Crystal Structure of an Antiviral Agent 5-[*N*-(L-Phenylalanyl)amino]uridine†

Helen M. Berman,* Walter C. Hamilton,‡ and Robert J. Rousseau

ABSTRACT: The crystal structure of 5-[*N*-(L-phenylalanyl)-amino]uridine, $C_{18}H_{22}N_4O_7$, has been determined by X-ray diffraction. There are two molecules in a monoclinic unit cell, space group $P2_1$, a = 6.944 (4), b = 8.179 (7), c = 16.662 (16) Å, β = 90.17° (6). The molecule is in an extended conformation. There is parallel intermolecular stacking of the uracil and phenyl rings, and the uracil is hydrogen bonded to the amino acid backbone of another molecule. These interactions between amino acids and nucleosides can serve as models for nucleic acid–protein interactions.

5-Aminouridine possesses a wide range of biological activity and has been shown to inhibit growth of fungi (Roberts and Visser, 1952), viruses (Visser *et al.*, 1952), and tumors (Visser, 1955). A number of aminoacyl derivatives of 5-aminouridine have recently been synthesized and tested as possible medicinal agents (Ivanovics *et al.*, 1971). 5-[*N*-(L-Phenylalanyl)amino]uridine, the subject of our study, inhibits herpes, parainfluenza, and rhinovirus multiplication in tissue culture.

Apart from its medicinal properties, this compound may serve as a model for understanding interactions of peptides and proteins with nucleotides and nucleic acids. Amino acids, peptides, and proteins interact with nucleic acids and their constituents in a variety of ways. Amino acids have been found to be covalently linked to both DNA and RNA and there are numerous examples of noncovalent interactions between proteins and nucleic acids (Huang, 1971). As will be shown below, two specific such interactions, ring stacking and hydrogen bonding, are operable in this compound.

Experimental Section

The material was prepared as described by Ivanovics *et al.* (1971). Triangular plate-like transparent colorless crystals were grown by evaporation from water. A crystal with maximum dimensions 0.4 × 0.2 × 0.05 mm was selected for data collection. 5-[*N*-(L-Phenylalanyl)amino]uridine ($C_{18}H_{22}N_4O_7$): for space group $P2_1$, a = 6.944 (4) Å, b = 8.179 (7) Å, c, = 16.662 (16) Å, β = 90.17 (6)°, d_m = 1.45 g/cm³, d_x = 1.43 g/cm³, μCu Kα = 9.5 cm^{-1}, λCu Kα = 1.5418 Å, and Z = 2.

The data were collected on a four-circle Syntex automatic diffractometer using variable rate θ–2θ scans; 1738 independent data, of which 469 had intensity less than $2\sigma(I)$, were collected to a maximum 2θ of 140° by the θ–2θ scan technique. Lorentz and polarization corrections were applied, but no absorption correction was deemed necessary.

Solution and Refinement of Structure. The structure factors were normalized and 277 E values above 1.3 were used to solve the structure by direct methods using the program MULTAN (Germain *et al.*, 1971). After the early cycles of full-matrix least-squares refinement there was some difficulty in convergence of the phenyl group atoms to reasonable geometrical positions. The phenyl group was thus constrained to have bond lengths of 1.39 Å and angles of 120° and the six parameters describing its orientation were refined along with positions and isotropic thermal parameters for all atoms.

A subsequent anisotropic refinement resulted in unusually large thermal parameters for the phenyl group atoms in a direction in the plane of the ring. From this and a difference Fourier which showed extra peaks, it was apparent that a structural model with disordered phenyl groups was most appropriate. A trial model which consisted of two phenyl groups with half-occupancy, differing by a rotation of about 15° around a vector passing through C(β) and perpendicular to the plane of the phenyl ring, was adopted for subsequent refinement. Again, six group parameters for each ring were refined along with the positions and anisotropic temperature factors of the other heavy atoms and isotropic temperature factors for the phenyl carbon atoms. Most of the hydrogen atoms were located on a difference Fourier. The positions of the hydrogen atoms attached to the phenyl ring and to N(3) were calculated and not refined. In the final cycles of refinement the positions of all atoms with the exception of these hydrogen atoms and the carbons of the phenyl groups were refined together with the anisotropic thermal parameters for the non-group heavy atoms only. Initial refinement of isotropic thermal parameters for hydrogen atoms indicated that these were not significantly different from those of the heavy atoms to which they are bonded, and in the final refinement they were fixed at the values presented in Table Ib. The weighting scheme used was, for $F < 50$, $w = 0.08$ and for $F > 50$, $w = 204.1/F^2$. The quantity minimized was

