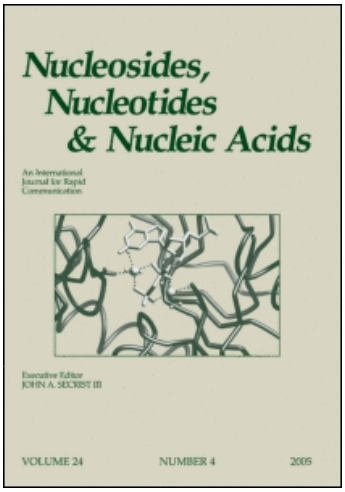

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Niedzwiecka, Anna , Darzynkiewicz, Edward and Stolarski, Ryszard(2005) 'Deaggregation of eIF4E Induced by mRNA 5' Cap Binding', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 507 — 511
To link to this Article: DOI: 10.1081/NCN-200061784
URL: http://dx.doi.org/10.1081/NCN-200061784

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):507–511, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200061784

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DEAGGREGATION OF eIF4E INDUCED BY mRNA 5′ CAP BINDING

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▫ *All eukaryotic mRNAs contain a 5′ terminal cap structure, which consists of 7-methylguanosine linked by a 5′-5′ triphosphate bridge to the first transcribed nucleoside (m^7GpppN). Specific recognition of the cap by the eukaryotic initiation factor eIF4E plays a key role in regulation of translation initiation as a rate-limiting step. Using dynamic light scattering (DLS), the apo-form of murine eIF4E (33–217) was shown to aggregate. After addition of m^7GTP, progressive deaggregation with the time of incubation in the presence of the cap analogue has been observed.*

Keywords eIF4E, Cap-binding, Deaggregation, DLS

INTRODUCTION

Translation initiation is a complex step of gene expression.[1] During initiation, the correct starting site on mRNA is identified and binding of the ribosome occurs. The 5′-terminal mRNA "cap" structure[2] (Scheme 1) is necessary for optimal mRNA translation initiation,[2,4] participates in the splicing of mRNA precursors[5,6] RNA nuclear export,[7–9] and affects mRNA stability.[8] Regular translation initiation is cap dependent, although there are two alternative ways to recruit the ribosome to mRNA, mediated by an internal ribosome entry site (IRES) or by the poly(A) tail.[10] The cap function in translation initiation is mediated by the eukaryotic initiation factor 4E (eIF4E).[11]

The eukaryotic initiation factor 4E (eIF4E) is a cytoplasmic, 25 kDa protein (217 amino acids) that acts as a the smallest subunit of the eIF4F initiation complex,[12]

We thank Prof. Stephen Burley for enabling access to his DLS facility (The Rockefeller University, New York). The work was supported by State Committee for Scientific Research KBN 3 P04A 021 25 and PBZ-KBN 059/T09/10.

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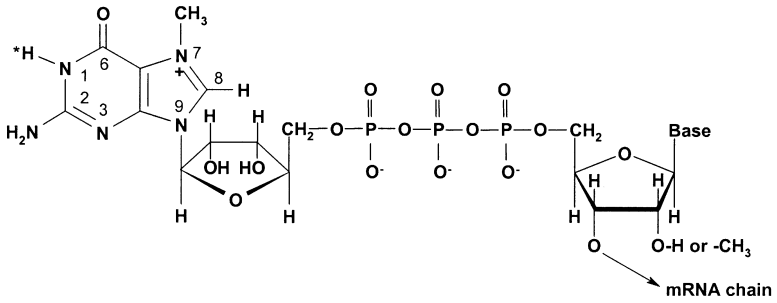

SCHEME 1 Chemical structure of the 5′ terminus of eukaryotic mRNA, called "cap." Proton that partially dissociates at physiological pH is marked with asterisk. According to recent results, the positive charge at the five-membered ring of the 7-methylguanosine moiety is localized at N^7. (From Ref. [3].)

containing the eIF4A and eIF4G proteins. Formation of the eIF4E complex is inhibited by eIF4E-binding proteins (4E-BPs[13,14]), which bind to this protein at the same site as eIF4G[15] eIF4F appears to be also required for the cap-independent translation of naturally uncapped mRNAs.[16] A fraction of eIF4E localizes also in the nucleus[17] and participates in nucleocytoplasmic transport of mRNA.[18] Since eIF4E colocalizes in nuclear speckles with splicing factors, it is also supposed to be involved in splicing and 3′ mRNA processing.[19] All these additional functions of eIF4E appear to be dependent on its intrinsic ability to recognize the mRNA 5′ cap. Thus, the biochemical activity of eIF4E is versatile enough to be exploited in divergent processes in different cellular compartments.

