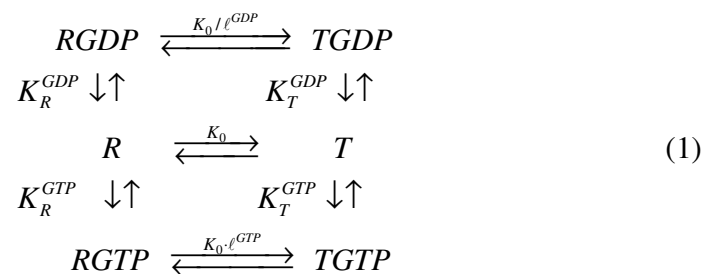


Additional data file 1 (added in proof)

Can free EF-G in solution change from a GDP to a GTP conformation?

The crystal structures of guanine-nucleotide-free- and GDP-bound EF-G are very similar [18,19], and small-angle X-ray scattering (SAXS) data suggest that the solution structures of EF-G and EF-G•GDP are virtually identical and very similar to their crystal structures [4]. Interestingly, the same SAXS data also suggest that the solution structure of GTP-bound EF-G might be indistinguishable from the guanine-nucleotide-free- and GDP-bound forms of the factor in solution as well as in crystal [4]. Very recently the notion that the solution structures of EF-G•GTP and EF-G•GDP are themselves similar yet are very different from the GDPNP-bound form of the factor on the post-termination ribosome [5] received support from a novel crystal structure of the GTP-bound form of a mutant of EF-G from *Thermus thermophilus* [17]. Could it then be that the GDP- and GTP-bound forms of the free factor are similar, while they are very different on the ribosome?

To examine the conditions for such a scenario, one may introduce a GDP-favoring (R) and a GTP-favoring (T) conformation of EF-G:



R and T are guanine-nucleotide-free R and T forms, respectively. RGDP is the R form in complex with GDP, TGDP is the T form in complex with GDP, RGTP is the R form in complex with GTP, and TGTP is the T form in complex with GTP. The parameters ℓ^{GDP} and ℓ^{GTP} are defined from the dissociation constants in Scheme 1 as

$$\ell^{GTP} = \frac{K_R^{GTP}}{K_T^{GTP}}, \quad \ell^{GDP} = \frac{K_T^{GDP}}{K_R^{GDP}} \quad (2)$$

We define effective dissociation constants for the binding of GTP or GDP to EF-G from the relations

$$\begin{aligned} \frac{([R] + [T])[GDP]}{K_{eff}^{GDP}} &= ([RGDP] + [TGDP]), \\ \frac{([R] + [T])[GTP]}{K_{eff}^{GTP}} &= ([RGTP] + [TGTP]) \end{aligned} \quad (3)$$

The effective dissociation constants K_{eff}^{GTP} and K_{eff}^{GDP} are given by the parameters of Scheme 1 through

$$\begin{aligned} K_{eff}^{GDP} &= \frac{K_R^{GDP} \ell^{GDP} (1 + K_0)}{\ell^{GDP} + K_0}, \\ K_{eff}^{GTP} &= \frac{K_T^{GTP} \ell^{GTP} (1 + K_0)}{1 + \ell^{GTP} K_0} \end{aligned} \quad (4)$$

The probability, $P(T)$, that EF-G is in the T form depends on the GDP and GTP concentrations through

$$P(T) = \frac{K_0 \left(1 + \frac{[GTP]}{K_T^{GTP}} + \frac{[GDP]}{\ell^{GDP} K_R^{GDP}} \right)}{K_0 \left(1 + \frac{[GTP]}{K_T^{GTP}} + \frac{[GDP]}{\ell^{GDP} K_R^{GDP}} \right) + 1 + \frac{[GTP]}{\ell^{GTP} K_T^{GTP}} + \frac{[GDP]}{K_R^{GDP}}} \quad (5)$$

The possibility that EF-G would switch in solution from its R to its T form by addition of pure GTP can be analyzed at zero GDP concentration in the limit of a large concentration of GTP in Equation (5):

$$P(T) = \frac{1}{1 + \frac{1}{\ell^{GTP} K_0}} \quad (6)$$

Accordingly, the condition for a switch is that $K_0 \ll 1$ and, yet that $\ell^{GTP} K_0 \gg 1$. If, in contrast $\ell^{GTP} K_0 \ll 1$, then EF-G will remain in the R form in solution even at a very high concentration of pure GTP.

When EF-G binds to the pre-translocation ribosome, this could hypothetically lead to a large increase in K_0 , so that $\ell^{GTP} K_0 \ll 1$ off and $\ell^{GTP} K_0 \gg 1$ on the ribosome, allowing for a conformational switch on but not off the ribosome.

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