Selective Binding of Guanine Base by a Tryptophan-containing Dipeptide

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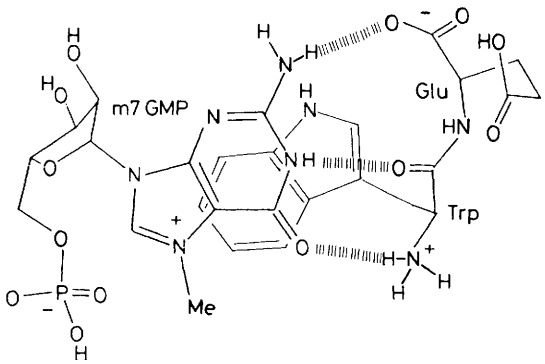
The crystal structure of the m7GMP–Trp-Glu (1 : 1) complex crystal shows the tight linkage of the guanine base with the peptide backbone chain *via* triple hydrogen bonds and a prominent stacking formation with the indole ring of tryptophan; this suggests the importance of a combination of these means for the selective binding of the guanine base by protein.

Recently much attention has been focused on the mechanism of recognition of guanine nucleotide by protein, because (i) many cell surface receptors have been demonstrated to exert their actions through specific guanine nucleotide regulatory proteins (G proteins),[1] and (ii) the cap structure (m7GpppX, where X is any nucleotide) at the 5′ end of most eukaryotic mRNAs, which plays an important role in several aspects of mRNA metabolism, has been shown to be specifically recognized by a ~24-kDA polypeptide, termed as cap binding protein (CBP) or eukaryotic initiation factor (eIF)-4E.[2,3] The results reported here could explain the essential factors for selective recognition.



(1)

Platelet single crystals of the complex m7GMP–Trp-Glu (1 : 1) (**1**)† were obtained as the trihydrate from aqueous methanol solution (30%) by slow evaporation at room

† M7GMP formate was synthesized by the methylation of GMP with dimethylsulphonic acid,[4] and converted to the OH form using a column of Amberlite IRA-401 anion-exchange resin. A series of Trp-containing dipeptides was synthesized by the usual liquid phase method. The purities of all samples synthesized were checked by HPLC and ^{1}H NMR spectroscopy.

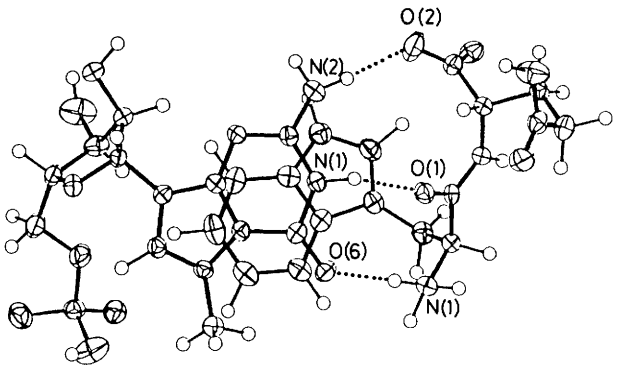


Figure 1. A perspective view of the co-operative binding mode consisting of triple hydrogen bond pairing and stacking interaction between the m7GMP and Trp-Glu molecules. The dotted lines represent hydrogen bonds.

temperature (20 °C), and the crystal structure was determined by *X*-ray crystallography.‡

As shown in Figure 1, m7GMP and Trp-Glu molecules were tightly linked by the two inherent characteristic interaction modes: (i) triple 'cyclic type' hydrogen bonds between the guanine and the dipeptide backbone chain, and (ii) stacking interaction between the guanine and the indole ring of tryptophan.

