A Spectroscopic Study of the Binding of m^{7}GTP and m^{7}GpppG to Human Protein Synthesis Initiation Factor 4E†

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Received March 31, 1989; Revised Manuscript Received June 13, 1989

ABSTRACT: The binding of analogues of the 7-methylguanosine-containing cap, m^{7}GTP and m^{7}GpppG, to eIF-4E from human erythrocytes as a function of pH, temperature, and ionic strength is described. From the pH-dependent binding of m^{7}GTP and m^{7}GpppG to eIF-4E, a new model describing the nature of the cap·eIF-4E interaction is proposed. The thermodynamic values and ionic strength dependence of binding are consistent with a binding site which is primarily hydrophobic. Fluorescence and circular dichroism data indicate that tryptophan residues may be involved in base-stacking interactions with the cap in a somewhat buried environment. The model presented here confirms the earlier proposal [Rhoads et al. (1983) *Biochemistry 22*, 6084–6088] that the enolate tautomer of the cap is preferred for interaction and further proposes that the interaction is with a protonated amino acid residue, such as histidine, while stacking with an aromatic amino acid, such as tryptophan.

The m^{7}G(5′)ppp(5′)N cap structure, found in all eukaryotic cellular mRNA, has been shown to facilitate ribosome binding to mRNA during initiation [reviewed by Rhoads (1985), Shatkin (1985), and Sonenberg (1988)] and impart stability to mRNA (Furuichi et al., 1977). Several structural features of the caps have been found to be crucial for effective recognition during the initiation of protein synthesis. These features include an N-7-substituted, positively charged guanosine (Adams et al., 1978; Furuichi et al., 1979), α-phosphate (Darzynkiewicz et al., 1981, 1987), and an electronic interaction between the positive charge at the N-7 position and the negatively charged phosphate, maintaining the cap in a rigid anti configuration (Kim & Sarma, 1978; Hickey et al., 1977; Darzynkiewicz et al., 1981, 1987). The 7-methylguanosine can exist as either a keto or an enolate tautomer in solution with a pK in the range of 7.5. Evidence has been presented (Rhoads et al., 1983; Rhoads, 1985) that cap recognition relies on the cap adopting the enolate form, which exists in an unstacked conformation in solution (Nishimura et al., 1980). Cap analogues incapable of forming the enolate tautomer are ineffective inhibitors of protein synthesis (Adams et al., 1978).

A further investigation of the function of the mRNA cap structure in the initiation process employed the isolation and identification of the initiation factor eIF-4E,[1] also known as the 24-kDa cap binding protein or CBP I. This factor has been shown to bind specifically to the mRNA cap (Tahara et al., 1981; Sonenberg, 1981; Sonenberg et al., 1981; Hellmann et al., 1982; Webb et al., 1984), although it can also be isolated as part of the larger eIF-4F (CBP II) complex, which also includes a 50-kDa (eIF-4A) and 220-kDa polypeptide (p220) in the rabbit reticulocyte system (Tahara et al., 1981; Grifo et al., 1983; Edery et al., 1983).

eIF-4E has been isolated from a number of sources, including yeast (Altmann et al., 1985), rabbit reticulocytes (Rychlik et al., 1986; Webb et al., 1984), and human erythrocytes (Rychlik et al., 1986); the amino acid sequence has been determined in the yeast (Altmann et al., 1987) and human (Rychlik et al., 1987) systems. On the basis of these findings, speculations have been made as to the location of the cap-binding site and the nature of the interaction, most recently in the yeast system (McCubbin et al., 1988). The binding site has been postulated to contain a tryptophan residue which is capable of stacking with the cap (Ueda et al., 1988; Ishida et al., 1983). Interactions of the cap keto N-1 proton and 2-amino groups with a nearby glutamic acid via hydrogen bonding has also been suggested (Ueda et al., 1988).

In order to further characterize the interaction between eIF-4E and the mRNA cap, we have measured the binding of m^{7}GTP and m^{7}GpppG to eIF-4E isolated from human erythrocytes as a function of pH, temperature, and ionic strength; the circular dichroism spectra of m^{7}GTP·eIF-4E and m^{7}GpppG·eIF-4E complexes are also reported. We propose, on the basis of these data, that the enolate tautomer of the cap is preferred for interaction with a protonated amino acid residue with a pK near 7 (such as histidine) and stacking with an aromatic amino acid (such as tryptophan) in a hydrophobic pocket. The data suggest that once binding occurs, the cap is locked in a rigidly stacked conformation.

