998 (7)
C(3′)		2575 (7)	459 (5)	−2128 (6)
C(4)		−89 (5)	−1776 (4)	−2410 (5)
C(4′)		1830 (6)	728 (4)	−2535 (6)
C(5)		313 (6)	−1420 (4)	−2964 (5)
C(5′)		1950 (9)	766 (5)	−3369 (7)
C(6)		770 (6)	−900 (4)	−2780 (5)
C(7)		−637 (6)	−3393 (4)	−750 (6)
C(8)		−1318 (6)	−3240 (4)	−197 (5)
C(9)		−1891 (6)	−2232 (5)	298 (5)
C(10)		−1950 (8)	−1539 (5)	134 (6)
C(11)		−2199 (6)	−3507 (5)	−453 (6)
C(12)		−2530 (6)	−3182 (4)	−1134 (6)
C(13)		−2241 (6)	−3343 (4)	−1828 (6)
C(14)		−2500 (6)	−3004 (5)	−2448 (5)
C(15)		−3039 (6)	−2488 (5)	−2370 (5)
C(16)		−3368 (6)	−2323 (5)	−1671 (5)
C(17)		−3111 (5)	−2677 (5)	−1070 (5)
O(W1)	1.0	−4084 (7)	−2231 (8)	984 (6)
O(W2)	1.0	−4531 (8)	−1099 (5)	−2811 (6)
O(W3)	0.5	−1283 (15)	0	5000
O(W4)	0.5	5000	−2887 (13)	0
O(W5)	1.0	−561 (11)	−4027 (9)	−3451 (9)
O(W6A)	0.67	−383 (2)	−16 (1)	−418 (1)
O(W6B)	0.33	−389 (3)	−1 (3)	−348 (2)
O(W7A)	0.15	189 (5)	0	0
O(W7B)	0.15	121 (5)	0	0
O(W7C)	0.20	318 (4)	0	0
O(W8)	0.34	500	411 (2)	0
O(W9)	0.15	500	−160 (4)	0
O(W10)	0.58	83 (2)	436 (1)	438 (1)

[a] Parameters are multiplied by 10^4 except those for oxygen atoms from disordered water molecules (W6–W10), which are multiplied by 10^3.

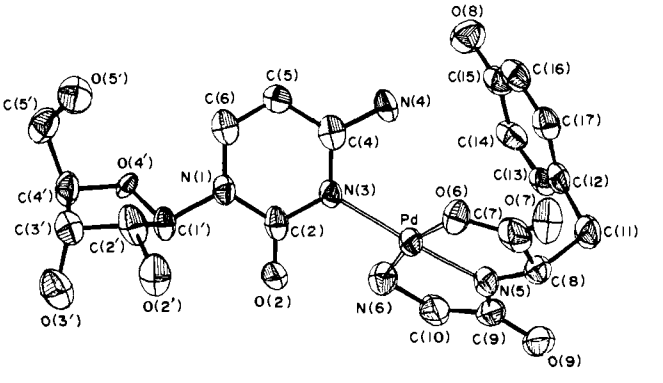

Figure 1. ORTEP drawing of the Pd(Gly-L-Tyr)(Cyd) molecule. The ellipsoids are drawn at the 50% probability level.

The weighting scheme used was of the form $w = (\sigma^2 + 0.0036F_o^2)^{-1}$. The final R index for the 3027 reflections was 0.065 and the R_w value[18]

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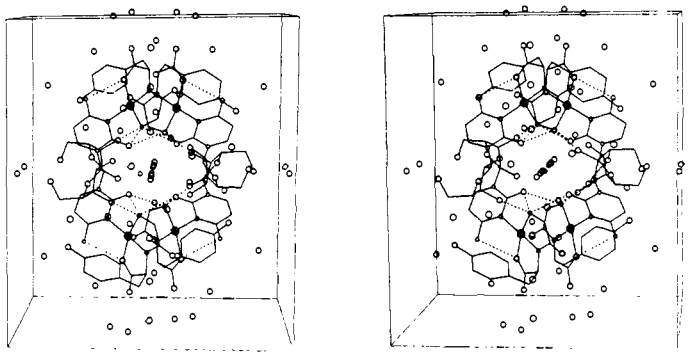

Figure 2. Stereoview of the unit cell looking down the *a* axis. Only four molecules of the complex distributed around ($^1/_2$, $^1/_2$, $^1/_2$) are included in order to present the hydration sphere. Possible hydrogen-bond interactions between atoms of the complex molecule are indicated by dashed lines.

was 0.091. Atomic scattering factors including anomalous scattering components for all non-hydrogen atoms were taken from the International Tables.[19]

The final coordinates of the non-hydrogen atoms are given in Table I. Table II[20] contains the temperature factors of the non-hydrogen atoms, while the parameters of the H atoms are listed in Table III.[20] Table IV[20] presents the values of $|F_o|$ and $|F_c|$ for the collected reflections.