Among the four polar atoms [N(1), N(2), N(3), and O(6)] of the 7-methylguanine base, three [N(1), N(2), and O(6)] were simultaneously hydrogen-bonded to the N and O atoms of the dipeptide backbone chain: N(1)···O(1) 2.762(4), N(2)···O(2) 3.001(5), and O(6)···N(1) 2.901(5) Å. Although this is the first example of this type of triple hydrogen bond formation to be established by *X*-ray crystallography, to the best of our knowledge, it appears to be as stable as the usual Watson–Crick G : C base pair. Thus, this becomes a good model for the interaction between the guanine and polypeptide, where N_i, O_i, and O_{i+1} atoms of the polypeptide backbone chain are simultaneously hydrogen-bonded to the guanine O(6), N(1), and N(2) atoms, respectively. The backbone chain of Trp-Glu is twisted in a suitable geometry so as to best form the pairing with the guanine base: the ψ_1, ω_1, and ϕ_2 torsion angles of Trp-Glu are 165.3(4), 176.9(4), and −105.7(4)°, respectively.

The guanine base prominently stacks on the indole ring of the tryptophan side chain. This stacking interaction is supposed to be strong because the aromatic rings are nearly parallel [dihedral angle 1.9(1)°] and their mean separation distance (3.301 Å) is shorter than the minimal van der Waals separation distance of 3.4 Å; this is a typical feature of the π–π charge-transfer complex.[6] In addition to this partial charge-transfer force, the stacking interaction was further stabilized by dipole–dipole coupling (angle between their calculated dipole moments 163.5°) and electrostatic interaction forces.

The co-operative binding mode of the triple hydrogen bond pairing and the stacking interaction observed in the m7GMP–Trp-Glu crystal appears to be specific and stable enough to tightly fix the guanine base by protein. Thus, this kind of binding mode may be essential for the binding of the mRNA cap structure by CBP, because this protein contains eight tryptophan residues,[7,8] two of which are essential for CBP activity;[9] the sequence Trp-Glu actually exists in CBP.

The present results could also provide a useful clue to the binding mechanism of guanine nucleotide by G proteins. m7GMP, instead of GMP, was used in this study in order to increase the stacking interaction with the tryptophan residue. This is based on experimental and theoretical evidence that the quaternization of the nitrogen atom of the nucleic acid base by protonation or methylation, significantly strengthens the stacking interaction with the aromatic amino acid.[6] However, because of the high nucleophilicity of the guanine N(7), protonation at this atom is highly likely in the local acidic environment in the protein, and consequently the neutral guanine base may become the most susceptible target for the simultaneous and co-operative interactions of the hydrogen bond with the acidic amino acid and the stacking with aromatic amino acids such as tryptophan.

It is noteworthy that there is a peptide sequence specificity for interaction with guanine base. Many attempts to cocrystallize m7GMP with other dipeptides have failed, and the measurement of association constants with 7-methylguanine derivatives by ^{1}H NMR and absorption spectroscopies has given the order Trp-Glu > Glu-Trp.[10] This implies that close co-operation between the hydrogen bond pairing and stacking interaction is essential for the specific recognition of guanine base.

In conclusion, the present study clearly shows that even a dipeptide can satisfy the structural requirements for tight binding with guanine base.

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‡ *Crystal data* for (**1**): $C_{11}H_{16}N_5O_8P{\cdot}C_{16}H_{19}N_3O_5{\cdot}3H_2O$, M = 764.64, monoclinic, space group $P2_1$, a = 6.558(1), b = 16.460(3), c = 15.395(4) Å, β = 96.70(2)°, U = 1650.5(5) Å^3, Z = 2, D_m = 1.524(1), D_x = 1.539 g cm^{-3}. The intensities of a total of 2924 independent reflections (sin θ/λ ≤0.588 Å^{-1}) were recorded on a Rigaku four-circle diffractometer with graphite-monochromated Cu-K_α radiation, and corrected for the Lorentz and polarization factors. The structure was solved by a combination of the heavy atom and direct methods using program MULTAN87,[5] and refined by a least squares method, nonhydrogen atoms anisotropic and hydrogen atoms isotropic, to R 0.048; R_w = 0.064. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

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