GTP PROTECTION OF A LABILE AMINO ACID POLYMERIZATION FACTOR

Nicholas W. Seeds and Thomas W. Conway
Department of Biochemistry, University of Iowa
Iowa City, Iowa

Received August 21, 1967

The amino acid polymerization activity of Escherichia coli extracts has been shown to be very heat labile (Allende et al., 1964). Fractionation of the extracts has led to the isolation of three transfer factors (Ts, Tu and G), and the most labile factor has been designated Tu (Lucas-Lenard and Lipmann, 1966). Since attempts to protect the more purified preparations of Tu have failed, the frequent fractionation of crude enzymes has been necessary.

The recent observation (Allende et al., 1967), that the heat lability and the behavior on DEAE-Sephadex chromatography of a very active GTP-binding activity in E. coli extracts resembles the Tu activity, suggested an interaction between GTP and Tu; however, a contaminating GTP-binding activity in the Tu fraction could not be ruled out. We have examined the possibility that a GTP-Tu interaction might lead to stabilization of Tu. It will be shown here that GTP protects the most labile transfer factor against heat and urea inactivation, thus indicating the identity of Tu and the GTP-binding activity.

Materials and Methods. E. coli B early log cells (Grain Processing Corp.) were the source of the ribosomes and the soluble amino acid polymerization enzymes. The preparation of NH_4Cl-washed ribosomes has been described previously (Seeds et al., 1967) as has the preparation of ^{14}C-phenylalanyl-tRNA (Conway, 1964). Sucrose-washed ribosomes free of the Ts transfer factor were isolated by the procedure of Lucas-Lenard and Lipmann (1966).

The preparation of the E. coli amino acid polymerizing enzymes (65% AS fraction), the γ-^{32}P-GTP, the assay for polyphenylalanine synthesis, and the assay for ribosome-dependent GTPase have been previously described (Conway and Lipmann, 1964). The assay mixtures for polyphenylalanine synthesis contained 12.5 μmoles of Tris-Cl, pH 7.4; 40 μmoles of NH_4Cl; 2 μmoles of $MgCl_2$; 1.75 μmoles of 2-mercaptoethanol; 50 mμmoles of GTP; 10 μg of poly U; 0.10 to 0.15 mg of NH_4Cl-washed ribosomes; 0.13 mg (6560 cpm) of ^{14}C-phenylalanyl-tRNA, 76 μc/μmole, and the indicated amounts of polymerizing enzymes in a final volume of 0.25 ml. The mixtures were incubated for 10 min at 30° and terminated by the addition of 2.5 ml of 5% trichloroacetic acid. The ribosome-dependent GTPase assay contained 12.5 μmoles of Tris-Cl, pH 7.4; 20 μmoles of NH_4Cl; 2.5 μmoles of $MgCl_2$; 1.75 μmoles of 2-mercaptoethanol; 10 μg of poly U; 0.18 mg of deacylated tRNA; 0.10 mg of NH_4Cl-washed ribosomes; 50 mμmoles of γ-^{32}P-GTP (24,000 cpm) and various amounts of enzyme in a final volume of 0.25 ml. Incubation was at 30° for 10 min.

The assay for Ts was identical to that for polyphenylalanine synthesis except sucrose-washed ribosomes replaced the NH_4Cl-washed ribosomes, and the enzyme fractions were heated at 55° for 4 min before being assayed. The formation of a GTP-protein complex which was retained by millipore membranes served as a measure of Tu activity (Allende et al., 1967). The enzyme fraction was allowed to react with 5 mμmoles of ^{3}H-GTP (1 mc/μmole) in 0.25 ml of a 0.05 M Tris-Cl pH 7.4, 0.05 M NH_4Cl, 0.01 M $MgCl_2$ buffer at 0° for 2 min.

Results and Discussion. GTP protects the amino acid polymerization activity from heat inactivation at 50° as illustrated in Figure 1. The small loss of activity in the presence of GTP may represent the partial inactivation of G or Ts which is known to occur at 50° (Lucas-Lenard and Lipmann, 1966). We find a similar GTP protection when the T (Ts and Tu) material from DEAE-Sephadex chromatography is heated. The G factor is not needed during the heat treatment of T, thus eliminating the possibility that stabilization of Tu requires the formation of a Tu-GTP-G complex.