Materials and Methods

Buffer A is 20 mM HEPES–KOH buffer, pH 7.6, containing 1 mM DTT. Buffer B is 10 mM sodium phosphate and 0.2 mM EDTA, pH 7.0. m^{7}GTP (lot OI614364) and m^{7}GpppG (lot QD814635) were purchased from Pharmacia Molecular Biologicals (Milwaukee, WI). m^{7}GTP–Sepharose was obtained from Pharmacia P-L (Piscataway, NJ).

eIF-4E was isolated from human erythrocytes as described by Webb et al. (1984) and Rychlik et al. (1986). Protein concentration was measured by the methods of Bradford (1976), using bovine serum albumin as a standard. A mo-

† D.J.G. was supported by grants from the National Science Foundation (NSF 8600 7070) and the American Heart Association (AHA-NYC Investigatorship) and by a PSC–CUNY faculty research award. R.E.R. was supported by a grant from the National Institutes of Health (GM 20818).

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[1] Abbreviations: eIF, eukaryotic initiation factor; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetate; CBP, cap binding protein; CD, circular dichroism; kDa, kilodalton(s).

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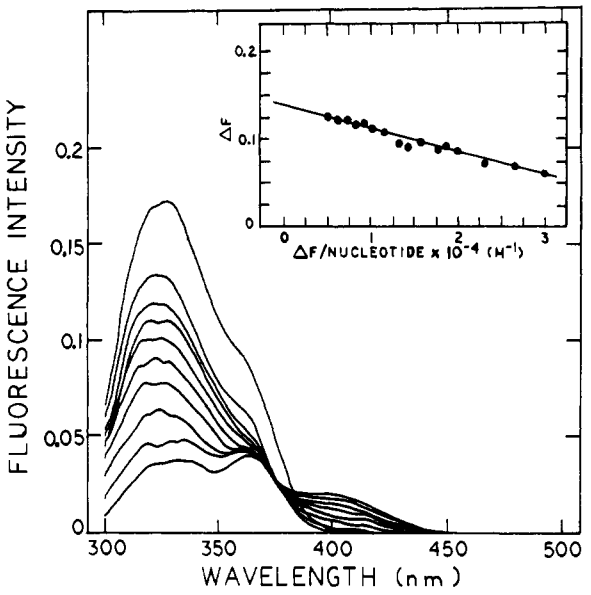

FIGURE 1: Fluorescence emission spectra of eIF-4E (4 μM) complexed with m^{7}GTP in buffer A, pH 7.6 at 23 °C. The m^{7}GTP concentration (top to bottom) was 0, 0.5, 1.0, 2.0, 3.0, 5.0, 9.5, 12, 16, and 22 μM. Intermediate nucleotide concentrations are omitted for clarity. The excitation wavelength was 258 nm, and a 1.4-mm slit was employed. Emission maxima were observed at 330, 370, and 400 nm. *Inset*: Eadie–Hofstee plot of fluorescence data. ΔF was calculated at 330 nm where $\Delta F = F_{eIF\text{-}4E} - F_{eIF\text{-}4E+m^7GTP}$.

lecular weight of 25 117 for eIF-4E was used.

Fluorescence measurements were performed on a custom-designed spectrophotometer utilizing an Oriel 200W Hg–Xe arc lamp. For all measurements, an excitation wavelength of 258 nm, a 1.4-mm slit, and a 1-cm path length were employed. The temperature of the sample was controlled by circulating thermostated water through the brass cuvette holder. The absorbance of 30 μM cap analogue m^{7}GpppG was 0.054 at the excitation wavelength of 258 nm and was negligible at 330 nm and higher wavelengths. Since the inner filter effect is less than 3% at the highest concentration, no correction was made (Lakowicz, 1983). Data were collected on a Zenith Z-100 computer utilizing software and interfacing by On-Line Instrument Systems (Jefferson, GA).

Fluorescence titrations were performed by adding 1–5-μL aliquots of m^{7}GTP or m^{7}GpppG to a 100-μL solution containing 4 μM eIF-4E in buffer A. All fluorescence measurements were corrected for the dilution of the sample in the course of the titration. The contribution of the individual components to the observed emission spectrum of the complex was monitored by parallel control experiments performed on the same day as the titration. In all experiments, the fluorescence intensity was monitored at 330, 370, and 400 nm (Figure 1); the change in eIF-4E fluorescence was calculated from data points at 330 nm, while the m^{7}GTP or m^{7}GpppG fluorescence contribution was monitored at both 370 and 400 nm. The equilibrium binding constants (K_{eq}) were obtained from the calculated slopes of Eadie–Hofstee plots as shown in Figure 1 (Eadie, 1942). ΔF was calculated from the difference in fluorescence intensity at 330 nm between eIF-4E before addition of nucleotide (F_o) and after each nucleotide addition (F_a), where $\Delta F = F_o - F_a$. For the inset of Figure 1, the x axis is ΔF/total nucleotide added. Slopes and intercepts of the plots were determined by least-squares linear regression analysis.

