Indole ring binds to 7-methylguanine base by π-π stacking interaction

Crystal structure of 7-methylguanosine 5′-monophosphate-tryptamine complex

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Strong π-π stacking interaction between the indole ring and 7-methylguanine base was shown by X-ray crystal analysis of the 7-methylguanosine 5′-monophosphate-tryptamine complex. This interaction appears to be strengthened by the attachment of ribose and phosphate groups to the base.

7-Methylguanosine 5′-monophosphate Tryptamine X-ray analysis Crystal structure π-π stacking interaction

1. INTRODUCTION

Insight into the geometry of nucleotide-amino acid interactions is of great significance for understanding the mechanism of specific nucleic acid-protein mutual recognition because such recognition is guaranteed by the direct interaction between the chemical groups constituting each of the 2 macromolecules.

Tryptophan and tyrosine, which have both π-electron-rich aromatic rings at their side chains, can bind with nucleic acid bases by π-π stacking interaction. Studies on this interaction have been done in solution by using various spectroscopic methods [1]. In contrast, there are few examples in crystal structure. This is due to the weakness of such an interaction force, and consequently the packing force accompanying the crystallization would predominate in the solid state.

On the other hand, stacking formation could be expected even in the crystal by quaternarization of the nitrogen atom of the nucleic acid base (especially purine bases) because the stacking interaction that arises from the interaction between the highest occupied molecular orbital (HOMO) of the donor aromatic ring and the lowest unoccupied molecular orbital (LUMO) of acceptor one is strengthened by protonation of the nucleic acid base as a result of the lowering of LUMO energy. This was ascertained in the crystal structures of the adenine-indole system [2] and the 7-methyl-9-ethylguanine–indole-3-acetic acid complex [3].

As a further development of this consideration we have prepared crystals of the 7-methylguanosine 5′-monophosphate (m^7GMP)-tryptamine (as a model for tryptophan) complex.

It is known that m^7GMP is a biologically important minor nucleotide isolated from a variety of RNAs, and inhibits, as a cap analogue, protein synthesis by competing with the 5′-terminal 'cap', m^7GpppN, of most eukaryotic mRNAs for the binding to cap-binding proteins essential for initiation [4–6]. Therefore crystal analysis of this complex may give some insight into the binding

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mechanism between the capped mRNAs and the cap-binding proteins.

2. MATERIALS AND METHODS

m^7GMP was synthesized by methylation of GMP with dimethylsulfonic acid [7]. Among the many combinations for the co-crystallization of the m^7GMP-tryptophan system, single crystals of m^7GMP-tryptamine complex were obtained from a m^7GMP:tryptamine (1:1) mixture dissolved in 70% aqueous methanol. Crystal data are as follows: $C_{11}H_{16}N_5O_8P \cdot C_{10}H_{13}N_2 \cdot 3H_2O$, triclinic P1, a = 6.741(3), b = 7.584(3), c = 12.904(5) Å, α = 97.31(11), β = 80.95(7), γ = 99.03(8)°, V = 639.8(5) Å^3 and Z = 1. Intensity data were collected on a Rigaku AFC-5 diffractometer with graphite-monochromated CuKα radiation. The crystal structure was analysed by a combination of heavy atom and direct methods, and refined by a least-squares method to a discrepancy R of 0.069 for 2138 ($F_0 > 0.0$) independent reflections.

3. RESULTS AND DISCUSSION

In this crystal the stacking layers consisting of alternate guanine and indole rings run in the a-axis direction, and are stabilized by the hydrogen bonds between the polar atoms of neighboring molecules and via waters of crystallization existing among these layers.

The indole rings are prominently associated with the guanine bases by π-π stacking interaction. The stacking mode of 2 up-and-down stacked indole rings with respect to the central guanine base is shown in fig.1. Both the upper and lower indole rings are well stacked on the central guanine base. The 2 respective stacked indole rings are related to each other by the a-axis translation operation. Both indole rings are almost parallel to the guanine base; the dihedral angles of the stacked pairs are both 3.9(1)°. The average interplanar spacings in the area of overlap are 3.385(7) and 3.352(7) Å for the upper and lower pairs, respectively. These values are significantly smaller than the normal van der Waals separation distance between 2 stacked aromatic rings (= 3.4 Å). Such a parallel stacking arrangement with an interplanar spacing less than 3.4 Å and with no direct hydrogen bond formation between both rings suggests the partial π-electron transfer of the indole ring to the unoccupied orbitals of guanine base even in its ground state. Thus, this π-π charge-transfer force could be thought of as being a major factor stabilizing the binding between the indole ring and guanine base. The degree of overlap in this complex was more prominent compared with that in 7-methyl-9-ethylguanine–indole-3-acetic acid complex (see fig.3 in [3]): both the upper and lower indole rings are stacked nearly to the center of the guanine base. This may imply that the stability of the π-π stacking interaction is increased more by the existence of ribose and phosphate groups.

