Vol. 99, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 16, 1981 Pages 16-22

STUDIES ON THE ELONGATION FACTOR Tu FROM STREPTOMYCES AUREOFACIENS PRODUCING TETRACYCLINE

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Received December 3,1980

SUMMARY

Polypeptide chain elongation factor Tu has been purified from the tetracycline producing strain of Streptomyces aureofaciens. The molecular weight of EFTu.GDP determined by dodecylsulphate-polyacrylamide gel electrophoresis is 49000 (± 2000). The factor spontaneously polymerized at a concentration of 1 mg/ml or higher. The binary complex EFTu.GDP from Str.aureofaciens is more thermolabile than that of Escherichia coli. After heating for 2 min at 50°C more than 50 % of the factor activity was lost. The partial immunological cross-reactivity between EFTu of E.coli and Str.aureofaciens indicates that both factors contain certain structural similarities.

1. INTRODUCTION

Polypeptide chain elongation involves the binding of aminoacyl-tRNA to the acceptor site of the ribosome. This binding is mediated by the elongation factor EFTu and GTP via the formation of the ternary complex EFTu.GTP aminoacyl-tRNA (1, 2). Up to the present time EFTu preparations have been purified and characterized from Pseudomonas fluorescens (3), Escherichia coli (4, 5), Bacillus stearothermophilus (6) and Thermus thermophilus (7-9). The EFTu from the thermophilic species are much more resistant to thermal denaturation than the factor of E.coli. Immunological studies with antibody

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0006-291X/81/050016-07$01.00/0

against EFTu of E.coli did not show any crossreactivity with the elongation factor from T.thermophilus (7).

Here, we report on the molecular properties of EFTu from Streptomyces aureofaciens, a producer of tetracycline antibiotics which grows optimally at 28°C. The streptomycete factor, although differing in many properties from E.coli EFTu.GDP, exhibits partial crossreactivity with antibodies raised against the latter protein.

2. MATERIALS AND METHODS

2.1. Materials

Streptomyces aureofaciens 84/25, a tetracycline-producing strain, was obtained from the Research Institute of Antibiotics and Biotransformations (Prague). The vegetative cells were grown at 28°C on a soya medium. After 16 hr of cultivation cells were rapidly chilled and washed with standard buffer containing: 10 mM Tris-HCl, pH 7.5, 10 mM $Mg(OAc)_2$, 60 mM NH_4Cl, 6 mM 2-mercaptoethanol and 0.1 mM phenylmethane sulphonylfluoride.

Escherichia coli A19 was grown at 37°C and harvested at the mid-log phase, chilled and washed with standard buffer. The cells were stored at -80°C until used.

^{3}H-GDP was purchased from the Radiochemical Centre (Amersham) and aminohexyl (AH)-Sepharose 4B from Pharmacia (Uppsala).

2.2. Purification of EFTu.GDP

EFTu.GDP from S.aureofaciens and E.coli was isolated by affinity chromatography on GDP-AH-Sepharose according to Jacobson and Rosenbusch (10). Coupling of GDP to aminohexyl-Sepharose 4B was as described earlier (11). EFTu.GDP preparations were stored in buffer S containing: 20 mM Tris-HCl, pH 7.6, 10 mM $Mg(OAc)_2$, 10 mM 2-mercaptoethanol and 250 mM sucrose at -80°C. EFTu.GDP from E.coli B isolated according to Arai et al. (5) was a gift from Dr. J. Jonák.

2.3. Assay of EFTu activity

The EFTu activity was determined by the exchange of ^{3}H-GDP using nitrocellulose membrane filtration (5) in a reaction mixture (0.1 ml) containing: 50 mM Tris-HCl, pH 7.5, 10 mM $Mg(OAc)_2$, 150 mM NH_4Cl, 10 mM 2-mercaptoethanol, 1.5 uM ^{3}H-GDP (specific activity 12.7 Ci/mmol) and EFTu.GDP as indicated. After 20 min of incubation at 0°C, the reaction was terminated with cold binding buffer. The radioactivity was determined in a liquid scintillation counter.