Results

The asymmetric part of the unit cell contains a discrete molecule of the Pd(Gly-L-Tyr)(Cyd) complex (Figure 1) and 6.5 water molecules. The distribution of the unit cell content is shown in Figure 2. The structure is rather highly hydrated, and some of the molecules of hydration exhibit significant disorder.

The disposition of the complex molecules within the unit cell leaves channels centered around the crystallographic 2-fold axes parallel to *x* (see Figure 2). The channels are filled with water molecules located on the 2-fold axis and disordered between three sites (see Table I). There is also another water molecule distributed between three sites: two of them on the 2-fold axis parallel to *y* (the refined occupancy factors for the sites are 0.34 and 0.15) and the third with the occupancy factor of 0.58 in general position. All molecules of hydration participate in an extensive network of hydrogen bonding (Table VII).

Earlier reports on the structure of Pd complexes with cytosine and its derivatives in solution at neutral pH[21] and in crystalline state[22-24] indicated invariably the coordination of palladium to nitrogen N(3) of cytosine. This kind of bonding was found also in the present complex (Figure 1 and Table V), though the Pd–N(3) bond length appears to be slightly longer than that of 2.031 (2) Å in *trans*-dichlorobis(1-methylcytosine)palladium(II).[22] The exocyclic O(2) atom of cytidine occupies an axial position in the coordination sphere, and one might expect some metal···O(2) interaction, as it has been observed in square-planar Cu complexes, where the N(3),O(2) chelation system was formed. Even though many other details of the chelate geometry are almost identical with those in $Cu(Cyt)_2Cl_2$[25] and Cu(Gly-Gly)(Cyd),[8] the Pd–O(2) distance of 3.113 (6) Å is too long for any significant bonding interactions. The remaining sites of the square-planar coordination sphere of the Pd(II) ion are occupied by the glycine amine nitrogen N(6), the deprotonated peptide nitrogen N(5), and the tyrosyl terminal carboxylate oxygen O(6).

The compound presented here is the first example of Pd(II) complex with peptide investigated by X-ray crystallography. A systematic comparison of the geometrical features can be carried out for this class of Pd compounds when further structural studies, some of which are currently in progress in this laboratory, have been completed. Nevertheless, it should be mentioned that the Pd–O and Pd–N bond lengths (see Table V) are not very different from the analogous distances found in transition metal–peptide complexes.[26] Similar bond lengths were also observed in Pd compounds with amino acids.[15,27]

The peptide side chain occurs in a gauche conformation characterized by the torsion angle $\chi_{C_\alpha-C_\beta}$ of 49.4° with the tyrosine

Table V. Bond Distances (Å) and Angles (deg) in Pd(Gly-L-Tyr)(Cyd)·6.5H_2O

Bond Distances			
Pd-O(6)	2.016 (6)	Pd-N(3)	2.087 (7)
Pd-N(5)	1.901 (8)	Pd-N(6)	2.011 (9)
N(1)-C(2)	1.38 (1)	C(2)-O(2)	1.25 (1)
C(2)-N(3)	1.38 (1)	N(3)-C(4)	1.37 (1)
C(4)-N(4)	1.31 (1)	C(4)-C(5)	1.40 (1)
C(5)-C(6)	1.35 (1)	C(6)-N(1)	1.36 (1)
N(1)-C(1')	1.47 (1)	C(1')-C(2')	1.50 (1)
C(2')-O(2')	1.43 (1)	C(2')-C(3')	1.51 (1)
C(3')-O(3')	1.46 (1)	C(3')-C(4')	1.48 (2)
C(4')-O(4')	1.43 (1)	O(4')-C(1')	1.41 (1)
C(4')-C(5')	1.54 (2)	C(5')-O(5')	1.43 (1)
O(6)-C(7)	1.30 (1)	C(7)-O(7)	1.24 (1)
C(7)-C(8)	1.49 (1)	C(8)-N(5)	1.49 (1)
N(5)-C(9)	1.34 (1)	C(9)-O(9)	1.25 (1)
C(9)-C(10)	1.49 (2)	C(10)-N(6)	1.50 (1)
C(8)-C(11)	1.54 (1)	C(11)-C(12)	1.51 (1)
C(12)-C(13)	1.39 (1)	C(13)-C(14)	1.40 (1)
C(14)-C(15)	1.38 (1)	C(15)-C(16)	1.42 (1)
C(16)-C(17)	1.39 (1)	C(17)-C(12)	1.40 (1)
C(15)-O(8)	1.39 (1)		

