gave a colorless oil (120 mg), which was purified by preparative TLC (chloroform–ethyl acetate (5:2)) to afford 26 (41 mg, 58%). Essentially the same result was obtained with Z-25.

Photoaddition of 5′-O-Acetyl-6-cyano-2′,3′-O-isopropylideneuridine (27) to 1-Hexyne. A solution of 27[14a] (188 mg, 0.55 mmol) and 1-hexyne (1.03 g, 12.5 mmol) in dry acetonitrile (100 mL) was irradiated under the standard conditions for 10 h at room temperature. After removal of the solvent, the oily residue was purified by preparative TLC (chloroform–ethyl acetate (5:1)) to yield **28** (86 mg, 37%).

5′-O-Acetyl-2′,3′-O-isopropylidene-5-(2-cyano-1-hexen-1-yl)uridine (28): viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, 3 H, J = 7 Hz), 1.76 (s, 3 H), 1.98 (s, 3 H), 1.60–2.10 (m, 4 H), 2.46 (s, 3 H), 2.75 (t, 2 H, J = 7 Hz), 3.65–3.80 (m, 1 H), 3.76 (d, 2 H, J = 1 Hz), 4.18–4.26 (m, 1 H), 4.38 (dd, 1 H, J = 6, 2 Hz), 5.30 (d, 1 H, J = 2 Hz), 6.42 (s, 1 H), 7.76 (s, 1 H), 9.50 (br, 1 H); mass spectrum, m/e (rel intensity) 433 (M^+, 18), 418 (30), 375 (17), 298 (5), 215 (100), 157 (60); high-resolution mass spectrum, calcd for $C_{21}H_{27}O_7N_3$: 433.1848, found 433.1816.

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Registry No. 1a, 49846-86-8; **1b**, 53293-11-1; **2**, 74226-53-2; **3**, 75156-34-2; **4**, 75156-35-3; **5**, 84175-00-8; **6a**, 75156-38-6; **6b**, 84175-01-9; **7a**, 75156-41-1; **7b**, 84175-02-0; **8b**, 75156-43-3; **9a**, 84175-03-1; **9b**, 84175-04-2; **21**, 75156-37-5; **22**, 75173-99-8; **23**, 75156-36-4; **24**, 75156-39-7; (*E*)-**25**, 74226-50-9; (*Z*)-**25**, 74226-51-0; **27**, 34351-92-3; **28**, 84275-39-8; 2-methyl-2-butene, 513-35-9; cyclopentene, 142-29-0; 1-propanethiol, 107-03-9; 1-hexene, 592-41-6; *cis*-cyclooctene, 931-87-3; 1-hexene, 643-02-7.

A Method for the Analysis of Puckering Disorder in Five-Membered Rings: The Relative Mobilities of Furanose and Proline Rings and Their Effects on Polynucleotide and Polypeptide Backbone Flexibility

E. Westhof† and M. Sundaralingam*

Contribution from the Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin—Madison, Madison, Wisconsin 53706. *Received August 14, 1981*

Abstract: In crystals, furanose or pyrrolidine rings are sometimes found disordered between different puckers. This puckering or pseudorotational disorder is classified into case I, three atoms anchored in the crystal (or one endocyclic bond angle constant), and case II, four atoms fixed in the crystal (or one endocyclic torsion angle constant). For each case, the geometrically possible disordered puckered states are investigated and analyzed in the framework of the pseudorotation concept. For case I, the pseudorotational domain available is generally restricted to one quadrant of the pseudorotational wheel. For case II, the pseudorotational domains available consist of narrow bands centered on the axis defined by the envelope states of the atom opposite the constant torsion angle. The method provides a knowledge of the possible disordered puckered states and their geometries that will be useful in crystallographic refinement of the nucleic acid monomers and oligomers as well as the polymers. The mobilities of the five-membered pyrrolidine and furanose ring in proline derivatives and nucleic acids, respectively, are compared from solid-state and solution data in the pseudorotation framework. In both systems, hydroxylation decreases flexibility (dR (DNA) vs. R (RNA); Pro vs. Hyp). In general, the proline ring is more flexible, and therefore more liable to disorder, than the furanose ring. However, the effects of the ring mobility or pseudorotational disorder on the polymer backbone are less pronounced in proline-containing polypeptides than in polynucleotides.

It is well-known that, in the solid state, the furanose ring of nucleic acid constituents is not planar but commonly puckered at either the C(2′) or C(3′) atom or at both atoms.[1,2] Usually, in nucleosides and nucleotides only one puckered state of the five-membered sugar ring is observed. In crystals of dinucleotides, the puckers of the two sugars are sometimes different but each nucleotide fragment has a precise pucker. When there is more than one molecule in the asymmetric unit, different conformations of the sugar,[3] the glycosyl bond torsion,[4] and the C(4′)–C(5′) exocyclic group,[5] are often observed. In these cases, each molecule has the same conformation in all of the unit cells of the crystal. Puckering disorder or pseudorotational disorder of a furanose ring with two different sugar conformations at the same position (crystallographic disorder) has been observed only recently.[6-8]

In contrast to furanose rings, the five-membered pyrrolidine ring of the imino acid proline is often found disordered in the solid state (e.g., see ref 9 and 10). The pyrrolidine ring is usually puckered at the C(γ) and/or C(β) carbon atoms,[11-15] and the disorder in the ring involves either C(γ) alone or both C(γ) and C(β) atoms.

