AFFINITY PURIFICATION OF ELONGATION FACTORS Tu AND Ts

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1. Introduction

Protein synthesis elongation factor Tu (cf. ref. [1] for a review) is coded for by 2 genes in *Escherichia coli* [2]. It exists in large amounts [3,4] and appears to be associated in part with the cell membrane [4]. These observations raise the question of whether the 2 gene products are identical, or whether they can be distinguished on the basis of structural, functional or topological properties. To answer these questions, the factor must be obtained by a purification procedure which renders any preselection unlikely, a condition not satisfied by conventional methods with recoveries of less than 45% [5,6]. We have therefore prepared a matrix-bound GDP derivative which allows a rapid and nearly quantitative purification of EF-Tu. Furthermore, in order to examine whether the factor may interact with proteins other than those involved in the transfer of aminoacyl-tRNA to ribosomes, we have in turn prepared immobilized EF-Tu. The potential of this method is demonstrated by the fact that it can be used to obtain homogeneous EF-Ts, a protein with which EF-Tu is known to interact during protein synthesis.

2. Materials and methods

2.1. *Protein preparation*

EF-Tu: GDP was purified by the procedure of Arai et al. [5]. Cell-free *E. coli* BE extracts from freshly grown cells (3 g) were made by spheroplast lysis which releases most membrane bound EF-Tu [7]. Proteins precipitated from the 105 000 × *g* supernatant by $(NH_4)_2SO_4$ between saturations of 37% and 64% were dissolved in buffer and used directly as described below.

Abbreviations: EF-Tu and EF-Ts, elongation factors Tu and Ts, respectively; SDS, sodium dodecyl sulfate; DTT, dithiothreitol

2.2. *Affinity matrices*

Immobilization of GDP to aminohexyl-Sepharose 4B (AH-Sepharose, Pharmacia) by oxidation, coupling and reduction was analogous to the procedure used for the covalent attachment of 5′-AMP to an insoluble matrix [8].

Periodate-oxidized GDP (0.1 mmol) was added to 20 ml AH–Sepharose and incubated with gentle stirring for 2 h at 4°C in total vol. 40 ml of 0.1 M Tricine, pH 8.2, 0.5 M NaCl, and was subsequently reduced with $NaBH_4$. In experiments designed to quantitate matrix substitution, [U-^{14}C]GDP (Amersham, England) was used. The final derivative, referred to as GDP–Sepharose, was stored at 4°C in H_2O with a drop of toluene and is stable for at least 3 months.

EF-Tu: GDP (2 mg) was attached to Sepharose 4B, activated [9] with CNBr (150 mg/ml packed resin) in 0.1 M $NaHCO_3$, pH 9.0, 10 mM $MgCl_2$, 10 μM GDP, 0.2 mM DTT and 0.2 M NaCl. The reaction was carried out at 4°C in tightly capped tubes under N_2 with gentle stirring. After 24 h, ethanolamine-acetate, pH 9.0, and DTT were added to final concentrations of 0.2 M and 5 mM, respectively. Stirring was continued an additional 12 h at 4°C. The EF-Tu–Sepharose was washed by centrifugation with four 5 ml portions of buffer A (50 mM Tris–HCl, 1 mM DTT, pH 8.0) containing 10 mM $MgCl_2$ and 10 μM GDP, and was stored in this buffer at 4°C. Before use, the immobilized EF-Tu was washed free of unbound Mg^{2+} and GDP with buffer A.

2.3. *Affinity purification of EF-Tu and EF-Ts*

EF-Tu was adsorbed to washed GDP–Sepharose by mixing equal volumes of packed resin and of $(NH_4)_2SO_4$-fractionated cell extracts, followed by dialysis (12 h, 20°C) against buffer A containing 10 mM $MgCl_2$ and 0.35 M NaCl. The mixture was then packed in a column (0.9 × 5 cm) at 20°C and washed with the same buffer until the absorbance at 280 nm reached baseline. One column volume of buffer containing 100 μM GDP was then allowed to flow into the column bed, and elution was interrupted for 1 h. Subsequent development with GDP-containing buffer yielded EF-Tu in homogeneous form. Purification of EF-Ts was performed after removing EF-Tu from the 37–64% $(NH_4)_2SO_4$ fraction either by heating in buffer A for 10 min at 55°C [10], or by using the unretarded eluate from the GDP–Sepharose (cf. above). After dialysis of the preparations into buffer A, an equal volume of packed EF-Tu–Sepharose was added, and the mixture was dialyzed at 4°C overnight against buffer A containing 0.35 M NaCl. The resin was then transferred to a column and washed with 6 column volumes of the same buffer at 20°C. Pure EF-Ts was eluted with buffer containing 100 μM GDP and 10 mM $MgCl_2$, as described above for EF-Tu.