Bond Angles			
O(6)-Pd-N(5)	82.3 (4)	O(6)-Pd-N(3)	97.3 (4)
N(3)-Pd-N(6)	96.8 (4)	N(5)-Pd-N(6)	83.7 (4)
Pd-O(6)-C(7)	112.9 (8)	O(6)-C(7)-O(7)	120 (1)
O(6)-C(7)-C(8)	121 (1)	C(7)-C(8)-N(5)	105.7 (9)
C(7)-C(8)-C(11)	110 (1)	C(8)-N(5)-Pd	117.5 (7)
C(9)-N(5)-Pd	120.6 (7)	N(5)-C(9)-O(9)	125 (1)
N(5)-C(9)-C(10)	122 (1)	C(9)-C(10)-N(6)	114 (1)
C(10)-N(6)-Pd	109.7 (8)	C(8)-C(11)-C(12)	113 (1)
C(12)-C(13)-C(14)	122 (1)	C(13)-C(14)-C(15)	120 (1)
C(14)-C(15)-C(16)	120 (1)	C(15)-C(16)-C(17)	119 (1)
C(16)-C(17)-C(12)	122 (1)	C(2)-N(1)-C(6)	121.4 (9)
N(1)-C(2)-N(3)	118.9 (9)	C(2)-N(3)-C(4)	119.4 (9)
N(3)-C(4)-C(5)	120.6 (9)	C(4)-C(5)-C(6)	119.1 (9)
C(5)-C(6)-N(1)	120.4 (9)	C(1')-N(1)-C(2)	119.5 (8)
C(1')-N(1)-C(6)	119.1 (9)	C(1')-O(4')-C(4')	110.5 (8)
O(4')-C(1')-C(2')	103.3 (9)	C(1')-C(2')-C(3')	103 (1)
C(1')-C(2')-O(2')	107 (1)	C(2')-C(3')-O(3')	109 (1)
C(2')-C(3')-C(4')	102 (1)	C(3')-C(4')-O(4')	107 (1)
C(4')-C(5')-O(5')	112 (1)		

(18) The R_w index was defined as $R_w = [\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$.
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Table VI. Torsion Angles (deg) in Pd(Gly-L-Tyr)(Cyd)·6.5H_2O

Pd-O(6)-C(7)-C(8)	−4 (1)
O(6)-C(7)-C(8)-N(5)	−2 (1)
C(7)-C(8)-N(5)-C(9)	−178 (1)
C(8)-N(5)-C(9)-O(9)	7 (1)
C(8)-N(5)-C(9)-C(10)	−169 (1)
N(5)-C(9)-C(10)-N(6)	−7 (1)
C(9)-C(10)-N(6)-Pd	6 (1)
N(5)-C(8)-C(11)-C(12) ($\chi_{C_\alpha-C_\beta}$)	49 (1)
O(4')-C(1')-N(1)-N(6) (χ_{CN})	47 (1)
C(4')-O(4')-C(1')-C(2') (τ_0)	−23 (1)
O(4')-C(1')-C(2')-C(3') (τ_1)	37 (1)
C(1')-C(2')-C(3')-C(4') (τ_2)	−36 (1)
C(2')-C(3')-C(4')-O(4') (τ_3)	22 (1)
C(3')-C(4')-O(4')-C(1') (τ_4)	1 (1)
C(3')-C(4')-C(5')-O(5') (Ψ)	55 (2)
O(4')-C(4')-C(5')-O(5')	−66 (2)

ring located above the complex plane. NMR studies of Pd(II) complexes with aromatic amino acids and peptides have indicated[13,14] that this conformation is favored also in solution. Furthermore, some metal–carbon distances less than the sum of the van der Waals radii have been observed in Pd(II)[15] and Cu(II)[16,17] complexes with tyrosine and its derivatives, suggesting an existence of weak metal ion–aromatic ring interactions. Crystal structure analysis of the potassium L-tyrosine *O*-sulfate[28] showed that the conformation of the tyrosine residue can be also influenced by main-group metal ions. In that structure, the potassium ion is coordinated to five ligands on one side, and the other side is occupied by the tyrosine ring with possible weak interaction between the K ion and π-electron cloud of the aromatic system.