The various puckered states of five-membered rings are best understood in the framework of the pseudorotation concept[16]

† Present address: Centre National de La Recherche Scientifique, Institut de Biologie Moléculaire et Cellulaire, 67084 Strasbourg, France.

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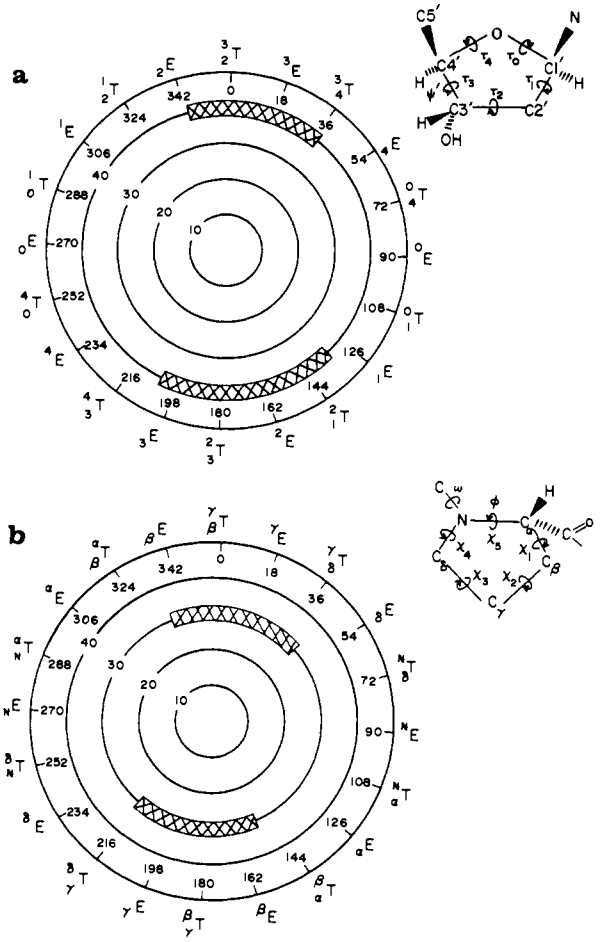
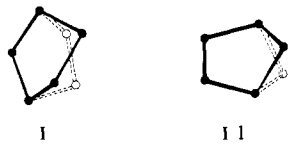


Figure 1. (a) Pseudorotation wheel of furanose rings with the nomenclature and the preferred puckered domains.[16] The phase angle of pseudorotation (in multiples of 18°) travels around the circle and the amplitudes of pucker radially increase from the center toward the edge (10°,..., 50°). Endo puckerings are indicated by an upper subscript and exo by a lower subscript. For the non symmetrical twist conformations occurring on each side of the envelope conformations (±9°), the most puckered atom is on the left (upper subscript) and the least one on the right (lower subscript). (b) Pseudorotation wheel of pyrrolidine rings. Analysis of proline ring conformations using the pseudorotation model had also been made earlier in these laboratories.[18] The nomenclature used here is analogous to that used in Figure 1a (see also ref 19). The preferred puckered domains are indicated. The extension of each domain depends on several factors (substitution, linear vs. cyclic peptides, etc.).[29,30] The endocyclic torsion angles χ_i are related to the endocyclic torsion angles τ_i of furanose by $\chi_i = \tau_i$, $i = 1,...,4$, $\chi_5 = \tau_0$. The phase of pseudorotation, P, and the amplitude of pucker, τ_m, are given by $\tan P = B/A$ and $\tau_m^2 = A^2 + B^2$, respectively, where $A = (2/5)\sum_{i=1}^{5}\theta_i \cos[4\pi(i-1)/5]$, and $B = -(2/5)\sum_{i=1}^{5}\theta_i \sin[4\pi(i-1)5]$, where $\chi_1 = \theta_5$, $\chi_2 = \theta_1$, $\chi_3 = \theta_2$, $\chi_4 = \theta_3$, and $\chi_5 = \theta_4$ (see ref 44). The backbone torsion angle ϕ, C–C(α)–N–C, is also shown.

according to which each pucker is described by two parameters, the phase angle of pseudorotation, P, and the amplitude of pucker, τ_m. Figure 1a shows a pseudorotational wheel for furanose rings with the nomenclature and the preferred domains as indicated by numerous crystal structures.[16,17] Figure 1b shows the corresponding pseudorotational wheel for pyrrolidine rings with the appropriate nomenclature for comparison purposes.[18,19] The



I II

Figure 2. Two main cases of disorder. In case I disorder (left), one bond angle is assumed constant; i.e., three consecutive atoms of the ring do not show residual difference electron densities. In case II disorder (right), one torsion angle is assumed constant; i.e., four consecutive atoms of the five-membered ring are anchored in the crystal.

conformations of the pyrrolidine ring in the solid state are distributed also in two broad preferred puckered domains.

