Discussion Letter

Evaluation and significance of kinetic parameters governing function of protein synthesis initiation factors eIF-2 and eIF-2B

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Received 10 December 1984

Published data dealing with the formation of the ternary complex eIF-2 · GTP · met-tRNA$_i$ involved in eukaryotic initiation have been evaluated to calculate the expected inhibition by GDP and the role of eIF-2B in limiting this inhibition. It is concluded that cellular levels of GDP are unlikely seriously to inhibit ternary complex formation if the reaction can proceed to equilibrium. However, derivation of 'on' and 'off' rates for the interaction of GTP and GDP with eIF-2 demonstrates that these are too slow in the absence of eIF-2B to support active protein synthesis, particularly if eIF-2 is released from ribosomes as eIF-2 · GDP. Whilst eIF-2 · GDP and eIF-2 · GTP appear to dissociate equally slowly, it is concluded that GDP binds to eIF-2 100-times faster than GTP. Addition of eIF-2B has the effect of raising k_{-1} for both GDP and GTP several hundred-fold and k_{+1} 50- and 7000-fold, respectively. Thus, a kinetic block can be relieved even if there is no change in the thermodynamic state. Phosphorylation of the α-subunit of eIF-2 appears to affect only those parameters influenced by eIF-2B. The reported rescue of inhibited lysates by addition of 1 mM GTP is not by mass action but by some other mechanism. Consideration of the kinetic parameters favours the formation of a ternary complex of eIF-2 · eIF-2B · GDP en route to eIF-2 · GTP as opposed to displacement of GDP from eIF-2 · GDP by eIF-2B.

Initiation factor eIF-2 *Initiation factor eIF-2B* *Ternary complex*

Formation of a ternary complex between the protein synthesis initiation factor eIF-2, GTP and methionyl-tRNA$_i$ is a vital first step in the initiation of eukaryotic protein synthesis [1,2]. The reactions of eIF-2, GRP, and met-tRNA$_i$ can be represented:

$$\text{eIF-2} \cdot \text{GDP} \overset{K_D}{\rightleftharpoons} \text{GDP} + \text{eIF-2} + \text{GTP} \overset{K_T}{\rightleftharpoons} \text{eIF-2} \cdot \text{GTP} + \text{met-tRNA}_i \overset{K_M}{\rightleftharpoons} \text{eIF-2} \cdot \text{GTP} \cdot \text{met-tRNA}_i$$

The formation of eIF-2 · GTP · met-tRNA$_i$ is believed to proceed by the prior attachment of GTP to eIF-2 before addition of met-tRNA$_i$. Early studies showed that GDP can also bind to eIF-2 and this binding prevents addition of met-tRNA$_i$. Since the binding of GDP is 100-times stronger than for GTP (dissociation constants K_D and K_T being 3×10^{-8} and 2.5×10^{-6} M, respectively [3]) GDP is a potent inhibitor of ternary complex formation. Moreover, the eIF-2 · GDP complex once formed appears to be very stable [4–6].

Recently, several groups have shown the existence of a further protein, named variously eIF-2B, SP, RF, GEF [4,5,7–9] that facilitates displacement of GDP from the eIF-2 · GDP complex by GTP. The role of eIF-2B becomes particularly important with the realisation that eIF-2 · GDP is formed from eIF-2 · GTP during each round of initiation. Thus, the rate of protein synthesis is potentially limited by the rate at which the GDP of

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00145793/85/$3.30

eIF-2·GDP can exchange with GTP. Safer and colleagues have shown that the dissociation constants for binding of GTP and GDP to eIF-2·eIF-2B are different from those for binding to eIF-2, becoming 1.7×10^{-7} and 1.8×10^{-7} M, respectively [2,7]. However, if the role of eIF-2B is catalytic, these figures do not explain how eIF-2B facilitates displacement of GDP except to indicate that the underlying rate constants for the interaction of eIF-2 and GDP and GTP are changed. The aim of this Discussion Letter is to use existing published data to make estimates of possible rate constants for formation of eIF-2·GDP and eIF-2·GTP in the presence and absence of eIF-2B in order to understand how eIF-2B may function.

1. TERNARY COMPLEX FORMATION

Table 1, from Konieczny and Safer [7], demonstrates (a) ternary complex formation on incubation together of eIF-2, GTP and met-tRNA$_i$, (b) the inhibition of ternary complex formation on addition of modest amounts of GDP, and (c) the ability of a limited amount of eIF-2B partially to relieve the inhibition produced by GDP provided that the α-subunit of eIF-2 is not phosphorylated.

