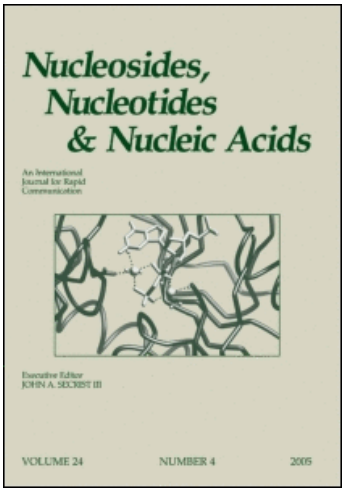

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Worch, Remigiusz , Stepinski, Janusz , Niedzwiecka, Anna , Jankowska-Anyszka, Marzena , Mazza, Catherine , Cusack, Stephen , Stolarski, Ryszard and Darzynkiewicz, Edward(2005) 'Novel Way of Capping mRNA Trimer and Studies of Its Interaction with Human Nuclear Cap-Binding Complex', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 1131 — 1134
To link to this Article: DOI: 10.1081/NCN-200061898
URL: http://dx.doi.org/10.1081/NCN-200061898

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):1131–1134, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200061898

NOVEL WAY OF CAPPING mRNA TRIMER AND STUDIES OF ITS INTERACTION WITH HUMAN NUCLEAR CAP-BINDING COMPLEX

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▫ *Binding of mRNA 5′ cap by the nuclear cap-binding complex (CBC) is crucial for a wide variety of mRNA metabolic events. The interaction involving the CBP20 subunit of CBC is mediated by numerous hydrogen bonds and by stacking of the tyrosine sidechains with two first bases of the capped mRNA. To examine a possible role of a longer mRNA chain in the CBC–cap recognition, we have synthesized an mRNA tetramer using a novel way of capping an RNA trimer and determined its affinity for CBC by fluorescence titration.*

Keywords Cap-Binding Complex, Capped mRNA, Fluorescence Titration

INTRODUCTION

Recognition of 5′ mRNA cap structure, $m^7G(5')ppp(5')N$, by the human nuclear cap-binding complex (CBC) plays a key role in pre-mRNA splicing, polyadenylation of the 3′ terminus, U snRNA transport, nonsense-mediated decay and translation initiation.[1] Crystal structures of *apo*-CBC[2,3] and CBC-m^7GpppG complex[3,4]

Supported by State Committee for Scientific Research KBN 3 P04A 021 25 and PBZ-KBN 059/T09/10.
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revealed a two-partite cap-binding center, in which both 7-methylguanine and guanine moieties stack with the protein tyrosines. Additionally, numerous hydrogen bonds and/or salt bridges to acidic and basic amino acid sidechains stabilize the sugar parts and the phosphate chain of m^7GpppG. To examine a possible role of a longer mRNA chain in specific recognition of CBC, we have performed synthesis of a tetranucleotide, $m^7GpppA^{m2'}pU^{m2'}pA^{m2'}$ using a new methodological approach and applied the product to fluorescence titration of CBC.

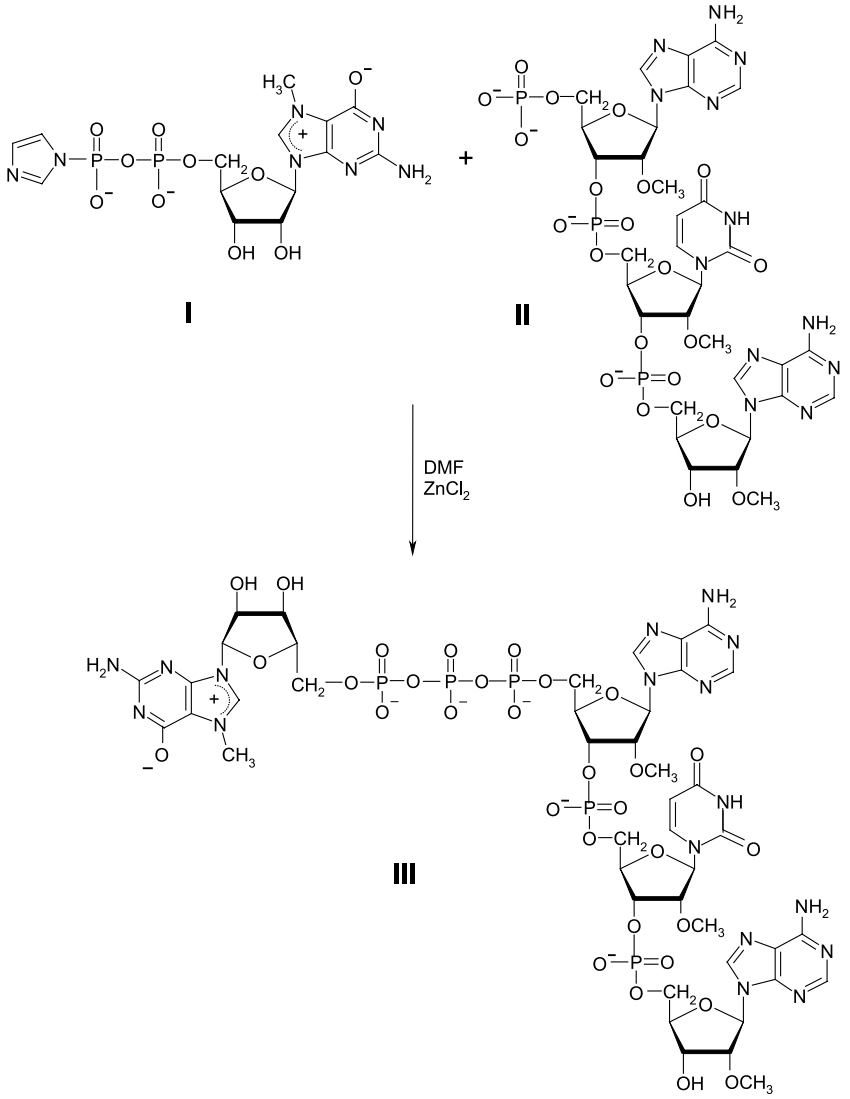

FIGURE 1 Chemical synthesis of the capped RNA tetramer (III).

