THE GTP-BINDING CENTER OF ELONGATION FACTOR G IS LOCATED IN ITS N-TERMINAL DOMAIN

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1. Introduction

A photo-affinity labelling approach has been used by us to study the detailed mechanism of interaction of the GTP molecule with the ribosome and elongation factor G (EF-G). In our previous communications we applied two types of GTP analogs with the photoactivated arylazide group bound to ribose or to the γ-phosphate residues of the nucleotide molecule [1–5]. It was shown that in the preformed 'ribosome·GTP·EF-G' ternary complex the GTP-binding center is located on EF-G [1–3]. Subsequently we found that EF-G is capable of forming a specific complex with GTP or GDP even without ribosomes [5] (see also [6–9]) and that the localization of the GTP molecule in the free and ribosome-bound EF-G is functionally identical [5].

This communication contains the result of limited trypsinolysis of EF-G labelled with the photoactivated GTP analogs, Guo*-5′-P-P-P and Guo-5′-P-P-P*, which permit one to conclude that the GTP-binding center is located in a discrete region of the EF-G, adjacent to the N-terminal and containing the functionally important SH-group.

2. Materials and methods

[^{14}C]Guo*-5′-P-P-P and [^{14}C]Guo-5′-P-P-P* (spec. act. approx. 500 Ci/mol) and EF-G from *E. coli* MRE-600 were obtained as described in [4] and [10], respectively; [^{14}C]iodoacetamide was supplied by the Radiochemical Centre, Amersham, spec. act. 58 Ci/mol; trypsin-TPCK was from Worthington (USA); diallyltartardiamide was prepared as described in [11].

Abbreviations: EF-G elongation factor G; Guo*-5′-P-P-P, (2-nitro, 4-azidobenzoyl) hydrazone of periodate oxidized GTP; Guo-5′-P-P-P*, *p*-azidobenzylamide of GTP

2.1. *Photo-affinity labelling of EF-G*

The irradiated mixture (1 ml) contained: 10 mM TEA–HCl, pH 8.0, 20 mM $MgCl_2$, 32.2 nmol EF-G and 8.8 nmol [^{14}C]Guo*-5′-P-P-P or [^{14}C]Guo-5′-P-P-P*. After irradiation for 3 min at 4°C (mercury lamp SVD-120A (USSR) with a filter to cut off radiation below 300 nm), the labelled EF-G was isolated on Sephadex G-50 with 10 mM Hepes, pH 8.0, as an eluant. The obtained product contained about 0.07 mol or 0.03 mol covalently-bound GTP/mol EF-G for the ribose or γ-phosphate analogs of GTP, respectively.

2.2. *Labelling of EF-G with iodoacetamide [7]*

Conditions of the reaction (1 ml): 50 mM Tris–HCl, pH 7.5, 1 mM EDTA, 12 nmol EF-G and 140 nmol iodo-[^{14}C]acetamide, 30 min at 25°C. The extent of the modification was about 0.6 mol reagent/mol EF-G according to a 5% TCA (trichloroacetic acid) precipitation test. After the reaction, EF-G was isolated on Sephadex G-50 with 10 mM Hepes, pH 8.0 as an eluant.

2.3. *Limited trypsinolysis of the modified EF-G*

This was done by a modification of the method described in [12] (1 ml): 1 mg EF-G, 0.02 mg trypsin-TPCK, 10 mM Hepes, pH 8.0, 37°C and time points (0.05 ml) from 0–60 min. Hydrolysis was