Table 1

Effect of eIF-2B on Met-tRNA$_i$ binding by eIF-2

	pmol met-tRNA$_i$ bound	
	eIF-2	eIF-2(αP)
1. 130 μM GTP 2 mM phosphocreatine 0.4 IU creatine phosphokinase	9.0	8.6
2. 125 μM GTP 5 μM GDP	1.8	1.9
3. 125 μM GTP 5 μM GDP $\simeq$1 pmol eIF-2B	6.4	2.1

eIF-2 (12 pmol) was incubated for 5 min at 30°C in a total volume of 100 μl with 15 pmol met-tRNA and the other additions as indicated, after which the amount of ternary complex formed was measured as described by Konieczny and Safer [7]

The ability of a relatively small amount of eIF-2B to reverse the inhibition produced by GDP is on the face of it surprising, since a catalyst cannot alter the equilibrium position of a reaction but only speed the attainment of equilibrium. Thus, it is not immediately clear whether in table 1 the role of eIF-2B is catalytic or more complex.

The dissociation constants for the three equilibria involved in table 1 are K_D, K_T and K_M as in the scheme shown above. The values of K_D and K_T are assumed to be 3×10^{-8} and 2.5×10^{-6} M, respectively [3,7]. From the amounts of met-tRNA$_i$, GTP and eIF-2 added in line 1 of table 1, the amount of ternary complex found and the assumed value of K_T it is possible to calculate K_M as 2.0×10^{-8} M [10]. Using this value of K_M it is possible to show that the amount of ternary complex formed in line 3 where both GDP and eIF-2B are present represents an equilibrium condition consistent with the value of the three constants [10]. Thus, under the conditions of table 1 addition of eIF-2B facilitates the attainment of an equilibrium which is not easily achieved in the presence of GDP but absence of eIF-2B (line 2) or when the α-subunit of eIF-2 is phosphorylated.

The data of table 1, lines 1 and 3 in the absence of α-subunit phosphorylation, appear to be consistent with the reaction scheme indicated above at values of K_D and K_T determined independently [3,7]. Altered values of K_D and K_T when eIF-2B binds to eIF-2 have no immediate relevance. Moreover, although K_D is 100-times lower than K_T, modest amounts of GDP, e.g., a GTP:GDP ratio of 25:1, suggested to be physiological [7], produce only 30% inhibition of ternary complex formation due to the effect of met-tRNA$_i$ and the value of K_M. Actual ratios of free GTP:GDP in cells are not known with any accuracy and if the GTP:GDP ratio is like that of ATP:ADP [11] the thermodynamic ratio is probably considerably greater than that measured by extraction. Even if GTP:GDP were lower than 25:1 the existence in many cells of higher concentrations of met-tRNA$_i$ than used in table 1 would tend to counteract inhibition by GDP. It thus seems unlikely that under most circumstances the concentration of GDP in cells seriously affects protein synthesis. Particularly in the much studied reticulocyte lysate the problem appears to be the kinetic one of the rate of exchange of GDP in eIF-2·GDP with GTP.

2. KINETIC DATA

Results of several groups [4–6] show that dissociation of the eIF-2·GDP complex is very slow and that the effect of eIF-2B is to enhance substantially the rate of release of GDP. Siekierka et al. describe experiments (fig.2A of [4]) in which addition of eIF-2B can raise the rate of exchange of free GDP with bound [^{3}H]GDP about 20-fold. However, in these experiments the ratio of eIF-2 to eIF-2B is about 10:1 and raising eIF-2B would be expected to increase the rate further. This can be seen in the experiment of Panniers and Henshaw (fig.7 of [5]) which show almost immediate dissociation on addition of excess eIF-2B.

Panniers and Henshaw [5] point out that to sustain active protein synthesis each eIF-2 molecule must be utilized once every 7 s. If there are about 3 eIF-2 to each eIF-2B it is possible that only about a third of the eIF-2 pool participates in this turnover and the rate will be correspondingly 3-times faster. Thus, the rate of displacement of GDP from eIF-2·GDP by eIF-2B or the dissociation of GDP from eIF-2·eIF-2B·GDP will have a rate constant (k_{-1} of [5]) of not less than 0.5 s^{-1}. If K_D for this complex is 1.8×10^{-7} M [7] then $k_{+1} \simeq 2.8\times10^{6}$ $M^{-1}\cdot s^{-1}$.