$$\Sigma w_{hkl}|F_o^{hkl} - F_c^{hkl}|^2$$

The final values of

$$R = \frac{\Sigma||F_o| - |F_c||}{\Sigma|F_o|}$$

† From the Institute for Cancer Research, Philadelphia, Pennsylvania 19111 (H. M. B.), the Brookhaven National Laboratory, Upton, New York 11973 (W. C. H.), and the ICN Nucleic Acid Research Institute, Irvine, California 92664. *Received December 11, 1972.* This research was supported by U. S. Public Health Service Grants CA-10925, CA-0927 and RR-05539 from the National Institutes of Health, by an appropriation from the Commonwealth of Pennsylvania and by the U. S. Atomic Energy Commission.

‡ Deceased.

TABLE I: Parameters.

a. Positional and Thermal Parameters ($\times 10^4$)[a]

Atom	x	y	z	u^{11}	u^{22}	u^{33}	u^{12}	u^{13}	B
N(1)	2277 (9)	−128	−3193 (3)	396 (38)	217 (37)	213 (32)	−21 (30)	−20 (26)	77 (29)
C(2)	2160 (10)	−1789 (11)	−3075 (4)	282 (43)	319 (47)	268 (38)	0 (36)	−89 (31)	−35 (38)
N(3)	2322 (9)	−2275 (9)	−2285 (3)	477 (40)	139 (31)	244 (31)	1 (30)	−63 (26)	35 (26)
C(4)	2589 (10)	−1251 (10)	−1611 (4)	350 (45)	214 (46)	257 (40)	−5 (36)	−41 (32)	−9 (34)
C(5)	2700 (10)	466 (10)	−1817 (4)	224 (41)	118 (38)	337 (42)	−42 (30)	−82 (30)	−32 (33)
C(6)	2591 (10)	950 (11)	−2568 (4)	389 (46)	224 (45)	235 (37)	−26 (35)	−58 (31)	−34 (33)
O(2)	1889 (8)	−2777 (9)	−3601 (3)	556 (35)	221 (30)	254 (26)	6 (26)	−140 (24)	−61 (26)
O(4)	2678 (8)	−1805 (9)	−935 (3)	594 (38)	372 (35)	206 (28)	−56 (30)	−74 (25)	44 (26)
C(1′)	2302 (10)	506 (10)	−4020 (3)	413 (45)	164 (38)	82 (31)	75 (34)	−32 (28)	4 (31)
C(2′)	892 (10)	1887 (11)	−4193 (4)	277 (39)	320 (47)	229 (35)	−50 (35)	−88 (28)	−21 (33)
C(3′)	1933 (11)	2774 (12)	−4871 (4)	323 (43)	373 (50)	307 (41)	46 (37)	25 (32)	1 (38)
C(4′)	4005 (11)	2625 (11)	−4632 (4)	428 (48)	255 (44)	279 (38)	11 (37)	28 (33)	64 (35)
C(5′)	4730 (11)	4065 (13)	−4111 (5)	366 (48)	365 (50)	419 (47)	−19 (40)	86 (36)	−20 (41)
O(1′)	4166 (7)	1112 (9)	−4182 (3)	268 (29)	262 (31)	379 (28)	9 (24)	30 (22)	11 (24)
O(2′)	−944 (7)	1302 (9)	−4379 (3)	360 (31)	337 (33)	410 (30)	−48 (27)	−118 (24)	−101 (29)
O(3′)	1658 (7)	1897 (9)	−5608 (3)	419 (32)	552 (40)	209 (25)	171 (31)	−75 (22)	31 (26)
O(5′)	6753 (8)	3911 (9)	−3979 (4)	330 (33)	342 (34)	729 (42)	−20 (29)	−23 (28)	13 (33)
N(2)	3067 (9)	1494 (9)	−1142 (3)	402 (39)	293 (40)	214 (33)	−46 (31)	−77 (27)	−72 (30)
C(1)	3049 (11)	3160 (11)	−1152 (4)	440 (49)	233 (44)	227 (40)	−60 (37)	−47 (32)	−50 (36)
O(1)	2638 (10)	3989 (9)	−1733 (3)	893 (50)	231 (29)	233 (30)	54 (33)	−210 (29)	51 (27)
C(α)	3567 (11)	3900 (12)	−330 (4)	456 (46)	327 (43)	160 (34)	−21 (39)	−78 (30)	−24 (36)
N(α)	4928 (10)	5227 (10)	−403 (4)	441 (41)	256 (38)	415 (38)	−77 (32)	−86 (31)	37 (31)
C(β)	1702 (12)	4493 (14)	89 (4)	470 (52)	617 (68)	288 (42)	−135 (49)	2 (37)	−66 (45)

