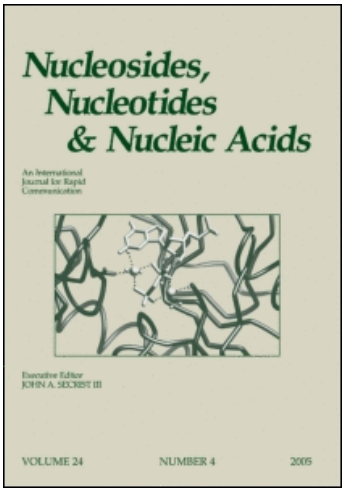

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Interaction Between Yeast Eukaryotic Initiation Factor eIF4E and mRNA 5′ Cap Analogues Differs from That for Murine eIF4E

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ABSTRACT

Measurements of interaction of 7-methyl-GTP eIF4E from *S. cerevisiae* were performed by means of two methods: Isothermal Titration Calorimetry (ITC) and fluorescence titration. The equilibrium association constants (K_{as}) derived from the two methods show significantly different affinity of yeast eIF4E for the mRNA 5′ cap than those of the murine and human proteins. The observed differences in the K_{as} values and the enthalpy changes of the association (ΔH°) suggest some dissimilarity in the mode of binding and stabilization of cap in the complexes with eIF4E from various sources.

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INTRODUCTION

Eukaryotic mRNA possesses a 5′-terminal cap, in which 7-methylguanosine is linked by a 5′-5′-triphosphate bridge to the first transcribed nucleoside of the polynucleotide chain. Recognition and binding of the cap by the eIF4E translation initiation factor enables the eIF4F protein complex to recruit the 40S ribosomal subunit to mRNA. It is thought to be a rate-limiting step for the cap-dependent translation.[1] Aberrations in the level of expression and activity of eIF4E result in changes of the cell morphology. Mammals are more sensitive to such aberrations than yeast, probably due to differences in the mechanism of the recruitment of the 43S preinitiation complex to mRNA.[2] However, this can be also related to structural differences in eIF4Es from various sources. The amino acid sequences of eIF4Es are highly conserved, e.g., a 31% sequence identity is observed for the yeast and murine proteins. The 3D structures of the murine and yeast eIF4Es, both in the complex with 7-methyl-GDP, were resolved by X-ray crystallography[3] and NMR,[4] respectively. Both proteins show a close similarity of the tertiary folds and have similar key amino acids in the cap-binding centres. However, some differences are observed in the stabilizing contacts between the cap and the protein. In the context of evolutionary changes in translation initiation, it is interesting to compare the modes of the cap binding to eIF4E from yeast and mouse.[5]

RESULTS AND DISCUSSION

The recombinant, His-tagged yeast eIF4E was expressed, purified and examined by means of Electrospray Ionisation Mass Spectrometry. Isothermal Titration Calorimetry (ITC) allowed for determination of two parameters: the equilibrium association constant (K^{cal}_{as}) and the standard total calorimetric enthalpy change (ΔH_{cal}°) of the association of 7-methyl-GTP with eIF4E. The fluorescence titration yielded the association constant (K^{flu}_{as}) (Table 1). The corresponding titration curves for the ITC and fluorescence experiments are shown in Fig. 1.

Table 1. Total calorimetric enthalpy change (ΔH_{cal}°) and equilibrium association constants (K^{cal}_{as} and K^{flu}_{as}) for binding of 7-methyl-GTP to yeast eIF4E obtained from calorimetric and fluorescence titrations, respectively. K^{flu}_{as} and the van't Hoff enthalpy change (ΔH_{vH}°) of murine eIF4E shown for comparison.

Thermodynamic parameter	His-tagged full length yeast eIF4E	Murine eIF4E (28-217)[a]
K^{cal}_{as} [μM^{-1}]	6.32 ± 3.17	n. d.
K^{flu}_{as} [μM^{-1}]	18.97 ± 2.59	108.7 ± 4.0
ΔH_{cal}° [kcal/mole]	-13.45 ± 0.06	n. d.
ΔH_{vH}° [kcal/mole]	n. d.	-17.76 ± 0.86

[a]Data from Niedzwiecka et al.[5]

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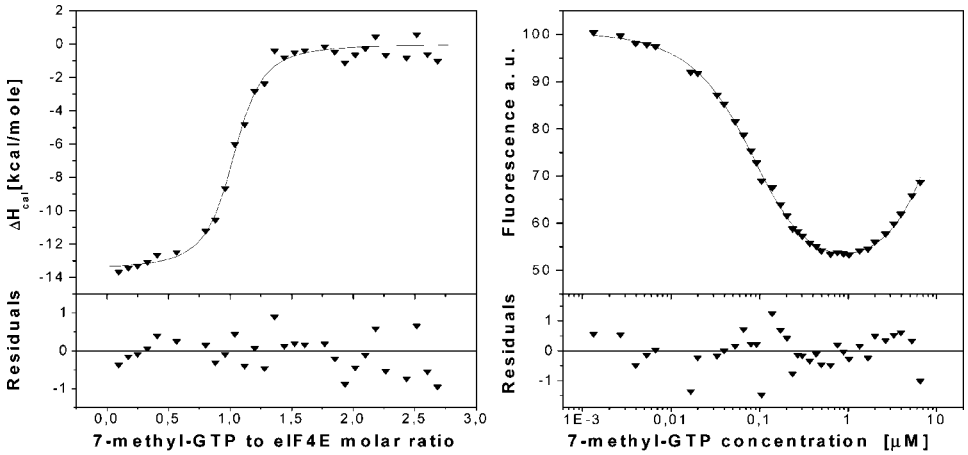

Figure 1. Titration curves and fitting residuals of the ITC (left, eIF4E concentration 13 μM) and fluorescence (right, eIF4E concentration 0.3 μM) experiments, both in 50 mM Hepes/KOH, 100 mM KCl, 1 mM EDTA buffer pH 7.2 at 20°C.

The association constants derived by both methods are in reasonable agreement, taking into account a difference of the protein concentration, two orders of magnitude, in the ITC and fluorescence experiments. The results clearly point to the lower stability of the complex with yeast eIF4E than that with the murine and human proteins.[5] This conclusion is supported by inspection of the 3D molecular structures. Although the general mode of binding seems to be similar, the geometry of cation-π sandwich stacking of 7-methylguanosine between two tryptophans (Trp58 and Trp104) is different inside the cap-binding slot of eIF4E from yeast and mammals. Moreover, the yeast protein forms only two hydrogen bonds involving 7-methylguanosine, while three hydrogen bonds occur in the mammalian complexes. The lack of the hydrogen bond between the carboxyl group of Glu-105 and the N(1) atom of 7-methylguanosine suggests that the ionic form of the latter should not modulate the binding affinity, in contrast to murine eIF4E, which prefers the cationic form.[5] Furthermore, the phosphate chain of the cap binds to the yeast protein less strongly, since Lys-114 and Lys-162 are too far from the phosphate groups to form even water-mediated hydrogen bonds. The enthalpy change for the yeast eIF4E is therefore less negative than that for the murine protein, by more than +4 kcal/mol. Even bearing in mind that the calorimetric and van't Hoff entalpies cannot be exactly compared in the quantitative way, this result indicates that in the phylogenetically earlier organism some polar eIF4E-cap contacts are lost.

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