Circular dichroism (CD) measurements were performed on a Jobin-Yvon Mark V circular dicrograph interfaced to an Apple IIe microcomputer with fast arithmetic processors. The solutions for CD measurements were prepared in buffer B.

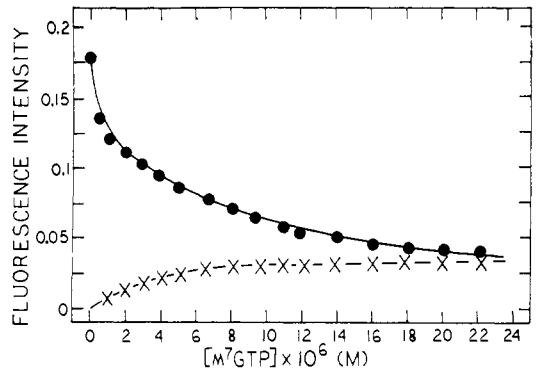

FIGURE 2: Fluorescence intensity of eIF-4E–m^{7}GTP complexes as a function on m^{7}GTP concentration. All solutions were prepared in buffer A, pH 7.6 at 23 °C; the eIF-4E concentration was 4 μM. An excitation wavelength of 258 nm and 1.4-mm slit were employed. The emission maximum of eIF-4E was monitored at 330 nm (●); m^{7}GTP contribution monitored at 370 nm (×).

Results

Fluorescence of eIF-4E·m^{7}GTP and eIF-4E·m^{7}GpppG Complexes. The fluorescence emission spectra of eIF-4E·m^{7}GTP complexes as a function of m^{7}GTP concentration are shown in Figure 1; similar results were obtained with m^{7}GpppG. Upon complex formation, there is a decrease in the protein fluorescence intensity at 330 nm and an increase in the m^{7}G fluorescence at 400 nm. Such protein fluorescence quenching has been attributed to the π–π stacking interactions between an aromatic amino acid residue and the nucleic acid base (Ishida et al., 1983; Brun et al., 1975; Lawaczek & Wagner, 1974). Tryptophan residues are most likely involved in this interaction since the position of the emission maximum of eIF-4E is similar to that of a free tryptophan residue (348 nm). Tyrosine and phenylalanine residues are unlikely to be major contributors due to the position of their emission maxima (303 and 282 nm, respectively) and the fact that the fluorescence contribution of these residues is extremely small.

The K_{eq} of m^{7}GTP·eIF-4E complex formation can be calculated from the relative fluorescence intensity changes in free and complexed eIF-4E emission spectra by construction of an Eadie–Hofstee plot, as shown in the inset of Figure 1. K_{eq} was found to be $(3.86 \pm 0.15) \times 10^5$ M^{-1}.

An alternative way to measure ligand binding to eIF-4E is to monitor the fluorescence of m^{7}GTP or m^{7}GpppG (Figure 2). m^{7}GTP and m^{7}GpppG have fluorescence emission maxima at both 370 and 400 nm; the former is attributed to the keto tautomer and the latter to the enolate (Nishimura et al., 1980; Rhoads, 1985). m^{7}GpppG has an overall lower fluorescence intensity relative to m^{7}GTP (data not shown), which is attributed to intramolecular base stacking between the guanosine residues. The change in fluorescence intensity of the 370-nm maximum of m^{7}GTP in the complex as a function of m^{7}GTP concentration is shown in Figure 2. The m^{7}GTP and eIF-4E data in Figure 2 fit a binding isotherm with values of K_{eq} determined to be 3.71×10^5 and 3.81×10^5 M^{-1}, respectively, at the midpoint of the respective curve. The coincidence of these values indicates that the same equilibrium step is being monitored. An increase in m^{7}GTP fluorescence for eIF-4F binding has been shown previously (Goss et al., 1987), although quantitative results were not obtained. The fluorescence results and K_{eq} values for the binding of m^{7}GTP and m^{7}GpppG to eIF-4E determined here are similar to those reported by McCubbin et al. (1988) for eIF-4E isolated from yeast.

