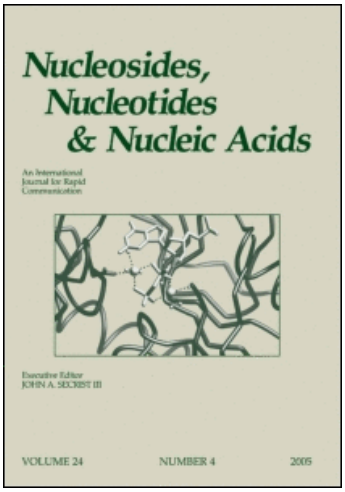

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Influence of the Length of the Phosphate Chain in mRNA 5′ Cap Analogues on Their Interaction with Eukaryotic Initiation Factor 4E

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ABSTRACT

The recognition of the 5′mRNA cap structure $m^7G(5')ppp(5')N$ by one of the components of the initiation translation machinery, the eIF4E factor, plays a pivotal role in regulation of the protein synthesis. In the present study we have shown two opposing roles of the cap phosphate chain in the specific eIF4E-cap interaction. The extension of the phosphate chain enhances the binding of the cap to the unphosphorylated eIF4E but destabilises the eIF4E-cap complex in case of the phosphorylated protein.

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INTRODUCTION

The dominant step during the translation initiation of eukaryotic mRNAs is the recruitment of the 40 S ribosomal subunit to the mRNA. In the mammalian cells the process occurs through binding of the mRNA 5′ cap structure to the cap-binding complex eIF4F which consists of three protein factors, eIF4A, eIF4E and eIF4G.[1] eIF4E, the smallest subunit of the complex, specifically recognizes and interacts with the cap.

Two structural features are of primary importance for the specific eIF4E-cap recognition: the negative charge of the phosphate chain, which depends both on the number of the phosphate groups and the presence of the second nucleotide, and the sandwich stacking of 7-methylguanine in between two tryptophan aromatic side chains.[2,3] Extension of the phosphate chain in the mononucleotide cap analogues, m^7GMP, m^7GDP, m^7GTP, results in a systematic increase of their association constants with eIF4E.[3] On the other hand, the activity of eIF4E is regulated by phosphorylation at Ser209,[4] and it was shown that the phosphorylation reduces the protein affinity for capped mRNA, probably due to electrostatic repulsion.[5,6]

RESULTS AND DISCUSSION

On exploring the subject how the negative electric charge of the phosphate chain of the cap influences the interactions with eIF4E, we have measured the affinity for eIF4E of a series of the cap analogues with different number of the phosphate groups via the fluorescence titration method.[3,6] In our studies we have used recombinant

Table 1. Equilibrium association constants (K_{as}) for the interaction of unphosphorylated and phosphorylated eIF4Es with mono- and dinucleotide cap analogues with different number of phosphate groups.

	K_{as} (μM^{-1})[a]		
Cap analogues	eIF4E(28–217)	eIF4E(28–217) T205C/S209phos	K_{as}(wt)/K_{as}(phos)
m^7GMP	1.5 ± 0.1	1.1 ± 0.1	1.4 ± 0.2
m^7GDP	20.9 ± 1.5	10.5 ± 0.3	2.0 ± 0.1
m^7GTP	119.7 ± 5.7	42.6 ± 1.8	2.8 ± 0.2
m^7Gp_4	734 ± 56	140.3 ± 16.1	5.2 ± 0.4
m^7Gp_5	1625 ± 195	202.4 ± 9.3	8.0 ± 1.0
m^7Gp_3G	12.5 ± 0.3	5.8 ± 0.3	2.2 ± 0.1
m^7Gp_4G	110.9 ± 6.0	27.3 ± 1.1	4.1 ± 0.3
m^7Gp_5G	543 ± 55	94.0 ± 3.8	5.8 ± 0.6

[a]From the fluorescence titration in 50 mM HEPES/KOH pH 7.2, 0.1 M KCl, 0.5 mM EDTA and 1 mM DTT at 20°C, by adding 1 μL of the cap analogue solution to 1400 μL of either 0.1 μM eIF4E(287–217) or eIF4E(28–217)T20C/S209phos.

