THIOSTREPTON: A RIBOSOMAL INHIBITOR OF TRANSLOCATION

by

Sidney Pestka
Roche Institute of Molecular Biology
Nutley, New Jersey 07110

Received June 24, 1970

SUMMARY

The peptide antibiotic thiostrepton inhibits translocation through inhibition of a 50S subunit function which is linked to or a part of the events involved in translocation. The antibiotic does not significantly inhibit aminoacyl-tRNA binding to ribosomes or peptide bond formation at concentrations which inhibit protein biosynthesis and translocation greater than 90%.

The peptide antibiotic thiostrepton (byramycin) has been reported to be an inhibitor of protein synthesis by Weisblum and Demohn (1). They also demonstrated that the antibiotic is a specific inhibitor of the 50S subunit of bacterial ribosomes (1) and that it inhibited protein biosynthesis in both cell-free extracts and intact cells of Bacillus megaterium. Genetic determinants of resistance to the antibiotic have been shown to be closely linked to genetic markers for resistance to other antibiotics which interfere with ribosomal functions (2). Recently, the chemical and x-ray crystallographic structure of thiostrepton has been reported (3).

This communication reports the effect of thiostrepton on various steps of protein biosynthesis. Thiostrepton was found to inhibit both enzymic and non-enzymic translocation in assays developed in this laboratory (4, 5). Thus, thiostrepton inhibits translocation through a direct action on the 50S subunit rather than through inhibition of supernatant factor G (6).

EXPERIMENTAL PROCEDURE

Ribosomes, supernatant enzymes, [^{14}C]Phe-tRNA and N-acetyl-[^{3}H]Phe-tRNA were prepared as previously described (4, 7). Assays for aminoacyl-

tRNA binding to ribosomes (8), N-acetyl-phenylalanyl-puromycin formation (7), protein synthesis (9), and enzymic and non-enzymic translocation (4, 5) have been reported previously. In this communication as well as prior reports from this laboratory factor G-independent oligophenylalanine synthesis is defined as non-enzymic translocation (4).

RESULTS

Thiostrepton is a potent inhibitor of polyphenylalanine synthesis by E. coli cell-free extracts (Table 1) as previously shown for extracts from B. megaterium (1). At 10^{-7} M thiostrepton, inhibition of polyphenylalanine synthesis was first detectable; at 10^{-6} M thiostrepton, protein synthesis was inhibited greater than 95%. Little or no inhibition of peptide bond

TABLE 1

EFFECT OF THIOSTREPTON ON POLYPHENYLALANINE SYNTHESIS BY CELL-FREE E. COLI EXTRACTS

THIOSTREPTON MOLARITY	pMOLES [^{14}C]Phe INCORPORATED	PERCENT OF VALUE IN ABSENCE OF ANTIBIOTIC
0	1.22	100
10^{-7} M	1.04	85
10^{-6} M	0.02	2
10^{-5} M	0.01	1
10^{-4} M	0.06	5

Each 0.050-ml reaction mixture contained the following components: 0.05 M Tris-acetate, pH 7.2; 0.02 M magnesium acetate; 0.05 M potassium acetate; 0.002 M GTP; 0.02 M phosphoenolpyruvate; 15 μg of phosphoenolpyruvate kinase from Sigma; 0.3 A_{260} unit of E. coli ribosomes; 29 nmoles of base residues of poly U; 30 μg of 100,000 x g supernatant and 5.3 pmoles of [^{14}C]Phe-tRNA (367 mCi/mmole). Reactions were incubated at 37° for 3 min and assays performed as described previously (4, 9). [^{14}C]Phe incorporated refers to incorporation into trichloroacetic acid insoluble material after alkaline hydrolysis of reaction mixtures.

