Vol. 136, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1986 Pages 294-299

STACKING AND HYDROGEN BONDING INTERACTIONS BETWEEN PHENYLALANINE AND GUANINE NUCLEOTIDE: CRYSTAL STRUCTURE OF L-PHENYLALANINE—7-METHYLGUANOSINE-5'-MONOPHOSPHATE COMPLEX

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Received March 3, 1986

SUMMARY: The crystal structure of title complex has been analyzed by X-ray diffraction method as a model for elucidating the possible interaction between the phenylalanyl residue of proteins and the N7-protonated or methylated guanine base of nucleic acids. The guanine base is associated with the benzene ring of phenylalanine by stacking interaction, and further connected with the carboxyl group by the formation of a pair of hydrogen bonds. These two interaction modes are suggested to be responsible for the specific recognition of base sequence by protein.

The specificity of nucleic acid-protein recognition is guaranteed by the direct interactions between nucleic acid bases and amino acid residues constituting each of the two macromolecules, in addition to their complementary spatial structures. Aromatic amino acids such as tryptophan have been demonstrated to bind with bases by ring-ring stacking interactions(1,2).

Evidence for such stacking interactions has come from solution studies by using spectroscopic methods such as nuclear magnetic resonance, fluorescence and circular dichroism. In contrast there are very few crystallographic data although these are undoubtedly important for understanding the nature of such interaction at the atomic level. As a series of crystallographic studies on nucleic base-amino acid interaction we have prepared crystals of L-phenylalanine(Phe)—7-methylguanosine-5'-monophosphate (m7GMP) complex and analyzed their structure by X-ray diffraction method. The elucidation of binding mode between both molecules is of interest

0006-291X/86 $1.50

because the importance of Phe residue in nucleic acid-protein interactions has been demonstrated(3-6). Furthermore these results would provide some insights as to the interaction between the cap structure of mRNA and the aromatic amino acid residue of cap binding protein, because m7GMP constitutes part of the cap structure, m7GpppN(m), of most eukaryotic viral and cellular mRNAs(7), and inhibits the binding of the mRNA to cap binding protein competitively(8,9).

MATERIALS AND METHODS

Phe was purchased from Nakarai Chemical Co. m7GMP was synthesized by the methylation of guanosine-5'-monophosphate with dimethylsulfate(10). Among many attempts for cocrystallization, transparent platelet crystals were obtained from equimolar quantities of Phe and m7GMP dissolved in 70% aqueous propanol by slow evaporation at about 25°C. The measurements of absorption spectra, electrophresis and Karl Fischer's titration showed that the crystals consisted of an equimolar ratio of the component molecules and contain six waters of crystallization per one complex pair. For the measurement of X-ray reflection intensities, a single crystal with dimensions of ca. 0.2x0.05x0.1 mm was sealed in a glass capillary tube under the presence of some mother liquid, because the complex crystals were very fragile and became to be rapidly amorphus on exposure to air. Crystal data are as follows: $C_{11}H_{16}N_5O_8P.C_9H_{11}NO_2.6H_2O$, space group $P2_1$, a=13.987(6), b=7.274(2), c=15.329(7) Å, β=104.25(3)°, V=1512(1) Å^3, Z=2, Dm(by flotation method in C_6H_6 /CCl_4 mixture)= 1.419(7) g.cm^{-3}, Dx=1.429 g.cm^{-3}. The intensities of a total of 1763 independent reflections($2°<2\theta<100°$) were measured on a Rigaku four-circle diffractometer with graphite-monochromated Cu Kα radiation. The structure was finally solved by a combination of Patterson vector search and Fourier techniques, and refined by a least-squares method up to the present discrepancy index R of 0.21, which is relatively high, probably due to the small size of complex crystal, the high thermal vibrations of respective atoms(especially 7-methyl and phosphate groups of m7GMP), and/or the disorder attendant on dehydration: we could not find out three out of six waters of crystallization clearly.

RESULTS AND DISCUSSION

Figure 1 shows the packing arrangement of Phe and m7GMP molecules in the complex crystal. The stacked layers consisting of alternating benzene and guanine rings are running parallel to b-axis, and these layers are stabilized by hydrogen bond formation between the polar atoms of neighboring molecules and via water of crystallization existing among the layers.

