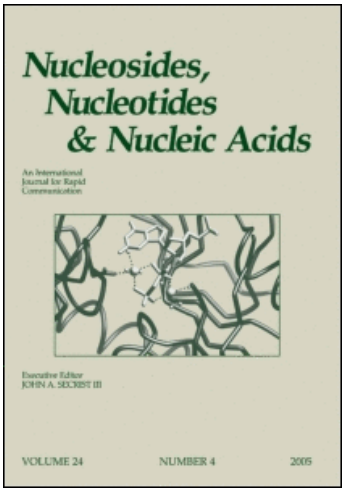

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Studies on Association of mRNA Cap-analogues with a synthetic Dodecapeptide *DGIEPMWEDEKN*

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STUDIES ON ASSOCIATION OF mRNA CAP-ANALOGUES WITH A SYNTHETIC DODECAPEPTIDE *DGIEPMWEDEKN*

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ABSTRACT: ^{1}H NMR and fluorescence were applied to a study of interactions between dinucleotide analogues of mRNA cap and a peptide *DGIEPMWEDEKN*. Structures of the m^{7}G-Trp complexes were determined by means of the ring current anisotropy theory, and their features were refered to the known X-ray structure of the eIF4E-m^{7}GDP associate.

Most eukariotic mRNAs possess at their 5'-termini a "cap", m^{7}G(5')ppp(5')N, N=G,A,U,C, necessary for optimal protein translation [1]. Stacking between m^{7}G and tryptophans plays a fundamental role in recognition of cap by the cap-binding protein eIF4E, as shown for the eIF4E-m^{7}GDP complex [2]. This paper presents results of the studies on interaction of three structurally various cap-analogues, N=G,C,m^{6}A [3], with a peptide *DGIEPMWEDEKN* [4], corresponding to the part of the binding center of eIF4E [2].

The chemical shift differences $\Delta\delta$ (Table 1) point to stacking between the 7-methyl-guanine and tryptophan rings, with the association constant K=50±10 M^{-1}, obtained by NMR (Fig. 1) and fluorescence. Distinct behaviour of $\Delta\delta$(H7) suggests additional, non-stacking interactions inside the complexes. Two types of such m^{7}G-Trp complexes (Fig. 2) were determined with the aid of a program GEOSHIFT [5]. This, and temperature studies of the m^{7}GpppG-peptide mixtures, show highly dynamic character of the stacking. Thermodynamic parameters for concurrent self-stacking of m^{7}GpppG: ΔH=15.0±0.4 kJ/mol and ΔS=-49±1 J/mol·K were also determined from NMR and fluorescence [6].

TABLE 1. ^{1}H chemical shifts differences Δδ=δ(mixture)-δ(free) (±0.003 ppm) due to stacking between the indole ring of the central Trp of the dodecapeptide and the m^{7}G ring of the cap-analogues, each at a concentration of 3 mM in a phosphate buffer pH 5.2, at 25 °C. Δδ for the protons of the second base in each cap-analogue are much smaller than those of m^{7}G, suggesting the Trp-m^{7}G stacking only.

	Tryptophan					m^{7}G	
Cap analogue	H2	H4	H5	H6	H7	H8	CH_3
m^{7}GpppG	-0.015	-0.012	-0.010	-0.009	+0.003	-0.007	-0.014
m^{7}GpppC	+0.005	-0.022	-0.020	-0.020	-0.055	+0.006	-0.013
m^{7}Gpppm^{6}A	+0.004	-0.026	-0.023	-0.024	-0.056	+0.067	+0.015

FIG. 1. Dependence of Δδ of the TRP protons on the concentration of m^{7}GpppG, pH 5.2, 25°C.

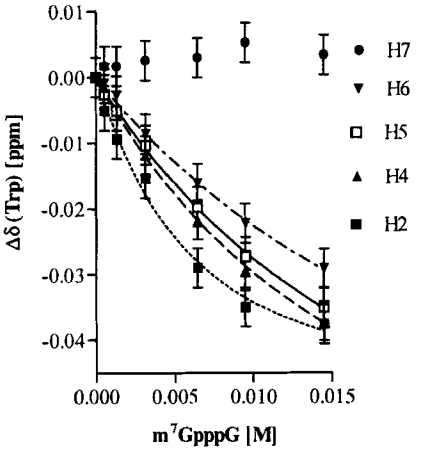
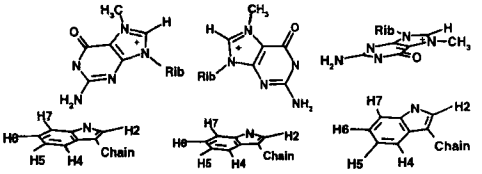


FIG. 2. Structures of the 7-methylguanine-indol associates for m^{7}G(5')ppp(5')G: **perpendicular:** (left) Euler's angle Θ≈70°, R≈4.9 Å, and (middle) Θ≈106°, R≈5.1 Å; **planar** (right), Θ≈20°, R≈3.4 Å. For m^{7}G(5')ppp(5')m^{6}A and m^{7}G(5') ppp(5')C the planar-type associates with Θ≈30° dominate, with mutual rotation of the rings around the vertical axis.



Sandwich stacking of 7-methyl-guanosine between two tryptophan indole rings in the eIF4E-m^{7}GDP complex [2] seems to prevent energetically more favourable perpendicular orientation of two aromatic rings usually observed inside the protein hydrophobic core [7].

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