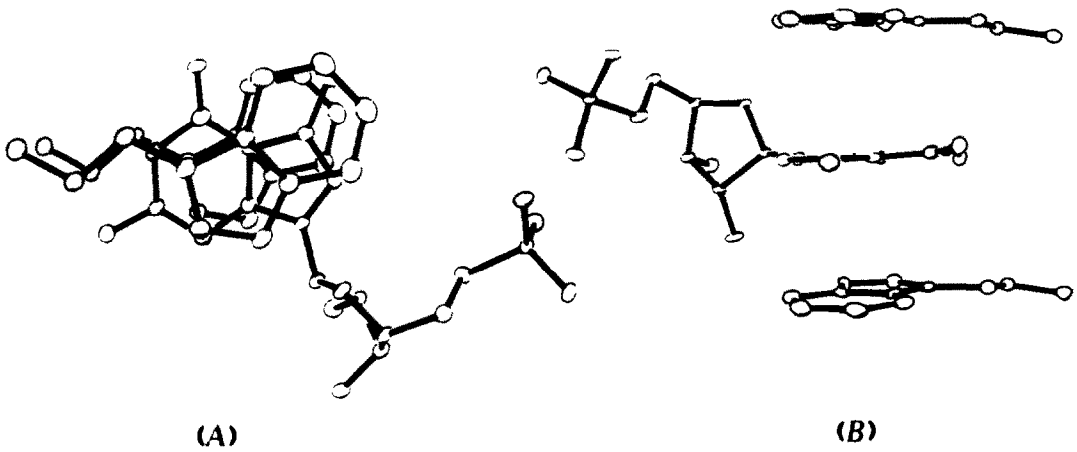

Fig.1. Stacking mode of nearest-neighboring indole-guanine pairs, projected perpendicular (A) or parallel (B) to the central guanine base.

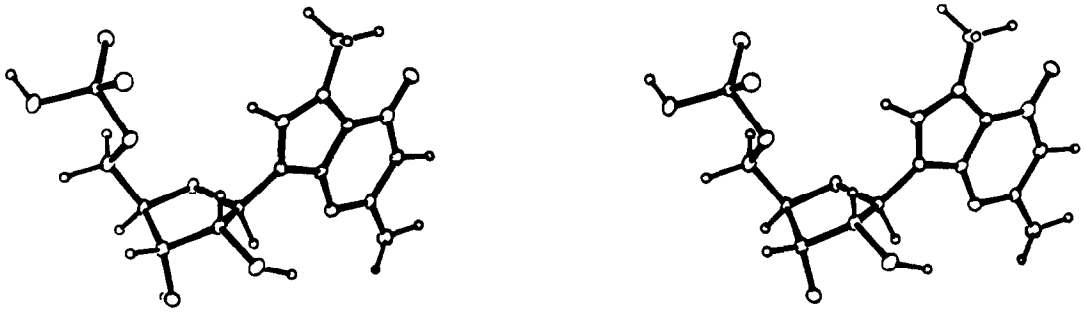

Fig.2. Stereoscopic drawing of m^7GMP molecule.

A structural feature of the stacked molecules is that the N7 atom of guanine, the center of positive charge, exists almost at the center of the benzene part of the indole ring, showing the strongest interaction site. Many atomic pairs with short contacts less than 3.4 Å are also observed in this stacking pair. On the other hand, no specific interaction between the anionic phosphate group and the positively charged N7 atom of guanine base was observed; an oxygen atom of phosphate was bifurcately hydrogen-bonded to the N1 and N2 atoms of the neighboring guanine base.

On the basis of NMR spectral analyses of 5′-phosphorylated 7-methylguanosine derivatives, Hickey et al. [8] reported that the introduction of N7-methyl and 5′-phosphate into guanosine induces the nucleotide to assume a rigid conformation in which the sugar moiety is preferentially C3′-*endo* with +*sc* (*gauche*·*gauche*) orientation around the exocyclic C4′–C5′ bond, and this conformation may be essential for inhibition of protein synthesis as result of the conformational similarity with the 5′-terminal capped structure of mRNA. Therefore it is interesting to investigate the conformation of m^7GMP observed in this complex crystal. The molecular conformation is shown in fig.2 and the conformational torsion angles are listed in table 1. The notation of torsion angles and the atomic numbering schemes are in accordance with the IUPAC-IUB Commission on Biological Nomenclature [9]. The conformation held very well for the phosphate group in the crystal as well as in solution: the bond sequence H4′-C4′-C5′-O5′-P is locked in a W arrangement. The sugar puckering took a C2′-*exo* form; the pseudorotation phase angle, *P*, was 341.7°. The slight deviation from the most probable C3′-*endo* pucker of m^7GMP may be due to the effect of the stacking interaction between the guanine base and indole ring. The orientation about the glycosyl bond was in the high *anti* region.

Table 1

Conformational angles (°) of the m^7GMP molecule

χ	O4′-C1′-N9-C4	−103.9(5)
ν_0	C4′-O4′-C1′-C2′	−25.1(5)
ν_1	O4′-C1′-C2′-C3′	39.8(5)
ν_2	C1′-C2′-C3′-C4′	−38.5(5)
ν_3	C2′-C3′-C4′-O4′	25.3(5)
ν_4	C3′-C4′-O4′-C1′	−0.4(5)
β	C4′-C5′-O5′-P	180.0(4)
γ	C3′-C4′-C5′-O5′	46.2(6)
δ	C5′-C4′-C3′-O3′	152.0(5)

It could be believed from many experiments that the N7-substituted, positively charged guanosine at the 5′-terminal cap of mRNAs apparently constitutes a part of the recognition site for cap-binding proteins which catalyse the binding of mRNAs to the ribosomes. An important finding obtained from this study is that the indole ring binds strongly with the N7-protonated guanine base by π-π stacking interaction. Therefore, an aromatic amino acid such as tryptophan or tyrosine contained in cap-binding proteins [10], although they have not yet been characterized sufficiently, may participate in the recognition of capped mRNAs.

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