MATERIALS AND METHODS

Expression and purification of CBC was performed according to Mazza et al.[4] The known tetranucleotide,[5] $m^7GpppA^{m2'}pU^{m2'}pA^{m2'}$, was prepared from the batch of the 5′-phosphorylated trimer $pA^{m2'}pU^{m2'}pA^{m2'}$ purchased from TriLink BioTechnologies (San Diego, CA). A mixture of ammonium salt of the trimer (1.1 mg, 1 μmol), sodium salt of P^2-imidazolide 7-methylguanosine 5′-diphosphate[6] (2.7 mg, 5 μmol), and $ZnCl_2$ (14 mg, 0.1 mmol) in dimethylformamide (0.3 mL) was stirred for 24 h at room temperature. The reaction mixture was diluted with 1.5 mL of EDTA (3.7 mg, disodium salt) solution in water. The product was isolated on HPLC (Spectra-Physics SP8800) using a reverse-phase Supelcosil LC-18-T column eluted for 15 min with a linear gradient of methanol, 0 to 25%, in 0.05 M ammonium acetate (pH 5.9), and for the next 15 min at 25% of methanol. After lyophilization, 0.73 mg (0.48 μmol) ammonium salt of $m^7GpppA^{m2'}pU^{m2'}pA^{m2'}$ was obtained (yield 48%, predicted molecular mass for free acid: 1463.9, ESI-MS measured mass: 1462.1).

The titration experiments (LS-50B fluorimeter, Perkin-Elmer Co., Norwalk, CT) were performed at 20°C, in 50 mM HEPES/NaOH pH 7.50, 200 mM NaCl, 10 mM DTT, and 0.2 mM EDTA, and the data were analyzed as described previously.[7]

RESULTS AND DISCUSSION

A novel procedure of capping[8] has been applied for a trimeric RNA fragment (Figure 1), i.e., coupling of the 5′-phosphorylated trimer (II) with the imidazolide derivative of 7-methylguanosine 5′-diphosphate (I) in dimethylformamide, with anhydrous zinc chloride as a promoter. The previously reported synthesis of tetranucleotide $m^7GpppA^{m2'}pU^{m2'}pA^{m2'}$ was achieved by a different approach,[5] i.e., acivation of nucleotide trimer $pA^{m2'}pU^{m2'}pA^{m2'}$ at 5′-phosphate group by imidazole, and coupling of the product with 7-methyl guanosine diphosphate, the yield 18.6% (Zuberek et al., personal communication, not cited in Ref. [5]). The new coupling method was found to be superior over the previous one regarding both the yield (48% *vs*. 18.6%) and reproducibility.

The value of the association constant (Kas) for CBC and a tetranucleotide, $m^7GpppA^{m2'}pU^{m2'}pA^{m2'}$ (Table 1), obtained by fluorescence titration (Figure 2), is similar to Kas for a control dinucleotide, $m^7GpppA^{m2'}$. The binding free energies ($\Delta G°$) do not differ within the experimental error. This suggests that only two first

TABLE 1 Association Constants (K_{as}) and Binding Free Energies ($\Delta G°$) Obtained by Fluorescence Titration

Ligand	$K_{as} \cdot 10^{-6}$ (M^{-1})	$\Delta G°$ (kcal/mol)
$m^7GpppA^{m2'}$	193 ± 40	−11.10 ± 0.12
$m^7GpppA^{m2'}pU^{m2'}pA^{m2'}$	128 ± 38	−10.90 ± 0.17

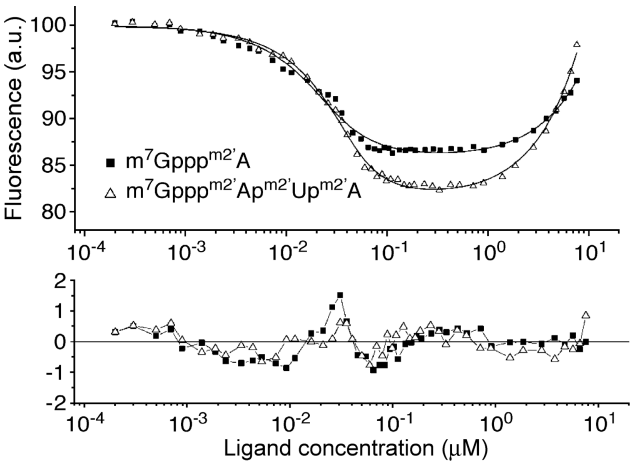

FIGURE 2 Titration curves for mRNA tetramer and a corresponding dinucleotide.

nucleotides at the mRNA 5′ terminus are responsible for the specific interaction with the CBC, while further nucleotides may be involved only in nonspecific contacts with the protein.

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