The intramolecular stacking in the Pd(Gly-L-Tyr)(Cyd) complex is thus realized through the tyrosine ring–coordination plane interactions, though both planes are far from being parallel as the dihedral angle between them is 41.0°.

The complexed cytosine ring is tilted 51.1° relative to the Pd coordination plane, and this makes it difficult for any closer approach between the plane and that of tyrosine aromatic ring to occur. The Pd–C (ring) distances range from 3.68 to 4.60 Å. However, in spite of the steric hindrance, the aromatic ring is again positioned above the coordination plane as in bis(L-tyrosinato)-palladium(II)[15] where such hindrances do not exist. The observed stacking pattern is rather different from that postulated by Sigel and Naumann[6] for metal–ATP–tryptophan systems where the interactions between adenine and tryptophan rings appear to prevail. Because of the lack of available data on the crystal structure of ternary metal–peptide–nucleoside (nucleotide) complexes, it is difficult to say whether and to what extent the base–aromatic side chain and metal–aromatic side chain stacking interactions are competitive. In fact, it has been found[6] that the intramolecular Trp–Ade stacking is more pronounced in Zn(ATP)(Trp)$^{3-}$ than in Cu(ATP)(Trp)$^{3-}$, where the square-planar coordination geometry is more favorable for metal–tryptophan interactions.

Conformational features of the cytidine moiety are typical for nucleosides coordinated to metal ions.[29] The ribose moiety is in the common C(2')endo puckering mode characterized by the torsion angles given in Table VI. The pseudorotation parameters τ_m and P have values of 38.1 and 160.9°, respectively. The configuration of the cystosine ring about the glycosyl C(1')–N(1) bond is anti with the torsion angle of 47.4°, and the conformation about the exocyclic C(4')–C(5') bond is gauche$^+$ (54.9°).

The crystal packing is accomplished by hydrogen-bonding and intermolecular stacking interactions. In addition to the stacking of the Pd coordination planes with Pd···Pd distance of 3.871 (1) Å (Figure 3b), there is an intermolecular interaction between the oxygen O(4') of ribose and the cytosine ring of a neighboring complex (see Figure 3a). The significance of such sugar–base

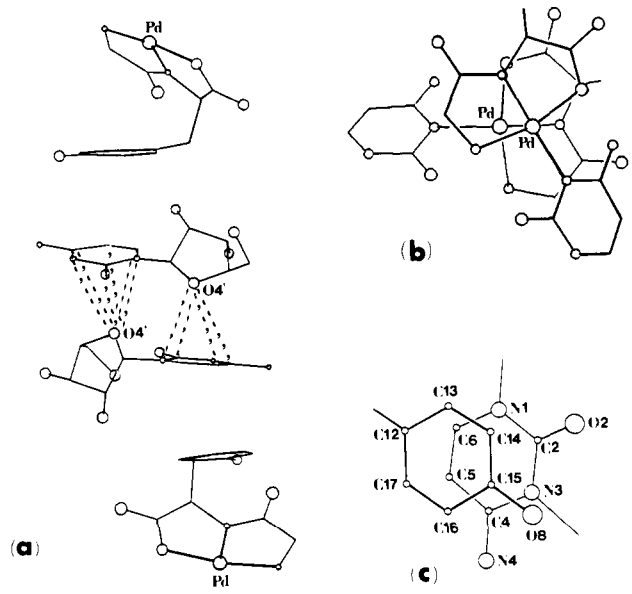

Figure 3. Stacking interactions in the Pd(Gly-L-Tyr)(Cyd)·6.5H_2O structure: (a) fragment of the structure showing the sugar O(4')-cytosine ring and the cytosine–tyrosine stacking interactions (the O(4')-cytosine distances less than 3.4 Å are marked by dashed lines, (b) stacking of the Pd coordination planes, (c) overlapping between the cytosine and tyrosine rings viewed perpendicular to the tyrosine ring.

