ON THE INTERACTION OF PERIODATE OXIDIZED GDP AND ITS BOROHYDRIDE REDUCTION PRODUCT WITH THE ELONGATION FACTORS Tu AND G FROM *ESCHERICHIA COLI*

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1. Introduction

Nothing is known about the nucleotide binding sites of polypeptide chain elongation factor EF-Tu or on the EF-G–ribosome complex (nomenclature as in ref. [1]). Also, so far no difference has been reported between their substrate specificities (reviewed in ref. [2]). We have been investigating derivatives of GTP and GDP as an attack on both of these questions.

In order to form a reactive derivative of GTP or GSP which may be useful as an active site label, we followed the strategy of Erlanger and Beiser [3]. They made an immunogenic adduct between nucleotides and serum albumin. First, they formed the dialdehyde derivative by periodated oxidation. Then they made the Schiff's base between this and the amino groups of the protein. Finally, they made a stable covalent link by borohydride reduction.

With this strategy in mind, we prepared periodate oxidized GTP (GTP^{ox}) and its borohydride reduction product (GTP^{ox-red}). We have found that both can substitute for GDP in the binding site of the EF-G–ribosome complex, but neither can bind to EF-Tu. While we have thus far been unable to form a covalent link between GDP^{ox} and the guanine nucleotide binding site, the experiments revealed an important difference between the substrate specificities of EF-Tu and EF-G.

2. Materials and methods

EF-Tu and EF-Ts were prepared by the method of Arai et al. [4] through their G-100 step. Ribosomes were prepared by the method of Bodley [5] and the preparation of EF-G is to be described elsewhere. Millipore binding assays were done by conventional procedures, except that the wash was with 10 mM potassium borate buffer, pH 9.0, 20 mM $MgCl_2$, and the binding was done in the same buffer with 1 mM dithiothreitol. Periodate oxidation of [^{3}H]GDP (5 Ci/mmole) was carried out following the protocol of Offengand and Chen [6] for ATP. This was in 0.1 M sodium acetate buffer pH 5.4, 10 mM $MgCl_2$, 8 mM $NaIO_4$ and 0.1 mM [^{3}H]GDP. After 30 min at 30°C, the excess periodate was reduced by addition of glycerol to 20 mM. Borohydride reduction of the [^{3}H]GDP^{ox} was carried out by the addition of an equal volume of 0.05 M KBH_4 in dimethylformamide followed by incubation at room temperature for 10 min. The resulting nucleotide was added directly to reaction mixtures. Thin layer chromatography of the nucleotides was on polyethyleneimine (PEI) cellulose plates in the system of Cashel et al. [7]. The solvent was 0.75 M potassium phosphate, pH 3.4.

3. Results

Periodate oxidation of [^{3}H]GDP gave a product which remained at the origin during PEI cellulose chromatography as is shown in fig. 1. It can be seen that the reaction went to completion. In addition, borohydride reduction gave the dialcohol which migrated ahead of the parent GDP. This reaction, too, was complete. These two modifications of the parent [^{3}H]GDP were then tested for their interaction with the elongation factors.

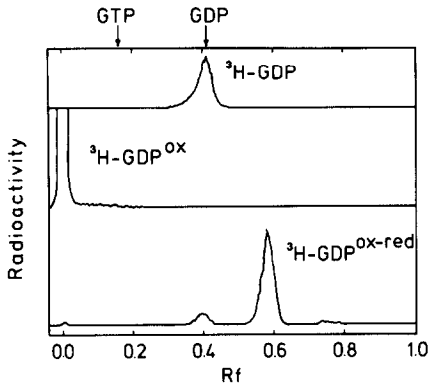

Fig. 1. Thin-layer chromatography of [^{3}H]GDP, [^{3}H]GDP^{ox} and [^{3}H]GDP^{ox-red}. The compounds were prepared as described in Materials and methods and analysed on PEI cellulose plates.

Table 1
Binding of GDP and derivatives to EF-G and the ribosome.

Reaction mixture	Substrate bound (pmoles)		
	[^{3}H]GDP	[^{3}H]GDP^{ox}	[^{3}H]GDP^{ox-red}
Complete	25.4	24.7	22.2
Complete EF-G	0.4	0.1	0.1
Complete ribosomes	<0.1	<0.1	<0.1

Each reaction (50 µl) contained 3 mM fusidic acid, 70 µg ribosomes, 37.5 units EF-G and 50 pmoles of the indicated nucleotide as well as the ionic components described in Materials and methods. Binding of ^{3}H-labelled nucleotide was conducted for 5 min at 0°C following which the samples were filtered and counted. The reported values are averages of triplicate determinations.

