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ELONGATION FACTOR Tu OF THE EXTREME THERMOPHILIC HYDROGEN OXIDIZING BACTERIUM CALDEROBACTERIUM HYDROGENOPHILUM

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Protein synthesis elongation factor Tu has been purified from an extreme thermophilic hydrogen oxidizing bacterium Calderobacterium hydrogenophilum. The molecular mass of EF-Tu .GDP is 51 000. The factor is heat stable and loses only 50 % of its activity after heating for 5 min at 80°C. Under mild conditions trypsin cleaved EF-Tu.GDP to four main fragments.Only one fragment of M_r = 20 000 had a mobility similar to the trypsin fragment "B" of Escherichia coli EF-Tu.Other peptide fragments of E.coli and C. hydrogenophilum EF-Tu differed in size,but native preparations of both factors are immunologically similar.

Protein synthesis elongation factor Tu from an extreme thermophile Thermus thermophilus differ from that of mesophilic E.coli in several important features.Complex EF-Tu.EF-Ts of T.thermophilus could not be dissociated by the surplus of GDP to EF-Tu and EF-Ts (1). The factor from T.thermophilus is fully active at 60°C, while EF-Tu of E.coli lost its activity after 5 min incubation at 55°C. Antibodies developed against EF-Tu of E.coli did not cross-react with T.thermophilus EF-Tu. However,recent reports on sequence determination of the tuf gene coding EF-Tu of T.thermophilus showed about 70 % homology with those of E.coli (2,3). Although the molecular basis of thermostability of EF-Tu remains unknown the high content of G+C in T.thermophilus (66 mol %) is implicated in the mechanisms of EF-Tu thermoresistance (2).

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Here, we report on molecular properties of EF-Tu from Calderobacterium hydrogenophilum containing 41 mol % G+C. The factor is structurally related to EF-Tu of E.coli and has been shown to be fully active at 70°C.

MATERIALS AND METHODS

Calderobacterium hydrogenophilum an extreme thermophilic,obligate chemoautotrophic hydrogen oxidizing bacterium was isolated from hydrotherms of Kamchatka.The cells were cultivated in a mineral medium in atmosphere of H_2/O_2CO_2 (7:2:1) at 75°C (4).After a 18 h cultivation the cells were washed twice with standard buffer (10 mM Tris-HCl,pH 7.5, 10 mM magnesium acetate, 60 mM NH_4Cl and 6 mM 2-mercaptoethanol) and frozen at -70°C.

Isolation of EF-Tu.GDP

Postribosomal supernatant proteins were precipitated with ammonium sulphate to 30 % and 70 % saturation.Protein fraction sedimented at 70 % saturation contains the bulk of EF-Tu.Electrophoretically homogeneous EF-Tu.GDP was isolated by affinity chromatography on GDP-Sepharose (5).

Limited tryptic digestion of EF-Tu.GDP

Digestion of EF-Tu.GDP with trypsin was performed as described previously (6). The ratio of the factor to trypsin was 40:1.The reactions were performed at 37°C or at 0°C.At the time intervals 10 µl samples were mixed with 40 µl solutions containing 60 mM Tris-HCl, pH 6.8, 2 % SDS, 10 % glycerol, 5 % 2-mercaptoethanol and 0.001 % bromophenyl blue. The samples were boiled at 95°C for 3 min and subjected to electrophoresis on 15 % SDS-polyacrylamide gels.

Terminal amino acids

The dansyl-Edman method with identification of the newly released N-terminal amino acid at each cycle with dansyl chloride was used to determination of N-terminal residues of EF-Tu and tryptic peptides (7).Carboxypeptidase A was used to determination of C-terminal sequences (8).

Assay of EF-Tu

EF-Tu.GDP (505 pmol) was incubated with 500 pmol $[^3H]$GDP at 0°C in a reaction mixture (0.1 ml) containing 50 mM Tris-HCl, pH 7.8, 10 mM magnesium acetate, 50 mM NH_4Cl and 1 mM dithiothreitol.After a 20 min incubation the reaction mixtures were stopped,filtered through membrane, washed and radioactivity of dry filters was determined.

EF-Tu dependent translation of poly(UG) was performed as in (9).