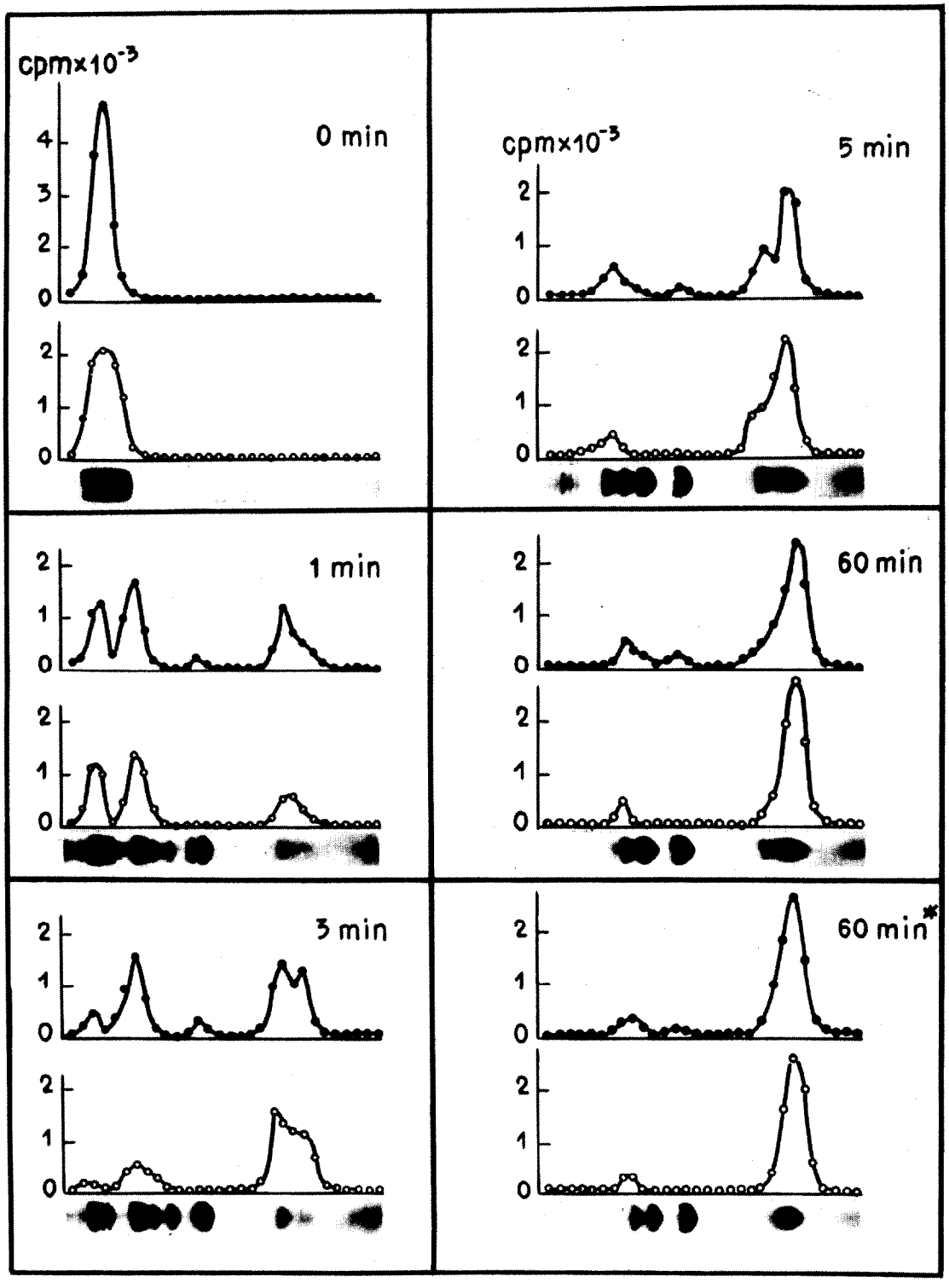

Fig.1. Comparative kinetic analysis of radioactive label distribution between the products of limited trypsinolysis of the EF-G labelled by [^{14}C]Guo*-5′-P-P-P (•——•) or by [^{14}C]iodoacetamide (○——○). The time point 60 min* at bottom right indicates that there was a double amount of trypsin (trypsin/EF-G weight ratio 1:25).

stopped by addition of 0.1 vol. 10% SDS and the product was precipitated by 9 vol. acetone without loss of the radioactive label.

2.4. *SDS–electrophoresis in polyacrylamide gel (10%)*

The acetone precipitate was dissolved in 0.05 ml buffer containing 0.5 mM sodium phosphate, pH 7, 0.1 mM β-mercaptoethanol, 0.1% SDS and after the addition of one drop of glycerol and tracer (methylene blue) was used for electrophoresis. SDS–electrophoresis was done in 10% polyacrylamide gel as described in [13] with diallyltartardiamide instead of the usual bis-acrylamide [11]. After staining with Coomassie brilliant blue R-250 and destaining with water containing 10% acetic acid and 20% methanol, the gel was cut into 2 mm slices and dissolved in 0.5 ml 1% periodic acid at room temperature for 2–3 h. Then 5 ml Triton X-100/toluene (1:2) scintillator was added and the radioactivity was counted on a Beckman LS-100.

