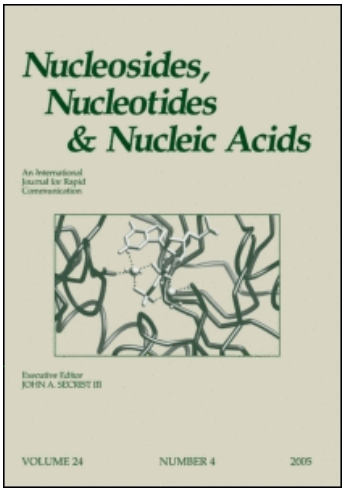

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Anna Niedzwiecka[a]; Janusz Stepinski[a]; Lajos Balaspiri[b]; Edward Darzynkiewicz[a]; Ryszard Stolarski[ac]
[a] Department of Biophysics, Institute of Experimental Physics, Warsaw University, Warszawa, Poland [b] Albert Szent-Gjorgyi University, Szeged, Hungary [c] Department of Biophysics, University of Warsaw, Warsaw, Poland

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Thermodynamics of 7-Methylguanosine Cation Stacking with Tryptophan upon mRNA 5′ Cap Binding to Translation Factor eIF4E

Anna Niedzwiecka,[1] Janusz Stepinski,[1] Lajos Balaspiri,[2] Edward Darzynkiewicz,[1] and Ryszard Stolarski[1,*]

[1]Department of Biophysics, Institute of Experimental Physics, Warsaw University, Warszawa, Poland
[2]Albert Szent-Gjorgyi University, Szeged, Hungary

ABSTRACT

All eukaryotic nuclear transcribed mRNAs possess the cap structure, consisting of 7-methylguanosine linked by the 5′-5′ triphosphate bridge to the first nucleoside. The goal of the present study is to dissect the enthalpy and entropy changes of association of the mRNA 5′ cap with eIF4E into contributions originating from the interaction of 7-methylguanosine with tryptophan. The model results are discussed in the context of the thermodynamic parameters for the association of eIF4E with synthetic cap analogues.

*Correspondence: Ryszard Stolarski, University of Warsaw, Department of Biophysics, ul. Zwirki i Wigury 93, 02-089 Warsaw, Poland; Fax: + 48 22 554 0001; E-mail: stolarsk@biogeo.uw.edu.pl.

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INTRODUCTION

The mRNA 5′ cap is recognized by the protein eukaryotic initiation factor eIF4E, a phylogenetically highly conserved subunit of the heterotrimeric eIF4F initiation complex.[1] The eIF4E-cap interaction plays a pivotal role in regulating translation initiation as a rate-limiting step.[2] The cap structure is stabilized in the initiation complex by cation – π sandwich stacking of 7-methylguanosine between two tryptophans (Trp56 and Trp102), Watson-Crick-type hydrogen bonds involving 7-methylguanosine, and interactions of the phosphate chain, i.e., salt bridges and direct or water-mediated hydrogen bonds.[3–5]

Beside the structural studies, investigation of molecular properties of the cap analogues in solution are of pharmacological importance because of the involvement of eIF4E activity in malignancy and apoptosis.[6] The thermodynamics of eIF4E binding to the cap is described in general by nonlinear van't Hoff plots as a result of the interplay of several processes.[7] The association is accompanied by disruption of intramolecular self-stacking of the cap,[8] proton uptake to ensure the cationic form of 7-methylguanosine, release of one potassium ion from the phosphate chain, hydration of the complex by ~65 water molecules, and a conformational change of eIF4E.[9]

RESULTS AND DISCUSSION

In order to extract information concerning the thermodynamics of the 7-methylguanosine moiety interactions, we used NMR spectroscopy to examine two model systems: 7-methylGMP and tryptophan, and a dinucleotide cap analogue, 7-methylGpppG, interacting with a dodecapeptide of the sequence related to a part of the eIF4E cap-binding site around Trp102 (DGIEPMWEDEKN).[10] Stacking between 7-methylGMP and tryptophan shifts the ^{1}H signals upfield, yielding microscopic equilibrium association constants at 298 K of 15.9 ± 3.8, 6.5 ± 1.6, 6.7 ± 2.0, 5.9 ± 1.8, 7.8 ± 1.3 M^{-1} for the H(2), H(4), H(5), H(6), H(7) tryptophan protons, respectively (Fig. 1, left). This indicates that the complex is almost parallel, in contrast with that of 7-methylGpppG with the dodecapeptide. In the latter case, the association constants of 242 ± 67 for H(2) but of $\sim 50 \pm 10$ M^{-1} for the remaining tryptophan protons were established and the complex assumed both parallel and perpendicular orientations in a dynamic equilibrium.[10]

Although stacking is a hydrophobic interaction, binding of cationic 7-methylGMP to tryptophan is enthalpy-driven and entropy-opposed, without any heat capacity change in the temperature range of 278–320 K, as determined on the basis of the temperature-dependent differences of ^{1}H chemical shifts of tryptophan (Fig. 1 right, Table 1). 7-methylGpppG interacts with the dodecapeptide tryptophan through its methylated base, and stacking is also described by the negative, constant values of the van't Hoff enthalpy change ΔH°_{vH} and the entropy change ΔS°.