In this paper, we will exploit the pseudorotational description of five-membered puckered states to describe the allowed combinations of the phase of pseudorotation, P, and of the amplitude of pucker, τ_m, for disordered furanose rings and pyrrolidine rings. Because of the closed structure of five-membered rings, the geometrically possible disordered puckered states depend on the number of atoms that are invariant. With only one or two atoms anchored in the crystal, all possible puckered states of the pseudorotational circle are in principle allowed. On the other hand, with three consecutive atoms fixed, only those combinations of the two pseudorotation parameters P and τ_m which keep the corresponding endocyclic angle constant are allowed. With four atoms fully ordered, only those combinations of P and τ_m which keep the corresponding endocyclic torsion angle constant are possible. The knowledge and use of these combinations will aid in restrained refinement of disordered crystal structures.[20]

This geometrical analysis also gives an insight on the relative free energies and entropy contents of the preferred puckers as well as the energies of activation for the possible modes of interconversion. A knowledge of these energies is a prerequisite for assessing the ring mobilities in polymers and their effects on the backbone flexibility and for simulating the dynamics and fluctuations of macromolecules.[21,22]

Crystallographic Pseudorotational Disorder

The endocyclic bond angles and, to a lesser extent, the bond lengths of five-membered rings observed in the crystal depends on the puckered state adopted.[1,2] Recently, the dependence of the endocyclic bond angles in furanose rings as a function of the pseudorotation parameters P and τ_m has been parametrized.[23] From the knowledge of the interrelationships between furanose geometry and ring pucker, it was possible to derive the coordinates of the closed furanose rings for any value of P and τ_m.[24] Using these coordinates we calculated the endocyclic bond and torsion angles for an ensemble of ring puckers and plotted them on (P, τ_m) plots (see figures below).

Here we consider two main cases of disorder found in the solid state as revealed by difference electron density maps (Figure 2). For the case I disorder three consecutive atoms in the five-membered ring do not reveal interpretable residual densities around them. This corresponds to one bond angle and the two associated bond distances being constant. For the case II disorder, four atoms of the five-membered ring are anchored in the crystal. This

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(17) In furanose rings, an endo displacement is toward the exocyclic group at C(5′), and in proline rings, it is toward the carbonyl group. Thus, the puckers of D sugars and L-proline rings are "enantiomerically" related; i.,e., a C(2′)-endo furanose pucker corresponds to a C(β)-exo proline pucker.
(18) S. T. Rao and M. Mallikarjunan, Abstract I11, American Crystallography Association summer meeting, University Park, PA, 1974.
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(23) (a) E. Westhof and M. Sundaralingam, *J. Am. Chem. Soc.*, **102**, 1493–1500 (1980). In Table I of the latter paper, the value of A at $\tau_m = 30°$ for O(4′) should be 110.27° and not 116.27°. (b) H. P. M. DeLeeuw, C. A. G. Haasnoot, and C. Altona, *Isr. J. Chem.*, **20**, 108–126 (1980).
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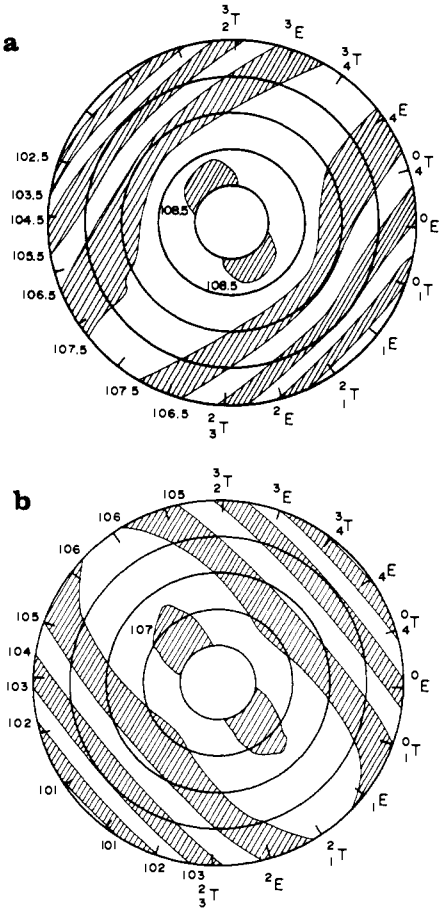

Figure 3. (a) Contours of constant endocyclic bond angle at C(1′) for furanose rings in a (P, τ_m) plot. (b) Contours of constant endocyclic bond angle at C(4′) for furanose rings in a (P, τ_m) plot.

corresponds to one torsion angle and the three associated bond distances and two bond angles being constant.

Case I Disorder

There are five possible case I disorders corresponding to the five endocyclic angles. Figure 3 is a (P, τ_m) plot representing the contours of constant endocyclic bond angles at C(1′) and at C(4′). In each case, there is a pseudomirror symmetry around the axis connecting the two envelope puckers ${}^1E$–${}_1E$ and ${}^4E$–${}_4E$, respectively. The contours of constant endocyclic angle run as bands perpendicular to this axis, i.e., along the lines connecting the twist puckers ${}^3_4T$–${}^4_3T$ and ${}^1_2T$–${}^2_1T$, respectively, except when approaching the planar state. In other words the interpretation of possible states should conform to those puckerings prescribed by the contours of constant endocyclic angle.

Common nucleo*sides* and nucleo*tides* usually do not occupy the western half of the pseudorotational wheel, especially the region $198° \leq P \leq 342°$ which leads to steric conflicts.[25] Thus, the possible puckered states with one constant endocyclic angle are restricted mainly to one quadrant: the southeastern quadrant for C(1′) (Figure 3a), and the northeastern quadrant for C(4′) (Figure 3b), including a small region on either side of the quadrant.