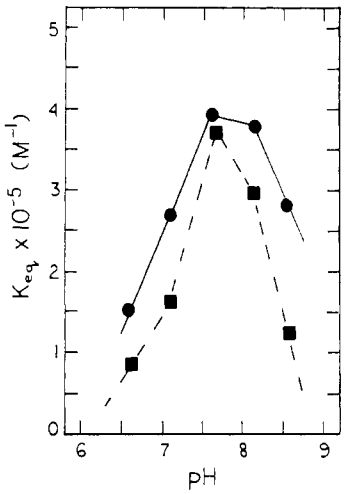

FIGURE 3: K_{eq} of m^7GTP (●) and m^7GpppG (■) binding to eIF-4E as a function of pH. All solutions were prepared in buffer A, adjusted to the appropriate pH at 23 °C. K_{eq} values were obtained from Eadie–Hofstee analysis of fluorescence data as described in the legend of Figure 1. The variance in the eIF-4E fluorescence intensity over the pH range was within experimental error (±10%).

Table I: Parameters of m^7GTP·eIF-4E and m^7GpppG·eIF-4E Complex Formation[a]

	T (°C)	m^7GTP·eIF-4E	m^7GpppG·eIF-4E
K_{eq} (M^{-1})	5	(1.17 ± 0.12) × 10^5	(0.98 ± 0.1) × 10^5
	15	(1.84 ± 0.3) × 10^5	(1.6 ± 0.2) × 10^5
	23	(3.87 ± 0.14) × 10^5	(3.70 ± 0.13) × 10^5
	35	(4.65 ± 0.2) × 10^5	(4.86 ± 0.34) × 10^5
ΔH (kcal/mol)		6.25 ± 0.25	8.12 ± 0.4
ΔS [cal/(mol·°C)]		46.1 ± 1.8	52.4 ± 2.6

[a] All samples were prepared in buffer A, pH 7.6. Temperature accuracy, ±0.2 °C.

pH Effects. The pK's for the N-1 proton of m^7GTP and m^7GpppG in aqueous solution were found to be 7.3 and 7.4, respectively (data not shown); the pK of m^7GTP has previously been reported as 7.5 (Hendler et al., 1970) and 7.4–7.8 depending on the salt concentration (Rhoads et al., 1983; Rhoads, 1985). The pH dependence of K_{eq} for m^7GTP and m^7GpppG binding to eIF-4E is shown in Figure 3. The optimum binding in both cases occurred at pH 7.6. The similar levels of m^7GTP and m^7GpppG binding to eIF-4E at this optimum pH parallels results obtained in studies of the inhibition of protein synthesis (Hickey et al., 1976) in which m^7GTP and m^7GpppG were found to be equally inhibitory at pH 7.6. Furthermore, the binding of cap analogues by eIF-4E (Figure 3) is in agreement with observations of the inhibition of globin mRNA synthesis by m^7GTP as a function of pH, where the efficacy of inhibition increased progressively from pH 6.6 to 7.9 (Rhoads et al., 1983; Rhoads, 1985). On the basis of those findings, it had been proposed that a specific electronic structure and charge distribution of the cap were required for recognition and binding.

Temperature Effects. Thermodynamic parameters can be obtained for the m^7GTP·eIF-4E and m^7GpppG·eIF-4E complexes from van't Hoff plots utilizing K_{eq} values obtained from fluorescence measurements at different temperatures. The values of entropy and enthalpy are obtained from the intercept and slope, respectively, and are given in Table I.

The fluorescence intensities of eIF-4E, m^7GTP, and m^7GpppG were found to be invariant over the 5–40 °C temperature range. It has been suggested that the invariance in m^7GpppG fluorescence is attributed to the increase in fluorescence due to destacking being compensated by the decrease due to deprotonation (Nishimura et al., 1980).

