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A POSSIBLE RECOGNITION MODE OF mRNA CAP TERMINAL STRUCTURE BY PEPTIDE: COOPERATIVE STACKING AND HYDROGEN-BOND PAIRING INTERACTIONS BETWEEN m7GpppA AND TRP-LEU-GLU

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^{1}H-NMR and fluorescence spectroscopic studies on the interaction between the Trp-Leu-Glu and m7GpppA have shown a specific binding mode, in which the π-π stacking interaction of the Trp indole ring and the hydrogen-bond pairing of Glu carboxyl side group with 7-methylguanine base are simultaneously formed.

The 5'-terminal portions of many eukaryotic mRNAs consist of 'cap' structures(1). This structure, described as m7GpppNp-, is formed by the triphosphate linkage between 5'-terminal hydroxyl groups of 7-methylguanosine(m7GuO) and mRNA, and plays important roles in the biological function of mRNA(2). Moreover, many initiation factors participate in the translation of mRNA (protein synthesis)(3). Among them, cap binding protein (CBP) is responsible for the specific recognition of the mRNA cap structure and consequently for conducting it to a ribosome(4).

Recently, it has been reported (5,6) that CBP is characterized by many tryptophan(Trp) and acidic amino acid residues. Therefore, the study on the interaction between these amino acids and the cap structure will be useful for understanding how CBP specifically recognizes the cap structure and why 7-methylguanine(m7G) is necessary for the cap terminal portion.

Bearing this in mind, the interactions between the cap analogues and peptides have been investigated by fluorescence and ^{1}H-NMR measurements. In

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this paper, we report a specific interaction mode between m7GpppA and Trp-Leu-Glu, where the latter amino acid sequence consists of a part of CBP.

MATERIALS AND METHODS

GpppA, as a precursor of mRNA cap structure analogues, was synthesized from GMP and ADP in accordance with the morpholidate method (7,8). m7GpppA was then synthesized by the methylation of GpppA with dimethylsulphate (9). Trp-Glu, Glu-Trp and Trp-Leu-Glu were also synthesized by the usual liquid phase method. The purities of all samples synthesized were checked by HPLC, paper electrophoresis and ^{1}H-NMR measurements.

Fluorescence spectra were measured on a Hitachi F-3000 spectrometer (a Xenon lump) with a 10-mm cell. For quantitative measurements, solutions of 0.1mM concentrations were prepared by dissolving the synthesized samples in 25mM phosphate buffer (pH 7.0 at 20°C). The emission spectra were measured at 20°C with 280-nm excitation and then were not corrected for monochrometer efficiency and photomultiplier response. The spectra were measured three times and averaged.

^{1}H-NMR measurements were carried out on a Varian XL-300 (300 MHz for ^{1}H; Fourier transform mode) spectrometer equipped with the temperature-control accessory (accuracy to ±1°C). Chemical shifts were measured with respect to internal reference to either TMS (tetramethylsilane) for DMSO-d_6 solvent or DSS (2,2-dimethyl-2-silapentane-5-sulphonate) for deuteriated 25mM-phosphate buffer (pD=6.7). The samples were lyophilized three times in 99.8% D_2O, and finally dissolved in the buffer. The sample concentrations were determined by their optical densities.

Possible molecular modellings on m7GpppA—Trp-Leu-Glu interaction mode were performed with an MMS program (10) operating on IRIS2400 graphics, and their molecular geometries were energetically optimized by using a molecular mechanics program MMFF (11) within CHEMLAB-II (12) operating on a Micro Vax II computer. The initial model building was carried out with present and related (13) spectroscopic data and the available crystal structures (14,15). The ^{1}H-NMR analyses and the temperature dependence of CD spectra (unpublished) showed the folded conformation of m7GpppA with having the stacking interactions between the m7G and adenine bases. The atomic charges and dipole moments were calculated by CNDO/2 method.

RESULTS AND DISCUSSION

The measurement of fluorescence quenching is a useful approach for assessing the ring stacking interaction. Therefore, the fluorescence intensities of Trp indole rings in peptides were quantitatively measured in the absence and presence of equimolar GpppA and m7GpppA. The degrees of fluorescence quenchings are given in Table 1.

The quenchings of Trp fluorescence are noticeably increased by the N7-methylation of the guanine base, suggesting the existence of a predominant indole—m7G stacking interaction; the same conclusion has also been drawn from X-ray crystallographic study(14,16). The quenching in the coexistence of m7GpppA is decreased in the order of Trp-Leu-Glu>Trp-Glu>Glu-Trp. This means that the stacking interaction is markedly strengthened by acidic amino acids such as Glu, which is connected at the C-terminal side of tryptophan, although this tendency is not observed in the interaction with GpppA.

Table 1
Average percentage fluorescence quenching of Trp accompanying stacking interaction with GpppA or m7GpppA in 25 mM phosphate buffer at λ=350 nm and 20°C λ_{excit} =280 nm [a,b]

Peptide	GpppA	m7GpppA
Glu-Trp	4.8	6.5
Trp-Glu	4.2	8.7
Trp-Leu-Glu	8.1	12.2

[a] The values are internally reproducible with 0.5%.
[b] All concentrations were adjusted to 0.01 mM.

