A Comparison of the Binding of Methylated Cap Analogues to Wheat Germ Protein Synthesis Initiation Factors 4F and (Iso)4F†

Susan E. Carberry,‡ Edward Darzynkiewicz,§ and Dixie J. Goss*,‡

Department of Chemistry, Hunter College of the City University of New York, New York, New York 10021, and Department of Biophysics, Institute of Experimental Physics, University of Warsaw, Warsaw, Poland 02-089

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ABSTRACT: The binding of the 5′-terminal cap analogues m^7GpppG and m^7GTP to wheat germ protein synthesis initiation factors eIF-4F and eIF-(iso)4F as a function of pH, ionic strength, and temperature is described. Equilibrium binding data indicate that eIF-4F and eIF-(iso)4F have different mechanisms for interacting with the 5′-cap structure, but the complexes formed between m^7GpppG and wheat germ factor eIF-(iso)4F more closely resemble complexes formed between this cap analogue and either mammalian eIF-4E or eIF-4F. The binding of these initiation factors to the hypermethylated cap analogues $m^{2,7}GMP$, $m^{2,7}GpppG$, and $m^{2,2,7}GpppG$ is also investigated. The differences in affinity of eIF-4F and eIF-(iso)4F for the hypermethylated 5′-terminal cap structures suggest that these factors may have discriminatory activity.

Protein synthesis initiation factors which bind directly to the 5′-terminal cap structure [$m^7G(5')ppp(5')N$][1] of mRNA have been isolated from several mammalian sources (Rychlik et al., 1986; Webb et al., 1984). Mammalian factors eIF-4E, a 24-kDa polypeptide, and eIF-4F, a complex consisting of eIF-4E and a 46-kDa and a 220-kDa polypeptide (Tahara et al., 1981; Grifo et al., 1983; Edery et al., 1983), have been shown to bind directly to the m^7G cap (Sonenberg et al., 1978; Tahara et al., 1981; Sonenberg, 1981; Sonenberg et al., 1981; Hellmann et al., 1982; Grifo et al., 1983; Webb et al., 1984; Shatkin, 1985; Goss et al., 1987, 1990; Carberry et al., 1989).

Two initiation factors isolated from wheat germ have also been shown to have cap-binding activity (Lax et al., 1985, 1986a,b). eIF-(iso)4F, which was designated eIF-4B in the early literature (Lax et al., 1985, 1986a,b), consists of a 28-kDa and a 82-kDa polypeptide in a 1:1 molar ratio (Lax et al., 1985) and eIF-4F, which consists of a 26-kDa and a 220-kDa polypeptide in a 4:1 molar ratio (Lax et al., 1985, 1986b). Specifically, the 28-kDa subunit of eIF-(iso)4F and the 26-kDa subunit of eIF-4F have been shown to cross-link to the 5′-terminal cap of oxidized mRNA in the absence of ATP (Lax et al., 1984), and this cross-linking is inhibited by the cap analogue m^7GDP (Browning et al., 1987).

Both wheat germ eIF-4F and eIF-(iso)4F have been found to have RNA-dependent ATPase activities which are additive, indicative of a structural and/or functional relationship between the two factors (Lax et al., 1986b). These data were also consistent with the finding that the presence of both factors was not required for ATP hydrolysis nor for stimulation of protein synthesis in an eIF-4F- or eIF-(iso)4F-deficient translation system (Lax et al., 1985, 1986b). Wheat germ eIF-(iso)4F, however, can substitute for mammalian eIF-4F in catalyzing RNA-dependent hydrolysis of ATP in the presence of mammalian eIF-4A and can substitute for mammalian eIF-4F in promoting the cross-linking of mammalian eIF-4A to the 5′-terminal cap of oxidized mRNA (Abramson et al., 1988).

Some natural mRNAs have been found to be capped with structures other than m^7G. Togaviruses, such as Sindbis and Semliki Forest viruses, synthesize mRNA capped with $m^{2,7}G$ and $m^{2,2,7}G$ in addition to m^7G during infection (HsuChen & Dubin, 1976; van Duijn et al., 1986). The trimethylated cap structure can also be found in snRNAs (Busch et al., 1982). The efficacy of di- and trimethylated cap analogues as inhibitors of globin synthesis in an in vitro reticulocyte lysate translation system has also been reported. The dimethylated cap analogue $m^{2,7}GMP$ was 2.2-fold more potent as an inhibitor than m^7GMP (Darzynkiewicz et al., 1988).