In order to maximize the possibility of formation of a Schiff's base, the binding reactions were carried out in the absence of Tris and at pH 9 (see section 2). The binding of [^{3}H]GDP to EF-Tu was indistinguishable from the conventional conditions (data not shown). In addition, as under normal conditions the addition of excess EF-Ts resulted in a very rapid initial rate of binding (fig. 2). However, neither the [^{3}H]GDP^{ox} nor the [^{3}H]GDP^{ox-red} were bound to the EF-Tu, nor was this binding stimulated after a temperature shift from 0°C to 30°C and prolonged incubation. Thus, the cleavage of the ribose moiety eliminates the capacity of GDP to bind to EF-Tu.

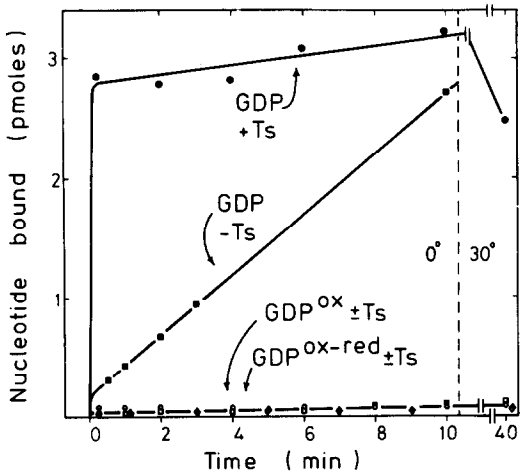

Fig. 2. Binding of [^{3}H]GDP and derivatives to EF-Tu in the presence (●—●—●, ○—○—○, △—△—△) or absence (■—■—■, □—□—□, ▽—▽—▽) of EF-Ts. Each 25 µl aliquot was taken from a reaction mixture at the time and temperature indicated. Each aliquot contained 2 µg EF-Tu, 2 µg EF-Ts and 0.4 µM of [^{3}H]GDP (●—●—●, ■—■—■), [^{3}H]GDP^{ox} (○—○—○, □—□—□), or [^{3}H]GDP^{ox-red} (△—△—△, ▽—▽—▽).

The EF-G binding reaction was also carried out at pH 9. Again, the results were indistinguishable from conventional assay conditions. In this case (table 1), the [^{3}H]GDP^{ox} and [^{3}H]GDP^{ox-red} bound as well as the parent nucleotide under conditions of limiting nucleotide. The requirement for both EF-G and ribosomes was seen with both derivatives. The binding in both cases was therefore specific.

In order to determine whether the EF-G–ribosome GDP^{ox} complex (table 1) involved the formation of a Schiff's base with the active site, the complex was treated with KBH_4. The reduction was performed under conditions where the nucleotide should remain bound and to ensure that this was the case, a large excess of unlabelled GDP was included in the reaction mixture. Control experiments indicated that the reduction of free [^{3}H]GDP^{ox} under these conditions was essentially complete (data not shown). The results in table 2 show that KBH_4 treatment had no significant effect on the amount of labelled nucleotide in complex but, like the untreated complex, the bound nucleotide was TCA unstable.

Table 2
Borohydride treatment in situ of bound GDP^{ox}

	Untreated	Borohydride treated
Buffer wash (pmoles)	22.6	19.9
TCA wash (pmoles)	<0.1	<0.1

The reaction mixtures were identical to that described for [^{3}H]GDP^{ox} in table 1. The untreated samples were incubated for 5 min at 0°C and then analyzed. The borohydride treated samples were first incubated for 5 min at 0°C after which 1 μl of 50 mM GDP and then 5 μl 0.25 M KBH_4 were added. Following these additions, the samples were further incubated for 5 min at 0°C and then analyzed. Millipore filtration analysis was performed either with the usual borate buffer wash (Buffer wash) or the filters were washed with 5% TCA (TCA wash).

4. Discussion

The last experiment (table 2) showed that, while GDP^{ox} participated efficiently in the formation of the EF-G–ribosome–nucleotide complex, no Schiff's base was formed, at least not in a form which was borohydride reducible under our experimental conditions. However, we would like to emphasize that dialdehyde derivatives of nucleotides are potentially useful as active site reagents for other systems. Moreover, this to our knowledge is the first report of the substitution of a ribonucleotide by its periodate oxidation product in the binding site of any enzyme. Since GDP^{ox} bound as well as the parent GDP, this is also a useful starting point for making further modifications of the nucleotide with other kinds of reactive groups.

Hitherto, there has been an exact parallel between the narrow range of nucleotide substrates accepted by EF-Tu and EF-G. In both cases, GDP is the strongest binding nucleotide, but clearly GTP can also bind, at least transiently and GDPCP will also interact with both. All these points are reviewed in ref. [2]. More recently, guanosine 5′-diphosphate, 3′-diphosphate (ppGpp) has been found to interact with both factors [8, 9]. No cases have been documented of interactions with other nucleotides with the exception of dGTP [10] and possibly ITP [11, 12]. These two nucleotides have been reported to substitute for GTP in overall polypeptide synthesis, and therefore can be presumed to interact with both EF-Tu and EF-G. The experiments reported here reveal an all-or-none difference between the substrate specificities of EF-Tu and EF-G. EF-Tu requires the intactness of the ribose moiety, while EF-G does not.

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