b. Parameters for Atoms Which Were Not Refined[b]

	Atom	10^4x	10^4y	10^4z	B
Phenyl Group 1	C(γ)	2011 (30)	4454 (26)	1020 (8)	3.3 (3)
	C(δ2)	2193 (33)	3064 (21)	1495 (11)	3.8 (4)
	C(δ1)	2140 (34)	5996 (22)	1368 (11)	4.4 (4)
	C(ε2)	2504 (34)	3215 (23)	2318 (10)	4.7 (4)
	C(ε1)	2450 (38)	6148 (22)	2190 (12)	6.0 (5)
	C(ζ)	2632 (35)	4757 (28)	2665 (8)	4.7 (5)
	H(Cδ1)	2099	6998	1026	6.0
	H(Cε2)	2100	1954	1245	6.0
	H(Cε1)	2543	7258	2441	6.0
	H(Cε2)	2635	2214	2659	6.0
	H(Cζ)	2856	4866	3257	6.0
Phenyl Group 2	C(γ)	2002 (25)	5103 (18)	905 (7)	2.1 (3)
	C(δ2)	2237 (28)	3924 (16)	1499 (10)	2.9 (3)
	C(δ1)	2034 (26)	6752 (21)	1106 (8)	2.8 (3)
	C(ε2)	2503 (20)	4394 (17)	2295 (9)	3.8 (4)
	C(ε1)	2299 (36)	7222 (24)	1902 (9)	5.7 (5)
	C(ζ)	2534 (37)	6043 (32)	2497 (7)	4.6 (4)
	H(Cδ1)	1865	7602	679	6.0
	H(Cδ2)	2215	2736	1354	6.0
	H(Cε1)	2322	8410	2047	6.0
	H(Cε2)	2672	3544	2723	6.0
	H(Cζ)	2725	6381	3069	6.0
	H(N3)	2268	−3420	−2199	2.8
	H(C6)	2863	2216	−2789	2.8
	H(C1′)	1973	−309	−4346	2.8
	H(C2′)	760	2577	−3668	2.8
	H(C3′)	1514	3953	−4978	2.8
	H(C4′)	4815	2536	−5132	2.8
	H(C5′)	3877	4126	−3537	2.8
	H(C5′)	4231	5151	−4318	2.8
	H(O2′)	−1474	2047	−4352	2.8
	H(O3′)	442	2024	−5966	2.8

TABLE I (*Continued*)

Atom	10^4x	10^4y	10^4z	B
H(O5′)	7511	4893	−4156	2.8
H(N2)	3521	884	−670	2.8
H(Cα)	4134	2950	6	2.8
H(Nα)	4651	5961	−692	2.8
H(Nα)	6099	5214	−645	2.8
H(Cβ)	761	3629	−24	3.7
H(Cβ)	1522	5458	−332	3.7

[a] The anisotropic Debye–Waller factor is given by $\exp[-2\pi^2\Sigma a_i^* a_j^* h_i h_j U^{ij}]$. [b] The standard deviations for the phenyl carbon atoms are derived from the refinement of the group parameters.

