LOCALIZATION OF THE GTP-BINDING CENTER ON THE ELONGATION FACTOR G

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1. Introduction

It is known that the translocation step at protein biosynthesis requires interaction of the ribosome with a specific protein elongation factor G (EF-G) in the presence of GTP. But the detailed chemical mechanism of the participation of the GTP molecule in this system has yet to be clarified. In the first place it is necessary to know the localization and nature of the GTP-binding center as well as the order of attachment of the individual components leading to the formation of the ternary complex 'ribosome · EF-G · GTP'. To solve this problem we applied the method of photo-affinity labelling using two types of GTP analogs with a photo-activated arylazide group bound to ribose (Guo*-5'-P-P-P) or γ-phosphate (Guo-5'-P-P-P*) residues of the nucleotide molecule [1–3]. It was shown that in the pre-formed ternary complex the GTP-binding center is located on EF-G. We concluded that it is not the ribosome but EF-G that specifically binds the GTP molecule in the ternary complex.

Here we report the results of photo-affinity labelling indicating that EF-G is capable of forming a specific complex with GTP or GDP even without ribosomes. It is shown that localization of the GTP molecule in the binary and ternary complexes is functionally identical.

2. Materials and methods

Ribosomes, EF-G from *E. coli* MRE-600 and photo-activated GTP analogs were obtained as described in [3].

Composition of the mixture for irradiation (0.5 ml) was the following: 1.2 nmol EF-G, 0.2 nmol (about 1.5×10^5 cpm) [^{14}C]Guo*-5'-P-P-P or [^{14}C]Guo-5'-P-P-P*, 10 mM TEA–HCl, pH 8.0 and 20 mM $MgCl_2$. Native nucleotides (GTP, GDP, GMP, ATP, UTP and CDP, Calbiochem) were added to this mixture in the amounts indicated in the legend to fig.1. The mixture was irradiated at 4°C for 3 min (conditions for complete photolysis of the azide group) with a mercury lamp SVD-120A equipped with a BC-4 filter to cut off radiation below 300 nm. The degree of EF-G labelling was determined by precipitating the protein with cold 5% TCA and analysing the radioactivity retained by GF/C glass ultrafilters (Whatman). Labelling of EF-G by the [γ-^{32}P]Guo*-5'-P-P-P analog was done under identical conditions and the modified EF-G was isolated by gel-filtration on Sephadex G-50.

$^{32}P_i$ release from [γ-^{32}P]Guo*-5'-P-P-P covalently bound to EF-G was analysed in the following mixture (0.2 ml): 0.12 nmol EF-G (about 1×10^4 cpm of the ^{32}P-label), 0.25 nmol ribosomes, 10 mM TEA–HCl, pH 8.0 and 20 mM $MgCl_2$. The mixture was incubated at 37°C and the amount of $^{32}P_i$ released was determined according to Kaziro et al. [4]. Hydrolysis of free [γ-^{32}P]GTP and [γ-^{32}P]Guo*-5'-P-P-P was determined under the same conditions.

3. Results and discussion

As a criterion of EF-G affinity labelling we chose the effect of different native nucleotides on the yield of radioactive GTP analogs covalently bound to EF-G. Figure 1 represents the results of EF-G labelling with

Abbreviations: Guo*-5'-P-P-P, (2-nitro, 4-azidobenzoyl) hydrazone of periodate oxidized GTP; Guo-5'-P-P-P*, γ-(4-azidobenzyl)amide of GTP

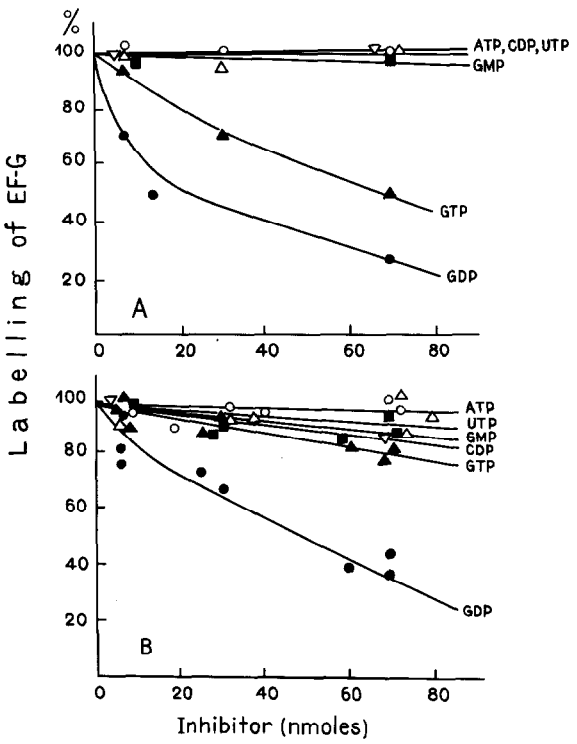

Fig.1. Effect of different nucleoside-5′-phosphates on the degree of EF-G photo-labelling by GTP analogs, [^{14}C]Guo-5′-P-P-P* (A) and [^{14}C]Guo*-5′-P-P-P (B). 100% of labelling corresponds to approx. 3×10^3 cpm and 8×10^3 cpm for A and B, respectively. (■) GMP, (●) GDP, (▲) GTP, (○) ATP, (△) UTP, (▽) CDP.

[^{14}C]Guo-5′-P-P-P* (A) and [^{14}C]Guo*-5′-P-P-P (B) in the presence of different concentrations of GTP, GDP, GMP, ATP, UTP and CDP. It is seen that GDP is the most efficient inhibitor of labelling. GTP possesses a noticeably lesser inhibiting ability in the case of the γ-phosphate analog (fig.1A) and is practically inactive in the case of the ribose analog (fig.1B). The different effects of GTP on EF-G labelling by the analogs may be due to the greater affinity of Guo*-5′-P-P-P for EF-G than that of Guo-5′-P-P-P*. In all the concentration ranges examined the other nucleotides do not inhibit photolabelling of EF-G by either of the analogs.