In Figure 4, the contours in the (P, τ_m) plot represent constant endocyclic bond angle at O(4′). The contours are much tighter and several elliptic bands are contained in the circle corresponding to $\tau_m = 50°$. It is, therefore, possible to cover the whole eastern half, and the possible states at constant amplitude of pucker are symmetrically disposed with respect to the ${}^0E$–${}_0E$ axis. With any one of the three angles C(1′), C(4′), or O(4′) constant, a disorder between ${}^1_2T$ and ${}^2_3T$ is possible at $\tau_m \sim 40°$. However, a ${}^3E$–${}^2E$ disorder is possible at constant amplitude with O(4′) constant but

(25) N. Yathindra and Sundaralingam, *Biopolymers*, **13**, 2061–2076 (1974).

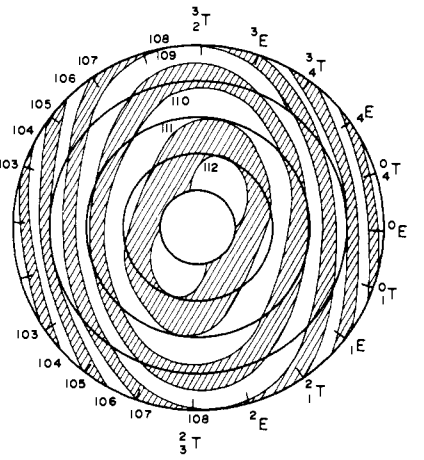

Figure 4. Contours of constant endocyclic bond angle at O(4′) for furanose rings in a (P, τ_m) plot.

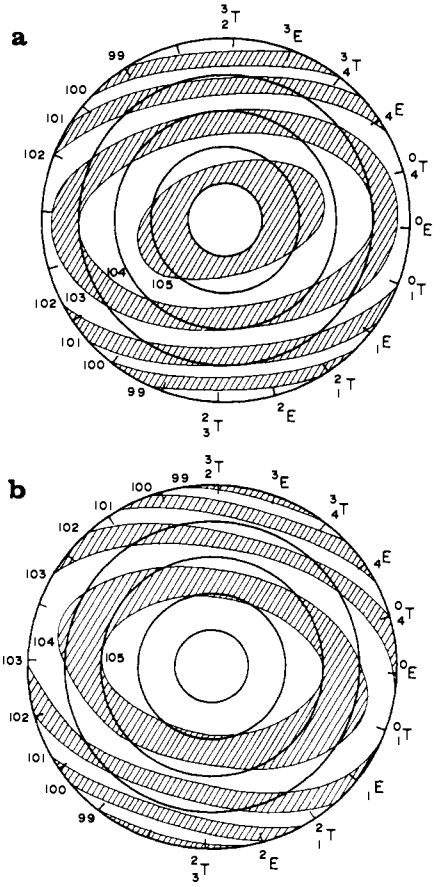

Figure 5. (a) Contours of constant endocyclic bond angle at C(2′) for furanose rings in a (P, τ_m) plot. (b) Contours of constant endocyclic bond angle at C(3′) for furanose rings in a (P, τ_m) plot.

not with either C(1′) or C(4′) constant.

Figure 5 gives the contours of constant endocyclic bond angles at the C(2′) and the C(3′) atoms. The contours are very tight at the periphery but quite broad toward the center. Thus, in a flattened ring ($\tau_m \sim 20°$–$30°$), whole domains of pseudorotation phase become accessible. The ${}^2_3T$–${}^3_2T$ and ${}^2E$–${}^3E$ disorder are possible at $\tau_m \sim 40°$, keeping the angle at C(2′) constant.

Except for the absolute values, the contours of Figures 3–5 are applicable for pyrrolidine rings. Thus, the disorder ${}_\gamma E$–${}^\gamma E$ and ${}^\gamma_\beta T$–${}^\beta_\gamma T$ can occur with the endocyclic angle at the nitrogen atom or at the C(α) carbon atom constant.

Case I Disorder in Furanose Systems. An example of case I disorder of a furanose ring occurs in the recently determined crystal structure of a 2:1 complex between adenosine and proflavine.[6]

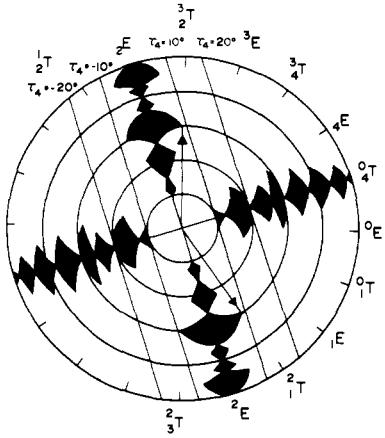

Figure 6. Contours (lines parallel to the ${}_2E$–${}^2E$ axis) of constant endocyclic torsion angle τ_4 and constant endocyclic (darkened regions) bond angles at C(4′) and O(4′) for furanose rings in a (P, τ_m plot. Pseudorotational disorder is possible either within a darkened region or between darkened regions of similar form. In addition, the line connecting the (P, τ_m) values of the disordered states must be parallel to the axis connecting the envelope states opposite the disordered atom (in this case ${}^2E$–${}_2E$). One of the most probable equilibrium between disordered states is indicated ${}^3_2 \rightleftharpoons {}^2_1T$ at $\tau_m \simeq 30°$ and $\tau_4 \sim 10°$.

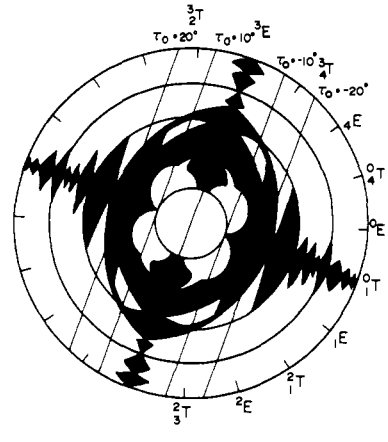

Figure 7. Contours of constant endocyclic torsion angle τ_0 and endocyclic bond angles at C(1′) and O(4′) for furanose rings in a (P, τ_m) plot.

