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STACKING INTERACTION BETWEEN TRYPTOPHAN AND URACIL IN A SYNTHETIC MODEL COMPOUND

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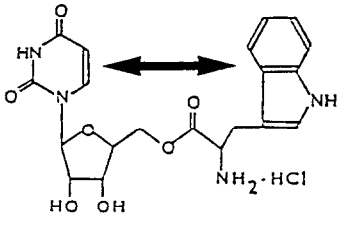
Summary: Stacking interaction between tryptophan and uracil in model compound 1 has been confirmed in solution by means of hypochromism and fluorescence emission, whereas 1 holds an unfolded conformation in a crystalline state as determined by X-ray analysis.

Specific interactions between amino acid residues and nucleic acid bases are involved in the selective recognition of base sequence by proteins. Among several types of specific interactions, stacking of pyrimidine bases with aromatic amino acids such as Trp has been demonstrated to take place in complexes involving nucleic acids and oligopeptides.[1] For example, Lys-Trp-Lys has been suggested to discriminate between single-stranded and double-stranded regions in a nucleic acid by such stacking interaction.[2] At the monomer level intramolecular stacking interactions in model compounds in which simple indoles are linked to pyrimidine bases by flexible chains have been described.[3] However, the stacking interaction involving Trp itself has not been demonstrated. In a continuation of our studies on the photochemistry of nucleic acids and proteins,[4] we investigated the ring-ring stacking interaction between Trp and uracil in a model compound by means of spectroscopy and X-ray crystallographic analysis.

Model compound 1 was prepared by reaction of t-Boc-L-tryptophan anhydride with 2',3'-O-isopropylidene-uridine (pyridine, 0 °C) followed by deprotection (HCl-methanol, room temperature, 6 h) in 51% overall yield. The monohydrochloride salt, mp 174-176 °C, was recrystallized from methanol. The intramolecular stacking between Trp and the pyrimidine ring in 1 was first assessed by hypochromism measurement by UV spectroscopy. The "percent hypochromism (% H)" which depends upon the degree and orientation of intramolecular stacking is known to be used as a measure of such interaction,[5] since very low



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concentration ($\sim 10^{-5}$ M) is used in order to avoid intermolecular contributions. Fig. 1 shows the UV spectra of 1 and a 1:1 mixture of two reference compounds, Trp methyl ester monohydrochloride and uridine, at same concentrations in acetonitrile. The spectrum of 1 exhibits a remarkable decrease in the absorption intensity in a region of 260-290 nm with the % H value of 11.1, indicating an appreciable contribution of intramolecular stacking between the two rings.

We next examined the fluorescence quenching of the Trp moiety by the uracil part in acetonitrile. Fig. 2 shows the comparison of the fluorescence intensity of 1 with that of a 1:1 mixture of the two reference compounds. Relative quenching efficiency (q_r) determined by comparison of the intensity is 0.041. The efficient quenching observed with 1 apparently involves either or both of the following two processes: (1) static quenching, time-independent process resulting from the proximity of the quencher prior rọ excitation, and (2) dynamic queching, a time-dependent process resulting from quenching during the excited state lifetime.[6] The result of the quenching experiments is suggestive of a strong intramolecular interaction between Trp and the uracil part in ground and excited states of 1 and is consistent with the observed hypochromic effect.

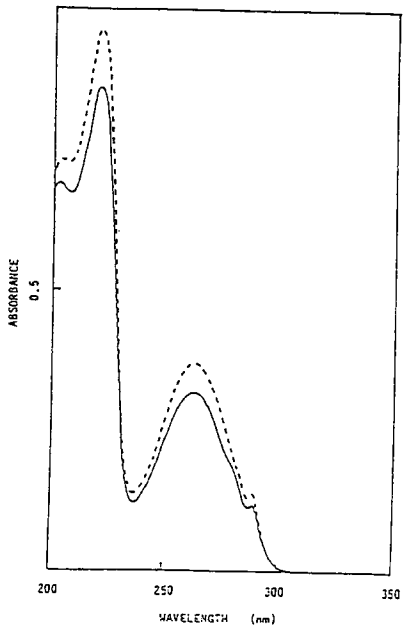

Fig. 1 UV spectra of 1 (—) (3 x 10^{-5} M) and a 1:1 mixture of Trp methyl ester and uridine (--) (each 3 x 10^{-5} M) in acetonitrile.