In order to characterize the interactions of wheat germ initiation factors eIF-4F and eIF-(iso)4F with the 5′-cap of mRNA, we have measured the binding of m^7GpppG and m^7GTP to these factors as a function of pH, ionic strength, and temperature. The interaction of eIF-4F and eIF-(iso)4F with the hypermethylated cap analogues $m^{2,7}GMP$, $m^{2,7}GpppG$, and $m^{2,2,7}GpppG$ is also reported. On the basis of these data, we conclude that wheat germ eIF-4F and eIF-(iso)4F interact differently with the 5′-cap analogues of mRNA. The complexes formed between m^7GpppG and wheat germ eIF-(iso)4F more closely resemble the complexes formed between this cap analogue and mammalian eIF-4E or eIF-4F, and wheat germ eIF-(iso)4F binds preferentially to dimethylated cap analogues.

Materials and Methods

m^7GpppG and m^7GTP were purchased from Pharmacia Molecular Biologicals; $m^{2,7}GMP$, $m^{2,7}GpppG$, and $m^{2,2,7}GpppG$ were provided by E. Darzynkiewicz and were synthesized as previously described (Darzynkiewicz et al., 1988). Buffer A, used for the fluorescence measurements,

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* Corresponding author.
‡ Hunter College of the City University of New York.
§ University of Warsaw.

[1] Abbreviations: m^7G, 7-methylguanosine; $m^{2,7}G$, 2,7-dimethylguanosine; $m^{2,2,7}G$, 2,2,7-trimethylguanosine; eIF, eukaryotic initiation factor; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetate, disodium salt; kDa, kilodalton.

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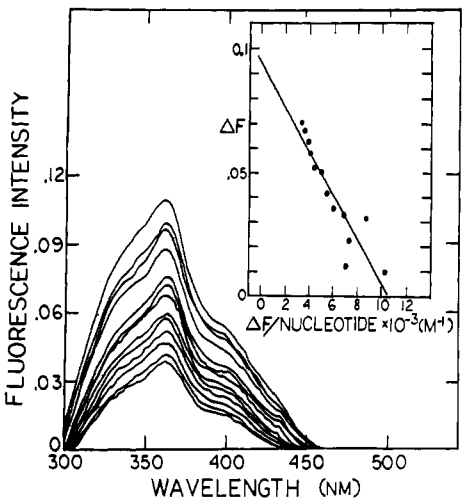

FIGURE 1: Fluorescence emission spectra of 3 μM wheat germ eIF-(iso)4F complexed with m^{7}GpppG in buffer A, pH 7.6 The m^{7}GpppG concentration was (top to bottom) 0, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, and 20 μM. Fluorescence was monitored at 360 nm and excited at 258 nm; a 1.4-mm slit and a 1-cm path-length cell were employed. (Inset) Eadie–Hofstee plot of the fluorescence data. A K_{eq} value of (1.21 ± 0.1) × 10^5 M^{-1} was obtained from the negative of the inverse of the slope.

consisted of 20 mM HEPES–KOH and 1 mM DTT and was adjusted to the appropriate pH. Buffer B, used in isolation of the wheat germ factors, consisted of 20 mM HEPES–KOH, pH 7.6, 0.1 mM EDTA, 1 mM DTT, 10% glycerol, and KCl as indicated. All chemicals were reagent grade or better.

Wheat germ initiation factors eIF-4F and eIF-(iso)4F were purified according to the protocol of Lax et al. (1986a, b) with the following modifications (J. Ravel, personal communication). The 40% ammonium sulfate fraction was loaded onto a DE-52 column, equilibrated with buffer B containing 40 mM KCl (B-40). The column was washed until the OD was less than 0.2. The buffer was then changed to B-80 (80 mM KCl), and the peak containing eIF-(iso)4F was collected. The buffer was then changed to B-120 (120 mM KCl), and the peak was collected. These peak fractions were concentrated with 80% ammonium sulfate precipitation and centrifuged at 11 000 rpm in a Sorvall centrifuge; the precipitate, which contains eIF-4F, was resuspended in 10 mL of B-120 and dialyzed overnight against B-120. The sample containing eIF-4F or eIF-(iso)4F was then applied to an m^{7}GTP–Sepharose column, and the pure factor was eluted as described by Lax et al. (1986a, b); in order to maximize yields, the m^{7}GTP solution used to elute the pure factors was freshly prepared. Molecular weight values of 108 000 and 250 000 were used to calculate the molar concentrations of wheat germ eIF-(iso)4F and eIF-4F, respectively.

Fluorescence measurements were carried out at 23 °C, unless otherwise noted, and data were collected and analyzed as previously described in detail (Carberry et al., 1989, 1990).

