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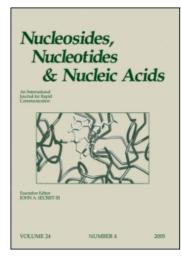
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Nucleosides, Nucleotides and Nucleic Acids

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

A. Niedżwiecka-Kornaś^a; R. Przedmojski^a; L. Balaspiri^b; Z. Wieczorek^c; J. Stępiński^d; M. Jankowska^d; H. Lönnberg^e; E. Darżynkiewicz^a; R. Stolarski^a

^a Department of Biophysics, University of Warsaw, Warszawa, Poland ^b Albert Szent-Gjörgyi-University, Szeged, Hungary ^c University of Agriculture and Technology, Olsztyn, Poland ^d Department of Chemistry, University of Warsaw, Warszawa, Poland ^e University of Turku, Turku, Finland

To cite this Article Niedżwiecka-Kornaś, A. , Przedmojski, R. , Balaspiri, L. , Wieczorek, Z. , Stępiński, J. , Jankowska, M. , Lönnberg, H. , Darżynkiewicz, E. and Stolarski, R.(1999) 'Studies on Association of mRNA Cap-analogues with a synthetic Dodecapeptide DGIEPMWEDEKN, Nucleosides, Nucleotides and Nucleic Acids, 18: 4, 1105 — 1106

To link to this Article: DOI: 10.1080/15257779908041660 URL: http://dx.doi.org/10.1080/15257779908041660

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

A. Niedźwiecka-Kornaś^a, R. Przedmojski^a, L. Balaspiri^b, Z. Wieczorek^c, J. Stępiński^d, M. Jankowska^d, H. Lönnberg^c, E. Darżynkiewicz^a and R. Stolarski^{a*}.

"Department of Biophysics. University of Warsaw, 02-089 Warszawa, Poland, bAlbert Szent-Gjörgyi-University, H-6720 Szeged, Hungary, University of Agriculture and Technology, 10-957 Olsztyn, Poland, Department of Chemistry, University of Warsaw, 02-089 Warszawa, Poland, University of Turku, FIN-20500 Turku, Finland.

ABSTRACT. ¹H NMR and fluorescence were applied to a study of interactions between dinucleotide analogues of mRNA cap and a peptide *DGIEPMWEDEKN*. Structures of the m⁷G-Trp complexes were determined by means of the ring current anisotropy theory, and their features were referred to the known X-ray structure of the eIF4E-m⁷GDP associate.

Most eukariotic mRNAs possess at their 5'-termini a "cap", m⁷G(5')ppp(5')N, N=G,A,U,C, necessary for optimal protein translation ¹. Stacking between m⁷G and tryptophans plays a fundamental role in recognition of cap by the cap-binding protein eIF4E, as shown for the eIF4E-m⁷GDP complex ². This paper presents results of the studies on interaction of three structurally various cap-analogues, N=G,C,m⁶A 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-methylguanine 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 m⁷G-Trp complexes (Fig. 2) were determined with the aid of a program GEOSHIFT ⁵. This, and temperature studies of the m⁷GpppG-peptide mixtures, show highly dynamic character of the stacking. Thermodynamic parameters for concurrent self-stacking of m⁷GpppG: Δ H=15.0±0.4 kJ/mol and Δ S=-49±1 J/mol·K were also determined from NMR and fluorescence ⁶.

TABLE 1. ¹H chemical shifts differences $\Delta\delta = \delta(\text{mixture}) - \delta(\text{free})$ (± 0.003 ppm) due to stacking between the indole ring of the central Trp of the dodecapeptide and the m⁷G ring of the cap-analogues, each at a concentration of 3 mM in a phosphate buffer pH 5.2, at 25 °C. $\Delta\delta$ for the protons of the second base in each cap-analogue are much smaller than those of m⁷G, suggesting the Trp-m⁷G stacking only.

	Tryptophan					m ⁷ G	
Cap analogue	H2	H4	H5	Н6	H7	H8	CH_3
Cap analogue 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 $\Delta\delta$ of the TRP protons on the concentration of m⁷GpppG, pH 5.2, 25°C.

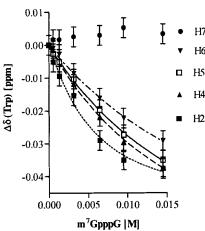


FIG. 2. Structures of the 7-methylguanine-indol associates for m⁷G(5')ppp(5')G: perpendicular: (left) Euler's angle $\Theta \approx 70^{\circ}$, $R \approx 4.9$ A, and (middle) $\Theta \approx 106^{\circ}$, $R \approx 5.1$ A; planar (right), $\Theta \approx 20^{\circ}$, $R \approx 3.4$ A. For m⁷G(5')ppp(5')m⁶A and m⁷G(5') ppp(5')C the planar-type associates with $\Theta \approx 30^{\circ}$ 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-m⁷GDP complex ² seems to prevent energetically more favourable perpendicular orientation of two aromatic rings usually observed inside the protein hydrophobic core ⁷.

Acknowledgements: Supported by the US-Polish M. Skłodowska-Curie Joint Fund II, MEN/NSF-96-257, and Polish Committee for Scientific Research, KBN 6PO4A03409 and partially BST-592/14/98.

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