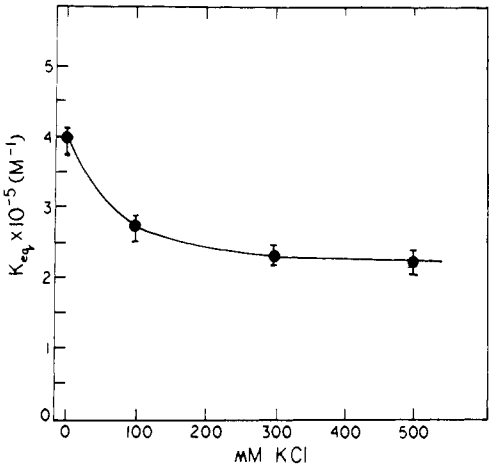

FIGURE 4: K_{eq} of m^7GTP·eIF-4E complexes (●) as a function of KCl concentration. All solutions prepared in buffer A, pH 7.6 at 23 °C, with varying amounts of KCl added. K_{eq} values were obtained from Eadie–Hofstee analysis of fluorescence data. Fluorescence conditions identical with those in Figure 1.

The interpretation of values obtained for ΔH and ΔS was suggested by Ross and Subramanian (1981): positive ΔH and ΔS values suggest either hydrophobic (Gill et al., 1967, 1976) or ionic, i.e., charge neutralization (Pimentel & McClellan, 1971), interactions between eIF-4E and m^7GTP or m^7GpppG. In order to distinguish between these two possibilities, the dependence of binding on ionic strength was investigated.

Ionic Strength Effects. The dependence of the K_{eq} on KCl concentration is shown in Figure 4. In experiments not shown, increasing KCl concentration was found to have no effect on the intensity of the eIF-4E fluorescence per se. The small (38%) decrease in the value of K_{eq} upon addition of 100–500 mM KCl suggests that ionic interactions are not dominant for the binding of m^7GTP to eIF-4E. Debye–Huckel theory [see Atkins (1982)] predicts that a plot of log K_{eq} vs the square root of the ionic strength gives a line with a slope of $1.02 z_A z_B$ for ionic interactions, where z_A and z_B are the respective charges of the reactants. Such an analysis yields a value of −0.31 for $z_A z_B$ as compared with an expected value of −1 for a single positive and negative charge interaction. The pK of m^7GTP decreases upon addition of KCl, as has been reported previously (Rhoads et al., 1983; Rhoads, 1985). This effect may lead to a small underestimate of the ionic interactions between eIF-4E and m^7GTP.

Circular Dichroism. The CD spectra of m^7GTP, eIF-4E, and the m^7GTP·eIF-4E complex are shown in Figure 5. eIF-4E has maxima at 283 and 290 nm; the m^7GTP signal (●) is small and broad. The CD spectrum of the m^7GTP·eIF-4E complex (Figure 5A, solid curve) is almost superimposable on the composite spectrum of the individual m^7GTP and eIF-4E spectra (×), suggesting that there is little perturbation of the m^7GTP or eIF-4E conformation upon binding. The contribution of eIF-4E to the complex is shown in Figure 5B; this spectrum was obtained by subtracting the m^7GTP spectrum from that of the complex. This spectrum is very similar to that of free eIF-4E (Figure 5B, dashed curve), with a small increase in the magnitude of the 283 and 290-nm maxima, and the appearance of a broad region from 310 to 340 nm. The m^7GTP contributions are shown in Figure 5C; the m^7GTP contribution in the complex is obtained by subtraction of the eIF-4E spectrum from that of the complex (solid curve). In the complex, a broad, small signal centered at 260

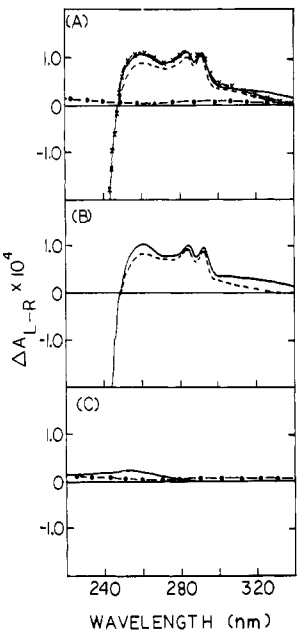

FIGURE 5: CD spectra of (A) (---) eIF-4E (7.06 μM), (●—●) m^{7}GTP (24 μM), and (—) m^{7}GTP·eIF-4E complex (containing 7.06 μM eIF-4E and 24 μM m^{7}GTP) and (×—×) composite spectrum of 7.06 μM eIF-4E plus 24 μM m^{7}GTP. (B) (---) eIF-4E (7.06 μM) and (—) eIF-4E in the m^{7}GTP·eIF-4E complex in which the m^{7}GTP spectrum has been subtracted from that of the complex. (C) (●—●) m^{7}GTP (24 μM) and (—) m^{7}GTP in the m^{7}GTP·eIF-4E complex in which the eIF-4E spectrum has been subtracted from that of the complex. All solutions were prepared in buffer B, pH 7.0 at 23 °C.

nm becomes apparent, but there is, overall, little difference between the free and complexed m^{7}GTP spectra.

