

Cooperative face-to-face and edge-to-face aromatic interactions of tryptophan indole ring with N7-quarternized guanine and neutral cytosine bases

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Received 15 July 1993; revised version received 10 September 1993

In order to investigate the effect of cytosine base upon the stacking interaction of N7-quarternized guanine base with tryptophan indole ring, the X-ray crystal structure of a 1:1 complex of model compounds **1** and **2** was carried out. Contrary to the expectation of the interaction of both molecules in aqueous solution, the crystal structure showed the first example of the simultaneous recognition of the Trp indole ring by guanine and cytosine bases by the coupling of the face-to-face and edge-to-face aromatic interactions, respectively.

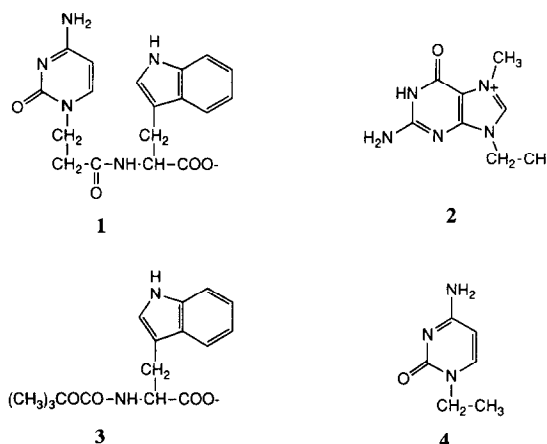
Tryptophan; Guanine; Cytosine; Face-to-face interaction; Edge-to-face interaction; X-ray analysis

1. INTRODUCTION

Strict molecular recognition is the first essential step for such biological processes as interactions of substrate/enzyme and nuclease/DNA base sequence. The precise recognition could be generally accomplished by multiple non-covalent interactions (such as hydrogen bonding, stacking, electrostatic and hydrophobic interactions). For better understanding of the strict recognition mechanism, it is of special importance to know various coupling modes of non-covalent interactions which serve to select the most suitable partner among many candidates. However, such information is very limited at present, although recent X-ray structural studies [1–3] showed that the specific recognition of guanine base by protein is accomplished by the coupling of multiple hydrogen bonding to the periphery of the base with aromatic/hydrophobic interaction perpendicular to the base.

Aromatic amino acids such as tryptophan (Trp) participate mainly in aromatic interactions with biomolecules [4]. Two kinds of interactions have been identified [5,6], i.e. the parallel face-to-face and perpendicular edge-to-face interactions. This paper describes the first example of the coupling of these two interactions for the recognition of the Trp indole ring by the nucleic acid bases of the N7-quarternized guanine and neutral cyto-

sine, which was observed in the crystal structure of **1** and **2** (1:1) complex (Scheme 1).



Scheme 1

2. MATERIALS AND METHODS

2.1. Materials

Model compounds **1** to **4** in Scheme 1 were synthesized from their component molecules according to the literature [7–10]. Here, **2** instead of the neutral guanine base, was used to increase the face-to-face stacking interaction with the indole ring, because the N7-quarternization of guanine base significantly strengthens the π - π stacking interaction with aromatic amino acids [11].

2.2. X-ray crystal analysis of **1** and **2** (1:1) complex

The complex crystals were obtained from aqueous methanol containing equimolar **1** and **2** by slow evaporation at room temperature. The content of water molecules of crystallization per asymmetric unit was estimated by the differential thermal analysis. Because of the very fragile crystal nature, they were sealed in glass capillaries with some mother liquid. A single crystal with dimensions $0.2 \times 0.2 \times 0.4$ mm

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Abbreviations: m7GMP, 7-methylguanosine-5'-phosphate; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital.

was used for the intensity data collection. Crystal data: $C_{18}H_{18}N_5O_4 \cdot C_8H_{12}N_5O \cdot 5H_2O$, $M_r = 652.66$, orthorhombic, space group $P2_12_12_1$, $a = 18.894(3)$, $b = 23.286(5)$, $c = 7.335(2)$ Å, $V = 3227.1(11)$ Å³, $Z = 4$, $\rho_{\text{calc}} = 1.343$ g·cm⁻³, $F(000) = 1384$, $\lambda = 1.5418$ Å, $T = 288$ K, $\mu(\text{Cu K}\alpha) = 7.56$ cm⁻¹. X-ray diffraction data were collected using a Rigaku AFC5 diffractometer. An $\omega/2\theta$ scan mode was used in the range $2^\circ \leq 2\theta \leq 130^\circ$. The intensity data were corrected for the Lorentz and polarization effects, but not for the absorption. Of the 2,920 collected reflections, 2,402 with $F_o > 2\sigma(F_o)$ were used in the structure solution and refinement. Structure was solved by direct methods (program SHELXS-86 [12], which located all nonhydrogen atoms of **1** and **2**. Five water molecules of crystallization were found on the successive Fourier syntheses. Hydrogen atom positions were calculated and were included for the calculations of structure factors, but not for the structure refinement. The structure was finally refined to $R = 0.070$ ($R_w = 0.063$) by the SHELX76 program [13]; this relatively high value is primarily due to the small crystal size and its poor crystallinity. The fractional atomic coordinates and other structural parameters have been deposited in Cambridge Data Center.