2.4. Antibodies against E.coli EFTu.GDP

Antibodies against EFTu.GDP from E.coli were isolated as described by Van de Klundert (12).

2.5. Polyacrylamide gel electrophoresis

Purified elongation factors Tu were analysed on dodecylsulphate-polyacrylamide gel slabs according to Laemmli (12) using 10 % acrylamide in the separation gel and 2.5 % in the stacking gel. Proteins were stained with Coomassie brilliant blue.

For the determination of molecular weicht the following standards were used: bovine serum albumin (M.W. 67,000), egg albumin (45,000), DNase I (31,000) and chymotrypsinogen A (25,000).

3. RESULTS AND DISCUSSION

3.1. Purification of EFTu

EFTu.GDP from S.aureofaciens and E.coli were purified by affinity chromatography on GDP-AH-Sepharose as described in Materials and Methods. In a single purification step virtually homogeneous EFTu.GDP was obtained from both organisms as judged by SDS polyacrylamide gel electrophoresis (Fig.1, lanes 2 and 4). EFTu of Streptomyces aureofaciens is abundantly represented among the postribosomal supernatant proteins (lane 5) as is the case with E.coli EFTu (lane 1). Elongation factors Tu-GDP from E.coli A19 and E.coli B displayed identical electrophoretic mobilities which differed from that of EFTu.GDP from S.aureofaciens.

We have found EFTu.GDP from S.aureofaciens to be stable for more than 12 months when stored at -80°C in buffer S at a concentration of 1 mg/ml. At this and higher concentrations the factor forms highly opalescent solutions due to polymerization of the protein as has also been described for E.coli EFTu (13). Polymerized EFTu.GDP from S.aureofaciens was as active in ^{3}H-GDP exchange as the unpolymerized factor (not shown).

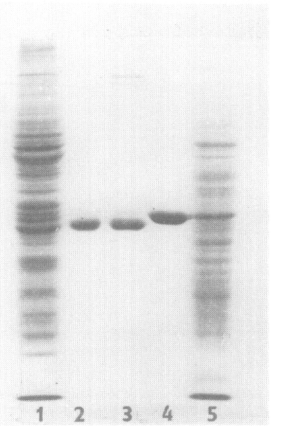
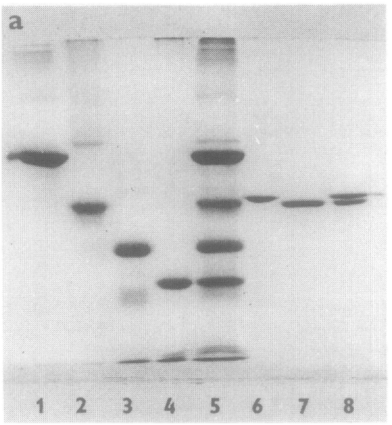


Fig. 1. Sodium dodecylsulphate gel electrophoresis of EFTu.GDP from S.aureofaciens and E.coli. Electrophoresis was carried out according to Laemmli (12). (1) 80 ug of postribosomal supernatant fraction from E.coli A19 before affinity chromatography. (2) 8 ug of EFTu.GDP from E.coli A19 purified by affinity chromatography. (3) 9 ug of EFTu.GDP from E.coli B isolated according to Arai et al. (5) (concentrated fraction after gel filtration on Sephadex G-100). (4) 8.5 ug of EFTu.GDP from S.aureofaciens purified by affinity chromatography. (5) 56 ug of postribosomal supernatant fraction from S.aureofaciens before affinity chromatography.

3.2. Determination of molecular weight

Previously (14) SDS polyacrylamide gel electrophoresis did not reveal significant differences in electrophoretic mobility in 15 % gels between EFTu.GDP from S.aureofaciens and E.coli. In 10 % acrylamide gels, however, we now find the two factors to migrate at different rates (Fig. 1 and 2a). This difference is brought out more clearly by running the two factors simultaneously in one run (Fig. 2a, lane 8).

