INHIBITION BY THIOSTREPTON OF THE FORMATION OF A RIBOSOME-BOUND GUANINE NUCLEOTIDE COMPLEX

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1. Introduction

Thiostrepton is an antibiotic inhibitor of protein synthesis. Its structure, determined by X-ray crystallography, was reported earlier this year by Anderson et al. [1] and we have recently characterized its action as an inhibitor of a function of the 50 S ribosome subunit [2]. In the present communication we wish to present evidence that thiostrepton interferes with 50 S subunit function in relation to the GTP hydrolysis reaction which is mediated by 70 S ribosomes and G-factor which was described by Nishizuka and Lipmann [3]; moreover, thiostrepton inhibits this reaction by inhibiting the formation of a ribosome–G-factor–guanine nucleotide complex which is trapped in the presence of fusidic acid and which was described previously by Bodley et al. [4].

2. Materials and methods

The assay conditions for G-factor-mediated GTP hydrolysis were based on those described by Nishizuka and Lipmann [3]; and the assay conditions for fusidic acid-dependent guanine nucleotide accumulation were based on those described by Bodly et al. [4]. The modifications used in adapting these reactions to this work are included in the respective figure legends. Fusidic acid was obtained from the Leo Pharmaceutical Co., Copenhagen through the courtesy of W.Gottfredsen and thiostrepton was obtained from the Squibb Co., New Brunswick, New Jersey through the courtesy of B.Stearns. ^{3}H-GTP, specific activity 1000 μCi/μmole was purchased from Schwartz Bioresearch, Orangeburg, New York.

3. Results and discussion

The properties of the system used in studying the effects of thiostrepton are shown in table 1. Increasing concentrations of thiostrepton are seen to inhibit progressively the GTPase reaction, and a sharp transition occurs between 10^{-7} and 10^{-6} M thiostrepton added to the system, fig. 1. Because the concentration of ribosomes used, 2.5×10^{-6} M also falls in this range, we attempted to reverse the inhibitory effect of 10^{-6} M thiostrepton by backtitration with individual components of the reaction mixture. As shown in fig. 2, only 70 S ribosomes and 50 S subunits are active in reversing the inhibitory effect of the antibio-

Table 1
GTP-Hydrolysis mediated by 70 S ribosomes and G-factor from *E. coli*.

	pmoles P_i released per 7 min
Complete system	48.8
minus G-factor	7.4
minus ribosomes	0.7

The reaction mixture contained in 50 μl: tris HCl, pH 7.5, 0.05 M; $MgCl_2$, 0.02 M; KCl, 0.05 M; β-mercaptoethanol, 0.007 M; ^{32}P-γ-labelled GTP, 5×10^{-5} M, specific activity 50 μCi/μmole; G-factor, 5 μg protein; ribosomes (*E. coli*), 125 pmoles. After incubation at 37° for 7 min, 0.5 ml 0.002 M K_2HPO_4 and 0.5 ml 5% ammonium molybdate in 4 N H_2SO_4 were added. The mixture was extracted with 2.5 ml isobutanol–benzene (1:1) and 0.1 ml of the upper phase was withdrawn and assayed for radioactivity in a liquid scintillation counter.

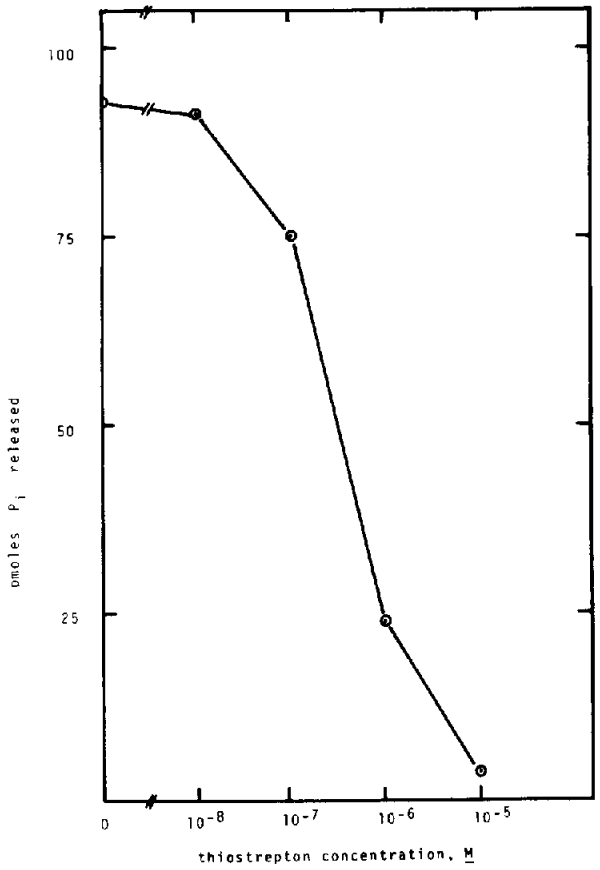

Fig. 1. Effect of thiostrepton on GTP hydrolysis. The complete system described in table 1 was supplemented with increasing concentrations of thiostrepton as indicated. Stock solutions of thiostrepton, 50-fold concentrated, were made using dimethyl-sulfoxide (DMSO) as solvent. At a final concentration of 2% DMSO in the reaction mixture, 92.0 pmoles P_i were released.