The ΔH°_{vH} values for stacking of the 7-methylG moiety with tryptophan are ~2-fold greater than those reported for adenine and uracil base stacking ($-3.0 \div -3.4$ kcal/mol per stack),[11–13] and for intramolecular self-stacking of the

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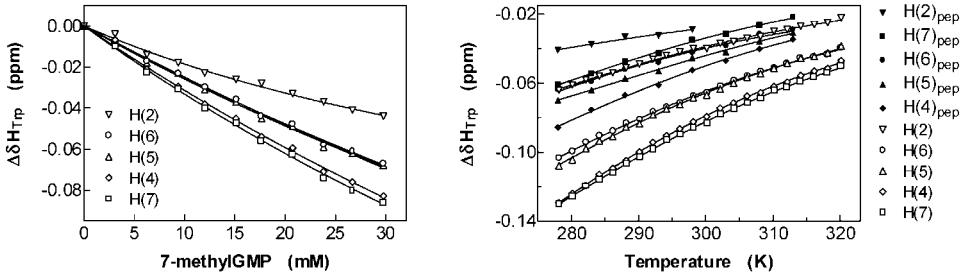

Figure 1. (left) Differences of ^{1}H chemical shifts of tryptophan N-acetylamid due to stacking upon titration with 7-methylGMP at 298 K; $\Delta\delta := \delta(\text{mixture}) - \delta(\text{free})$; $\Delta\delta = \Delta\delta_u \cdot c_u/c_{trp} + \Delta\delta_{st} \cdot (1 - c_u/c_{trp})$; (right) Temperature dependence of differences of ^{1}H chemical shifts of tryptophan N-acetylamid (open symbols) at 1 mM in the presence of 29.8 mM 7-methylGMP, in 1/15 M phosphate buffer, pH 5.6, 10% D_2O for spin locking; and of the dodecapeptide tryptophan (filled symbols) at 2.8 mM in the presence of 17.2 mM 7-methylGpppG, at pH 5.2; $\Delta\delta = \Delta\delta_{st} \cdot (1 - c_u/c_{trp})$; $c_u = (K \cdot (c_{trp} - c_{cap}) - 1 + \text{sqrt}(K^2 \cdot (c_{trp} - c_{cap})^2 + 2 \cdot K \cdot (c_{cap} + c_{trp}) + 1))/(2 \cdot K)$; $K = \exp(\Delta S^\circ/R - \Delta H^\circ_{vH}/RT)$. Spectra recorded on a Varian UNITY plus 500 MHz vs. internal TSP, ± 0.001 ppm.

dinucleotide cap analogues[8] (Table 1). The enthalpy and entropy changes of the stacking seem to provide a significant contribution to the overall thermodynamic parameters of 7-methylGpppG binding to eIF4E upon translation initiation. The large, negative values of both ΔH°_{vH} and ΔS° for stacking of 7-methylG with the second G within the cap or with the protein tryptophan should be interpreted in terms of the cation – π interactions. The latter are dominated by the Coulombic component resulting from the attraction between the cation and the quadrupole charge distribution of the aromatic ring.[14]

The 7-methylG moiety represents a unique example of a cation which is concurrently a heteroaromatic ring. The model results point to a diversity of permissible spatial structures of stacked complexes. Similar diversity is observed among the sandwich configurations in the eIF4E cap-binding site. The structures become more parallel in the course of evolution, from primitive eukaryotes (yeast)[4] to higher ones (mouse or human).[3,5]

Table 1. Thermodynamic parameters for stacking of mRNA 5′ cap analogues with tryptophan, peptide, and eIF4E and for self-stacking of the cap.

System	ΔH°_{vH}	ΔS°	ΔC°_P
(cal/mol · K)	(kcal/mol)	(cal/mol · K)	
7-methylGMP-Trp	−6.18 ± 0.19	−15.31 ± 0.46	n.d.
7-methylGpppG-Trp(peptide)	−7.69 ± 0.47	−18.58 ± 1.30	n.d.
7-methylGpppG-eIF4E [7]	−13.4 ± 6.2	−13.6 ± 11.2	+460 ± 220
7-methylGpppG self-stacking [8]	−3.64 ± 0.12	−11.24 ± 0.48	n.d.

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