When m^{7}GpppG binds to eIF-4E, the effects on the CD spectra are much more dramatic (Figure 6A). m^{7}GpppG (●) has small negative peaks at 250 and 290 nm and a postive maximum at 276 nm. The m^{7}GpppG·eIF-4E complex (Figure 6A, solid curve) is very different from the composite spectrum (×), indicating that there is a significant change in the m^{7}GpppG and/or eIF-4E conformation upon complex formation. These changes in the spectrum may arise from changes in the eIF-4E CD spectrum, m^{7}GpppG CD spectrum, or both. To examine these possibilities, we have constructed difference spectra as described above. The complexed eIF-4E signal intensity (Figure 6B, solid curve) has doubled at 283 and 290 nm, and, as was shown for eIF-4E complexed with m^{7}GTP (Figure 5B), an increase in the intensity at 310–340 nm is apparent. Large changes at 283 and 290 nm would suggest tryptophan interactions. However m^{7}GTP does not produce such changes, which would be expected if the binding modes of m^{7}GTP and m^{7}GpppG are similar as suggested by the pH dependence and magnitude of the K_{eq} values. The magnitude of the changes is unreasonably large for protein conformational changes. A more likely explanation is that the changes in the CD spectrum upon complex formation can be attributed to changes in the cap conformation. The m^{7}GpppG contribution to the spectrum is shown in Figure 6C (solid curve) and is compared with the spectrum of m^{7}GpppG (●). The negative signal at 250 nm has increased 8-fold, and the maximum at 276 nm has increased slightly and red-shifted to 280 nm; a broad 310–340-nm signal also appears. The bisignate nature of the 250- and 280-nm peaks of complexed m^{7}GpppG suggests that the m^{7}GpppG has "locked in" to a specific, presumably stacked, conformation involving tryptophan residues on eIF-4E. This type of spectral change is also seen at low temperature (data not shown) where the m^{7}GpppG is more tightly stacked.

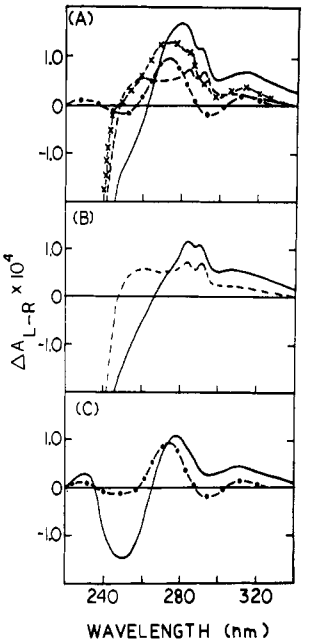

FIGURE 6: CD spectra of (A) (---) eIF-4E (7.06 μM), (●—●) m^{7}GpppG (24 μM), and (—) m^{7}GpppG·eIF-4E complex (containing 7.06 μM eIF-4E and 24 μM m^{7}GpppG) and (×—×) composite spectrum of 7.06 μM eIF-4E plus 24 μM m^{7}GpppG. (B) (---) eIF-4E (7.06 μM) and (—) eIF-4E in the m^{7}GpppG·eIF-4E complex in which the m^{7}GpppG spectrum has been subtracted from that of the complex. (C) (●—●) m^{7}GpppG (24 μM) and (—) m^{7}GpppG in the m^{7}GpppG·eIF-4E complex in which the eIF-4E spectrum has been subtracted from that of the complex. All solutions were prepared in buffer B, pH 7.0 at 23 °C.

Discussion

The binding of m^{7}GTP or m^{7}GpppG to eIF-4E under various conditions provides insight into the nature of the cap·eIF-4E interaction and allows speculation on possible binding sites. The involvement of a tryptophan residue is suggested by the fluorescence data. The fluorescence emission maximum (330 nm; Figure 1) is consistent with a somewhat buried tryptophan residue. The 50% quenching of the fluorescence intensity of eIF-4E (at 5 molar excess of m^{7}GTP or m^{7}GpppG) may indicate that the tryptophan residues are involved in base-stacking interactions with the m^{7}G as suggested by several groups (Brun et al., 1975; Ishida et al., 1983; Ueda et al., 1988).