RESULTS

Polyacrylamide gel electrophoretic analysis under denaturating conditions of the elongation factor Tu from C.hydrogenophilum is shown in Fig.1. The purity of the factor was at least 97 % (Fig.1A lane 1).This factor differs from analogous preparations of E.coli in electrophoretic mobility (lane 4) or when both EF-Tu.GDP prepa-

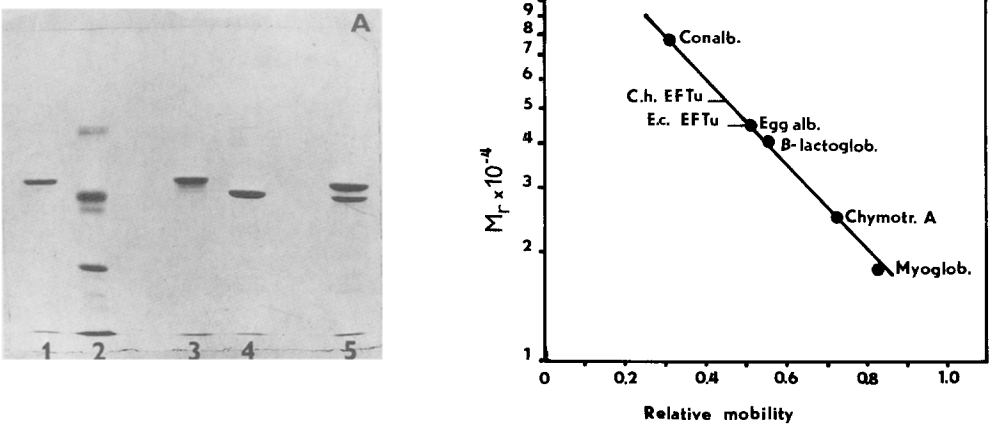


Fig.1.SDS-polyacrylamide gel electrophoresis of C.hydrogenophilum EF-Tu.GDP
(A) Lane 1-C.hydrogenophilum EF-Tu.GDP (5 µg); lane 2-reference proteins (from top: conalbumin M_r=78 000, egg albumin M_r=45 000, β-lactalbumin M_r=40 000,chymotrypsinogen M_r=25 000, myoglobin (M_r=17 000); lane 3-partially purified preparation of C.hydrogenophilum EF-Tu (25 µg); lane 4-E.coli EF-Tu.GDP (10 µg); lane 5-C.hydrogenophilum and E.coli EF-Tu were mixed and run together.
(B) Relative mobilities of the C.hydrogenophilum EF-Tu.GDP and reference proteins.

rations were mixed and run together (lane 5).The relative molecular mass of the EF-Tu.GDP from C.hydrogenophilum (M_r= 51 000) was calculated from mobilities of reference standard proteins plotted against their molecular mass (Fig.1B).

In order to gain some insight into molecular organization of the factor,we decided to dissect the protein with trypsin.The fragments generated were compared with well characterized tryptic digests of E.coli EF-Tu (Fig.2).Trypsin cleaves (at 0°C or 37°C) EF-Tu of C.hydrogenophilum to the main fragments of M_r= 43 000, 41 000, 20 000, 17 000 and 10 000. The high molecular mass fragment (M_r= 43 000) and small fragment (M_r= 10 000) were produced after 1 min incubation at 37°C. The fragment of M_r= 43 000 was then cleaved to a more resistant peptide of M_r= 41 000.Kinetics of trypsinolysis suggest that the fragment of M_r= 41 000 was further digested to fragments of M_r= 20 000 and M_r= 17 000. The data pre-

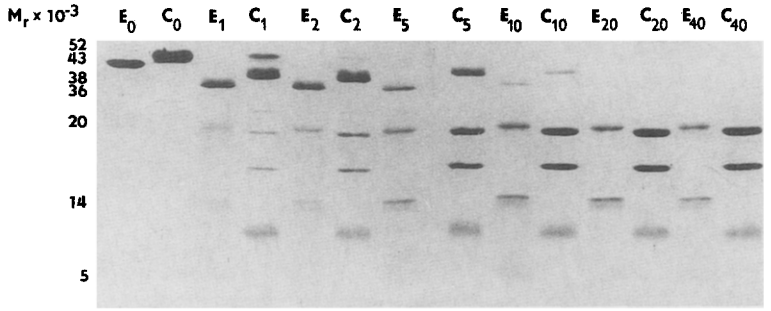
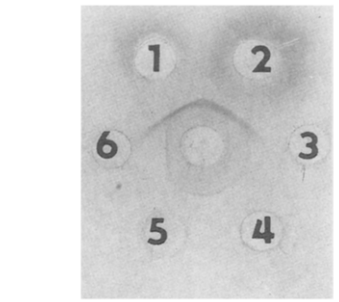


Fig.2.Electrophoretic pattern of limited digestion of E.coli and C.hydrogenophilum EF-Tu.GDP. E_0-E.coli EF-Tu.GDP; E_1 to E_{40} -time dependent trypsinolysis of the factor (min). C_0-C.hydrogenophilum EF-Tu.GDP; C_1 to C_{40}-time dependent digestion of the factor.