Table VII. Intermolecular Contacts (Å) in Pd(Gly-L-Tyr)(Cyd)·6.5H_2O

Hydrogen-Bonding Contacts			
O(2)···N(6)[a]	2.94 (1)	O(2)···O(W7B)[b]	2.70 (4)
O(2')···O(W5)[c]	2.87 (2)	O(2')···O(W7A)[b]	3.05 (3)
O(2')···O(W7C)[b]	2.88 (4)	O(3')···N(6)[d]	2.94 (1)
O(3')···O(W10)[e]	2.77 (3)	O(5')···O(W3)[h]	2.80 (1)
O(5')···O(W6A)[d]	2.73 (3)	O(5')···O(W6B)[d]	2.71 (5)
O(6)···N(4)[a]	3.01 (1)	O(6)···O(W2)[c]	3.03 (1)
O(7)···O(W2)[c]	2.82 (2)	O(7)···O(W6A)[f]	2.79 (2)
O(7)···O(W6B)[f]	2.77 (3)	O(8)···O(9)[g]	2.65 (1)
O(8)···O(W2)	2.85 (2)	O(9)···O(W1)	2.78 (1)
N(4)···O(W1)[g]	2.91 (1)	N(6)···O(W7A)[h]	3.03 (2)
N(6)···O(W7B)[h]	2.91 (1)	O(W1)···O(W4)[i]	2.67 (2)
O(W1)···O(W5)[j]	2.90 (3)	O(W2)···O(W5)[k]	2.81 (2)
O(W3)···O(W8)[l]	2.73 (3)	O(W5)···O(W10)[b]	2.83 (3)
O(W9)···O(W10)	2.65 (7)		
Some Contacts Less Than 3.6 Å			
O(4')···N(1)[d]	3.11 (1)	O(4')···N(3)[d]	3.32 (1)
O(4')···C(2)[d]	3.12 (1)	O(4')···C(4)[d]	3.40 (1)
O(4')···C(5)[d]	3.33 (1)	O(4')···C(6)[m]	3.20 (1)
C(4)···C(15)[m]	3.55 (1)	C(5)···C(15)[m]	3.49 (1)
C(5)···C(16)[m]	3.41 (1)	C(5)···C(17)[m]	3.56 (1)
C(6)···C(14)[m]	3.56 (1)		

[a] Intramolecular hydrogen bond. [b] $x, -y, -z$. [c] $1/2 + x, 1/2 - y - 1, 1/2 - z - 1$. [d] $-x, -y, z$. [e] $1/2 - x, 1/2 - y, 1/2 + z - 1$. [f] $1/2 - x - 1, 1/2 + y - 1, 1/2 - z - 1$. [g] $1/2 - x - 1, 1/2 - y - 1, 1/2 + z - 1$. [h] $-x, y, -z$. [i] $x - 1, y, z$. [j] $1/2 - x - 1, 1/2 - y - 1, 1/2 + z$. [k] $1/2 + x - 1, 1/2 - y - 1, 1/2 - z - 1$. [l] $1/2 - x, 1/2 - y, 1/2 + z$. [m] $1/2 + x, 1/2 - y - 1, 1/2 - z - 1$.

stacking interactions for stabilization of nucleic acid structures has been pointed out earlier,[30] and it has gained great interest after the discovery of its presence in the left-handed *Z* DNA structure.[31] There is also a significant overlapping between the tyrosine ring and the cytosine ring of the adjacent complex (Figure 3c). Thus, the base ring is sandwiched between the sugar moiety on one side and the tyrosine ring on the other side. Both rings are almost parallel (the dihedral angle between least-squares planes through

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the rings is 15.5°). The closest contacts between the atoms of the rings are 3.49 (1) and 3.41 (1) Å for C(5)···C(15) and C(5)···C(16), respectively. Stacking interactions between purine and pyrimidine bases and tyrosine rings are thought to be of significance for protein–nucleic acid recognition. Studies on crystal structure of the aminoglycosyl antibiotic puromycin containing the *p*-methoxyphenylalanyl ring and the adenine base have shown[32] that the bases and the aromatic rings form columns of alternating stacks with an interplanar separation of about 3.4 Å. However, the structures containing both pyrimidine bases and tyrosine residues studied so far exhibit rather different modes of interaction. In the staphylococcal nuclease–thymidine 3′,5′-diphosphate–Ca^{2+} complex, the thymine ring and the phenyl ring of Tyr 113 are almost parallel but not stacked with each other.[33] Another pattern is observed in the 1-thyminylacetic acid–tyramine (1:1) complex,[34] where the hydroxy group of the tyramine molecule, and not the aromatic ring, is located above the pyrimidine ring. The stacking sequence in the crystal structure of Pd(Gly-L-Tyr)(Cyd) can be described by the scheme