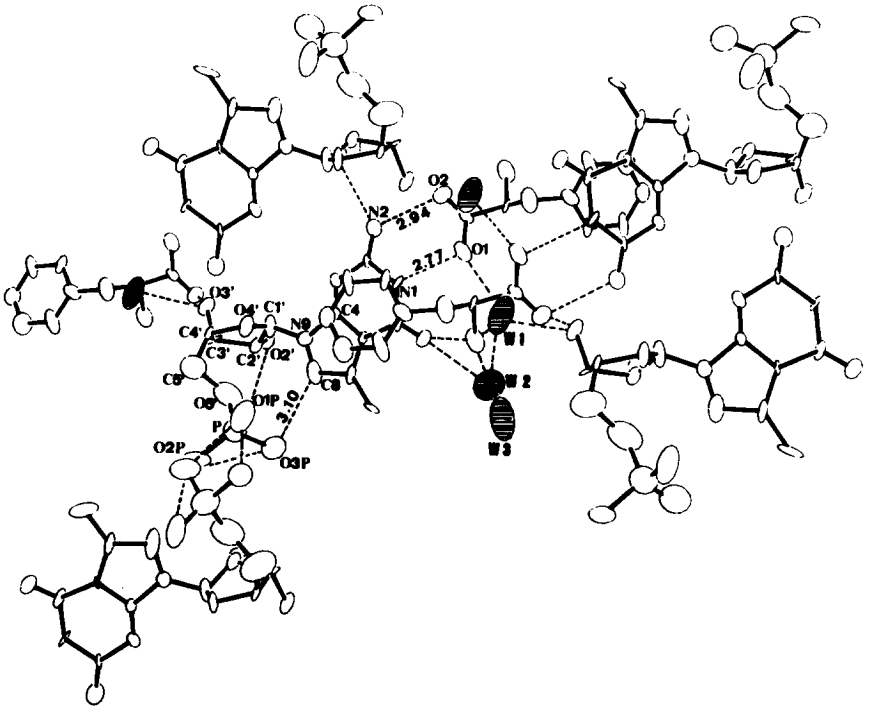

Fig.1. Crystal packing arrangement of Phe and m7GMP molecules, viewed along b-axis. Hydrogen bonds are indicated by the broken lines. W1, W2 and W3 indicate three water molecules.

Several interesting features are evident from this figure. The first is the formation of a hydrogen bond pair between the carboxyl group of Phe and the guanine base of m7GMP: O(1)---N(1)=2.77 Å, and O(2)---N(2)=2.94 Å. Since the unpaired guanine is the only base which possesses two donor groups in a suitable position to form a complex involving two hydrogen bonds with ionized carboxyl group, this type of hydrogen bonds could be involved in the selective recognition of ionized glutamic acid and aspartic acid side chains by the unpaired guanine base(11). Indeed the same hydrogen bonding pattern has been observed in the crystal structure of 7-methyl,9-ethylguanine —indole-3-acetic acid complex(12) and in aqueous solution containing carboxylate ions and guanine derivatives(13,14). The second feature of the co-crystal is the prominent stacking interaction between the benzene ring of Phe and the guanine base of m7GMP. The stacking

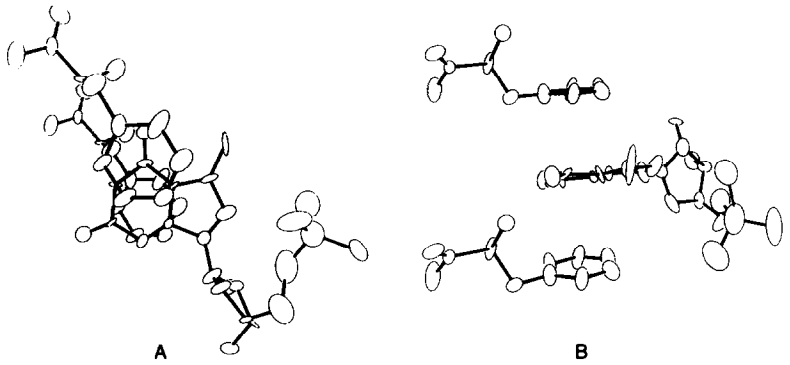

Fig.2. Stacking mode between the guanine and its two nearest neighboring benzene rings, projected perpendicular to the central guanine base(A) and parallel to the ring(B).