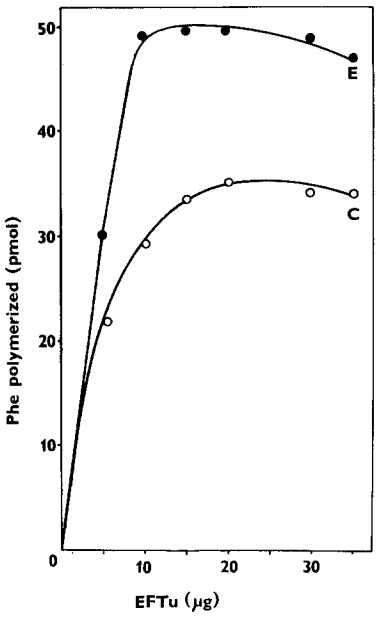
sented in Fig.2 also show that only one fragment of M_r= 20 000 had a mobility similar to the trypsin fragment "B" of E.coli EF-Tu.

We further attempted to locate trypsin fragments with structure of EF-Tu. The following N-terminal sequences were found: the small fragment M_r= 10 000 contains Ala-Lys; the fragments M_r=41 000 and M_r= 20 000 possessed identical sequences Gly-Ile-Thr-Ile-Asn-Thr-. The fragment M_r= 17 000 contains sequences Leu-Ile-. Since the N-terminal amino acid of native EF-Tu contains free Ala, the fragment of M_r= 10 000 is presumably derived from the N-terminal part of EF-Tu. Sequence homologies between N-terminus of fragments M_r= 41 000 and M_r= 20 000 suggest that the last fragment arose from that N-terminal part of the peptide M_r= 41 000. C-terminal amino acids of native EF-Tu,fragments of M_r= 10 000 and M_r= 17 000 were analyzed after Carboxypeptidase A digestion (8).The tentative C-terminal sequences of intact EF-Tu as well as those of fragments M_r= 17 000 are OH-Gly-Val-Ala-.These data indicate that the peptide M_r= 17 000 is derived from C-termini of EF-Tu.

It was shown previously(1) that antibody developed against E.coli EF-Tu did not show any cross-reactivity with the EF-Tu of thermophile microorganisms T.thermophilus. We found immunological similarity between EF-Tu of C.hydrogenophilum and EF-Tu of E.coli (Fig.3).Clear precipitin lines were visible already after a 10 h



(3)



(4)

Fig.3. Immunological reactivity of C.hydrogenophilum EF-Tu with anti-EF-Tu of E.coli. Central well contained the anti EF-Tu of E.coli and peripheral wells 1 and 2 E.coli EF-Tu.Wells 3-6 contained an increasing amount of C.hydrogenophilum EF-Tu (1,5,10,and 20 μg).

Fig.4. Elongation factor Tu dependent translation of poly(UG) on ribosomes of E.coli. Synthesis of polyphenylalanine from $|^{14}C|$Phe-tRNA was carried out in 0.1 ml reaction mixtures as described in (9). E-E.coli EF-Tu; C- C.hydrogenophilum EF-Tu.

of incubation at room temperature. We thus conclude that EF-Tu of E.coli and C.hydrogenophilum are structurally related proteins.

The factor dependent translation of poly(UG) was employed to study EF-Tu specificity. Synthesis of polyphenylalanine from $|^{14}C|$Phe-tRNA on ribosomes of E.coli was examined at 37°C.Results presented in Fig.4 show that C.hydrogenophilum EF-Tu can substitute about 75 % of the activity E.coli EF-Tu. As expected at the temperature optimal for translation of poly(UG) on ribosomes of C.hydrogenophilum (70°C) (10),E.coli EF-Tu was inactive.

In the following experiments the activity of both EF-Tu preparations was examined as a function of temperature. The factors were preincubated for 5 min at different temperatures (35°C to 95°C) and EF-Tu.GDP/$|^{3}H|$GDP exchange was estimated using membrane filter assay. As shown in Fig.5A, E.coli EF-Tu.GDP lost about

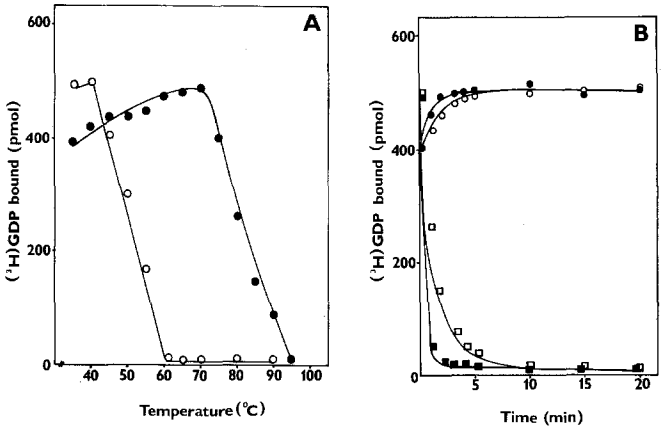


Fig.5. Thermal stability of C.hydrogenophilum and E.coli EF-Tu GDP.
(A) Elongation factors were preincubated at temperatures from 35°C to 95°C for 5 min and incubated for 20 min at 0°C. C.hydrogenophilum EF-Tu.GDP (●); E.coli EF-Tu.GDP (○).
(B) Kinetics of activation of EF-Tu.GDP of C.hydrogenophilum at 60°C (○) and 70°C (●). Temperature dependent inactivation of EF-Tu.GDP of E.coli at 60°C (□) and 70°C (■).