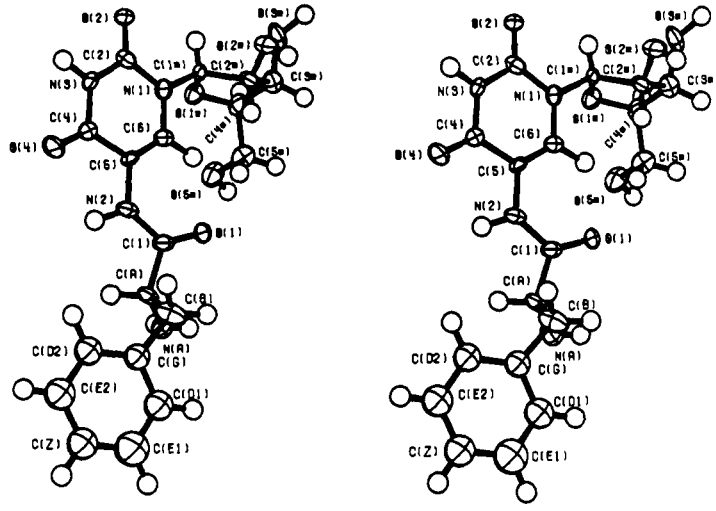

FIGURE 1: A stereo view of a single molecule of phenylalanylaminouridine showing atomic labeling and thermal elliposids. (The hydrogen atoms have been assigned an arbitrary radius of 0.16 Å.) A = α, B = β, G = γ, D = δ, E = ϵ, Z = ζ in Figures 1, 2, and 3.

TABLE II: Conformation Angles in Ribose Portion of Phenylalanylaminouridine (in degrees).

χ	O(1′)-C(1′)-N(1)-C(6)	63.4
ψ	C(3′)-C(4′)-C(5′)-O(5′)	−174.2
τ_0	C(4′)-O(1′)-C(1′)-C(2′)	−14.2
τ_1	O(1′)-C(1′)-C(2′)-C(3′)	+31.7
τ_2	C(1′)-C(2′)-C(3′)-C(4′)	−36.5
τ_3	C(2′)-C(3′)-C(4′)-O(1′)	+29.6
τ_4	C(3′)-C(4′)-O(1′)-C(1′)	−10.0

[a] The nomenclature used is that used by Sundaralingam (1969).

and

$$wR = \left(\frac{\Sigma w(F_o - F_c)^2}{\Sigma wF_o^2}\right)^{1/2}$$

were 0.067 and 0.083. The scattering factors for the heavy atoms were taken from Cromer and Liberman (1970) and

TABLE III: Conformation Angles in Peptide Unit of Phenylalanylaminouridine (PAU) as Compared with Those in Glycylphenylalanylglycine (GPG).

					PAU (deg)	GPG (deg)
φ_1^1	HN(α)	N(α)	C(α)	C(1)	55.1	
φ_1^2	HN(α)	N(α)	C(α)	C(1)	−54.9	
ψ_1	N(α)	C(α)	C(1)	N(2)	134.8	132.0
ω_1	C(α)	C(1)	N(2)	C(5)	−178.2	179.7
φ_2	C(1)	N(2)	C(5)	C(4)	−173.1	84.3
ψ_2	N(2)	C(5)	C(4)	N(3)	−177.0	179.3 (ψ_T), −4.6
ω_2	C(5)	C(4)	N(3)	C(2)	−0.3	
$\chi_1^{1,1}$	N(α)	C(α)	C(β)	C(γ)	−83.3[a]	−174.7
					−61.1	
$\chi_1^{2,1}$	C(α)	C(β)	C(γ)	C(δ1)	109.4	102.5
					103.1	
$\chi_1^{2,1}$	C(α)	C(β)	C(γ)	C(δ2)	−69.4	−81.5
					−77.9	

[a] For the χ angles the first number given refers to the value for phenyl group 1 and the second for phenyl group 2.