The data in fig.1 show that the GTP analogs label EF-G at a site which binds only GDP and GTP. In other words, EF-G has a specific affinity for these guanine nucleotides. This result agrees with our earlier conclusion concerning the inherent ability of EF-G to bind GTP or GDP within the ternary complex 'EF-G · GTP(GDP) · ribosome [1–3]. The existence of a specific affinity of GDP or GTP for free EF-G has been also demonstrated by other methods [5–7]. The feature of our approach is the direct labelling of the GTP-binding center of EF-G needed for its analysis within the EF-G primary structure.

A logical consequence of the demonstrated affinity of GTP for free EF-G is the assumption that the binary complex of EF-G with GTP may be the first stage of formation of the ternary complex 'ribosome · EF-G · GTP'. The formulation of such a mechanism of interaction of the ribosome, EF-G and GTP seems attractive. However, in our opinion, it is correct only if the localization of the GTP-binding centers in the free and the ribosome-bound EF-G is identical, i.e., if there are no considerable shifts in the position of the GTP molecule during transition of the binary complex to the ternary.

To investigate this problem we applied a functional approach. It consists of the analysis of the ability of the GTP molecule, covalently fixed at the GTP-binding center of free EF-G, to serve as a substrate of GTPase appearing at the formation of the ternary complex with the ribosome. For this experiment we chose the analog Guo*-5′-P-P-P, shown in [3] to be the substrate of EF-G- and ribosome-dependent uncoupled GTPase (see also fig.3). To observe the process of γ-phosphate release the analog was synthesized from [γ-^{32}P]GTP. The general scheme of the experiment is given in fig.2.

The result of the analysis is shown in fig.3. It is seen that after 20 min of incubation at 37°C the GTP and the free analog are completely hydrolysed. Under the same conditions the analog, covalently bound to EF-G, 'loses' almost half of its radioactivity in the form of $^{32}P_i$. In the absence of ribosomes hydrolysis does not occur. An analogous negative effect is observed in a complete reaction mixture when the magnesium ion concentration is decreased to 0.5 mM, i.e., under conditions which block the ribosome-dependent GTPase [4,8]. The antibiotic thiostrepton, a GTPase inhibitor affecting the ribosomal component, also blocks the appearance of $^{32}P_i$. The functional activity of GTP, covalently bound to EF-G, is not an artefact due to the instability in the chosen conditions of the covalent bond between the analog and EF-G which

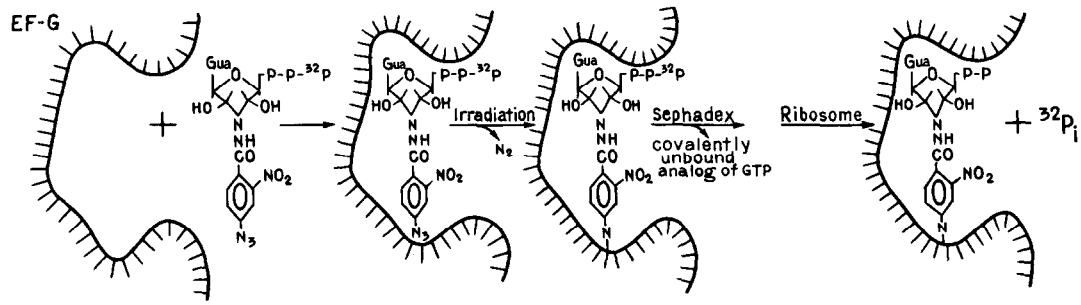

Fig.2. Analysis of the ability of [γ-^{32}P]Guo*-5'-P-P-P covalently bound with EF-G to function as a substrate of the ribosome-dependent uncoupled GTPase (scheme of the experiment).

could lead to the appearance of a free analog in the mixture: after 20 min of incubation at 37°C, EF-G loses, according to TCA precipitation and gel filtration data, no more than 5–10% of the label. Thus the observed hydrolysis of the β–γ-pyrophosphate bond in the GTP analog, covalently fixed to EF-G, is a true ribosome-dependent GTPase reaction. It is noteworthy that the rate of this reaction differs little from that of the free analog.

This result is evidence for the identical localization of the GTP molecule in free and ribosome-bound EF-G and, consequently, is a serious argument in favour of the scheme according to which the interaction of GTP with EF-G precedes the binding of EF-G to the ribosome.

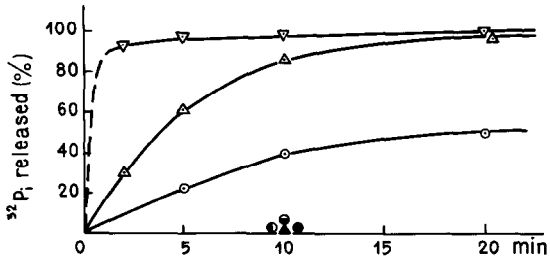

Fig.3. Kinetics of $^{32}P_i$ release at 37°C in a reaction mixture including [γ-^{32}P]GTP (▽), free [γ-^{32}P]Guo*-5'-P-P-P (△) or the same analog covalently bound with EF-G (⊙). (▲) the same as (△), but without ribosomes; (●) the same as (⊙), but without ribosomes; (◐) the same as (⊙), but with 0.5 mM $MgCl_2$; (◒) the same as (⊙), but in the presence of 6×10^{-6} M thiostrepton.

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