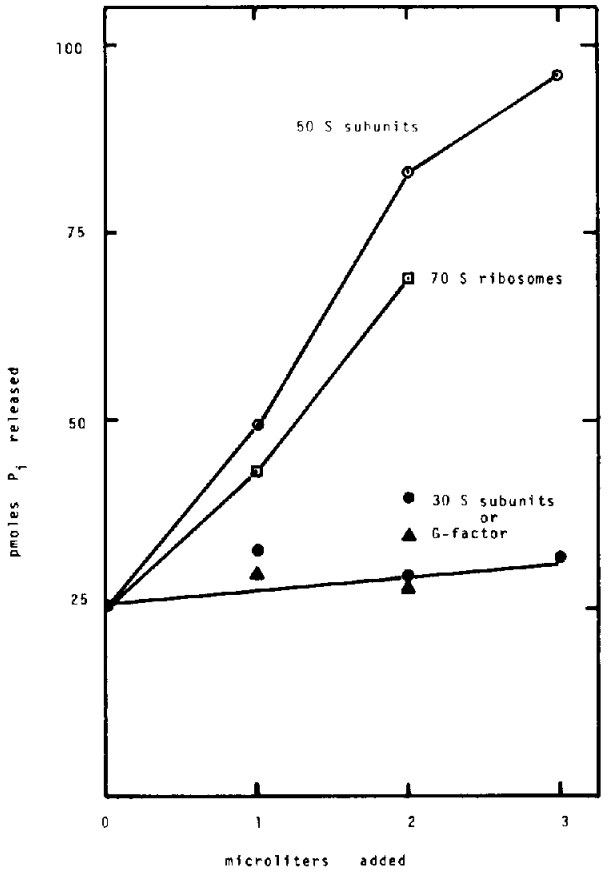

Fig. 2. Reversal of inhibition in a thiostrepton-inhibited reaction. The complete system, containing 10^{-6} M thiostrepton was supplemented with additional G-factor, 30 S or 50 S subunits, or 70 S ribosomes as indicated. For these supplemental components, 1 microliter contained: G-factor, 2.5 μg protein; 30 S subunits, 0.1 A_{260} unit; 50 S subunits, 0.17 $O.D._{260}$ units, or 70 S ribosomes, 0.5 A_{260} units, respectively.

Table 2
Effects of fusidic acid and thiostrepton on ribosome–guanine nucleotide complex formation.

Experimental conditions	pmoles guanine nucleotide retained on filter
a) Complete	2.55
minus fusidic acid	1.42
minus fusidic acid plus thiostrepton (10^{-6} M)	1.13
plus thiostrepton (10^{-7} M)	2.07
plus thiostrepton (10^{-6} M)	1.49
plus thiostrepton (10^{-5} M)	1.00
b) Complete	3.98
minus G-factor	1.46
plus thiostrepton (5×10^{-6} M)	1.97

The complete reaction mixture contained in 50 μl: tris HCl pH 7.4, 0.01 M; Mg acetate, 0.01 M; ammonium chloride 0.01 M; dithiothreotol, 0.001 M; ^{3}H-GTP (specific activity 1000 μCi/μmole) 5×10^{-5} M; G-factor, 20 μg protein; ribosomes (*E. coli*), 125 pmoles; and fusidic acid, 10^{-3} M. The complete reaction mixture was prepared with omission of GTP, and incubated for 5 min at 37°. The reaction mixture was then chilled on ice and ^{3}H-GTP was added. After incubation on ice for 5 min, the reaction mixture was diluted to 1 ml with a buffer containing the salts used in the reaction mixture and supplemented with 10^{-3} M fusidic acid. Bound guanine nucleotide was isolated by adsorption onto a millipore filter.

tic; 30 S subunits and G-factor are without effect. Thus, in contrast to the mechanism of action of fusidic acid which is apparently mediated by inhibition of a function of the G-factor, as shown by Kinoshita et al. [5], thiostrepton works on a different component of the system, the 50 S ribosome subunit. These results confirm and extend the results recently reported by Pestka [6], in which it was noted that thiostrepton inhibited GTP hydrolysis and translocation.

Fusidic acid can be used as a tool in studying further the mechanism of thiostrepton action. Bodley et al. [4] have shown that in the presence of fusidic acid, a Millipore-adsorbable complex containing GDP was formed on incubation of GTP, ribosomes, and G-factor. Presumably, fusidic acid prevents release of GDP after hydrolysis of a single GTP. We therefore inquired whether the inhibitory effect of thiostrepton might be observable in relation to the formation of this complex. As shown in table 2, thiostrepton, not only did not support accumulation of this complex, but also inhibited formation of the fusidic acid-induced complex. It is apparent that, under conditions of the assay, only about 5% of the ribosomes are active in formation of the complex. It is not yet clear whether this represents a loss of activity of the ribosomes, heterogeneity of ribosome function, or lack of optimization of one of the parameters in the reaction.

In conclusion, thiostrepton and fusidic acid appear to act in opposite ways with respect to their inhibitory effect on GTP hydrolysis. Whereas fusidic acid traps a ribosome-bound GDP intermediate, thiostrepton appears to destabilize or to inhibit formation of guanine nucleotide-containing complexes.

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