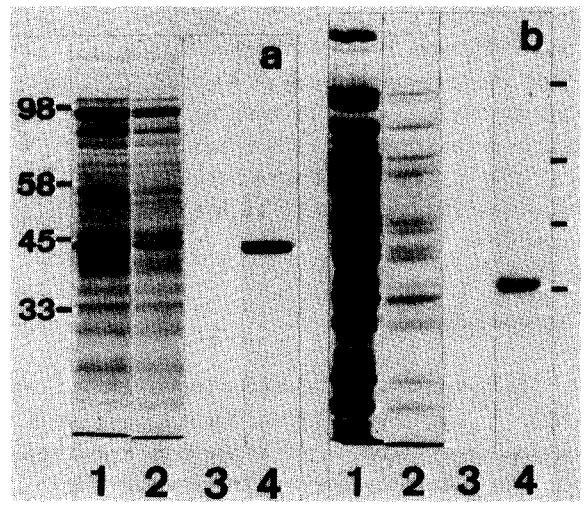

Fig.1. SDS–Polyacrylamide gel electrophoretic analyses of column fractions during affinity purification of EF-Tu and EF-Ts from cell-free *E. coli* extracts. The identities of the purified proteins were confirmed by assays of GDP binding [11] for EF-Tu, and of stimulation of GDP exchange into EF-Tu: GDP for EF-Ts (conditions quoted in ref. [4]). (a) EF-Tu purified on GDP–Sepharose: (1) 37–64% $(NH_4)_2SO_4$ fraction; (2) column wash; (3) column eluate before buffer change; (4) elution of homogeneous EF-Tu with buffer containing 100 μM GDP. (b) EF-Ts purified on EF-Tu-Sepharose: (1) 55°C treated extract (cf. text), purposely overloaded to allow direct comparison with the amount of EF-Ts in panel (4); (2) column wash; (3) eluate before buffer change; (4) elution of EF-Ts with buffer containing 100 μM GDP. The molecular weight scales, indicated in kilodaltons, were obtained with phosphorylase a, catalase, ovalbumin and the catalytic chain of aspartate transcarbamoylase.

3. Results and discussion

Purification of EF-Tu and EF-Ts by affinity chromatography is illustrated in fig.1 by the analyses of the respective column eluates by SDS–Polyacrylamide gel electrophoresis (details quoted in ref. [12]). The coupling of oxidized GDP to AH–Sepharose yielded 0.5 μmol nucleotide covalently attached to 1 ml of the resin, which in turn was found to bind 0.5 mg pure EF-Tu. The final yield of EF-Tu was 80–90% with this procedure, as judged both by GDP binding [11] and by the comparison of gel electrophoretic patterns with those of unbroken cells. It can therefore be concluded that if subpopulations of EF-Tu exist, they all bind guanine nucleotides. The coupling efficiency of EF-Tu to activated Sepharose was > 95%, as determined by SDS–gel electrophoresis of the reaction supernatants and washes. The capacity of EF-Tu–Sepharose for EF-Ts was 0.57 mg/ml packed resin and recovery of EF-Ts after the affinity step was 20% of the amount present in the extract. Optimization has not been attempted in this instance. In both procedures, removal of GDP initially bound to EF-Tu by dialysis of the respective mixtures of extracts and resin greatly increased the efficiency of adsorption of either EF-Tu to GDP-Sepharose, or of EF-Ts to matrix-bound EF-Tu. The relatively high salt concentrations used in both procedures are necessary to prevent nonspecific ionic adsorption of proteins to the matrices. It is also worth noting that the AH–Sepharose-bound GDP derivative is effective in retaining EF-Tu, whereas periodate-oxidized GDP or oxidized and subsequently reduced GDP appears to be a very poor ligand in solution [13].

The procedures described in this report allow purification to homogeneity of EF-Tu and EF-Ts within 24 h. In principle, both should be applicable

to large scale purifications. We have found them particularily suitable for the purification of radioactive EF-Tu [14] and for homogeneous EF-Ts. Also, since the protein-synthesis apparatus is similar in most procaryotes, and in certain respects also in eucaryotes, affinity chromatography may be a convenient method for purifying factors corresponding to EF-Tu and EF-Ts from other organisms.

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