O(4′)···Cyd···Tyr···Pd

with quite extensive overlapping between the cytidine and tyrosine aromatic rings. As mentioned earlier, the tyrosine residue can interact with nucleosides both in a stacking mode and by formation of hydrogen bonding through its OH group. However, there is no hydrogen-bonding interaction between the hydroxyl group of tyrosine and the nucleoside in the present structure. Instead, the OH group is hydrogen bonded to the carbonyl of the glycine residue, with O(8)···O(9) distance of 2.65 (1) Å. The tyrosine OH group is also involved in the hydrogen bonding (see Table VII) with water of crystallization $H_2O(2)$ and the D···A distance [O(W2)···O(8)] is 2.86 (1) Å.

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Supplementary Material Available: Tables of thermal parameters and structure factors for all atoms of Pd(Gly-L-Tyr)-(Cyd)·6.5H_2O (17 pages). Ordering information is given on any current masthead page.

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The Bicarbonate Proton in Carbonic Anhydrase Catalysis

Y. Pocker* and Thomas L. Deits

Contribution from the Department of Chemistry, BG-10, University of Washington, Seattle, Washington 98195. *Received March 1, 1982*

Abstract: Substitution of an alkyl group (methyl through *n*-pentyl) for the proton of HCO_3^- dramatically alters the properties of the resultant alkyl carbonate esters, $ROCO_2^-$, toward bovine carbonic anhydrase (BCA). While HCO_3^- is the natural substrate of BCA, with a turnover number of $\sim 10^6\ s^{-1}$, the alkyl carbonates show no detectable substrate activity under conditions designed to detect catalysis of substrates with turnover numbers less than $10^1\ s^{-1}$. The alkyl carbonates bind efficiently to BCA, however, acting as typical anionic inhibitors of BCA-catalyzed CO_2 hydration, with K_i values comparable to those of the alkyl carboxylates, RCO_2^-, under the same conditions. We hypothesize the substitution of an alkyl group inhibits a proton transfer essential in the BCA-catalyzed dehydration of HCO_3^-. We further show that the proton of HCO_3^- also permits a unique binding interaction with BCA. At high pH (pH 9.0), HCO_3^- inhibits BCA-catalyzed CO_2 hydration by a linear mixed noncompetitive mechanism, distinct from the linear uncompetitive mechanism observed for the alkyl carbonates and other anions of diverse structure. We demonstrate the congruence of these results with our previous rapid-equilibrium kinetic analysis of BCA catalysis and offer a molecular mechanism for carbonic anhydrase that provides an appealing physical model for catalysis.

Carbonic anhydrase (CA) is a widely distributed Zn(II) metalloenzyme of exceptional catalytic efficiency.[1,2] Its physiological activity, the catalysis of the reversible hydration of CO_2 and dehydration of HCO_3^-, is carried out with turnover numbers approaching $10^6\ s^{-1}$. A critical question that remains unanswered in the elucidation of the molecular details of catalysis is the role of the HCO_3^- proton in the catalytic cycle.

The net uptake and release of a proton from the enzyme during the course of a complete catalytic cycle are required by the reaction stoichiometry. The function, if any, of the proton of the HCO_3^- ion itself in the catalytic cycle is a quite distinct question, which, if understood, could strongly constrain acceptable mechanisms for CA catalysis. To probe this question, we have initiated the first substrate analogue study of the catalysis by CA of HCO_3^- dehydration, employing as structural analogues the alkyl carbonates $ROCO_2^-$.

We have previously characterized the solution kinetic parameters for alkyl carbonate decomposition under physiological conditions, demonstrating the full congruence of the mechanism of decomposition of the alkyl carbonates and HCO_3^- in the absence of CA.[3] The alkyl carbonates therefore appear to be excellent structural and mechanistic analogues of HCO_3^-.

In order to understand the interaction of the alkyl carbonates with carbonic anhydrase, account must be taken of the anionic nature of these compounds. To this end, we have recently completed and reported upon a comprehensive analysis of the inhibition of CA-catalyzed CO_2 hydration by monoanions, the results of which have led us to propose a novel mechanism of catalysis for CA.[4,5]

From the results of our previous work and the present study, we find, on the basis of the alkyl carbonates as substrate analogues, that the HCO_3^- proton is critical in catalytic turnover, and, on the basis of the alkyl carbonates and HCO_3^- as inhibitors of

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