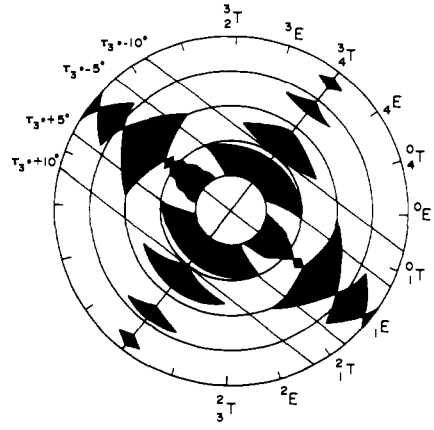

Figure 8. Contours of constant endocyclic torsion angle τ_3 and endocyclic bond angles at C(3′) and C(4′) for furanose rings in a (P, τ_m) plot. Notice how a ${}^2E \rightleftharpoons {}^3E$ equilibrium is not possible, since the eastern hemisphere leads to steric interactions in nucleic acids.

The difference electron density maps revealed that the ribose of one adenosine molecule of the asymmetric unit presents puckering disorder. The major puckering was identified as being nearly ${}^0E$, and the disorder corresponds to a constant endocyclic angle of 104 ($\sigma = 2°$) at C(1′). Difference electron density maps calculated by subtracting the contribution of the major ribose pucker revealed residual electron densities which could be interpreted as corresponding to a puckering close to ${}^2E$. The observed puckering disorder ${}^0E$–${}^2E$ is consistent with Figure 3a for $\tau_m \sim 44°$.

By use of the constrained–restrained refinement of Hendrickson and Konnert,[20] the two conformers were refined against the idealized geometries for a ${}^0E$ pucker and a ${}^2E$ pucker at $\tau_m = 44°$. After refinement, the puckerings were $P = 99°$, $\tau_m = 39°$ for the major pucker (occupancy 78%) and $P = 158°$, $\tau_m = 44°$ for the minor pucker (occupancy 22%). In this structure the relatively high occupancy of the minor pucker (22%) allowed the characterization of it from the electron densities. However, in a case where the occupancy of the minor pucker is low, then such a characterization would be difficult and the information in the plots would be crucial to define the possible puckerings.

Case I Disorder in Pyrrolidine Systems. The structure of *N*-acetyl-L-prolyl-L-lactylmethylamide shows a similar disorder type in the pyrrolidine ring of the prolyl residue.[26] Difference Fourier synthesis revealed two elongated regions of positive density around C(β) and C(γ). Thus, in this case, the angle at the nitrogen atom is constant. This case corresponds to the constant endocyclic angle at the O(4′) atom in furanose rings. As expected for this kind of disorder, the disorder involves the enantiomerically related twist conformation ${}^\gamma_\beta T$ and ${}^\beta_\gamma T$.

Leung and Marsh reported a disorder of the prolyl ring in the crystal structure of L-leucyl-L-prolylglycine[9] which we would assign as a case II disorder with the endocyclic torsion angle around N–C(α) constant (corresponding to τ_0 in the furanose nomenclature and equal to −11.1°). However, further refinement[10] showed the disorder to be really case I with the endocyclic angle at the N atom constant; i.e., both C(β) and C(γ) atoms are disordered. The pseudorotation parameters are $P = 174°$, $\tau_m = 38°$ (${}^\beta_\gamma T$), and $P = 15°$, $\tau_m = 47°$ (${}^\gamma_\beta T$).

Case II Disorder

With one torsion angle constant or four atoms fixed in the crystal, there is only one atom of the five-membered ring which is disordered with possibly also the substituents on the first and last atom defining the torsion angle. The contours are straight lines running parallel to the axis connecting the enantiomeric envelope states of the disordered atom, which is the zero contour. For example, in Figure 6 are represented the contours with the torsion angle τ_4, C(3′)–C(4′)–O(4′)–C(1′), constant. The axis connecting the puckers ${}^2E$–${}_2E$ is the contour for $\tau_4 = 0$. It is also the only contour along which, in addition to having τ_4 constant and equal to zero, the endocyclic bond angles at O(4′) and C(4′) can be kept constant if the amplitude of puckering is identical for the two disordered states. Thus, when the invariant torsion angle is zero (or close to zero), the disorder can only involve the enantiomeric envelope states with identical amplitudes of puckering. Also, the possible disordered states are very much restricted when the invariant torsion angle is nonzero since, in addition to the torsion angle, the adjacent two endocyclic angles have to be invariant. The available domains are shown in Figures 6 and 7 for τ_4 or τ_0 constant with the endocyclic angles respectively at C(4′) and O(4′) or O(4′) and C(1′) invariant. It appears that, even when the torsion angle is not zero, the endocyclic angles can be kept constant if the amplitude of pucker is the same for the two disordered states (at least within ±5°, depending on the precision desired for the endocyclic angles). Thus, as a rule, *when one torsion angle is zero, any amplitude of pucker is, in principle, possible and is dictated by the values of the constant endocyclic bond angles.* When the torsion angle is nonzero, the values of the constant endocyclic bond angles determine the amplitude, and the contour of constant torsion angle determines the possible phases of pucker.