TABLE 2

EFFECT OF THIOSTREPTON ON N-ACETYL-PHENYLALANYL-PUROMYCIN SYNTHESIS

ANTIBIOTIC	pMOLE N-ACETYL-PHE-PUROMYCIN	PERCENT CONTROL
NONE	0.47	100
THIOSTREPTON	0.44	94

Each 0.05-ml reaction mixture contained the following components: 0.05 M Tris-acetate, pH 7.2; 0.4 M KCl; 0.04 M $MgCl_2$; 0.06 M NH_4Cl; 4.1 A_{260} units of ribosomes; 1.5 pmoles of N-acetyl-[^{3}H]Phe-tRNA (0.27 A_{260} unit and 3948 mCi/mmole); 10^{-3} M puromycin; and 10^{-4} M thiostrepton where indicated. Assays were performed as described previously (7).

formation as measured by N-acetyl-phenylalanyl-puromycin formation was observed at 10^{-4} M concentration of the antibiotic (Table 2). In addition, thiostrepton did not affect Phe-oligonucleotide binding to ribosomes (10). Although not shown here binding of Phe-tRNA to ribosomes in response to poly U was inhibited about 30 percent by an antibiotic concentration of 10^{-5} M; however, little inhibition was observed at 10^{-6} M; and no inhibition of Phe-tRNA binding to 30S ribosomes in response to poly U was seen. Furthermore, little or no inhibition of Lys-tRNA binding in response to poly A or of [^{14}C]tRNA binding to 70S ribosomes was observed.

Oligophenylalanine can be synthesized by ribosomes in the presence or absence of transfer factor G and GTP (4). Effect of inhibitors on factor G-dependent and -independent translocation can be used to localize the site of action of inhibitors of translocation. In the absence of factor G the synthesis of oligophenylalanine (designated non-enzymic translocation) represents the ribosomal events of translocation. Thus, inhibitors of factor G itself such as fusidic acid, p-chloromercuribenzenesulfonate or guanylyl 5'-methylene diphosphonate inhibit factor G-depen-

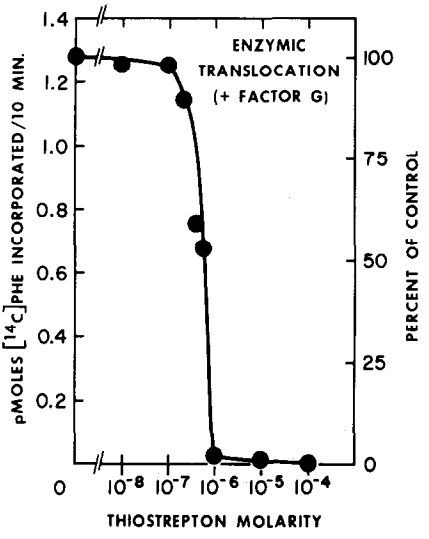

Fig. 1

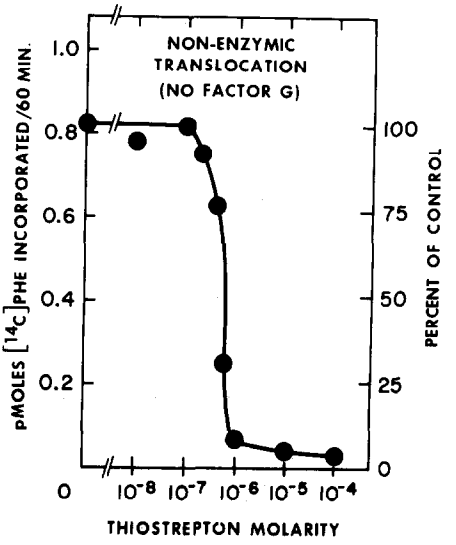

Fig. 2

Fig. 1. Rate of enzymic translocation as a function of thiostrepton concentration. Each 0.050-ml reaction mixture contained the following components: 0.05 M Tris-acetate, pH 7.2; 0.05 M potassium acetate; 0.02 M magnesium acetate; 0.6 A_{260} unit of ribosomes; 0.2 A_{260} unit of poly U; 1.3 μg of factor G; 2 x 10^{-4} M GTP; 4.8 pmoles of [^{14}C]Phe-tRNA (367 mCi/mmole); and thiostrepton concentration as indicated on the abscissa. Reactions were incubated at 37° for 10 min. [^{14}C]Phe incorporation into trichloroacetic acid precipitable material was assayed as reported previously (4).

