Fluorescence Studies on Association of Human Translation Initiation Factor eIF4E with mRNA cap-Analogues

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Binding of a long series of mono- and dinucleotide analogues of the 7-methylguanosine containing 5′-mRNA-cap to human protein translation initiation factor eIF4E has been investigated by means of fluorescence. A new methodological approach in gathering and analysis of the fluorescence data provided us with very accurate values of the association equilibrium constant K and normalized, maximal quenching of the protein fluorescence ΔF_{max}, during titration of eIF4E by various cap-analogues. The results confirm participation of at least two conserved tryptophan residues of eIF4E in interaction with 7-methylguanine, as has been described recently for murine eIF4E, complexed with 7-methyl-GDP in crystal (Marcotrigiano *et al*., 1997, Cell 89, 951), and for yeast eIF4E, complexed with the same ligand in solution (Matsuo *et al*., 1997, Nature Struct. Biol. 4, 717). On the other hand binding by eIF4E of unmethylated guanine nucleotides and N^2,N^2,7-trimethylguanine containing nucleotides differ substantially from the way of binding of the regular mRNA-cap. Influence of the structural features of the cap-analogues, especially the type of the second nucleoside in the dinucleotide caps, on their association with eIF4E and biological activities in *in vitro* protein translation systems has been discussed in light of the known structures of the eIF4E–7-methyl-GDP complexes in crystal and solution.

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