The contours for the torsion angle τ_3 constant with the endocyclic angles at C(3′) and C(4′) invariant are shown in Figure 8. It appears that, except for the small domain and its variant ${}^4_3T$ (${}^4_3T$) puckers, puckers around the ${}_1E$ in the southeastern quadrant are

(26) C. Lecomte, A. Aubry, J. Protas, G. Boussard, and M. Marrand, *Acta Crystallogr., Sec. B*, **B30**, 1992–1996 (1974).

Table I. Relationships between the Allowed Disordered States, the Pucker Amplitude, and the Invariant Torsion Angle for Furanose and Pyrrolidine Rings

invariant torsion angle, deg	pucker amplitude, deg	allowed disordered states[a]
$\tau_0 = 0$	any	${}^3E \rightleftharpoons {}_3E$
±5	~38	${}^4T_3 \rightleftharpoons {}_2T^3$, ${}^3T_4 \rightleftharpoons {}_3T^2$
±10	~30	${}^4_3T \rightleftharpoons {}^3_2T$, ${}^3_4T \rightleftharpoons {}^2_3T$
$\tau_4 = 0$	any	${}^2E \rightleftharpoons {}_2E$
±5	~30	${}_2T^3 \rightleftharpoons {}^2T_1$, $({}^2T_3 \rightleftharpoons {}^1T_2)$
±10	~30	${}^3_2T \rightleftharpoons {}^2_1T$, $({}^2_3T \rightleftharpoons {}^1_2T)$
$\tau_3 = 0$	any	${}_1E$
±5	~30 (or 40)	${}_1T^2$, ${}_1T^0$
±10	~30	${}^2_1T$, ${}^0_1T$
$\chi_5 = 0$	any	${}^\gamma E \rightleftharpoons {}_\gamma E$
±5	~30	${}^\delta T_\gamma \rightleftharpoons {}_\beta T^\gamma$, ${}^\gamma T_\delta \rightleftharpoons {}_\gamma T^\beta$
±10	~30	${}^\gamma_\beta T \rightleftharpoons {}^\delta_\gamma T$, ${}^\gamma_\delta T \rightleftharpoons {}^\beta_\gamma T$
$\chi_4 = 0$	any	${}^\beta E \rightleftharpoons {}_\beta E$
±5	~30	${}_\beta T^\gamma \rightleftharpoons {}^\beta T_\alpha$, ${}^\beta T_\gamma \rightleftharpoons {}^\alpha T_\beta$
±10	~30	${}^\gamma_\beta T \rightleftharpoons {}^\beta_\alpha T$, ${}^\alpha_\beta T \rightleftharpoons {}^\beta_\gamma T$

[a] When the torsion angle in consideration is nonzero, the first set refers to the positive sign and the second one to the negative sign.

the only ones possible for polynucleotides and the disorder ${}^2E$–${}_3E$ cannot occur.

With the torsion angle τ_2 constant, the contours would run along the ${}^0E$–${}_0E$ axis. Since the western sector is sterically disallowed in nucleic acids, the disorder is restrained to small domains around ${}^0E$ or around ${}^2_3T$ and ${}^3_2T$ but, evidently, the ${}^2E$–${}_3E$ disorder is not possible.

In the case of the pyrrolidine ring, the two torsion angles χ_4 and χ_5 (Figure 1b) are of interest. The latter because it is common with the polypeptide backbone and the former because the C(δ) mobility is often restrained due to steric hindrance with the preceding residue in a trans peptide bond configuration.[27,28] By analogy with the contours of the furanose ring, it seems that disorder between the preferred puckered domains are possible especially for χ_5 (see Table I).

Case II Dissorder in Furanose Systems. The literature contains two other recent examples of pseudorotational disorder in a furanose ring: one is the crystal structure of adenosine-5′-diphosphoric acid[7] and the other is the structure of the intercalative complex of proflavine and deoxycytidylyl-3′,5′-guanosine.[8] In adenosine-5′-diphosphoric acid we calculated the pseudorotation parameters from the coordinates kindly made available to us. Here the ribose ring presents a case II disorder with τ_4 constant (τ_4 = 15°), the two states have pseudorotation parameters P = 9°, τ_m = 29° and P = 145°, τ_m = 39° and represents a ${}^3_2T$–${}^2_1T$ mixture. However, the pseudorotation values as well as the endocyclic angles are outside the ranges predicted by our curves[23] and Figure 6. We believe that the use of restrained refinement would improve the geometry.

Not much information is available to us on the intercalative structure, except that it is a disorder between C(3′)-endo and C(2′)-endo involving the 3′ end deoxyribose ring. Since the sugar is a deoxyribose, disorder of the C(2′) atom can be expected. Thus, it is probably a case II disorder with τ_4 constant similar to adenosine-5′-diphosphoric acid, involving a mixture of ${}^3_2T$ and ${}^2_1T$ at $\tau_m \sim 30°$. ${}^3_2T$–${}^2_3T$ disorder with the bond angle at O(4′) constant (case I) is also conceivable.

Since a 3′ end sugar can be disordered between the C(2′)-endo and C(3′)-endo puckers without affecting the torsion angle τ_4 (and, consequently, the exocyclic group at C(4′)), it appears that the pucker at the 3′ end of a nucleic acid fragment is not a stereochemical determinant. It has not yet been shown whether a sugar disordered between the states ${}^3_2T$ and ${}^2_1T$ at $\tau_m \sim 30$ can exist within a polynucleotide chain. In any case, the disregard of such a pseudorotational disorder would lead to a distorted furanose geometry with a flattening of the ring, and ψ' around 120°.