The molecular weights were determined by plotting the relative mobilities of reference proteins against the logarithms of their molecular weights as 49,000 ($\pm$ 2000) for EFTu.GDP from S.aureofaciens and 46,000 (- 2000) for EFTu.GDP from E.coli (Fig. 2b). The latter value is in good agreement with that published by others (15).



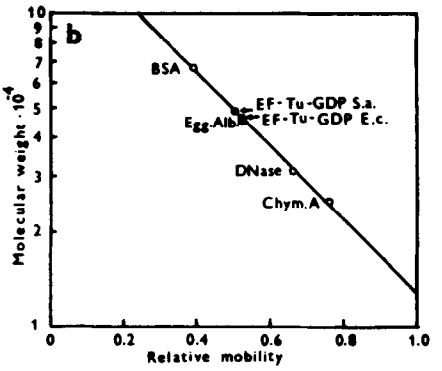


Fig. 2. Sodium dodecylsulphate gel electrophoresis (a) and molecular weight determination (b) of S.aureofaciens and E.coli EFTu.GDP. (a) Electrophoresis was carried out according to Laemmli (12). (1) bovine serum albumin (BSA) 8.8 ug (M_r 67,000). (2) egg albumin (Egg Alb.) 9.5 ug (M_r 45,000). (3) deoxyribonuclease I (DNase I) 8 ug (M_r 31,000). (4) chymotrypsinogen A (Chym. A) 8.5 ug (M_r 25,000). (5) BSA 8.8 ug; Egg Alb. 9.5 ug; DNase I 8 ug; Chym. A 8.5 ug. (6) 4 ug of EFTu.GDP from S.aureofaciens. (7) 7 ug of EFTu.GDP from E.coli A19. (8) 4 ug of EFTu.GDP from S.aureofaciens and 7 ug of EFTu.GDP from E.coli A19. (b) The relative mobilities of reference proteins were plotted against the logarithms of their molecular weights.

3.3. Thermal stability of the factors

Since vegetative cells of S.aureofaciens grow optimally at temperatures around 28°C, we compared the thermal stabilities of EFTu.GDP from S.aureofaciens and E.coli. To this aim the two factors were incubated at 50°C for various periods of time whereafter ^{3}H-GDP exchange was measured at 0°C for 20 min.

As Fig. 3 shows 50 % of S.aureofaciens EFTu.GDP was inactivated after 2 min at 50°C whereas the E.coli factor retained more than 80% of its initial activity under these conditions. Apparently EFTu.GDP from S.aureofaciens is more thermolabile than EFTu.GDP from E.coli.

3.4. Immunological studies

In order to study the question whether the EFTu proteins

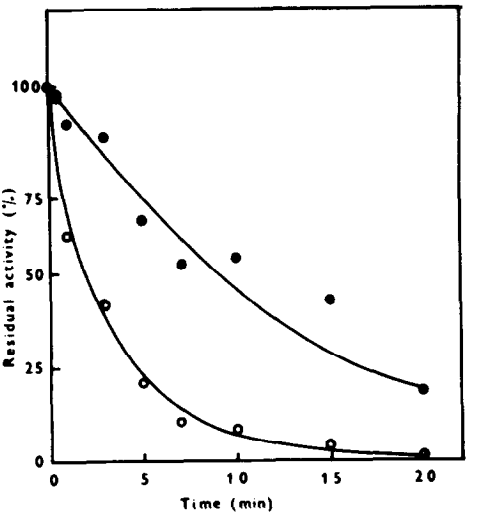


Fig. 3. Thermal inactivation of EFTu.GDP from S.aureofaciens and E.coli. 60 pmol of EFTu.GDP purified either from S.aureofaciens (O) or E.coli (O) were incubated at 50°C in 120 ul of the buffer S. At indicated time intervals 10 ul aliquots were withdrawn and assayed for ^{3}H-GDP exchange at 0°C for 20 min as described in Materials and Methods.