50 % of activity at 55°C and after preincubation at 60°C the factor was inactive. In contrast, the activity of C.hydrogenophilum EF-Tu increases as a function of temperature up to 70°C and at 80°C the factor lost only 50 % of its activity. The complete thermal inactivation of the factor was observed after a 5 min at 95°C. The binding of $|^3H|$GDP stimulated by C.hydrogenophilum EF-Tu was enhanced by thermal activation of the factor. Kinetic of the reaction at 60°C and 70°C is shown in Fig.5B. When the factor activity was examined at 0°C (without activation) the factor possessed about 75-80 % of its maximum activity (after activation).To eliminate possible artifacts of the assay system,analogous experiments with E.coli EF-Tu were simultaneously performed.

DISCUSSION

The experiments reported here are consistent with conception that the thermal stability of C.hydrogenophilum EF-Tu is not accompanied by an extensive structural changes of the factor.This suggestion is supported by results of immunological experiments (Fig.3) demonstrating a structural relationship between C.hydrogenophilum

and E.coli EF-Tu.The thermal stability of EF-Tu of T.thermophilus is connected with a higher G+C content in the DNA (2).However, there are data indicating that the high G+C rate may not be exclusively connected with thermal stability of proteins.Thus,Streptomyces aureofaciens contains 72 mol % of G+C and temperature optimum for growth is 28°C and the EF-Tu lost 50 % activity after 2 min at 50°C (11).On the other hand,Methanothermus fervidus or Methanococcus janaschii contais 33 and 31 mol % of G+C and optimum temperatures for growth are 83°C and 85°C respectively (12).

An interesting difference between the EF-Tu of C.hydrogenophilum and T.thermophilus was found in response to elevated temperatures. The EF-Tu from T.thermophilus had the same activity when heated for 5 min at 20°C to 60°C and then assayed at 0°C for the residual activity (13).In contrast, the preincubation of C.hydrogenophilum EF-Tu at temperatures from 50°C to 70°C (Fig.5A and B) enhanced the rate of $|^{3}H|$GDP exchange significantly.These results indicate that temperatures required for the optimum growth may induce conformational changes of EF-Tu influencing its functional state. Further study using NMR and oligonucleotide directed mutagenesis of the tuf gene should provide more stringent evidence whether this assumption is correct.

REFERENCES

1.Arai,K.,Ota,Y.,Nakamura,S.,Henneke,Ch.,Oshima,T and Kaziro,Y. (1978) Eur.J.Biochem. 92 509-519.
2.Kushiro,A.,Shimizu,M and Tomita,K. (1987) Eur.J.Biochem. 170 93-98.
3.Seidler,L.,Peter.M.,Meissner,F and Sprinzl,M. (1987) Nucl.Acid.Res. 15 9263-9277.
4.Kruykov,V.R.,Savelyeva,N.D and Puscheva,M.A. (1983) Mikrobiologija. 52 781-788.
5.Jacobson,G.R and Rosenbusch,J.P. (1977) FEBS Lett. 79 8-11.
6.Nakamura,S.,Arai,K.,Takahashi,K and Kaziro,Y. (1977) Biochem.Biophys.Res.Commun. 77 1418-1424.
7.Gray,W.M. (1972) Methods Enzymol. 25 121-138.

8. Ambler,R.P. (1972) Methods Enzymol. 25 262-272.
9. Mikulík,K.,Jiráňová,A.,Weiser,J.,Janda,I.,Šťastná,J and Nguyen,Q. (1983) Biochim.Biophys.Acta. 740 99-107.
10. Mikulík,K.,Maňas,J.,Jiráňová,A.,Qiao,Ch-L. and Zavarzin,G.A. (1987) in Lithotrophy (Schlegel,H.G and Gottschalk,G eds) p.40, Golze-Druck,Göttingen.
11. Weiser,J.,Mikulík,K and Bosch,L. (1981) Biochem.Biophys.Res. Commun. 99 16-22.
12. Jones,W.J.,Nagle,D.P.and Whitman,W.B. (1987) Microbiol.Rev. 51 135-177.
13. Nakamura,S.,Ohta,S.,Arai,K.,Arai,N.,Oshima,T and Kaziro,Y. (1978) Eur.J.Biochem. 92 533-543.