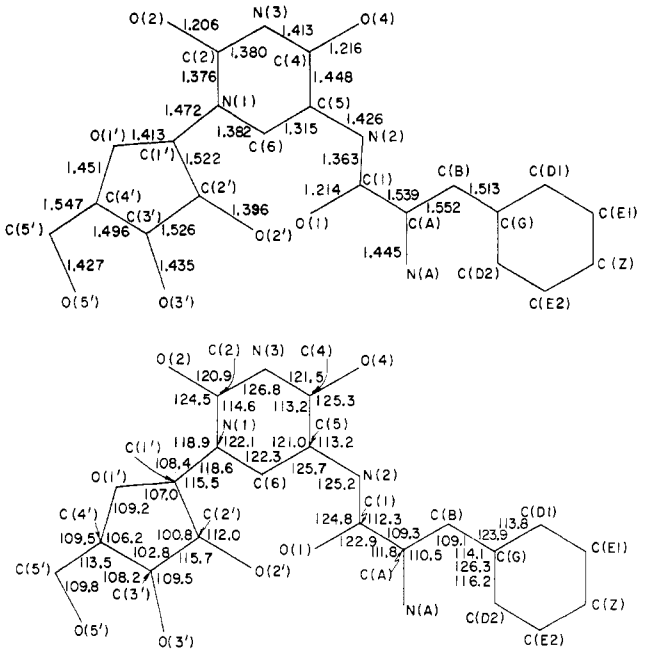

FIGURE 2: (a, top) A schematic drawing of phenylalanylaminouridine showing distances. The esd's of the bond lengths are 0.008–0.010 Å with the exception of O^{β}–$C\gamma$, which has an esd of 0.04 Å, and the phenyl ring distances which were not refined. (b, bottom) Bond angles: esd's are 0.5–0.7°. Two values are given for the angles at C(γ), corresponding to the two phenyl group atoms.

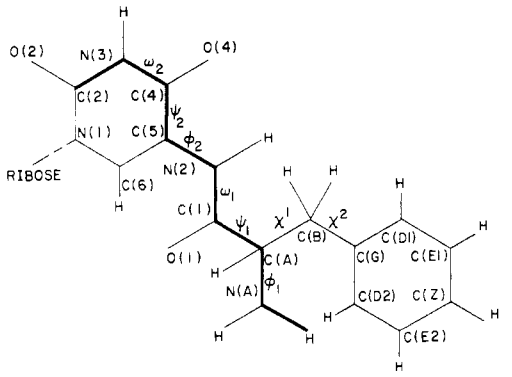

FIGURE 3: A schematic drawing showing torsion angles in peptide portion of phenylalanylaminouridine. The heavy bonds can be interpreted as a portion of a polypeptide chain.

unusual features. The bond distances and angles (Figure 2) are each within one standard deviation of the average found in other uracil structures (Voet and Rich, 1970). The ring is planar and is in the anti conformation (χ = 63.4°) with respect to the ribose (Table II).

The ribose ring is in the C(2') endo C(3') exo conformation. The ribose ring is puckered (Table II) with the C(2') atom displaced by 0.5 Å from the plane of the ring and on the same side of the plane as C(5'). This conformation is less usual than the C(3') endo C(2') exo conformation. The bond distances and angles in the ribose moiety are consistent with this conformation: the C(2')–O(2') bond distance is 0.03 Å shorter than the C(3')–O(3'), and the C(3')–C(2')–C(1') angle is the smallest in the ring. The C(4')–C(5') bond is in the unusual trans conformation (φ = −174.17°). This is probably because in the more usual gauche conformation the O(5') atom would be well within van der Waals contact distance of the peptide oxygen O(1).

Conformation of the Phenylalanyl Peptide. The phenylalanyl moiety and a portion of the uracil ring have a structure which is analogous to a small portion of polypeptide chain (see Figure 3 in which the torsion angles are defined). The entire chain from N(3) to C(α) is approximately planar (and thus coplanar with the uracil ring). The value of 134.5° for ψ_1 is not uncommon for peptides and is that found in the antiparallel chain pleated sheet (Pauling and Corey, 1951). Other conformation angles are presented in Table III, where

TABLE IV: Hydrogen Bonds and Contacts in Phenylalanylaminouridine.