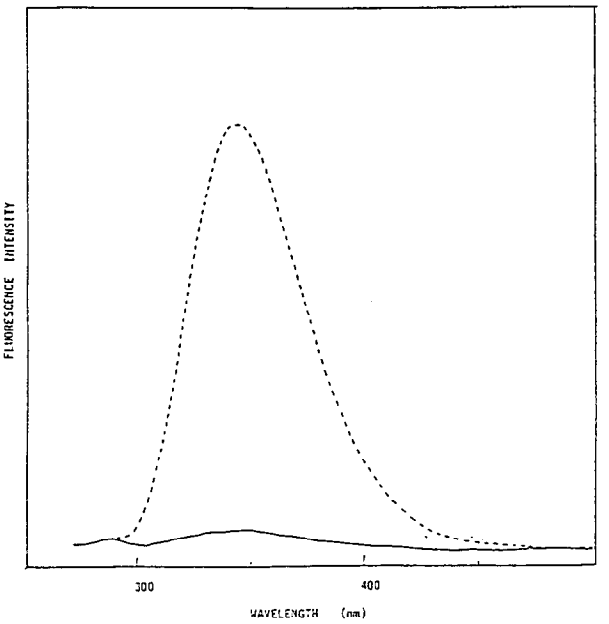

Fig. 2 Fluorescence spectra of 1 (—) (3 x 10^{-5} M) and a 1:1 mixture of Trp methyl ester and uridine (--) (each 3 x 10^{-5} M) in acetonitrile with excitation at 290 nm at room temperature.

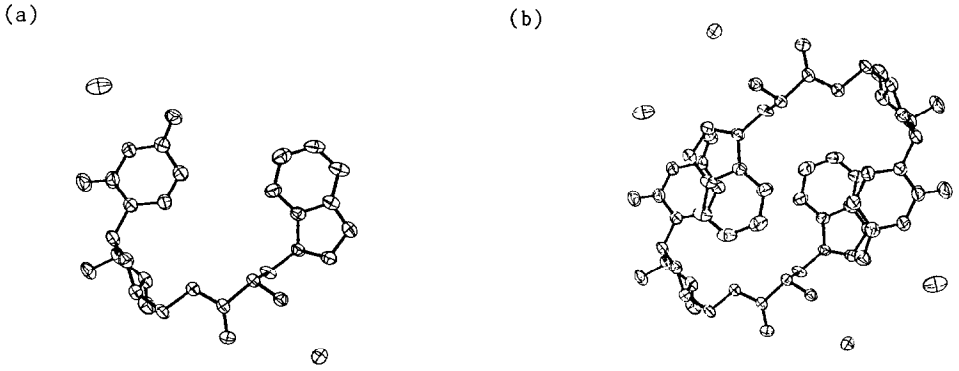

Fig. 3 (a) Perspective drawing of 1. (b) Overlapping mode of two molecules of 1.

While the spectroscopic date support the existence of such stacking interaction in solution, direct structural information of the mode of stacking is not available from such studies. In order to know whether or not such stacking interaction exists in a crystalline state, crystal structure of 1 was determined by X-ray analysis. Fig. 3a illustrates the molecular structure of 1 in crystals.[7] The ribose unit has C-3' endo conformation and the orientation of the pyrimidine base with respect to the sugar is anti. Unexpectedly, 1 does not hold a folded conformation in crystals but exists as an extended form. However, the indole ring is stacked with the uracil rings of neighboring molecules. The intermolecular stacking between indole and uracil rings is illustrated in Fig. 3b and 4. Both the upper and lower uracil rings are stacked with the central indole ring. Average interplanar spacing distance in the overlapping region is about 3.4 Å, a normal van der Waals separation.

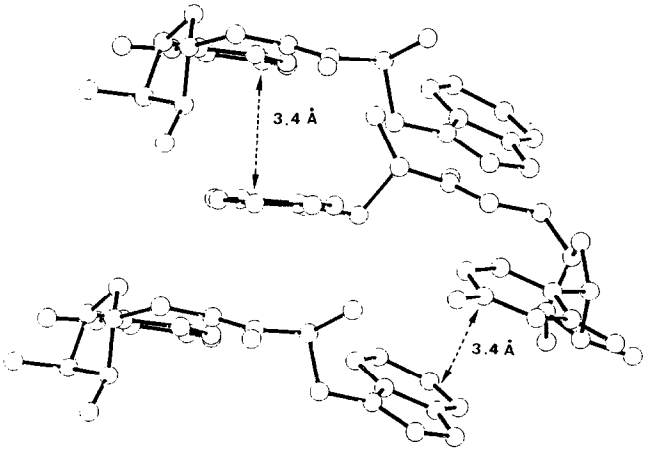

Fig. 4 Stacking of the indole ring of 1 with neighboring uracil rings projected parallel to the indole ring.

The spectroscopic studies of the model compound 1 indicate a singificant contribution of a stacked conformation in solution. In a crystalline state, however, 1 exists as an unfolded form with the indole ring being associated with the uracil rings of adjacent molecules, probably through a normal stacking interaction. The present results, particularly the spectroscopic evidence, indicate that π-π stacking interaction between Trp in a protein and a pyrimidine base of a nucleic acid is to be expected if these are in close proximity in a complex. These findings will be of use in investigating specific recognition of base sequences by Trp residues of proteins.

References and Notes

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7. Crystal date: monoclinic, space group $P2_1$, a = 11.694 (3), b = 6.948 (2), c = 13.325 (3) Å, β = 97.55, Z = 2, V = 1073.2 Å, D_c = 1.5 g cm^{-3}, μ(CuKα) = 20.9 cm^{-1}. The structure was solved by direct methods.[8] The current R value is 0.04. Details will be publiched elsewhere.
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