Case II disorder in a pyrrolidine ring has not yet been observed.

Table II. Measured and Calculated Energy Barriers to the Inversion and Pseudorotation Processes for Various Five-Membered Rings

	inversion barrier[a]		pseudorotation barrier[g]	
compd	measd	calcd	measd	calcd
cyclopentane	5.5[c]	4.1[d]	0[c]	0[d]
tetrahydrofuran	3.9[c]	2.6[d]	~0.14[c]	0.6–1.3[d]
pyrrolidine			0.3[f]	1.2[e]
proline		2.7,[g] 3.2[h]		>3.0[g,h]
furanose		>3.5[k]		2.5–3.0[i]
				<2.0[b,i]
				4.0[j]
				~0.5[k]
nucleosides			4.0–5.0[l]	4.0[i]
				2.5[h,i]
				5.0–6.0[j]

[a] In kcal mol^{-1}. [b] For a deoxyribose sugar. [c] Reference 33. [d] Reference 34. [e] Reference 35. [f] Reference 36. [g] Reference 12. [h] Reference 13. [i] Reference 37. [j] Reference 38. [k] Reference 39. [l] Reference 40.

Concluding Remarks

Up to now, the discussion was essentially geometrical. However, the occurrence of disorder (and different puckered forms) in the solid state reflects the fact that the interconversion barriers between the conformers involved are low.

In solution, the five-membered ring of both proline and furanose sugars exhibits a dynamic conformational equilibrium between the two preferred puckering domains observed in the solid state: ${}^\gamma E \rightleftharpoons {}_\gamma E$[19,29,30] and ${}^3E \rightleftharpoons {}_2E$,[31,32] respectively. The mechanism of interconversion between these two broad energy minima might be either pseudorotation (at constant or variable amplitude of puckering) or inversion through the planar state.[23] Some values for interconversion barriers in relevant compounds are given in Table II. It can be seen that *energy calculations favor interconversion via the pseudorotation path in furanose rings and interconversion via the planar state in pyrrolidine rings.*

Thus, internal motions in proline residues consist of quasi-free ($E \leq 1$ kcal mol^{-1}) partial pseudorotation in each of the preferred puckered domains (${}_\beta E \rightleftharpoons {}_\delta E$ and ${}^\delta E \rightleftharpoons {}^\beta E$) as well as interconversions through the planar state (${}^\gamma E \rightleftharpoons {}_\gamma E$) with an energy barrier between 2 and 3 kcal mol^{-1}.[33] The internal motions in furanose rings of nucleic acids involve mainly quasi-free partial pseudorotation in one of the preferred puckered domains, C(3′)-endo and C(2′)-endo, with interconversions between the preferred domains being less frequent than in proline, and the interconversions in

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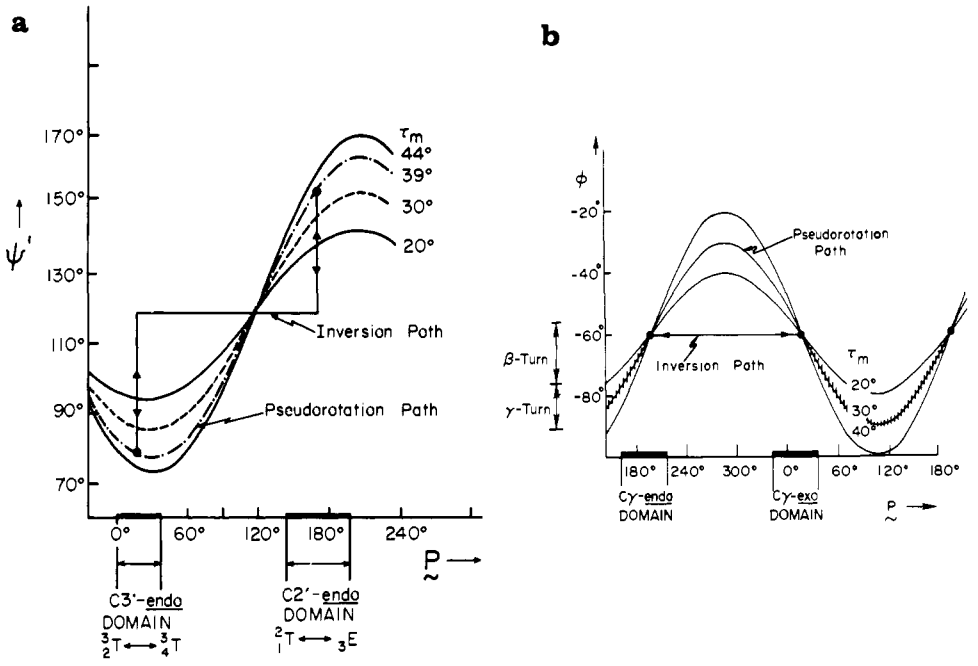