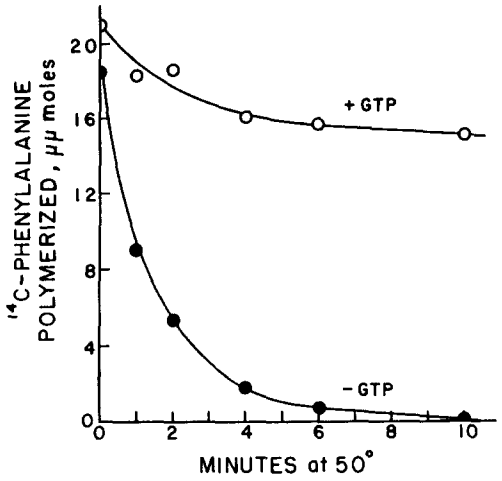

Figure 1. GTP protection of the heat labile transfer activity. The amino acid polymerizing enzymes (16.5 μg protein of a 65% AS fraction) were heated at 50° for various times in 50 μl of a 0.01 M Tris-Cl pH 7.4, 0.01 M $MgCl_2$, 0.05 M NH_4Cl buffer which contained 50 mμmoles of GTP where indicated. After heating, the mixtures were assayed for phenylalanine polymerization ability as described in Methods.

Ribonucleoside triphosphates other than GTP were assayed for their ability to protect the labile polymerizing enzyme against heat inactivation, and Table I shows the high specificity of GTP in this reaction. The possibility that other guanosine phosphates would protect the labile factor was also examined. Since GDP (Conway and Lipmann, 1964) and 5'guanylyl methylene-diphosphonate (GMP-PCP) (Hershey and Monro, 1966) inhibit polymerization, the heated enzyme fractions were dialyzed before they were assayed for amino acid polymerization activity. As shown in Table II, guanosine and GMP offer very little protection while GDP protects as well as GTP; the analog GMP-PCP shows some protection. A similar specificity has been shown for GDP and GMP-PCP for inhibiting the binding of ^{3}H-GTP to a soluble E. coli protein (Allende et al., 1967).

Since we had earlier observed a loss of amino acid polymerization activity in urea solutions, we examined the stability of the individual factors in differ

TABLE I

Nucleoside Triphosphate Specificity for Protection of a Heat Labile Transfer Factor

Nucleoside triphosphate	^{14}C-phenylalanine polymerized ($\mu\mu$moles)	
	50°	0°
None	7.8	27.8
CTP	4.3	26.6
UTP	9.5	29.8
ATP	3.8	24.5
ITP	8.7	28.1
GTP	27.7	31.9

Reaction mixtures contained 0.5 μmoles of Tris-Cl, pH 7.4; 0.5 μmoles of $MgCl_2$; 2.5 μmoles of NH_4Cl; 16.5 μg protein of a 65% AS fraction, and 50 mμmoles of nucleoside triphosphate in a final volume of 50 μl. The mixtures were incubated at 50° or 0° for 4 min. The incubated mixtures (50 μl) were assayed for polyphenylalanine synthesis as described in Methods.

TABLE II

Protection of the Heat Labile Transfer Factor by Guanosine Derivatives

Guanosine derivative	Per cent of control[a]
None	38%
Guanosine	47%
GMP	35%
GDP	83%
GTP	76%
GMP-PCP	62%

[a] % of control = $\frac{\mu\mu\text{moles of phenylalanine polymerized with heated enzyme}}{\mu\mu\text{moles of phenylalanine polymerized with unheated enzyme}}$ (100)

30 $\pm$ 3 $\mu\mu$moles of phenylalanine was polymerized by the unheated enzyme.

Reaction mixtures contained 5 μmoles of Tris-Cl, pH 7.4; 5 μmoles of $MgCl_2$; 25 μmoles of NH_4Cl; 0.33 mg protein of a 65% AS fraction, and 0.5 μmoles of a guanosine derivative in a final volume of 0.5 ml. Duplicate reaction mixtures were prepared; one was incubated at 50° for 4 min and the other was kept at 0°. After the heat treatment each reaction mixture was dialyzed overnight against 200 volumes of 0.01 M Tris-Cl, pH 7.4; 0.001 M dithiothreitol (DTT), and 10^{-4} M EDTA. The dialyzed samples were assayed for amino acid polymerization activity as described in Methods.

ent concentrations of urea. Table III shows that the Tu activity is the most sensitive to urea, G less sensitive and Ts the least sensitive. Thus the factors show the same order of sensitivity to urea as they do to heat. Consequently, we tested to see if GTP would protect the labile amino acid polymerizing factor in 4 M urea. Table IV indicates that GTP can also protect the urea-labile polymerization activity.