3. RESULTS AND DISCUSSION

Previously, we proposed the importance of cooperative coupling of hydrogen bond pairing and parallel face-to-face stacking interactions for the molecular recognition between **1** and **2**, based on comparative fluorescence and NMR measurements on the interactions of **1** with **2**, **3** and **4** [14]. In contrast with the interaction mode proposed in the solution state, the X-ray crystal structure of the 1:1 complex and **1** and **2** showed an alternative interaction mode (Fig. 1), which also explains well the spectral behaviors of aromatic rings in the solution, except for those of N2-amino protons of **2**.

Both molecules are linked with hydrogen bond pairing between backbone amide NH and carboxyl O of **1** and guanine O(6) and N(1)H of **2**: 3.017(7) and 2.779(8) Å, respectively. As expected, the indole ring is sandwiched between neighboring guanine bases with face-to-face stacking interactions, in which both aromatic rings are nearly parallel with a dihedral angle of $1.4(2)^\circ$ and mean separations of 3.428 Å (upper pair) and 3.442 Å

CC(lower pair); the crystal structure is mainly stabilized by this continuous ...indole-guanine-indole-guanine... stacking, rather than 1:1 interaction of molecules **1** and **2**, leaving little possibility for the less energetically favorable face-to-face indole-cytosine interaction. As observed in the m7GMP-Trp [15] and m7GMP-Trp-Glu complexes [16], the present extensive stacking with an interplanar spacing near to the minimal van der Waals separation of 3.4 Å is typical for the partial π -electron interaction between the HOMO of the indole ring and the LUMO of the guanine base. In addition, this stacking interaction/orientation is stabilized by (a) electrostatic interactions and (b) dipole-dipole coupling (the angle between the calculated dipole moments is 153°).

Simultaneously, the Trp indole ring is fixed by the cytosine base with an edge-to-face interaction, although its molecular orientation in **1** may be resulting from the covalent linkage of both aromatic rings via an amide bond. The aromatic rings are in close proximity to each other with a distance of 4.75 Å (the vertical distance from the center of the benzene moiety of the indole ring to the cytosine plane), and in a nearly orthogonal orientation, with an angle of $94.5(2)^\circ$ between their average planes. This results in short contacts between the indole CH groups and the cytosine base, indicating electrostatic $C^{\delta-}-H^{\delta+} \cdots \pi$ interactions. Similar T-shaped intra-/intermolecular aromatic-aromatic interactions have frequently been observed in protein crystal structures and shown to be enthalpically favorable [5,6,17,18].

The complex crystals contain five water molecules of crystallization per asymmetric unit. They are located at the cavity formed by the crystal packing of molecules **1** and **2** and stabilize the simultaneous formation of the face-to-face and edge-to-face aromatic-aromatic interactions by two to four hydrogen bond and/or short contact formations with neighboring **1** and **2** polar atoms.

To the best of our knowledge, this result provides the

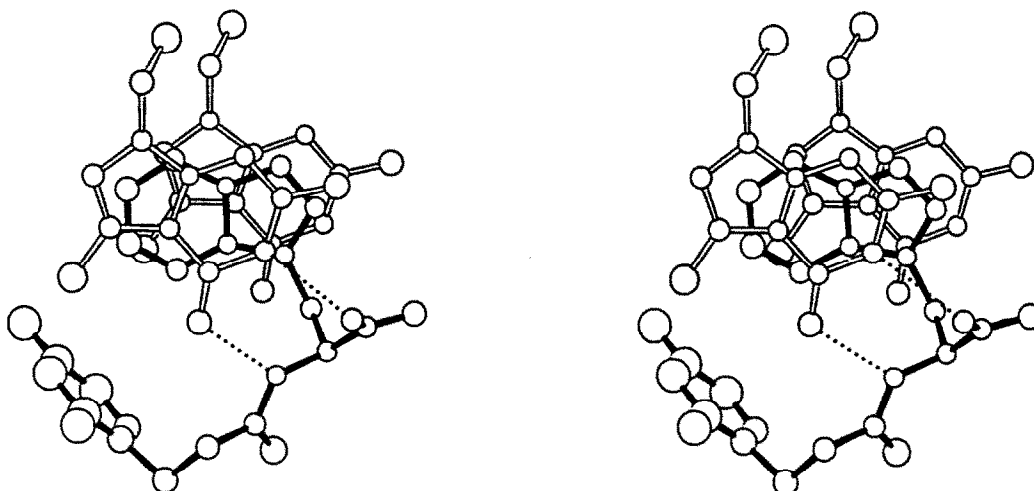


Fig. 1. Stereoscopic X-ray structure of the 1:1 complex of **1** and **2**. The dotted lines represent hydrogen bonds. The molecule **1** is shown with heavy lines.

first example in the recognition of Trp indole ring by N-quarternized guanine and a neutral cytosine base, in which the hydrogen bonding, aromatic face-to-face and edge-to-face interactions are cooperating, although this interaction is of course stabilized by hydrogen bondings with the surrounding water molecules. Such an intimate coupling of non-covalent interactions may be important for the selective recognition of aromatic amino acids by nucleic acid bases in protein-DNA interactions.

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