One model of the eIF-4E·cap interaction can be designated as *model 1*. This model requires the keto form of the m^{7}G to interact with a negatively charged amino acid residue, such as aspartic acid or glutamic acid; a stacking interaction between m^{7}GTP and the tryptophan residue also occurs. The m^{7}GTP residue interacts through the N-1 proton and 2-amino group with the carboxylate anion of the glutamic residue (Ueda et al., 1988).

There are two kinds of data supporting this model. The first is from studies with tripeptides (Ueda et al., 1988) which has been adopted by McCubbin et al. (1988) to explain the binding of eIF-4E derived from yeast to m^{7}G cap analogues. The second set of evidence in support of this model is based upon the finding that the 2-amino group is important for cap recognition (Hickey et al., 1977; Adams et al., 1978).

Model 1, however, fails mainly in its inability to interpret the pH dependency of binding shown in Figure 3. The curves in Figure 3 suggest that two pK values are involved in the interaction: one from the cap and one from eIF-4E. Since the deprotonated form of aspartic or glutamic acid would interact with the N-2 position of the cap, the *decrease* in

affinity at higher pH is not accounted for by this model. Furthermore, this model does not explain the reported differences in the ability of m^7GTP and GTP to inhibit protein synthesis (Hickey et al., 1976; Canaani et al., 1976), since both can be found in the keto form at pH 7.6.

Model 2. This model states that the enolate tautomer of the m^7G moiety is required for interaction with an amino acid residue with a p*K* of 7–8, such as histidine. In this model, the negatively charged O-6 of the m^7G is hydrogen bonded to the protonated nitrogen of this amino acid residue. The stacking interaction of m^7GTP with a tryptophan also occurs.

The pH dependence of binding (Figure 3) is correctly described by this model as follows: (a) the increase in binding (pH 6.0–7.6) is due to the preferred interaction of the enolate tautomer (which becomes the dominant species in solution as the pH is raised) with the protonated amino acid residue; (b) the decrease in binding (pH 7.6–9.0) occurs as the amino acid residue becomes deprotonated. Thus, the model takes into account ionization of both the cap and an amino acid residue in eIF-4E. Furthermore, this model can explain the difference in the observed inhibitory activity of m^7GTP and GTP. At pH 7.6, m^7GTP can lose the N-1 proton (p*K* 7.3–7.5), and so it can exist in the enolate form. GTP cannot lose this proton (p*K* 9.4) and so remains in the keto form.

Model 2 is supported by various studies of the inhibition of protein synthesis by modified m^7GDP analogues as well. Adams et al. (1978) prepared $m^{1,7}GDP$ and 6-Cl-m^7GDP, both of which were found to be inactive as inhibitors; the former cannot lose the N-1 proton, and the latter cannot bear a negative charge on O-6. Thus, neither analogue can exist in the enolate form. Furthermore, Kozarich and Deegan (1979) prepared a tricyclic analogue by bridging the N-1 and 2-amino groups of m^7GDP using methylglyoxal; the analogue is thus unable to lose the N-1 proton. This derivative was also reported to be an ineffective inhibitor.

Additional information on the conformation of the cap when bound to eIF-4E can be obtained from the CD data. The change in the protein conformation upon binding of a 3.5 molar excess of m^7GTP is small (Figure 5B), suggesting that the m^7G residue acts in a symmetric fashion. It may be concluded that there is little overall perturbation of the eIF-4E conformation upon binding of m^7GTP.

The change in the CD spectrum of m^7GpppG upon binding to eIF-4E is very dramatic (Figure 6C). The presence of a bisignate spectrum (250 and 280 nm) for m^7GpppG in the complex suggests that there is a major conformational change in the m^7GpppG residue upon binding. This spectrum is similar to that obtained for m^7GpppG alone at 8 °C (data not shown), where an increased stacking of the guanosine bases is known to occur. The interaction of m^7GpppG with eIF-4E can be described by a two-step mechanism: (1) The enolate form of the cap is unstacked in solution (Nishimura et al., 1980); the m^7G moiety of the cap (analogous to m^7GTP) binds via stacking interactions, possibly with a tryptophan residue(s), and hydrogen bonding to a protonated (e.g., histidine) residue as described by model 2. This interaction causes little perturbation of the microenvironment, as the binding is qualitatively similar to that of m^7GTP to eIF-4E. (2) The second base of the cap is now ready to "lock in" to place, stacking onto the m^7G or an aromatic amino acid side chain. The stacking of this second base is primarily responsible for the dramatic change in the CD spectrum of m^7GpppG by rendering the m^7GpppG conformationally rigid in the binding pocket.