Results

pH Dependence. The fluorescence emission spectra of wheat germ eIF-(iso)4F–m^{7}GpppG complexes as a function of m^{7}GpppG concentration are shown in Figure 1; similar spectra are obtained for eIF-4F and m^{7}GpppG–eIF-4F complexes (data not shown). eIF-(iso)4F has a fluorescence maximum at 360 nm and a shoulder at 336 nm; however, the relative fluorescence intensities at these wavelengths appeared to be preparation-dependent. In comparison, mammalian eIF-4E and eIF-4F were found to have a fluorescence emission maximum at 330 nm and a shoulder at 360 nm (Carberry et al., 1989, 1990; Goss et al., 1990); these peaks were attributed to tryptophan residues (Carberry et al., 1989). An equilibrium binding constant (K_{eq}) for the m^{7}GpppG–eIF-(iso)4F interaction can be obtained from an Eadie–Hofstee analysis of the data (Eadie, 1942). This analysis is shown in the inset of Figure 1; a K_{eq} value of (1.21 ± 0.1) × 10^5 M^{-1} was obtained; the K_{eq} values determined are independent of the protein concentration.

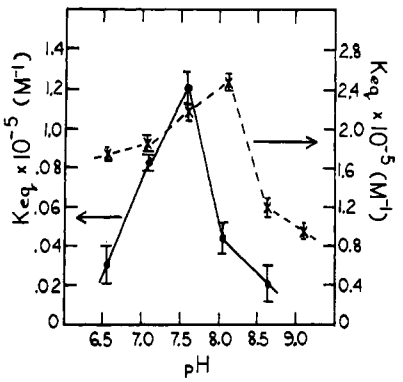

FIGURE 2: Binding of m^{7}GpppG to eIF-4F (×) and eIF-(iso)4F (●) as a function of pH. All solutions were prepared in buffer A, adjusted to the appropriate pH at 23 °C.

Table I: Comparison of K_{eq} Values for Interaction of the Cap Analogues m^{7}GpppG, m^{2,7}GMP, m^{2,7}GpppG, and m^{2,2,7}GpppG to Wheat Germ Initiation Factors eIF-4F and eIF-(iso)4F and to Human eIF-4E

	K_{eq} × 10^{-5} (M^{-1})		
cap analogue	eIF-4F[a]	eIF-(iso)4F[b]	eIF-4E[c]
m^{7}GpppG	2.32 ± 0.10	1.21 ± 0.10	3.77 ± 0.11
m^{2,7}GMP	0.65 ± 0.05	1.53 ± 0.08	3.27 ± 0.20
m^{2,7}GpppG	1.37 ± 0.03	2.24 ± 0.07	2.25 ± 0.24
m^{2,2,7}GpppG	0.85 ± 0.10	0.77 ± 0.03	0.54 ± 0.05

[a] Measurements made at pH 8.013, 23 °C. [b] Measurements made at pH 7.6, 23 °C. [c] Values taken from Carberry et al. (1990); conditions same as for eIF-(iso)4F.

The pH dependence of m^{7}GpppG binding to wheat germ eIF-4F and eIF-(iso)4F is shown in Figure 2. The pH optimum for m^{7}GpppG binding was found to be 8.0 for eIF-4F and 7.6 for eIF-(iso)4F; at the respective pH optima, the binding of m^{7}GpppG to eIF-4F was 1.9-fold greater than that to eIF-(iso)4F. m^{7}GpppG–eIF-4F complex formation was less pH dependent than m^{7}GpppG–eIF-(iso)4F complex formation for pH values lower than the optimum; the falloff in K_{eq} values at pH greater than optimum was also less for m^{7}GpppG–eIF-4F complexes than for m^{7}GpppG–eIF-(iso)4F complexes. The pH-dependent binding profile reported here for the m^{7}GpppG–eIF-(iso)4F complexes is similar to that of m^{7}GpppG–eIF-4E complexes (Carberry et al., 1989), except that at the pH optimum of 7.6 eIF-4E had 3.1-fold higher affinity for m^{7}GpppG than wheat germ eIF-(iso)4F (Table I).

