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PROTEIN SYNTHESIS ELONGATION FACTOR EFTu FROM STREPTOMYCES COLLINUS PRODUCING KIRROMYCIN

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Elongation factor EFTu of Streptomyces collinus has been purified. The molecular weight of the factor determined by SDS-polyacrylamide gel electrophoresis is 52,500 (± 1,500). The factor shares common immunochemical determinants with EFTu of Streptomyces aureofaciens and Escherichia coli. The factor is not involved in the mechanism of resistance to the drug produced, since translation of poly(U) in the presence of EFTu and ribosomes of S. collinus is sensitive to the kirromycin effect.

1. INTRODUCTION

Cells of Streptomyces collinus produce the antibiotic kirromycin (1,2). The drug inhibits peptide bond formation of sensitive bacteria by binding to the elongation factor Tu. Kirromycin induces a conformational alteration of EFTu in which it does not dissociate from the ribosomes (3-5). In Escherichia coli genetic analysis (6) and transcription studies (7,8) have revealed the existence of two genes for EFTu. One of them is located near the str-spc region (tuf A) and the second one is near to the rif region (tuf B). However, in cells of Bacillus subtilis only a single functional active gen was found (9). A mutant of E. coli was isolated, in which tuf A and tuf B gene products are resistant to kirromycin (10-13).

In this paper we extend the study of molecular properties of EFTu.GDP from the kirromycin producing strain of S.

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collinus. Our results show that this factor has the highest molecular weight for EFTu.GDP reported up to now. In spite of differences in molecular weights between EFTu.GDP of E. coli 46,000 (14) and S. collinus 52,500, both factors are functionally interchangable in poly(U) translation on ribosomes of E. coli and S. collinus, respectively.

2. MATERIALS AND METHODS

2.1. Material

Escherichia coli A19 was used for isolation of tRNA (15) and aminoacyl tRNA synthetase fraction. For the charging of tRNA with ^{14}C-phenylalanine (360 mCi/mMol) a procedure described in (16) was used.

Kirromycin was isolated from fermentation fluid after cultivation of S. collinus as previously described (1). Purity of the preparation was compared with that of the standard.

2.2. Isolation of ribosomes

Ribosomes of E. coli and S. collinus were isolated according to (17).

2.3. Purification of EFTu.GDP from S. collinus

Electrophoretically homogeneous EFTu.GDP was isolated from postribosomal supernatant fraction (S150) by affinity chromatography on GDP-AH-Sepharose. This method was applied for isolation of EFTu of E. coli (18). The factors used in this study were stored in buffer containing 20 mM Tris-HCl pH 7.6; 10 mM $Mg(OAc)_2$; 10 mM 2-mercaptoethanol and 250 mM sucrose at -70 °C.

2.4. Double immunodiffusion

Ouchterlony immunodiffusion was performed in 1 % Agarose gel containing 10 mM Tris-HCl pH 7.6; NaCl 0.9 % and NaN_3 0.01 % at room temperature. Antibodies against EFTu.GDP from E. coli were prepared as described in (19).

2.5. Determination of molecular weight

Molecular weight of purified EFTu was determined according to mobility in sodium dodecylsulphate-polyacrylamide gel (20). The following molecular weight standards were used: bovine serum albumin (67,000); egg albumin (45,000); DNAase I (31,000) and chymotrypsinogen A (25,000).

2.6. Translation of poly(U)

The poly(U) directed polyphenylalanine synthesis from ^{14}C Phe-tRNA was carried out in 0.1 ml reaction mixtures containing 50 mM Tris-HCl, pH 7.4; 8 mM $MgCl_2$; 60 mM NH_4Cl; 6 mM 2-mercaptoethanol; 0.2 mM GTP; 5 mM phosphoenolpyruvate; 1 µg pyruvate kinase; 40 µg poly(U); 50 pmol ^{14}C Phe-tRNA, 25 pmole ribosomes; 50 pmol EFTu and kirromycin when indicat-