mode of two benzene rings with a central guanine base is shown in Fig.2. The two stacked benzene rings are related to each other by a b-axis translation operation. The dihedral angles between the benzene and guanine rings are both 11°, and their average interplanar spacings in the overlapping area are 3.48 Å and 3.54 Å for the upper and lower pairs, respectively. Since these distances are both near the van der Waals separation distance(=3.4 Å), these stacked pairs are stabilized by normal van der Waals forces. This type of stacking interaction has also been observed in indole—N(1)methylated adenine(15) and —N(7)methylated guanine base system(12,16), whereas crystallographic data on the stacking interaction between aromatic amino acids and the neutral nucleic bases have not been published yet. This could be due to the quarternization of base nitrogen atom by protonation or alkylation, because the π-orbital interaction between the aromatic ring and the nucleic acid base is strengthened by the quarternization(12,15). Therefore it could mean that stacking interaction is used by aromatic amino acids to recognize nucleic bases in acidic condition(pKa=3.2 for guanine base) or alkylated bases.

It is interesting to note that the formation of a hydrogen bond pair and stacking interaction observed in this crystal could in part explain why the positive charge and N(2) amino group of 7-methyl-

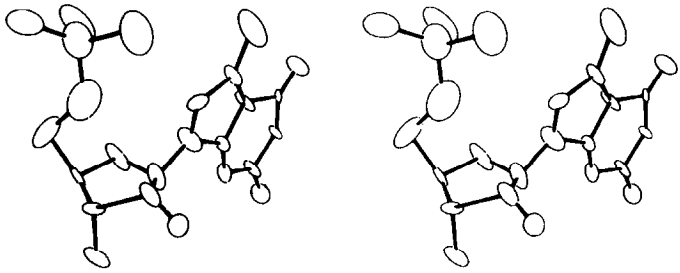

Fig.3. Stereoview of m7GMP molecular conformation.

guanine at the 5'-terminus of capped mRNA are necessary for the effective binding to the cap binding protein(17), if this protein contains phenylalanine and acidic amino acid residues at the binding site of mRNA although this is unclear at present.

It is important to investigate the conformation of m7GMP, because it could be thought to inhibit protein synthesis as a result of the conformational similarity to the 5'-terminal cap mRNA (18,19). The molecular conformation of m7GMP is shown in Fig.3, and the relevant torsion angles and ribose conformational descriptions are given in Table 1. The ribose ring is in the C2'-endo puckering (P=150°) and takes the anti conformation relative to the base(χ=-105°). The conformations about the exocyclic C(4')-C(5') and C(5')-O(5') bonds are in gauche.gauche and trans regions, respectively. Consequently the bond sequence of H(4')-C(4')-C(5')-O(5')-P is locked as a W arrangement. Such conformation has also been observed in the

Table 1. Torsion angles and ribose pseudorotation parameters

χ	O(4')-C(1')-N(9) -C(4)	-105°
α	O3P -P -C(5')-C(5')	180
β	P -O(5')-C(5')-C(4')	160
γ	O(5')-C(5')-C(4')-C(3')	67
δ	C(5')-C(4')-C(3')-O(3')	147
ν_0	C(4')-O(4')-C(1')-C(2')	-23
ν_1	O(4')-C(1')-C(2')-C(3')	29
ν_2	C(1')-C(2')-C(3')-C(4')	-24
ν_3	C(2')-C(3')-C(4')-O(4')	13
ν_4	C(3')-C(4')-O(4')-C(1')	4
P	phase angle of pseudorotation	150
τ_m	max amplitude of pseudorotation	28

crystal of m7GMP—tryptamine complex(16) and is in agreement with the NMR data in solution(18,19). Irrespective of whether it is in the isolated solution state or in the crystalline state complexed with proper amino acids, m7GMP exhibits a common conformational feature, implying that this conformation is a rigid and stable form for m7GMP. This could be due in part to the formation of an intramolecular hydrogen bond between C(8) and O(3)P atoms, as a result of the increase of acidity of H(8) proton by the N7 methylation.

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