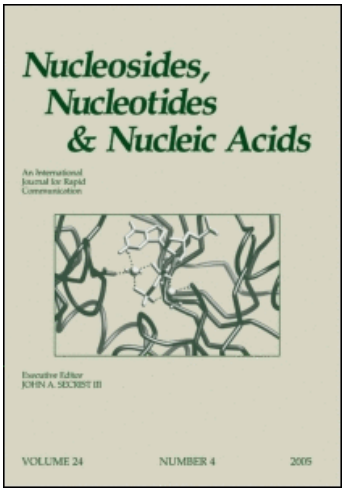

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 09 August 2003

To cite this Article Zuberek, Joanna , Jemielity, Jacek , Stepinski, Janusz , Lewdorowicz, Magdalena , Niedzwiecka, Anna , Haber, Dorota , Stolarski, Ryszard , Rhoads, Robert E. and Darzynkiewicz, Edward(2003) 'Binding Studies of Eukaryotic Initiation Factor eIF4E with Novel mRNA Dinucleotide Cap Analogues', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1703 — 1706
To link to this Article: DOI: 10.1081/NCN-120023118
URL: http://dx.doi.org/10.1081/NCN-120023118

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1703–1706, 2003

Binding Studies of Eukaryotic Initiation Factor eIF4E with Novel mRNA Dinucleotide Cap Analogues

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ABSTRACT

Studies on the interaction of the murine translation initiation factor 4E with two new-synthesized cap-analogues, modified at C2′ of 7-methylguanosine, have been performed by means of the fluorescence titration method. No difference in the binding affinity for eIF4E was observed compared with the ''anti reversed'' cap analogues, possessing the analogous modifications at C3′. Potential significance of the novel caps as research tools for examination of the nuclear cap binding complex CBC80/20 has been discussed.

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DOI: 10.1081/NCN-120023118
1525-7770 (Print); 1532-2335 (Online)

INTRODUCTION

The 5′ mRNA cap structure m7G(5′)ppp(5′)N, which consists of 7-methylguanosine linked via a 5′-to-5′ triphosphate bridge to the first transcribed nucleotide N, plays a crucial role in many cellular processes, such as splicing of pre-mRNA, intracellular RNA export to cytoplasm, and facilitation of the cap-dependent translation. For that reason, the ability to synthesise capped mRNA transcripts in vitro constitutes a useful research tool. The most frequently employed technique is to transcribe a DNA template with bacteriophage RNA polymerase in the presence of all four ribonucleotide triphoshates and a dinucleotide cap analogue, m^7GpppG.[3,4] However, it was found that 30–50% of the m^7GpppG is incorporated in the reverse orientation,[5] i.e., with the m^7Guo moiety linked by a 3′–5′ phosphodiester bond to the mRNA chain. Recently, two novel Anti-Reverse Cap Analogues (ARCAs) have been synthesised,[6] in which 3′OH of m^7G was either replaced by 3′OCH_3 or changed to 3′deoxy. The modifications protect against incorporation of cap in the reverse orientation to the mRNA transcripts. It was shown that the ARCA-capped transcripts are translationally more active than the traditional ones.[6]

RESULTS AND DISCUSSION

The recognition of the 5′mRNA cap by one of the components of the translation machinery, i.e., the eIF4E factor, is a crucial, rate-limiting step in initiation of protein synthesis.[1] To compare the binding affinity of eIF4E for the ARCAs and for the novel cap-analogues, possessing these same modifications at C2′ in m7G we have determined the association constants K_{as} for the eIF4E-cap complexes (Fig. 1 and Table 1) by means of the fluorescence quenching method.[7]

The values of the association constants (K_{as}) for both classes of the tested cap analogues: $m^7$3′dGpppG, $m_2{}^{7,3'O}$GpppG, $m^7$2′dGpppG, and $m_2{}^{7,2'O}$GpppG as well