A	B	C	d(AC) (Å)	d(AB) (Å)	d(BC) (Å)	ABC (deg)	Symmetry Code for C
				a. Hydrogen Bonds			
O(2')	H	O(5')	2.750	0.71	2.058	164.1	$-1+x, y, z$
O(3')	H	O(2)	2.803	1.04	1.78	168.8	$-x, \frac{1}{2}+y, -1-z$
O(5')	H	O(3')	2.767	1.00	1.78	166.0	$1-x, \frac{1}{2}+y, -1-z$
N(α)	H	O(4)	3.019	0.79	2.32	147.6	$x, 1+y, z$
N(3)	H	O(1)	3.199	0.95	2.27	165.6	$x, -1+y, z$
N(2)	H	N(α)	3.101	0.98	2.15	161.8	$1-x, -\frac{1}{2}+y, -z$
				b. Close Contact			
C(3')	H	O(2')	3.218	1.02	2.24	160.8	$-x, \frac{1}{2}+y, -1-z$

for hydrogen from Stewart (1965). The position and thermal parameters are presented in Table I. The observed and calculated structure factors are on microfilm.[1]

Description of the Structure. 5-[*N*-(L-Phenylalanyl)-amino)uridine (Figure 1) is a covalently linked amino acid and nucleoside; we will discuss the important features of each part of the molecule, how these parts are related in one molecule and, finally, how the molecules are related in the crystal.

The uracil ring is not unusual. The uracil ring displays no

[1] *Structure factor data* will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number BIO-73-1809.

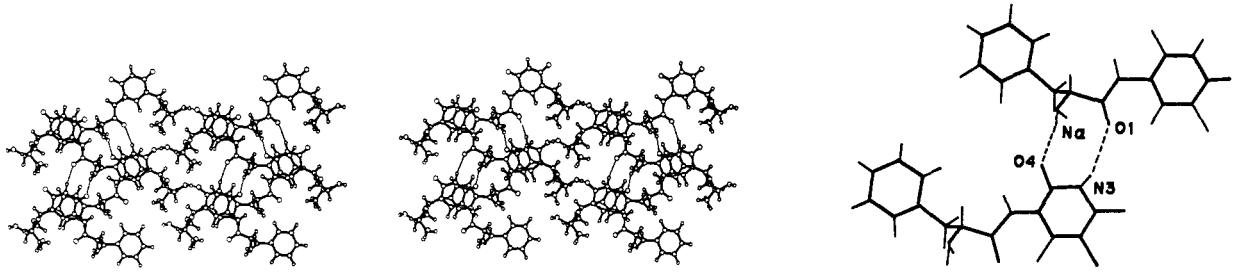

FIGURE 4: (a, left) A stereo view of the crystal structure down the *b* axis showing the hydrogen bonding between the uracil ring and the amino acid as well as the stacking of the uracil and phenyl rings. The phenyl group is shown in configuration 1 of the two disordered forms. (b, right) One hydrogen-bonded dimer with the nitrogen and oxygen atoms labeled.

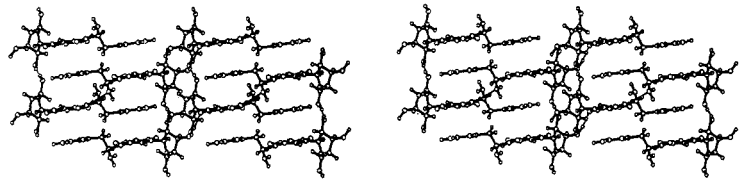

FIGURE 5: A stereo view of the crystal structures down the *a* axis. The ring stacking is seen edge-on, and hydrogen bonds involving the ribose are seen.

they are compared to analogous angles in the tripeptide glycylphenylalanylglycine (Glusker and Marsh, 1961).

The phenyl and uracil rings are parallel. C(γ) is trans to C(1) (torsion angle = 166°), and the resulting orientation of the phenyl ring is such that it and the uracil ring of the same molecule lie in nearly parallel planes 1.2 Å apart; the dihedral angle between the two planes is 174°.