Ionic Strength Dependence. The binding of m^{7}GpppG to wheat germ eIF-4F and eIF-(iso)4F as a function of KCl concentration is shown in Figure 3A, and the treatment of these data according to Debye–Huckel theory is shown in Figure 3B. A plot of log (K/K_o) versus the square root of the ionic strength yields a straight line passing through the origin with a slope of $1.02z_Az_B$, where K and K_o are the equilibrium constants in the presence and absence of KCl, respectively, and z_A and z_B are the respective charges of the reactants. For the interaction of a single positive and negative charge, z_Az_B is equal to −1. For the interaction of m^{7}GpppG with eIF-4F, a value of −1.33 was obtained for z_Az_B, and a value of −0.211

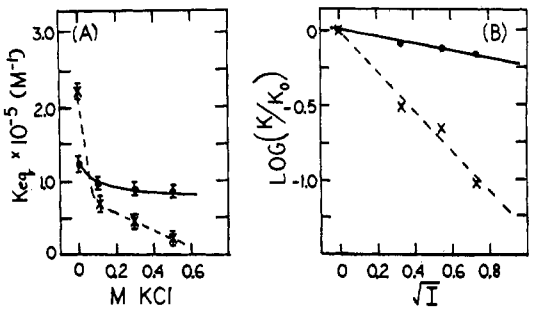

FIGURE 3: (A) K_{eq} values of m^{7}GpppG–eIF-4F (×) and m^{7}GpppG–eIF-(iso)4F (●) complexes as a function of KCl concentration. All solutions were prepared in buffer A, which was adjusted to pH 7.6 for eIF-(iso)4F-containing samples and pH 8.01 for eIF-4F-containing samples. (B) Debye–Huckel analysis of the data in (A).

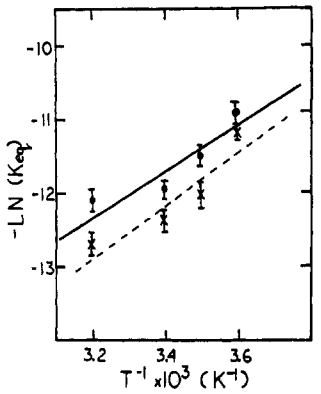

FIGURE 4: van't Hoff plot for m^{7}GTP–eIF-4F (×) and m^{7}GTP–eIF-(iso)4F (●) complexes. All experimental conditions as in Figure 3.

was obtained for eIF-(iso)4F–m^{7}GpppG complexes. These $z_A z_B$ values compare with a value of −0.31 obtained for the m^{7}GTP–eIF-4E complexes under similar conditions (Carberry et al., 1989).

Temperature Dependence. The K_{eq} values for m^{7}GTP–eIF-(iso)4F and m^{7}GTP–eIF-4F complexes as a function temperature were also measured; Figure 4 shows the van't Hoff plot constructed from these data. For the m^{7}GTP–eIF-(iso)4F interaction, values of 5.81 ± 0.5 kcal/mol and 42.9 ± 4.0 cal/(mol °C) were obtained for ΔH and ΔS, respectively, while for the m^{7}GTP–eIF-4F interaction ΔH was found to be 6.84 ± 0.7 kcal/mol and ΔS was 47.4 ± 5.0 cal/(mol °C). These values are very similar to those previously reported for the m^{7}GTP–eIF-4E interaction, where ΔH was found to be 6.25 ± 0.25 kcal/mol and ΔS was 46.1 ± 1.8 cal/(mol °C) (Carberry et al., 1989).

Interaction of Hypermethylated Cap Analogues. We have previously described the interaction of the hypermethylated cap analogues m^{2,7}GMP, m^{2,7}GpppG, and m^{2,2,7}GpppG with human eIF-4E (Carberry et al., 1990). In Table I, the interaction of these cap analogues with wheat germ eIF-4F and eIF-(iso)4F is reported and compared with that of eIF-4E. Wheat germ eIF-(iso)4F appears to prefer an interaction with the dimethylated cap analogues: the affinity of eIF-(iso)4F for m^{2,7}GMP and m^{2,7}GpppG is 1.3- and 1.9-fold greater, respectively, than that for m^{7}GpppG. The trimethylated cap analogue m^{2,2,7}GpppG has only 1.6-fold lower affinity than m^{7}GpppG for eIF-(iso)4F, as compared to a 7-fold lower affinity (relative to m^{7}GpppG) for eIF-4E. Overall, wheat germ eIF-4F forms weaker complexes with the hypermethylated caps: the trimethylated cap analogue has 2.7-fold less affinity for eIF-4F, and m^{2,7}GMP and m^{2,7}GpppG have 3.3- and 1.7-fold less affinity (all values relative to m^{7}GpppG).

Discussion

The binding of m^{7}GpppG to wheat germ eIF-4F and eIF-(iso)4F, measured under various conditions, indicates differences in the cap–initiation factor interaction. The fluorescence emission spectra of wheat germ eIF-4F and eIF-(iso)4F suggest the involvement of tryptophan residue(s) in the cap-binding site, as was previously described in detail for human eIF-4E (Carberry et al., 1989). In the case of the wheat germ initiation factors, however, the fluorescence maximum is red shifted to 360 nm, suggesting the involvement of a more solvent-exposed tryptophan residue (Lakowicz, 1983).