In order to clarify to what extent the m7G within m7GpppA interacts with Trp as compared to the adenine base (A), and to consider the structural stability at the complex state, the association constant and its thermodynamic parameters (ΔH and ΔS) were determined from the temperature dependence (20° C — 70°C) of the upfield shift changes of A and m7G H1' protons as a function of peptide concentration (10mM — 40mM). The determination from the base H8 protons was impossible because of the fast H-D exchange in m7G. The experimental details were carried out according to the method of Kamiichi et al (16). The results for Trp-Glu and Trp-Leu-Glu peptides are given in Table 2. As is obvious from these data, the Trp indole ring

Table 2
Association constants K and thermodynamic data for m7GpppA—peptide complexes in deuteriated 25 mM phosphate buffer (pD=6.7)[a]

Position/ peptide	m7GpppA		
	$K(M^{-1})$	-ΔH(kcal/mol)	-ΔS(cal/mol.deg)
Adenosine H1'			
Trp-Glu	3.97	2.43	5.19
Trp-Leu-Glu	2.43	3.62	10.04
Guanosine H1'			
Trp-Glu	8.79	5.49	13.38
Trp-Leu-Glu	7.61	9.30	26.53

[a] The concentrations used were 8 mM for m7GpppA. The concentrations of peptides varied from 10 mM to 40 mM, and the temperature variation was in the range from 20°C to 70°C. All data were computed by a linear least-squares fit followed by an error estimation: the mean errors are up to 10%. The K and -ΔS values listed correspond to those at 30°C.

interacts with m7G about three times more than with A, which is in accordance with the fluorescence data. Furthermore, $-\Delta H$ and $-\Delta S$ values, which are closely related to the magnitude of the force of ring stacking formation and the order of the stacking structure, respectively, imply that the interaction between Trp-Leu-Glu and m7GpppA stabilizes the complex structure significantly. In other words, the acidic Glu in this peptide sequence strongly affects the complex formation.

Therefore, it is of interest to elucidate how the Glu residue participates in the Trp-Leu-Glu—m7G interaction. Since the unpaired guanine base is the only base which possesses two hydrogen-bond donor groups, it could be supposed the pairing between the carboxyl anion and guanine base via two NH...O hydrogen bonds. Indeed, this hydrogen-bond pairing has been observed in the solid (17,18) and solution (19) states of a guanine (or m7G)—carboxylate system. In order to investigate the possibility of this type of pairing in the present system, the chemical shift movements of m7G N1 imino and N2 amino protons were measured in the absence and presence of the peptides. Unfortunately, none of the signals of these protons in m7GpppA and related compounds were observed in the aqueous buffer solution because of the fast exchange with the solvent. For this study, therefore, we used 7-methylguanosine (m7Guo) in DMSO-d_6; the other related coumpounds such as m7GpppA were all insoluble in this solvent. The results are given in Table 3.

The addition of Trp-Glu moves the chemical shifts of m7GuO N1 imino and N2 amino protons to the upfield side, due to the effect of indole—guanine base stacking interaction. Conversely, the coexistence of Trp-Leu-Glu causes large downfield shifts of both protons. This clearly reflects the formation of hydrogen-bond pairing between the Glu carboxylate side group and m7G base.

Judging from the present results, it could be concluded that Trp-Leu-Glu interacts with m7G in m7GpppA by the combination of stacking formation and

Table 3
Interaction shifts (in Hz) of guanine N1 imino and N2 amino protons of m7GuO in the presence of Trp-Glu or Trp-Leu-Glu at 30°C [a]

	H1	H2
m7GuO		
+ Trp-Glu	-16.1	-12.1
+ Trp-Leu-Glu	14.9	44.3

[a] All concentrations were 8mM in DMSO-d_6. The standard error is ±0.3 Hz.

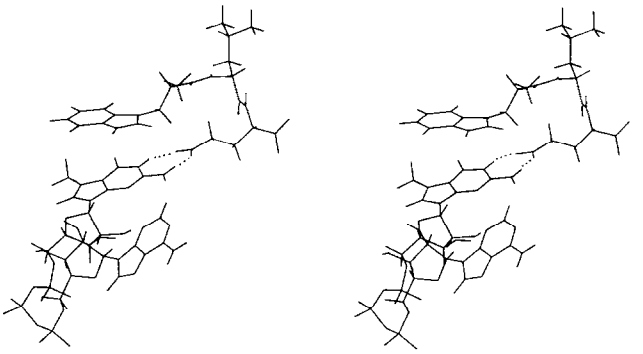

Figure 1 Stereoscopic view of a possible binding mode between m7GpppA and Trp-Leu-Glu
The dotted lines represent hydrogen bonds between m7G base and Glu carboxyl group.

hydrogen-bond pairing. This interaction mode appears to be important in considering the specific recognition mechanism of the mRNA cap structure by CBP, because the Trp-Leu-Glu sequence consists of a part of this protein. Therefore, we considered the reasonable spatial interaction mode between Trp-Leu-Glu and m7GpppA by using molecular mechanics force field (MMFF) calculations.

All possible conformers built up for each molecule were separately optimized and then jointed to each other so as to form the reasonable stacking and hydrogen-bond pairing interactions. The conformations of all possible complex pairs were further optimized by the MMFF method. Figure 1 shows a stereoscopic view of the most stable complex pair (-100.56 kcal/mol), where the dotted lines represent hydrogen bonds. Trp-Leu-Glu takes a stable conformation with α-helix fashion, and interacts with m7GpppA; the m7G base is fixed by the stacking formation with Trp indole ring (average interplanar spacing =4.0 Å and dihedral angle = 15°) and the hydrogen-bond pairing with Glu carboxyl group (N1...O = 2.75 Å and N2...O' = 2.68 Å). No short contact was observed between the molecules. The molecular conformation of m7GpppA is not largely different from the one estimated from the ^{1}H-NMR and CD measurements(unpublished).

This figure may provide a possible situation for the interaction between CBP and mRNA cap structure, because the recognition mechanism of guanine nucleotide by ribonuclease T_1 (Arni et al unpublished results) is achieved by the essentially same interaction mode to the present one: guanine base is stacked with Tyr42 and 45, and hydrogen bonding pairing is formed between the guanine N1 and N2 and carboxyl oxygens of Glu46 side chain.

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