If GDP is to be exchanged for GTP the rate of eIF-2·GTP formation must be equal to the rate of GDP displacement or dissociation. If the concentration of eIF-2·eIF-2B is about 45 nM [5] then the rate of binding of GTP is at least 22 $nM\cdot s^{-1}$. From this rate and the concentration of GTP and free eIF-2·eIF-2B, calculated for the latter to be 6×10^{-11} M using the binding constants of 1.8 and 1.7×10^{-7} M for GDP and GTP [2], k_{+1} for GTP binding is likewise not less than 2.9×10^{6} $M^{-1}\cdot s^{-1}$ and k_{-1} at least 0.5 s^{-1}.

According to Siekierka et al. (fig.2A of [4]) the rate of dissociation of eIF-2·GDP in the absence of eIF-2B can be calculated to be about 1.5×10^{-3} s^{-1}. This is roughly 300-fold less than the postulated rate in the presence of saturating amounts of eIF-2B. Given that K_D is 3×10^{-8} M, k_{+1} under these conditions will be $1.5\times10^{-3}/3\times10^{-8} = 5\times10^{4}$ $M^{-1}\cdot s^{-1}$, i.e., 50-times less than for eIF-2·eIF-2B.

A possible explanation of the results in line 2 of table 1 is that on initiation of the ternary complex reaction in the presence of GDP, eIF-2·GDP forms more rapidly than eIF-2·GTP and having formed dissociates too slowly for equilibrium to be attained during the time of the incubation. Calculation as before [10] suggests that 5-times more eIF-2 is linked to GDP than to GTP, including that in the ternary complex. Since [GTP]:[GDP] is 25:1, k_{+1} for GDP binding could be of the order of $125\times k_{+1}$ for GTP. If k_{+1} for GDP is 5×10^{4} as calculated in the previous paragraph, then k_{+1} for GTP would be $\simeq$ 400 $M^{-1}\cdot s^{-1}$. A value for k_{+1} of 400 would imply that k_{-1} is $400\times2.5\times10^{-6} = 1\times10^{-3}$ s^{-1} which indicates that the eIF-2·GTP complex is as stable as eIF-2·GDP.

The various values of k_{+1} and k_{-1} are summarized in table 2. Those in the presence of eIF-2B will be affected by the actual cellular ratio of eIF-2B to eIF-2, assumed to be about 1:3. In order

Table 2

Kinetic parameters for the binding of GDP and GTP to eIF-2 and eIF-2·eIF-2B

	Dissociation constant (M)	k_{+1} ($M^{-1}\cdot s^{-1}$)	k_{-1} (s^{-1})
eIF-2·GDP	3×10^{-8}	5×10^{4}	1.5×10^{-3}
eIF-2·GTP	2.5×10^{-6}	4×10^{2}	1×10^{-3}
eIF-2·eIF-2B·GDP	1.8×10^{-7}	2.8×10^{6}	0.50
eIF-2·eIF-2B·GTP	1.7×10^{-7}	2.9×10^{6}	0.50

Dissociation constants are from Walton and Gill [3], Konieczny and Safer [7] and Safer [2]

to achieve rates of initiation compatible with rates of protein synthesis in vivo it appears that eIF-2B must enhance both on and off rates, the latter 500-fold and the former 50-fold for GDP and almost 10^4 times for GTP.

Though a value of k_{-1} for eIF-2 · GTP similar to that for eIF-2 · GDP seems at first sight inconsistent with the stability of eIF-2 · GDP and the difficulty reported in dectectin eIF-2 · GTP [1,12], the difference between K_T and K_D resulting from a much smaller k_{+1} for eIF-2 · GTP vs eIF-2 · GDP formation is consistent with published data. Thus, in figs 3A and B of Siekierka et al. [8] GTP is of the order of 500 times less effective than GDP in displacing [^{3}H]GDP from its complex with eIF-2. Whilst the apparent failure of Chaudhuri et al. (fig.1 of [12], refered to in [1] to detect eIF-2 · GTP formation at first sight suggests a high k_{-1}, it can also be explained as the consequence of a low k_{+1}. The authors' conclusion that such binary complex as formed was with GDP contaminating their GTP is to be expected if the GDP has a much higher on rate than does GTP.