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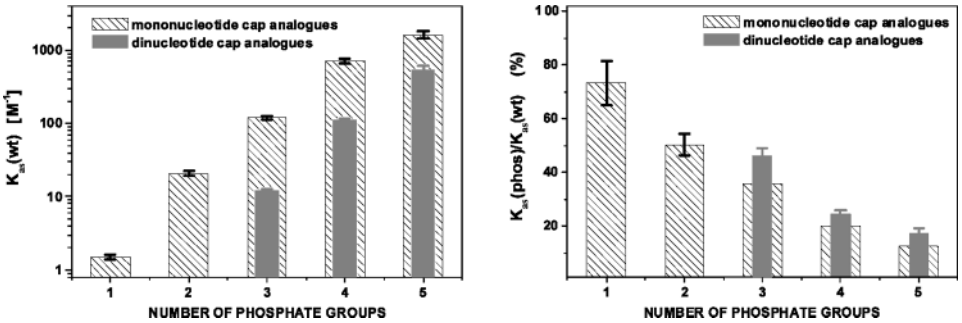

Figure 1. Effect of negative charge of the cap analogues on the interaction with eIF4E. Left panel; the association constants K_{as}(wt) for unphosphorylated eIF4E vs. the number of the phosphate groups in the mono- and dinucleotide cap analogues. Right panel; comparison of the binding affinity of phosphorylated eIF4E with its unphosphorylated counterpart.

murine eIF4E(28–217), and its Ser209 phosphorylated counterpart obtained by Intein-Mediated Protein Ligation.[6]

The elongation of the phosphate chain in each class of the cap analogues, i.e., from one to five phosphate groups in the mononucleotides, and from three to five in the dinucleotides, results in a systematic, well-marked enhancement of the binding affinity for the phosphorylated and unphosphorylated eIF4E (Table 1, Fig. 1).

The α-, and β-phosphates of the cap exert the strongest influence on the binding to eIF4E.[2,3] The subsequent addition of two phosphate groups to m^7GDP (m^7GTP, m^7Gp_4) results in the same, *ca.* six-fold, increase of the affinity for the unphosphorylated eIF4E. Most probably, δ-phosphate interacts directly with the protein forming one hydrogen bond, as it occurs for the γ-phosphate.[2] Only twofold increase of the association constant for m^7Gp_5 ($K_{as} = 1625 \pm 195 \times 10^9\,M^{-1}$) in comparison with m^7Gp_4 ($K_{as} = 734 \pm 56 \times 10^9\,M^{-1}$) suggests that the ε-phosphate is not involved in the creation of the intermolecular contacts with eIF4E inside the cap-binding centre. The addition of the second guanosine to a mononucleotide cap analogue reduces the affinity for eIF4E to the level of that of the mononucleotide cap analogue with one phosphate less in the chain (Fig. 1, left panel). This is due to reduction of the negative charge in the phosphate chain, and is also consistent with the observation that the second guanosine does not bind directly to eIF4E.[3]

In case of the phosphorylated form of eIF4E a systematic increase of the affinity for the cap analogues with the extended phosphate chain is also observed, but the values of K_{as}(phos) are lower in comparison with those for unphosphorylated eIF4E. The decrease of K_{as} depends considerably on the negative charge of the cap (Fig. 1, right panel). The association constant of phosphorylated eIF4E for m^7GMP is about 1.4-fold lower compared with the unphosphorylated protein while for m^7Gp_4 and m^7Gp_5 it is 5.2- and 8.0-fold lower, respectively. This confirms the hypothesis that the electrostatic repulsion between the negatively charged phosphate group at Ser209 and the phosphate chain of the cap analogue can play a dominant role in destabilisation of the complex.[6]

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