from both sources were structurally similar, antibodies against E.coli EFTu were raised in a rabbit. As is illustrated in Fig. 4a the Ouchterlony double diffusion reaction revealed a partial

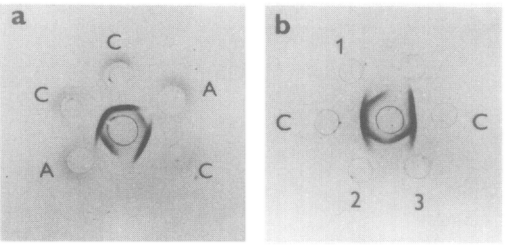


Fig. 4. Double-immunodiffusion test with antiserum against E.coli EFTu.GDP. (a) The center well contained antiserum against E.coli EFTu.GDP (580 ug). The peripheral wells contained 15 ug of EFTu.GDP from E.coli (C) and 15 ug of EFTu.GDP from S.aureofaciens (A).
(b) 870 ug of antiserum against E.coli EFTu.GDP was placed in the center well and the peripheral wells contained 12 ug of E.coli EFTu.GDP (C) and 10, 15 and 30 ug of S.aureofaciens EFTu.GDP (L), (2) and (3), respectively.

cross reactivity between EFTu.GDP from S.aureofaciens and antibodies against E.coli EFTu.GDP. This indicates that the two factors share common immunological determinants, the positions of the precipitation zone being dependent on the concentration of EFTu.GDP (Fig. 4b).

We conclude that in spite of the differences in molecular weights and thermal stabilities the two factors are structurally related.

Further studies on the peptide map and the functional properties of EFTu from S.aureofaciens are in progress.

ACKNOWLEDGEMENTS

We are greatly endebted to Drs. J.A.M. Van de Klundert for providing us with antibodies against E.coli EFTu, and to Dr. J. Jonák for a gift of EFTu.GDP from E.coli B.

4. REFERENCES

1. Gordon, J. (1968) Proc.Natl.Acad.Sci. U.S.A. 59, 179-183.
2. Shorey, R.L., Ravel, J.M., Garner, C.W., and Shive, W. (1969) J.Biol.Chem. 244, 4555-4564.
3. Lucas-Lenard, J., and Lipmann, F. (1967) Proc.Natl.Acad. Sci. U.S.A. 55, 212-219.
4. Miller, D.L., and Weissbach, H. (1970) Arch.Biochem.Biophys. 141, 26-37.
5. Arai, K., Kawakita, M., and Kaziro, Y. (1972) J.Biol.Chem. 247, 7029-7037.
6. Wittinghofer, A., and Leberman, R. (1976) Eur.J.Biochem. 62, 373-382.
7. Arai, K., Ota, Y., Arai, N., Nakamura, S., Henneke, Ch., Oshima, T., and Kaziro, Y. (1978) Eur.J.Biochem.92, 509-519.
8. Arai,K., Arai, N., Nakamura, S., Oshima, T., and Kaziro, Y. (1978) Eur.J.Biochem. 62, 521-531.
9. Nakamura, S., Ohta, S., Arai, K., Arai, N., Oshima, T., and Kaziro, Y. (1978) 62, 533-543.
10. Jacobson, G.R., and Rosenbusch, J.P. (1977) FEBS Letters 79, 8-10.
11. Van de Klundert, J.A.M. (1978) Ph.D. Thesis, Department of Biochemistry, State University of Leiden, The Netherlands.
12. Laemmli, U.K. (1970) Nature 227, 680-685.
13. Beck, B.D. (1979) Eur.J.Biochem. 97, 495-502.
14. Weiser, J., Mikulík, K., and Van de Klundert, J.A.M. (1978) 12th FEBS Meeting, Dresden, Abstract No. 3129.
15. Arai, K., Kawakita, M., Kaziro, Y., Kondo, T., and Ui, N. (1973) J.Biochem. 73, 1095-1105.