3. PHOSPHORYLATION OF eIF-2α AND EFFECT OF [GTP]

The data of table 1, line 1, suggest that α-subunit phosphorylation does not inhibit the equilibria involved in ternary complex formation from eIF-2, GTP and met-tRNA$_i$. Likewise, line 2 of table 1 suggests that α-subunit phosphorylation does not affect the rate constants for nucleotide binding to eIF-2. This point is indirectly confirmed by both Konieczny and Safer [7] who found that K_D remained the same and by Siekierka et al. [13]. When the α-subunit is phosphorylated there is no change of rate constants on addition of eIF-2B.

Konieczny and Safer [7] attribute the 'rescue' of a haem-deficient lysate by 1 mM GTP to a mass action effect. However, if initiation in a haem-deficient lysate is primarily limited by the rate of GDP dissociation from eIF-2, increasing [GTP] is unlikely to have any direct result. It is more likely that GTP can in some way inhibit for example, HCR formation or activity as suggested by Balkow et al. [14].

4. MECHANISM OF ACTION OF eIF-2B

Slightly different reaction sequences have been proposed by Siekierka et al. [4,13] and Konieczny and Safer [7] for the mechanism of action of eIF-2B. Siekierka et al. [4,13] have argued that since isolated eIF-2 · eIF-2B does not contain GDP, eIF-2B is not likely to form a ternary complex with eIF-2 · GDP as proposed by Konieczny and Safer [7] but will displace GDP, being itself in turn displaced by GTP. However, if the rate of GDP dissociation from eIF-2 · eIF-2B is much faster than from eIF-2 it is possible that the GDP will be lost during purification.

Gross et al. [15] have recently measured the dissociation constants for the reaction of eIF-2B with eIF-2 and eIF-2 · GDP to be 5×10^{-11} and 2×10^{-10} M, respectively. These figures imply tight binding of eIF-2B to both eIF-2 and eIF-2 · GDP. Combining the two dissociations gives

$$\text{eIF-2} \cdot \text{eIF-2B} + \text{eIF-2} \cdot \text{GDP} \rightleftharpoons \text{eIF-2} \cdot \text{eIF-2B} \cdot \text{GDP} + \text{eIF-2}$$

which has an equilibrium constant of 0.25. Since any free eIF-2 is likely to be sequestered by GDP (or eIF-2B) there will be a strong pull towards formation of the ternary complex. Gross et al. [15] also provide direct evidence against displacement of GDP by eIF-2B as proposed by Siekierka et al. [4]. Moreover, from the figures of Gross et al. [15] the equilibrium constant for the displacement of GDP from eIF-2 · GDP can be calculated to be about 600 [10]. Although such a constant appears to favour displacement of GDP by eIF-2B, the much higher concentration of GDP in comparison with that of eIF-2B would probably result more readily in eIF-2 · GDP formation. This is shown by the data of Siekierka et al. (fig.3 of [13]) where GDP is observed readily to displace eIF-2B from its complex with eIF-2. Such a reaction will inhibit rather than favour initiation. Thus, available evidence is more consistent with the formation of the ternary complex eIF-2 · eIF-2B · GDP than a mechanism involving displacement.

The results of Gross et al. [15] do not support the displacement of GDP from the ternary complex with eIF-2 · eIF-2B by GTP, but the dissociation of the complex according to the equation

$$\text{eIF-2} \cdot \text{eIF-2B} \cdot \text{GDP} + \text{GTP} \rightleftharpoons \text{eIF-2} \cdot \text{GTP} + \text{GDP} + \text{eIF-2B}$$

The dissociation constant for this reaction can be calculated to be 2.4×10^{-12} M [10]. Although this number appears rather forbidding, if the concentration of free eIF-2B is very low and the ratio of [GTP]:[GDP] high it is possible that [eIF-2 · GTP] could approach [eIF-2 · eIF-2B · GDP]. In the presence of met-tRNA$_i$ capable of sequestering eIF-2 · GTP such a reaction may be able to operate from left to right. Thus, although it is not possible to exclude other hypotheses, the available data appear to be consistent with the asymmetrical model proposed by Gross et al. [15] in which a ternary complex formed from eIF-2 · eIF-2B · GDP is dissociated by GTP to yield eIF-2 · GTP directly.

ACKNOWLEDGEMENT

I am grateful to Dr M. Minks for many helpful discussions.

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