Figure 9. (a) Dependence of the sugar phosphate backbone torsion angle ψ' with the phase angle of pseudorotation, P, for various values of the amplitude of pucker, τ_m (20°, 30°, 39°, 44°). It can be seen that a flattening of the furanose ring decreases the range available to ψ', regardless of P. A value of ψ' around 120° is obtained for both a flat furanose ring (i.e., ψ' independent of P) and for the C(1′) envelope conformations, ${}_1E$ (P = 126°) and ${}^1E$ (P = 306°), (i.e., ψ' independent of τ_m). Thus, in nucleic acids incorporating furanose rings with C(1′) envelope conformation, the orientation of the bases relative to the sugar phosphate backbone can be altered not only by changes in the glycosyl torsion angle but also by mere variations in τ_m. Consequently, the C(1′) envelope puckered nucleic acids will have strongly dampened conformational transmissions or correlations between side-chain base and sugar phosphate backbone. The variation of ψ' during a ${}^2E \rightleftharpoons {}^3E$ interconversion through the pseudorotation path or through the inversion path is illustrated. During inversion, the amplitude of pucker decreases steadily toward the planar ring ($\psi' \sim 120°$) wherefrom it increases again at some other phase of pseudorotation. In the pseudorotation path, the ring stays puckered during interconversion but the pucker type varies. Both interconversion paths induce a large variation in ψ' (from ref 43). (b) Dependence of the polypeptide backbone torsion angle ϕ with the phase angle of pseudorotation, P, for various values of the amplitude of pucker, τ_m (20°, 30°, 40°). These curves were obtained from $\phi = \chi_5 - 120°$ and are approximate (±5–10°). The values given by Madison[14] correspond closely to the values predicted by the curve for τ_m = 30° (average amplitude of pucker in proline derivatives). The ϕ range in each preferred domain are roughly the same. A flat pyrrolidine ring yields the same value of ϕ (−60°) than the C(γ) envelope puckers at any amplitude of pucker. Thus, the backbone torsion angle is not influenced by the mobility of a C(γ) puckered ring. Partial pseudorotation in each of the preferred domains has only minor effects on ϕ. Also, *in contradistinction to nucleic acids, while interconversion through the inversion path does not change ϕ, the pseudorotation path requires a large variation in ϕ.* The domains of the torsion angle ϕ compatible with a β turn structure[45,46] or a γ turn structure[47] are shown. Besides energetic contributions, those β turn structures which allow a ${}^\gamma E \rightleftharpoons {}_\gamma E$ interconversion through the planar state might be entropically favored. In the γ turns, since the torsion angle ϕ of the central amino acid should be between −70° and −85°, the allowed puckers are ${}^\beta_\alpha T$ (at $\tau_m \sim 30°$) or ${}^\beta E$ and ${}_\beta E$ at higher amplitudes of pucker ($\tau_m \sim 40°$). Therefore, the γ turns do not allow mobility in the proline ring. The hatched line shows the most probable pseudorotation path (through the ${}^N E$ pucker at P = 90°).

deoxyribose ring of DNA ($E \geq 2$–3 kcal mol^{-1}) more frequent than in the ribose ring of RNA ($E \geq 3$–4 kcal mol^{-1}).[41]

What is the importance of the inherent mobility of the proline and furanose rings for the internal motions and dynamics of proteins and nucleic acids? It is now widely recognized that fluctuations and flexibility in macromolecular structure are important for the activity of biological macromolecules. Thermal fluctuations are expected to capitalize on the pseudorotation property of five-membered rings.

In polynucleotides and in proline-containing polypeptides, (e.g., hormones, collagen, etc.), one bond is common to the five-membered ring and to the polymer backbone, C(3′)–C(4′) and N–C(α), respectively. It is, therefore, expected that the propagation of the ring mobility to the backbone depends on the relationships between the linkage bond and the preferred puckering domains. In nucleic acids, the torsion angle about the common bond is ψ', O(3′)–C(3′)–C(4′)–C(5′) (roughly $\psi' = \tau_3 + 120°$), and, in polypeptides, it is ϕ, C–C(α)–N–C (roughly, $\phi = \chi_5 - 60°$).

We have shown that, *in nucleic acids, there is a sugar-pucker-dependent flexibility: when incorporated in a polynucleotide, C(3′)-endo sugars confer relatively greater "rigidity" to the polynucleotide chain (small degree of freedom for ψ') than C(2′)-endo sugars (large degree of freedom for ψ').*[42,43] This is illustrated in Figure 9a which shows how partial pseudorotation in each of the preferred puckered domains leads to a small variation for ψ' in the C(3′)-endo domain and to a larger one in the C(2′)-endo domain. Also, interconversion between the two preferred domains by pseudorotation or inversion induces a very large change in the backbone torsion angle ψ' (70°) in nucleic acids.

Similar curves relating ϕ and P, in proline-containing polypeptides, are shown in Figure 9b. *The relation between ϕ and P does not reveal a ring-pucker-dependent flexibility in proline-containing polypeptides.* Partial pseudorotation in each of the preferred domains leads to similar but large variations in ϕ, and this would induce mobility of the adjacent polypeptide main chain bonds. Naturally, with a more restricted pseudorotation (${}_\gamma E \rightleftharpoons {}^\gamma_\beta T$ or ${}_\gamma E \rightleftharpoons {}^\beta_\gamma T$) the variation in ϕ becomes quite small ($\sim 10°$) and not very dependent on the amplitude of pucker. Interconversion between the two preferred domains by pseudorotation requires a large change in the backbone torsion ($\sim 30°$). However, interconversion through the planar state has either no

(41) It is difficult to find experimental support for the contention (ref 39) that the furanose ring of nucleic acids is extremely flexible in RNA and DNA. Despite corrections (*J. Am. Soc.*, **103**, 1879 (1981)) to the published coordinates (ref 39), the relaxed geometries of the sugar rings are still quite off.

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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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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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