Fig. 2. Rate of non-enzymic translocation as a function of thiostrepton concentration. Each 0.050-ml reaction mixture contained the components indicated in the legend to Fig. 1 except for the following changes: GTP and factor G were omitted; 0.8 A_{260} unit of ribosomes; 0.1 A_{260} unit of poly U. Thiostrepton concentration is given on the abscissa. Reactions were incubated at 37° for 60 min and [^{14}C]-Phe incorporation into trichloroacetic acid precipitable material assayed as reported previously (4).

---

dent, but not factor G-independent translocation (4). Thiostrepton inhibited both enzymic and non-enzymic translocation as shown by the data of Figs. 1 and 2 respectively. At 10^{-6} M, thiostrepton inhibited both factor-dependent and -independent translocation greater than 90%; 50% inhibition occurs at about 6 x 10^{-7} M thiostrepton, a concentration at which there are about 1.5 molecules of thiostrepton per ribosome. Inhibition of translocation by thiostrepton can be reversed by addition of excess 50S

TABLE 3

EFFECT OF THIOSTREPTON ON RIBOSOME

AND FACTOR G GTPase ACTIVITY

COMPONENTS	pMOLES [^{32}P] RELEASED		% OF CONTROL IN ABSENCE OF THIOSTREPTON
	NO THIOSTREPTON	10^{-6} M THIOSTREPTON	
+ RIBOSOMES - FACTOR G	15	15	100
- RIBOSOMES + FACTOR G	12	13	108
+ RIBOSOMES + FACTOR G	183	15	8

Each 0.050-ml reaction mixture contained the following components: 0.05 M Tris-acetate, pH 7.2; 0.05 M potassium acetate; 0.02 M magnesium acetate; 10^{-4} M 2-mercaptoethanol; 2 x 10^{-5} M γ-[^{32}P]GTP; 1.4 μg factor G where noted; 0.26 A_{260} unit of ribosomes where indicated; and 10^{-6} M thiostrepton where designated. Reaction mixtures were incubated at 37° for 5 min and assays performed for release of inorganic [^{32}P] according to Nishizuka and Lipmann (6). In the absence of ribosomes or factor G, there was a blank, which was subtracted from all values, of 20 pmoles of inorganic phosphate. The values for GTPase activity in the presence of both ribosomes and factor G are corrected for the non-specific GTPase activity contributed by each of these components alone. The column "% of control" refers to GTPase activity in the presence of 10^{-6} M thiostrepton as a percentage of the GTPase activity in the absence of the antibiotic. Specific activity of γ-[^{32}P]GTP was 160 cpm/pmole.

---

subunits (unpublished observations). As shown by the data of Table 3, 10^{-6} M thiostrepton inhibits factor G-ribosome-dependent GTPase activity about 90%. Little or no inhibition of non-specific GTPase activity associated with either ribosomes or factor G was detectable.

DISCUSSION

The present results indicate that thiostrepton inhibits translocation. The finding that non-enzymic translocation is inhibited as well as enzymic

translocation, indicates that it is directly blocking a ribosomal dependent event involved in the translocation processes. In contrast, fusidic acid, another inhibitor of translocation, interferes with the process of enzymic (5, 11), but not with that of non-enzymic translocation (4); fusidic acid is an inhibitor of supernatant factor G (5, 11-15). Neither antibiotic inhibits peptide bond formation itself.