The described procedure of staining-destaining is not suitable a priori for EF-G labelled with [^{14}C]Guo-5′-P-P-P* because of the lability of the phosphoamide bond between the γ-phosphate residue and the *p*-azidobenzylamine in the analog molecule. In this case the sample (0.1 ml) was divided into two equal parts, one of which was stained after electrophoresis and the other was not. The length of the unstained gel was measured immediately after electrophoresis and it was cut into slices to determine the radioactivity as described above. It is known that polyacrylamide gel swells during the staining–destaining procedure. Therefore the stained gel after destaining was photographed and its length was made equal to the length of unstained gel during printing of the picture. This permitted the correct identification of the labelled peptide bands in the gel column.

3. Results and discussion

A direct photo-affinity labelling of the GTP-binding center of EF-G [5] permits analysis of this site in the EF-G structure. We used the method of limited EF-G trypsinolysis which, according to [12], leads to a stepwise disruption of some especially sensitive peptide bonds and the formation of discrete fragments. EF-G, photo-affinity labelled with [^{14}C]-Guo*-5′-P-P-P, was treated with trypsin and samples were taken at definite time intervals and subjected to SDS–electrophoresis in polyacrylamide gel to determine the distribution of label in the fragments. A parallel analogous experiment was made with native EF-G, labelled with radioactive iodoacetamide. Such an experiment was particularly interesting for us, since a number of authors have shown that in native EF-G there is a unique SH-group highly sensitive to thiol agents and very important for the functional activity of EF-G [7,8,14]. Therefore it seemed expedient to compare the localization of this SH-group with that of the GTP-binding center in the primary structure of EF-G.

The results are shown in fig.1 where solid circles represent the EF-G labelled with the GTP analog, and open circles the EF-G labelled with iodoacetamide. It is seen that for both reagents the radioactive label is distributed identically and synchronously (coincides at equal time intervals), accumulating at the end of hydrolysis in a single and most mobile peptide which is the same for the GTP analog and for the iodoacetamide. The molecular weight of this peptide determined from its amino acid sequence is 7511 [15]. Use of the γ-phosphate analog of GTP with the photoactivated arylazide group in another site of the nucleotide molecule ([^{14}C]Guo-5′-P-P-P*) resulted in the labelling of the same peptide (fig.2). It follows that the photo-affinity labelled GTP-binding center and the functionally important SH-group are found in the same discrete region of EF-G. This result correlates with the decrease of the chemical reactivity of this SH-group [7,8], or with a change of its environment [9] in the presence of guanine nucleotides.

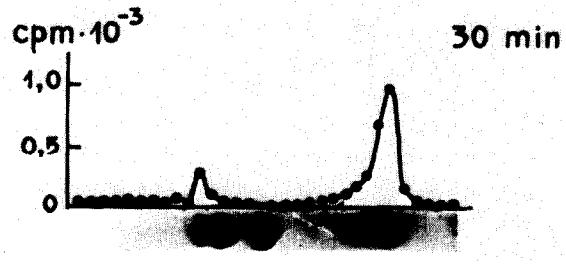

Fig.2. Distribution of the radioactive label between the products of limited trypsinolysis of the EF-G labelled by [^{14}C]Guo-5′-P-P-P*.

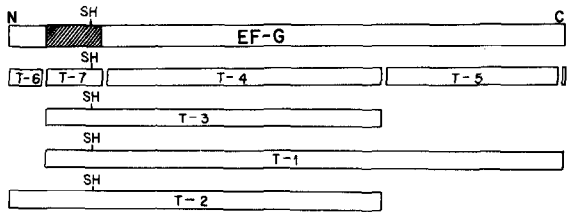

Fig.3. Scheme of arrangement of the products of limited trypsinolysis in the EF-G sequence [10]. The peptide T-7, affinity labelled with the photoactivated GTP analog, is hatched.

In our laboratory [10], an analysis of N-terminal amino acid sequences of intermediate and final products of EF-G limited trypsinolysis was done which permitted them to be arranged along the EF-G chain. The data are represented in the scheme (fig.3). It is seen here that the peptide in question (T-7) is adjacent to the N-terminal of EF-G.

Thus, we can conclude that the GTP-binding center is localized in the N-terminal domain of EF-G containing the functionally important SH-group.

Acknowledgements

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