MATERIALS AND METHODS

Synthesis and purification of m^7GTP as sodium salt was performed as described previously.[20] Murine eIF4E (residues 33–217) was expressed in *Escherichia coli* (strain BL21(DE3)pLys)[21,22] and purified without contact with cap.[23]

The DLS measurements were run on a DynaPro-801 Molecular Size Detector (Protein Solutions Inc., Charlottesville, VA) for eIF4E (residues 33–217) at a concentration of 1 mg/mL, in the absence and in the presence of 50-fold excess of m^7GTP, in 50 mM Hepes/KOH pH 7.20, 100 mM KC1, 1 mM dithiothreitol (DTT), and 0.5 mM disodium ethylenediaminetetraacetate (EDTA). Molecular weight calculations were performed by means of a logarithmic function based on experimental data for several well-characterized proteins, provided by the manufacturer of the DLS equipment.

RESULTS AND DISCUSSION

The *apo*-form of eIF4E (33–217) formed huge aggregates immediately after filtration with use of membranes of the 10-nm pore size and the protein solution was polydispersed. Only rough estimates of the molecular weight (MW) and the

hydrodynamic radius (R_h) of the eIF4E aggregates could be determined by means of DLS. The influence of cap binding on the aggregation state of eIF4E have been investigated. For this purpose, 7-methylguanosine 5′ triphosphate (m^7GTP) has been used as the cap analogue due to its very high affinity for eIF4E, described by the equilibrium association constant $K_{as} = 108.7 \pm 4.0 \cdot 10^6\ M^{-1}$.[23] After addition of m^7GTP to the eIF4E samples progressive deaggregation with the time of incubation has been observed. The time-dependent results of the DLS measurements are shown in Figure 1. The estimates of the molecular weight and the hydrodynamic radius of the protein aggregates dropped after several hours down to the well-defined, reliably measured values. After 24 h of incubation with m^7GTP, the protein solution was monodispersed, and the protein was finally characterized by the molecular weight of MW = 23.3 kDa and the hydrodynamic radius of R_h = 2.36 nm. These parameters obtained for the hydrated complex correspond exactly to the monomeric state of the globular eIF4E protein in the complex with m^7GTP, which has the molecular weight of MW_{cx} = 22.2 kDa and approximate diameters of 4.1 nm × 3.6 nm × 4.5 nm, which were determined by crystallography.[24] However, the *apo*-protein remained totally aggregated, even after sonication of the sample. Hence, the deaggregation and stabilization of the eIF4E monomeric, globular structure occured under the influence of interaction with cap.

Although the investigated form of the eIF4E protein was shorter than the form used for the earlier crystallogaphic and binding studies[15,23–30] only by 5 amino acids located at the unstructured N-terminal tail,[15,24] the tendency to aggregation of these two species was completely different: the 1 mg/mL solution of *apo*-eIF4E (residues 28–217) is monodispersed,[24] whereas *apo*-eIF4E (residues 33–217) is prone to immediate aggregation.

The unstructured N-terminus is close to the hydrophobic region of the eIF4E molecular surface, which is specifically recognized by the eIF4G and 4E-BP proteins.[15] It can be supposed that the presence of the unstructured N-terminal tail

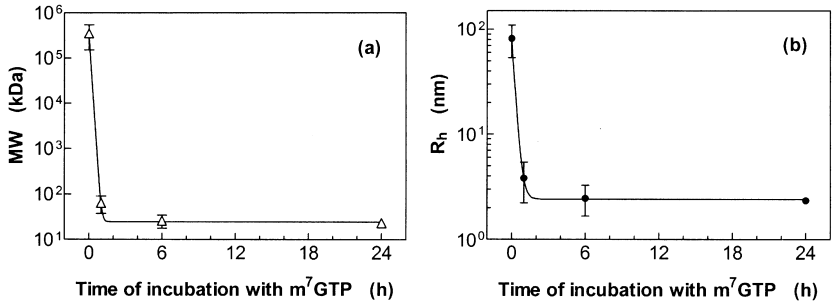

FIGURE 1 Deaggregation of eIF4E (33–217) at 1 mg/mL induced by the cap analogue binding. (a) The estimates of the molecular weight (MW, △) and (b) the estimates of the hydrodynamic radius (R_h, ●) of nonspecific protein aggregates decrease systematically with the time of incubation with m^7GTP by several orders of magnitude to the final values that are characteristic for the single, globular eIF4E-cap complex.

in the proximity of the eIF4G/4E-BP-binding site may have a regulatory function on interactions of eIF4E with other proteins.

It was shown that the two active sites of eIF4E, i.e., the cap-binding site and the eIF4G/4E-BP-binding site, work cooperatively.[23,31] Our present data concerning the deaggregation of eIF4E, forced by cap binding, confirm some earlier suppositions[23] that eIF4E undergoes significant conformational changes upon interaction with mRNA 5′ cap. These changes can propagate on the opposite side of the protein, in relation to the cap-binding center, and can modify the eIF4E dorsal surface responsible for recognition of eIF4G and 4E-BP.

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