The amino acid and uracil interact. The most striking features of this structure are the parallel intermolecular stacking of the uracil and phenyl rings (Figures 4–6) and the hydrogen bonding (Table IV) between the amino acid backbone of one molecule and the uracil of another molecule. The uracil ring is sandwiched with a 3.4-Å spacing between the two sets of disordered phenyl rings at $-x$, $^1/_2 + y$, $-z$ and $1 - x$, $-^1/_2 + y$, $-z$; its plane is an average of 0.06 Å closer to the rings at $-x$, $^1/_2 + y$, $-z$. This type of stacking of aromatic amino acids and bases has also been observed in the crystal structure of another antibiotic, puromycin (Sundaralingam and Arora, 1969), where the phenylalanyl and adenine rings are also 3.4 Å apart. There are hydrogen bonds between O(1) and N(α) of the amino acid portion of one molecule and O(4) and N(3) of a translationally related uracil to form cyclic dimers similar to the ones found in nucleic acid base pairs. Other examples of hydrogen bonding between base and amino acid have been seen in the actinomycin–guanosine complex (Sobell *et al.*, 1971) where guanine is hydrogen bonded to threonine and in ribonuclease–oligonucleotide complexes (Richards *et al.*, 1970), where, for example, uridylic acid and cytidylic acid hydrogen bond to threonine.

Thus we see in one structure two types of interactions between amino acid and nucleoside. These interactions can probably be generalized for proteins and nucleic acids. The specific interaction between the aromatic phenyl group and the base is no doubt prevalent in a variety of complexes. This particular type of hydrogen bonding between the amino acid backbone and the uracil can occur in any case where one has a stretched out conformation for a polypeptide chain, as for example in a β pleated sheet.

Other hydrogen bonding in this structure occurs between the ribose rings and O(2) of uracil. The ribose oxygen O(2′) is not the acceptor in a normal hydrogen bond, but it is in unusually short contact distance with the hydrogen atom on C(3′) of another molecule. The ribose ring oxygen is not involved in any hydrogen bonding.

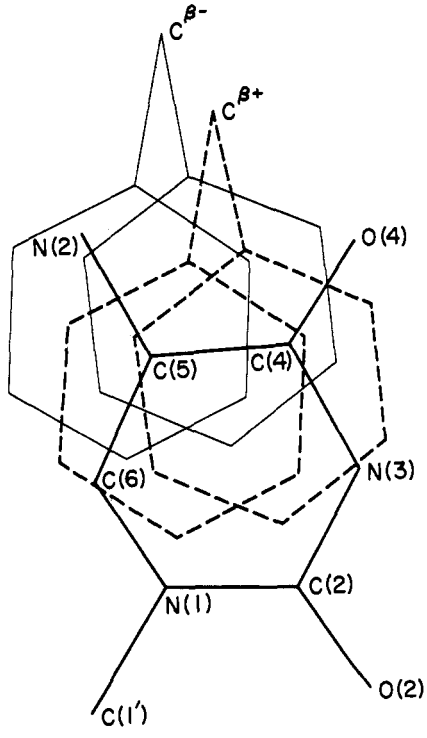

FIGURE 6: Details of the stacking of phenyl and uracil rings; projection perpendicular to the uracil rings. The dashed lines represent the two disordered phenyl groups at $-x$, $-^1/_2 + y$, $-z$, and the light weight lines those at $1 - x$, $-^1/_2 + y$, $-z$. The two disordered configurations are denoted by superscripts on the β carbon atom.

Acknowledgment

Computer programs used in addition to MULTAN were written by Dr. H. L. Carrell or were part of the BNL Crystallographic program library.

References

Cromer, D. T., and Liberman, D. (1970), *J. Chem. Phys. 53*, 915.
Germain, G., Main, P., and Woolfson, M. M. (1971), *Acta Crystallogr. A 27*, 368.
Glusker, J. P., and Marsh, R. M. (1961), *Acta Crystallogr. 14*, 1110.
Huang, P. C. (1971), *Progr. Biophys. Mol. Biol. 23*, 105.
Ivanovics, G. A., Rousseau, R. J., and Robins, R. K. (1971), *J. Med. Chem. 14*, 1155.
Pauling, L., and Corey, R. B. (1951), *Proc. Nat. Acad. Sci. U. S. 37*, 729.
Richards, F. M., Wyckoff, H. W., and Allewell, N. (1970), The Neurosciences, New York, N. Y., Rockefeller University Press, p 901.
Roberts, M., and Visser, D. W. (1952), *J. Biol. Chem. 194*, 695.
Sobell, H. M., Jain, S. C., Sakore, T. D., and Nordman, C. E. (1971), *Nature (London) 231*, 200.
Stewart, R. F., Davidson, E. R., and Simpson, W. T. (1965), *J. Chem. Phys. 42*, 3175.
Sundaralingam, M. (1969), *Biopolymers 7*, 821.
Sundaralingam, M., and Arora, S. K. (1969), *Proc. Nat. Acad. Sci. U. S. 64*, 1021.
Visser, D. W. (1955), *Antimetabol. Cancer Symp. 1953*, 59.
Visser, D. W., Langenborg, D. L., and Pearson, H. E. (1952), *Proc. Soc. Exp. Biol. Med. 79*, 571.
Voet, D., and Rich, A. (1970), *Progr. Nucl. Acid Res. Mol. Biol. 10*, 183.