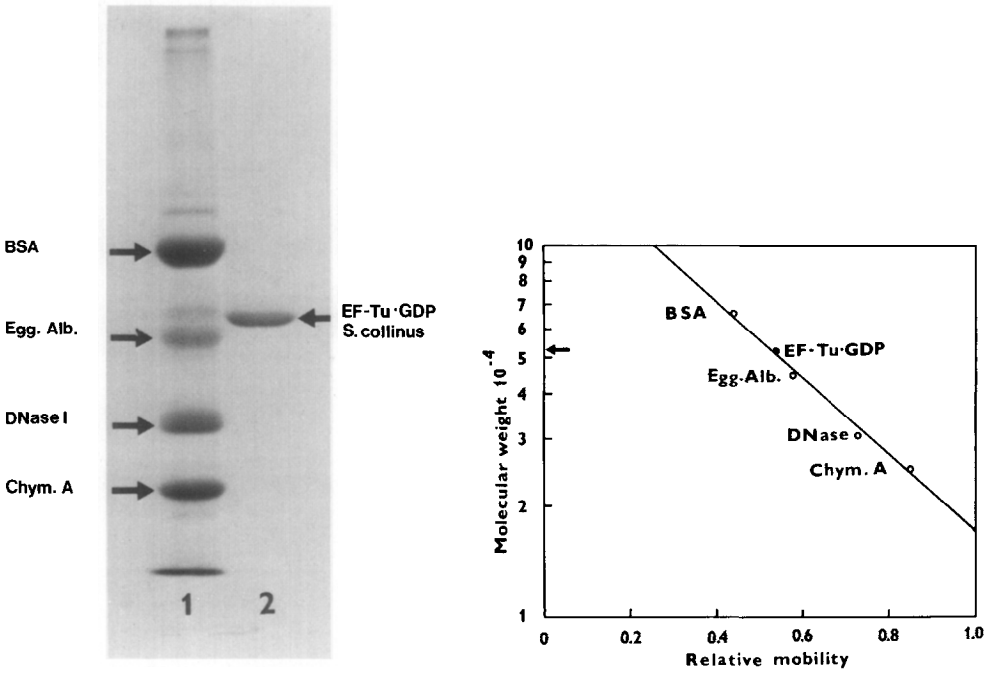

Fig. 1 Sodium dodecylsulfate gel electrophoresis (A) and determination of molecular weight (B) of S. collinus EFTu.GDP. Electrophoresis was performed in 10 % SDS-polyacrylamide gel (20).

A - Slot 1 - molecular weight standards, 8 µg each. Slot 2 - electrophoretic mobility of purified EFTu.GDP (10 µg).

B - The relative mobilities of reference proteins were plotted against the logarithms of their molecular weights.

ed. Incubation was 10 min at 37 °C. Reactions were stopped with 1 ml of 5 % TCA heated for 20 min at 95 °C. The samples were assayed for the incorporation of ^{14}C-label into the TCA-precipitable material.

3. RESULTS AND DISCUSSION

Elongation factor Tu of S. collinus was isolated from the postribosomal supernatant fraction by affinity chromatography on GDP-AH-Sepharose as described in Material and Methods. The purity of the factor, as estimated according to sodium dodecylsulfate gel electrophoresis (Fig. 1A), is at least 95 %.

Molecular weight of the EFTu.GDP (52,500 ± 1,500 $gmol^{-1}$) was calculated from relative mobilities of the factor and re-

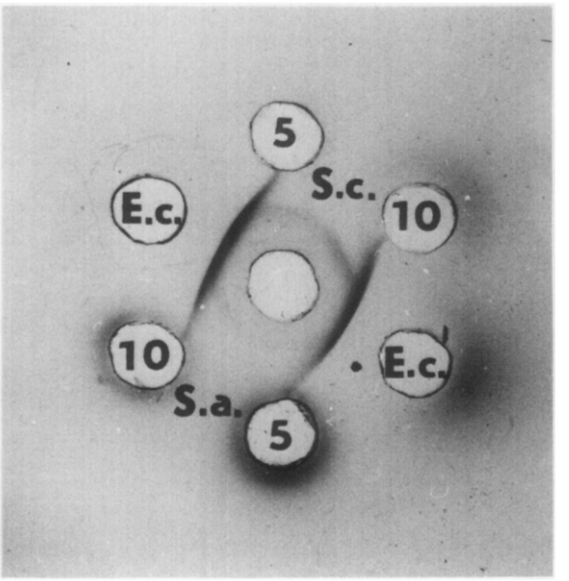

Fig. 2 Double immunodiffusion test with antiserum against EFTu.GDP of E. coli. The center well contained anti-EFTu.GDP (600 ug). The peripheral wells - E.c. 10 ug E. coli EFTu.GDP; S.c. - 5 and 10 ug EFTu.GDP of S. aureofaciens.

ference standard proteins plotted against their molecular weights (Fig. 1B). The data indicate that EFTu.GDP of S. collinus has significantly higher molecular weight than analogous preparations from ordinary Gram-positive or Gram-negative bacteria (21). The factor is stable for one year when stored at -70 °C.