The pH dependence of binding of m^{7}GpppG to eIF-4F and eIF-(iso)4F suggests that another residue or other residues in the cap-binding site are different, or are in different environments, for the two factors. The pH-dependent binding profile observed for the m^{7}GpppG–eIF-(iso)4F interaction, with optimum binding at pH 7.6, is similar to that previously reported for the m^{7}GpppG–eIF-4E interaction (Carberry et al., 1989). This latter interaction was previously characterized in terms of a model which proposed that the increase in K_{eq} at pH values below the optimum was due to the increased affinity of a protonated histidine residue for the enolate form of the cap while the decrease in K_{eq} values at pH values greater than optimum was due to the decrease in affinity of the enolate form of the cap for the deprotonated histidine residue. The data reported in Figure 2 for the m^{7}GpppG–eIF-(iso)4F interaction as a function of pH are also consistent with this model. The binding of m^{7}GpppG to eIF-4F as a function of pH, however, is very different: the pH optimum is 8.0, and there is a less dramatic dependence of K_{eq} on pH at values below the optimum. This profile suggests that (1) the enolate form of the cap is interacting with a histidine residue that is in an environment different than that found in either wheat germ eIF-4F or human eIF-4E and/or (2) other residues are involved in the interaction; one possible residue is cysteine (pK 8.0). The difference in the fluorescence spectrum for the proteins suggests that the environment around the binding site may be different.

The ionic strength dependence of the interaction of wheat germ factors eIF-4F and eIF-(iso)4F with m^{7}G-cap is also different. The binding of m^{7}GpppG to these factors as a function of KCl concentration indicates that electrostatic interactions do not play a dominant role in the formation of the cap–eIF-(iso)4F complex but are important for the cap–eIF-4F interaction.

Wheat germ eIF-4F and eIF-(iso)4F differ in their binding to m^{7}GpppG as a function of pH and ionic strength. However, eIF-(iso)4F and eIF-4E have similar characteristics in their affinity for m^{7}G cap analogues, although the overall levels of binding of m^{7}GTP or m^{7}GpppG to eIF-4E are higher than those to eIF-(iso)4F. The pH optimum and ionic strength dependence profiles and the thermodynamic parameters are similar for the interaction of the m^{7}G cap analogues with either eIF-4E or eIF-(iso)4F. At the optimum translation temperature for the reticulocyte system, the K_{eq} value for the m^{7}GTP–eIF-4E interaction is 4.1 × 10^5 M^{-1}, while at the optimum temperature for translation of the wheat germ system (23 °C) the K_{eq} for the m^{7}GTP–eIF-(iso)4F interaction is 1.2 × 10^5 M^{-1}. This represents an overall 3.3-fold greater affinity of eIF-4E for the m^{7}G cap relative to eIF-(iso)4F at the respective temperature optima.

In order to investigate selectivity of cap binding proteins, the binding of hypermethylated cap analogues to eIF-4F and eIF-(iso)4F was measured (Table I). Although eIF-(iso)4F

and eIF-4E have been shown to have similar affinity for m^7G-containing cap analogues, their relative affinities for the hypermethylated caps are very different. eIF-(iso)4F appears to prefer the dimethylated cap analogues, by 1.3–1.9-fold, over the single methyl group at the N-7 position. This contrasts with human eIF-4E, which had 1.2–1.7-fold *lower* affinity for the dimethylated cap analogues relative to m^7GpppG and a 7-fold lower affinity for the trimethylated analogue (Table I; Carberry et al., 1990). eIF-4F has even lower (1.7–3.6-fold) affinity for the hypermethylated cap analogues, relative to m^7GpppG, than eIF-4E. Since eIF-(iso)4F is more abundant than eIF-4F, this result suggests a competitive advantage for the hypermethylated cap analogues.

These data suggest that the cap-binding pockets for human eIF-4E and wheat germ eIF-4F and eIF(iso)4F are distinct; however, data presented here indicate that there are more similarities between the cap-binding sites of eIF-(iso)4F and eIF-4E than between those of eIF-4F and eIF-(iso)4F. This work has also shown that, although both eIF-4F and eIF-(iso)4F interact with the 5′-cap of mRNA, the mechanisms of interaction are different; this suggests that these factors may have discriminatory or regulatory roles in selecting mRNA for translation.

Registry No. m^7GpppG, 78193-13-2; m^7GTP, 26554-26-7; $m^{2,7}GMP$, 72051-11-7; $m^{2,7}GpppG$, 131066-18-7; $m^{2,2,7}GpppG$, 131066-19-8.

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