The CD spectrum of eIF-4E in a complex formed with m^7GTP (Figure 5B, solid curve) is qualitatively similar to that obtained for eIF-4E from yeast in a complex with m^7GDP (McCubbin et al., 1988, viz. Figure 4). The spectra obtained upon binding of m^7GpppG in the two systems, however, are somewhat different. We report an increase in the eIF-4E maxima at 283 and 290 nm upon binding to m^7GpppG, while the spectrum reported for the yeast eIF-4E in an analogous complex shows little change in these maxima, but rather the appearance of a new maximum at 259 nm. McCubbin et al. have assumed that the changes occurred only in the protein component of the spectrum. The 259-nm maximum could equally be attributed to an m^7GpppG contribution. Such an effect is negligible in the case of m^7GTP or m^7GDP but becomes increasingly important for m^7GpppG (viz. Figure 6A,C).

In summary, the model proposed here for the interaction of the mRNA cap with eIF-4E differs significantly from model 1. Data presented in this study indicate that the binding of the enolate form of the cap to eIF-4E proceeds via a possible tryptophan stacking interaction with the cap, and an association with a protonated histidine residue.

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High-Affinity Transport of L-Glutamine by a Plasma Membrane Preparation from Rat Brain†

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Received December 27, 1988; Revised Manuscript Received June 9, 1989

ABSTRACT: Plasma membrane vesicles prepared from rat brain contain a saturable, high-affinity transport system for L-glutamine that exhibits the following characteristics: (1) The rate of L-glutamine transport is linear up to 200 μg/mL membrane protein. (2) Transport of [^{3}H]-L-glutamine is linear with time for at least 10 min, is significantly reduced by lowering the assay temperature to 4 °C, and is essentially abolished by the addition of excess unlabeled L-glutamine. (3) The transport rate is optimal in the range of pH 7.4–8.2. (4) The system exhibits a K_m for L-glutamine of ~1.7 μM and a V_{max} of ~46 pmol/(min·mg of protein). (5) The system is not highly dependent upon the addition of monovalent or divalent cations. (6) Inhibitor studies reveal that the amino acid amides exhibit the highest affinity for the system and that there is a high specificity for the L-isomers.

The amino acid L-glutamine serves as an important extracellular carrier of amino acid nitrogen in the central nervous system and also as a primary metabolic precursor of the neurotransmitter pool of L-glutamate (Kvamme, 1983). Because glutamine is inactive as a neurotransmitter, it is tolerated in the extracellular field of the central nervous system in relatively high (0.2–0.5 mM) levels, in contrast to amino acid neurotransmitters or modulators such as glutamate, aspartate, γ-aminobutyrate, and glycine, which occur at much lower concentrations (<0.01 mM). The initial metabolic step in glutamine catabolism involves the action of glutaminase, which is active in neurons and also in glial cells (Kvamme et al., 1985).

The transport of glutamine into brain cells has been investigated by using brain slices, cultured neurons and glia, and crude synaptosomal preparations. Because of its high extracellular concentration, there is no obvious need for high-affinity glutamine transport in the central nervous system. In keeping with this, a number of workers have detected glutamine transport system that exhibit low affinity (K_m = 0.2–1.0 mM) and relatively high capacity [1–10 nmol/(min·mg of protein)] in both glia and neuronal cell cultures (Cohen & Lajtha, 1972; Baldessarini & Yorke, 1974; Balcar & Hauser, 1978; Walum & Weiler, 1978; Schousboe et al., 1979; Weiler et al., 1979; Benjamin et al., 1980; Yu & Hertz, 1982; Minn & Besagni, 1983; Johansen et al., 1987). However, other investigators have identified high-affinity (K_m ~ 10–50 μM) glutamine transport systems that exhibit a relatively low capacity for glutamine (Roberts & Keen, 1974; Balcar & Johnston, 1975; Roberts, 1976; Shank & Campbell, 1982). These systems may represent minor carriers under physiological conditions.

An energy source of glutamine transport has not been determined, but energy input may be required at some level since the intracellular level of glutamine is at least 10 times higher than the extracellular levels (Kvamme, 1983). Most workers have concluded that low-affinity, high-capacity glutamine transport across the plasma membrane of CNS cells does not involve sodium ion symport (Cohen & Lajtha, 1972; Bal-

†Supported by NIH Grant NS 17944 and grants from the Minnesota Medical Foundation and Graduate School, University of Minnesota.
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0006-2960/89/0428-8083$01.50/0