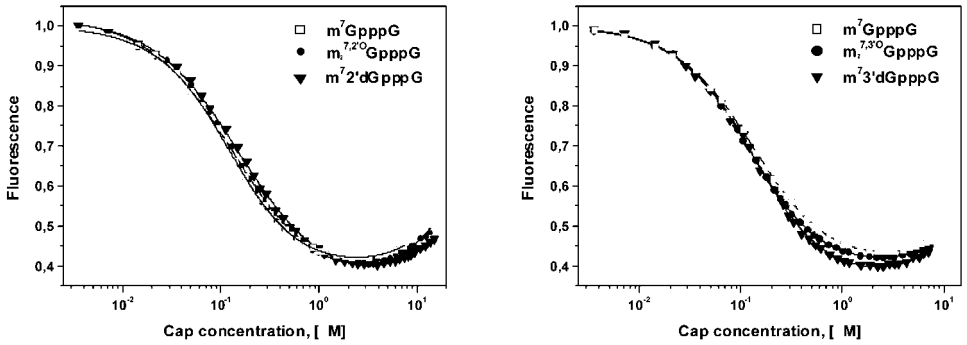

Figure 1. Fluorescence titration curves for binding of the Anti-Reverse Cap Analogues to eIF4E. Comparison of the analogues modified at C2′ and C3′ of m^7G with m^7GpppG, left and right panels, respectively. The titrations were performed in 50 mM HEPES/KOH pH 7.2, 0.1 M KCl, 0.5 mM EDTA, 1 mM DTT, at 20°C, by adding 1 μL of the cap analogue solution to 1400 μL of 0.1 μM eIF4E(28–217).

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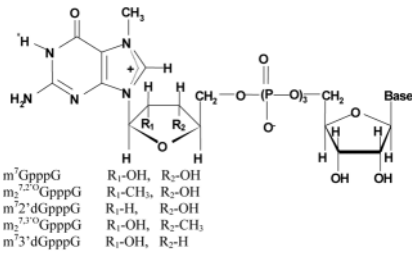
Table 1. The values of K_{as} for the association of murine eIF4E(28–217) with the dinucleoside triphosphate cap analogues.

Cap analogues	K_{as} (μM^{-1})
m^7GpppG	12.5 ± 0.3
$m_2{}^{7,2'O}GpppG$	10.8 ± 0.3
$m^72'dGpppG$	9.1 ± 0.5
$m_2{}^{7,3'O}GpppG$	10.2 ± 0.3
$m^73'dGpppG$	13.1 ± 0.7



as the parent m^7 GpppG, are very similar (Table 1). The observed differences are within 20%; $K_{as.} = 9.1 \pm 0.5 \cdot 10^6\ M^{-1}$ for $m^72'dGpppG$ and $K_{as} = 12.5 \pm 0.3 \cdot 10^6\ M^{-1}$ for m^7GpppG. The results are consistent with the crystallographic study of the murine eIF4E-m7GDP complex,[8] which showed that C2′ and C3′ do not interact directly with the protein and are located outside the protein surface.

Since the modifications at the C3′ or C2′ of m^7Guo do not affect the eIF4E-cap interaction, the mRNA transcripts capped with novel ''ARCAs'' could be more effective templates for protein synthesis. Moreover, the novel cap analogues are very useful for studying the interactions with the Nuclear Cap Binding Complex.[9] This functional hetero-dimer, consisting of a small, CBC20, and a large, CBC80 subunits, binds with high affinity to the 5′ cap of nascent RNA polymerase II transcripts, and on the contrary to eIF4E, CBC20 forms three direct hydrogen bonds with the 2′- and 3′-hydroxyl groups of m7G.[10] The novel analogues are therefore a good tool to probe the contribution of the m7G ribose ring to the stabilization energy in the CBC-cap complex.

ACKNOWLEDGMENT

This work was supported by KBN 6 P04A 055 17, PBZ-KBN 059/T09/10, BST-718/BF, BW-1525/BF.

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