A Collagenolytic Protease from the Hepatopancreas of the Fiddler Crab, *Uca pugilator*. Purification and Properties†

Arthur Z. Eisen,* Kenneth O. Henderson, John J. Jeffrey, and Ralph A. Bradshaw‡

ABSTRACT: A collagenolytic protease has been isolated from extracts of the hepatopancreas of the fiddler crab, *Uca pugilator*, and purified to homogeneity by a variety of chromatographic procedures. The enzyme acts both on native collagen fibrils and on collagen in solution and is capable of degrading the collagen molecule under conditions that do not denature the protein. Unlike the vertebrate collagenases the purified crab hepatopancreas collagenase also demonstrates specificities against synthetic substrates for mammalian trypsin and chymotrypsin. These latter enzymes, however, are incapable of cleaving the native collagen helix. In certain respects this collagenase resembles the vertebrate trypsins in that it is inhibited by diisopropyl fluorophosphate, tosyllysyl chloromethyl ketone, and soybean trypsin inhibitor, has the same pH optimum, an approximate minimum molecular weight of 25,000, and a similar amino acid composition. It differs in that it is inactivated at acid pH, is not stabilized by calcium, is an acidic protein, has chymotryptic activity, and has aspartic acid as its apparent amino-terminal group. The findings presented indicate that this unusual enzyme possesses not only specific collagenase activity but also trypsin- and chymotrypsin-like activities as an inherent part of the same molecule.

Although a number of digestive proteases have been demonstrated in a variety of lower animals (Prahl and Neurath, 1966; Gibson and Dixon, 1969; Zwilling *et al.*, 1969; Winter and Neurath, 1970; Camacho *et al.*, 1970), little attention has been given previously to the presence of collagenases in these species even though many of these animals, such as the crustaceans, are predaceous scavengers that feed on animal tissues frequently containing collagen. However, a collagenolytic enzyme has been obtained from the hepatopancreas of the fiddler crab, *Uca pugilator* (Eisen and Jeffrey, 1969), which, unlike most vertebrate collagenases, is readily extractable in its active form (Eisen *et al.*, 1970a).

In common with other collagenases, this enzyme acts on native collagen in solution and is characterized by the cleavage of the native collagen helix at loci 75, 70, and 67% from the amino terminal of the molecule. In addition, there is a marked decrease in the original intramolecular cross-linked β component of collagen with a corresponding increase in the monomeric α chains (Eisen and Jeffrey, 1969).

Early attempts to purify the hepatopancreas collagenase were complicated by the persistence of enzymatic activities possessing specificities normally associated with trypsin and chymotrypsin. The existence of such activities is not unexpected in view of the digestive function of the crab hepatopancreas. Nonetheless, it has been shown that neither vertebrate trypsin nor chymotrypsin, in quantities equal to or greater than that present in hepatopancreas extracts, is capable of cleaving the native collagen helix in either soluble or fibrillar form (Eisen and Jeffrey, 1969).

The first indication that the trypsin and chymotrypsin activities of the collagenase preparations might be inherent to the same enzyme was revealed by inhibitor studies using

† From the Division of Dermatology, Department of Medicine, and the Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri 63110. *Received November 29, 1972.* This investigation was supported by U. S. Public Health Service Grants AM 12129, AM 05611, and AM 13352.

‡ R. A. B. is a recipient of Research Career Development Award AM 23968.