An attempt was made to detect possible structural homologies between EFTu.GDP of S. collinus and Escherichia coli. Antiserum against EFTu of E. coli was tested with EFTu's for immunoprecipitation. The test was performed using Ouchterlony's double diffusion assay (Fig. 2). Center well contained the antibody against EFTu of E. coli and peripheral wells elongation factors Tu. The results showed that EFTu of S. collinus is a protein structurally related to EFTu of E. coli, similarly to EFTu from S. aureofaciens.

TABLE I.
Effect of postribosomal supernatant fractions (S150) on poly(U) directed polyphenylalanine synthesis.

Ribosomes	S150 fractions	Poly(Phe) polymerized (pmol)
E.c.	-	1.2
E.c.	E.c.	42.7
E.c.	S.c.	1.8
E.c.	S.a.	43.4
S.c.	-	1.4
S.c.	S.c.	2.0
S.c.	S.a.	16.8
S.c.	E.c.	17.2

Incubation mixtures contained 25 pmol of ribosomes and S150 fraction (80 ug protein) of E.coli (E.c.); S.collinus (S.c.) or S.aureofaciens (S.a.). Other components of the reaction mixtures were as given in Materials and Methods.

To investigate, whether EFTu from S.collinus is susceptible to kirromycin, we first compared stimulation of poly(U) translation by the postribosomal supernatant fraction (S 150) from S.collinus; S.aureofaciens and E.coli. As shown in Table I, polyphenylalanine synthesis from ^{14}C-Phe-tRNA was stimulated by S150 fractions of S.aureofaciens and E.coli, but S150 fraction from S.collinus has a little stimulatory effect. When the S150 fractions were examined for the presence of EFTu by means of the anti-EFTu of E.coli, precipitation lines analogous to those presented in Fig. 2 were obtained. The S150 fraction of S.collinus was tested for the presence of kirromycin. An ethylacetate extract of the S150 fraction was chromatographed on silica gel plates in three different solvent systems (1). The chromatograms were transferred on an agar plate with the test organism and inhibition zones at the positions corresponding to R_f of kirromycin indicated

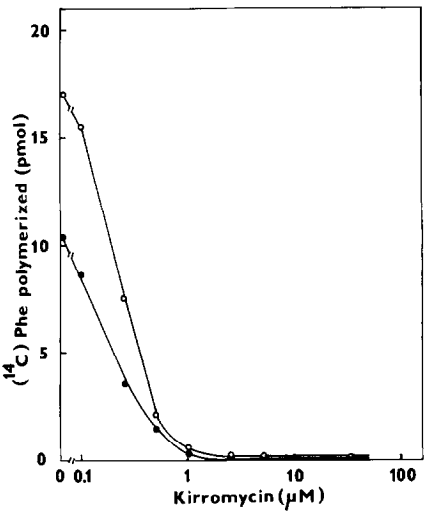

Fig. 3 Effect of kirromycin on polyphenylalanine synthesis stimulated by EFTu.GDP of S. collinus and E. coli. Assays were performed with ribosomes of S. collinus as described in Materials and Methods. (●) EFTu.GDP from S. collinus; (o) EFTu.GDP of E. coli. The factor activity was determined as described in (14).

the presence of the antibiotic (not shown). These experiments suggest that the low activity of the S150 fraction of S. collinus might be induced by kirromycin that probably inactivates EFTu during preparation of cell-free extract.

In the last set of experiments the effect of kirromycin on EFTu.GDP of S. collinus and E. coli at poly(U) directed polyphenylalanine synthesis was determined. The data from this experiment (Fig. 3) indicate again that purified EFTu.GDP of S. collinus (50 pmol) has a lower stimulatory effect on poly(U) translation than an equal amount of the factor of E. coli. The nature of the effect remains to be studied in more detail. The rate of polypeptide synthesis on ribosomes of S. collinus was reduced to 50% by 0.25 μM kirromycin in the presence of EFTu.GDP either of E. coli or S. collinus. We therefore conclude that EFTu from S. collinus is sensitive to kirromycin and is not involved in the mechanism of self-resistance to the drug produced.

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