TABLE III

The Effect of Urea on the Amino Acid Polymerization Factors

Urea concentration	Per cent activity remaining			
	Amino acid polymerization	Tu	Ts	GTPase
0	100(949 cpm)	100(504 cpm)	100(2608 cpm)	100(17906 cpm)
2 M	81	84	93	85
3 M	67	51	86	82
4 M	46	17	74	52

Reaction mixtures contained 1 μmole of Tris-Cl, pH 7.4; 0.1 μmole of DTT; 0.6 mg protein of a 65% AS fraction, and the indicated amounts of urea in a final volume of 0.1 ml. The reactions were kept at 4° for 4 hr. Aliquots of 5 μl were assayed for Ts, GTPase, and amino acid polymerization activity, and 20 μl aliquots for GTP binding to Tu. All assays were performed as described in Methods.

TABLE IV

GTP Protection of Urea-Labile Transfer Activity

Additions	^{14}C-phenylalanine polymerized (μμmoles)
None	38.5
0.001 M GTP	37.5
4 M Urea	17.5
4 M Urea + 0.001 M GTP	41.0

Reaction mixtures contained 2 μmoles of Tris-Cl, pH 7.4; 2 μmoles of $MgCl_2$; 0.2 μmoles of DTT; 1.3 mg protein of a 65% AS fraction, and the appropriate additions in a final volume of 0.2 ml. The reaction mixtures were kept at 4° for 5 hr. The amino acid polymerization activity of 5 μl aliquots was determined as described in Methods.

Allende et al., (1967) have gathered a considerable amount of experimental evidence that indicates a relationship between the Tu polymerization factor and a GTP-binding activity; however, they could not rule out the possibility that ^{3}H-GTP might be binding to a protein that chromatographed like Tu, had a similar heat lability, and was washed from the ribosomes with 0.5 M NH_4Cl. The high degree of specificity of GTP for protecting the Tu enzyme from inactivation by heat and urea strongly supports the identity of the GTP binding factor and Tu.

Because the lability of Tu has frustrated attempts to purify it, formation of a stable Tu-GTP complex may facilitate the isolation of this activity. Studies now in progress are aimed at exploring the possibility of an interaction between the Tu-GTP complex and ribosomes, tRNA, or other transfer factors in the hope that we may learn more about the function of this complex in protein synthesis and its relationship to other reactions requiring GTP (Nishizuka and Lipmann, 1966; Seeds and Conway, 1966; Lucas-Lenard and Lipmann, 1967; Allende and Weissbach, 1967; Ravel, 1967; Skogerson and Moldave, 1967; Gordon and Lipmann, 1967).

Acknowledgements

The authors are grateful for the technical assistance of Therese Cheng and for support of this work by the National Science Foundation, Research Grant No. GB-4703.

References

Allende, J. E., R. Monro, and F. Lipmann, Proc. Natl. Acad. Sci. 51, 1211 (1964).
Allende, J. E., N. W. Seeds, T. W. Conway, and H. Weissbach, Proc. Natl. Acad. Sci. 58, (1967); in press.
Allende, J. E. and H. Weissbach, Biochem. Biophys. Res. Comm. 28, 82 (1967).
Conway, T. W., Proc. Natl. Acad. Sci. 51, 1216 (1964).
Conway, T. W. and F. Lipmann, Proc. Natl. Acad. Sci. 52, 1462 (1964).
Gordon, J. and F. Lipmann, Proc. Natl. Acad. Sci. 58, (1967); in press.
Hershey, J. W. B. and R. E. Monro, J. Mol. Biol. 18, 68 (1966).
Lucas-Lenard, J. and F. Lipmann, Proc. Natl. Acad. Sci. 55, 1562 (1966).
Lucas-Lenard, J. and F. Lipmann, Proc. Natl. Acad. Sci. 57, 1050 (1967).
Nishizuka, Y. and F. Lipmann, Proc. Natl. Acad. Sci. 55, 212 (1966).
Ravel, J. M., Proc. Natl. Acad. Sci. 57, 1811 (1967).
Seeds, N. W. and T. W. Conway, Biochem. Biophys. Res. Comm. 23, 111 (1966).
Seeds, N. W., J. A. Retsema, and T. W. Conway, J. Mol. Biol. 27 (1967); in press.
Skogerson, L and K. Moldave, Biochem. Biophys. Res. Comm. 27, 568 (1967).