Recently, Tanaka et al. (17) have reported that a similar peptide antibiotic, siomycin, inhibits translocation dependent on factor G, but does not inhibit N-acetyl-phenylalanyl-puromycin formation. They localized its action also to the 50S subunit of E. coli ribosomes. Analogy with the results described for thiostrepton suggest that siomycin also may be an inhibitor of non-enzymic translocation. In addition, erythromycin, which binds to 50S subunits, has been suggested to be an inhibitor of translocation (18, 19); at relatively high concentrations (10^{-4} M) we also have found it to inhibit non-enzymic translocation about 30%. Caution should be used in interpreting effects of antibiotics and other agents used at concentrations substantially higher than necessary for their in vivo action, for many antibiotics affecting ribosomal function exhibit pleotropic rather than specific effects at high concentrations (20-22).

Enzymic translocation involves the movement of mRNA and ribosome with respect to each other and the rejection of deacylated tRNA (23, 24); it requires factor G, GTP and both subunits of ribosomes (6). Precisely how all these molecular movements are coupled to GTP hydrolysis is unclear. From these and other studies it is apparent that certain molecular events of translocation depend primarily on a 50S subunit function. Rejection of deacylated tRNA may be one of these. The use of thiostrepton as a specific inhibitor of a 50S function related to translocation should help to elucidate this function as well as other events coupled to translocation and hydrolysis of GTP.

ACKNOWLEDGEMENT

I wish to thank Mr. Henry Hintikka and Miss Elena Smith for expert technical and secretarial assistance, respectively; and Dr. N. Brot for supplying purified factor G. Thiostrepton was generously supplied by Miss Barbara Stearns of Squibb.

REFERENCES

1. Weisblum, B., and V. Demohn, J. Bact., 101, 1073 (1970).
2. Dubnau, D., C. Goldthwaite, I. Smith, and J. Marmur, J. Mol. Biol., 27, 163 (1967).
3. Anderson, B., D. C. Hodgkin, and M. A. Viswamitra, Nature, 225, 233 (1970).
4. Pestka, S., J. Biol. Chem., 244, 1533 (1969).
5. Pestka, S., Proc. Nat. Acad. Sci., U.S., 61, 726 (1968).
6. Nishizuka, Y., and F. Lipmann, Arch. Biochem. Biophys., 116, 344 (1966).
7. Pestka, S., Arch. Biochem. Biophys., 136, 80 (1970).
8. Pestka, S., J. Biol. Chem., 241, 367 (1966).
9. Pestka, S., J. Biol. Chem., 243, 2810 (1968).
10. Lessard, J. L., T. Hishizawa, and S. Pestka, in preparation.
11. Pestka, S., Arch. Biochem. Biophys., 136, 89 (1970).
12. Tanaka, N., T. Kinoshita, and H. Masukawa, Biochem. Biophys. Res. Commun., 30, 278 (1968).
13. Kinoshita, T., G. Kawano, and N. Tanaka, Biochem. Biophys. Res. Commun., 33, 769 (1968).
14. Brot, N., and C. Spears, Fed. Proc., 29, 862 Abs (1970).
15. Bodley, J. W., F. J. Zieve, L. Lin, and S. T. Zieve, Biochem. Biophys. Res. Commun., 37, 437 (1969).
16. Haenni, A-L., and J. Lucas-Lenard, Proc. Nat. Acad. Sci., U.S., 61, 1363 (1968).
17. Tanaka, K., S. Watanabe, H. Teraoka, and M. Tamaki., Biochem. Biophys. Res. Commun., in press.
18. Cundliffe, E., and K. McQuillen, J. Mol. Biol., 30, 137 (1967).
19. Igarashi, K., H. Ishitsuka, and A. Kaji, Biochem. Biophys. Res. Commun., 37, 499 (1970).

20. Pestka, S., Proc. Nat. Acad. Sci., U.S., 64, 709 (1969).

21. Pestka, S., Cold Spring Harbor Symp. Quant. Biol., 34, 395 (1969).

22. Schlessinger, D., and D. Apirion, Annual Review of Microbiology, 23, 387 (1969).

23. Kuriki, Y., and A. Kaji, Proc. Nat. Acad. Sci., U.S., 61, 1399 (1968).

24. Lucas-Lenard, J., and A-L. Haenni, Proc. Nat. Acad. Sci., U.S., 63, 93 (1969).