STREPTOTHRICIN F, AN INHIBITOR OF PROTEIN SYNTHESIS WITH MISCODING ACTIVITY

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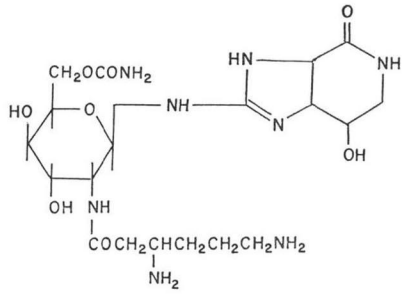
The effect of streptothricin F on macromolecular syntheses in intact cells and cell-free protein synthesis of *E. coli* was studied. The results indicate that protein synthesis is the primary site of inhibition by streptothricin F in growing *E. coli* cells. Cell-free polypeptide synthesis from *E. coli* directed by poly (U) was inhibited, while poly (A) and poly (C) directed polypeptide syntheses were both stimulated by the drug. Furthermore, streptothricin F caused misreading of translation of poly (U), poly (A) and poly (C) directed protein syntheses in *E. coli* systems. The extent of misreading by streptothricin F increases with increasing drug concentrations. The results are compared with those of other miscoding antibiotics. In rat liver extracts protein synthesis directed by poly (U) or endogenous mRNA was not inhibited.

Streptothricin antibiotics, isolated by WAKSMAN and WOODRUFF[1] are produced by a number of *Streptomyces* strains mostly as mixtures with varying amounts of the single components. Under acidic conditions the antibiotics are hydrolized to three characteristic degradation compounds: streptolidine, L-gulosamine, and β-lysine. On the basis of these results VAN TAMELEN *et al.*[2] established a general chemical structure of β-lysine-containing streptothricins. In continuing these efforts RESHETOV *et al.*[3,4,5] and KHOKHLOV and SHUTOVA[6] made an exact chromatographic separation combined with analytical investigations of the single streptothricin components occurring in several natural mixtures. It was concluded that the isolated streptothricin components F, E, D, C, B, A, and X are homologous compounds of the same general structure but differ in their amino acid content varying from 1 to 7 β-lysine moieties.

Streptothricin antibiotics are active against Gram-positive, Gram-negative bacteria and mycobacteria.

It was demonstrated by TELESNINA *et al.*[7] that streptothricins inhibit poly(U) directed polyphenylalanine synthesis, while MISRA and SINHA[8] have shown that boseimycin, a streptothricin-like antibiotic, preferentially inhibited protein synthesis in intact bacterial cells. Additional information concerning the mode of action of streptothricin antibiotics is now presented.

Fig. 1. Structure of streptothricin F



In the present paper the action of streptothricin F (ST-F; Fig. 1) on DNA-, RNA- and protein syntheses of intact cells of *E. coli* is described. Furthermore studies are reported of the effects of ST-F on polypeptide synthesis directed by synthetic homopolynucleotides in *E. coli* and rat liver systems. The results indicate

that ST-F is a specific inhibitor of protein synthesis in *E. coli* and induces misreading of translation of synthetic homopolynucleotides.

Materials and Methods

Production and isolation of ST-F

Streptomyces lavendulae JA 2254, which produces ST-F free of other streptothricin components, was cultivated under submerged conditions in a medium containing 1% soybean meal, 2% glucose, 0.5% sodium chloride, 0.3% calcium carbonate, and 0.5% corn steep liquor at 28°C for 96 hours.

The harvested mash (70 liters) was acidified to pH 3 with dilute hydrochloric acid and the mycelium removed by centrifugation. The resulting filtrate was passed through a column of the weakly acidic cation-exchange resin Wofatit CP-300 (Na^+ form) after adjustment to pH 7 with dilute sodium hydroxide. The resin was thoroughly washed with water followed by 0.01 N acetic acid according to RESHETOV and KHOKHLOV[3]. The antibiotic was eluted with 0.05 N hydrochloric acid and the acidic effluent neutralized by addition of the anion-exchange resin Wofatit L-150 (OH^- form). The active fractions of the eluate were combined and concentrated *in vacuo* to 1/10 volume. The concentrate was decolourized by treatment with activated carbon (EPN-Kohle), filtered and evaporated to dryness. The resulting residue was reprecipitated twice with methanol-acetone. Yield 2.1 g.

The isolated antibiotic was identified with an authentic sample of streptothricin F, kindly supplied by Prof. KHOKHLOV, Moscow, by using microbiological and chemical methods described by RESHETOV *et al.*[9].

ST-F was dissolved in water immediately before use.

Synthesis of cellular macromolecules

Escherichia coli 15 TAU^- was grown in glucose-salts medium as previously described[10]. For incorporation experiments 2 μCi of either ^{14}C-leucine (125 mCi/mMol) or ^{14}C-uracil (25 mCi/mMol) or ^{14}C-thymidine (44 mCi/mMol) were added to 10 ml of the culture. After preincubation of 30 minutes the antibiotic was added to the culture and samples of 0.5 ml were withdrawn at appropriate time intervals. The samples were cooled in ice and made 5% with respect to trichloroacetic acid. The acid-insoluble fractions were collected on membrane filters and counted in 5 ml toluene-scintillator in a Packard Tricarb scintillation spectrometer. Samples with ^{14}C-leucine were heated for 20 minutes at 90°C before filtration.

In vitro protein synthesis of *E. coli*

The method for the preparation of the cell-free amino acid incorporation system of *E. coli* A 19 was described earlier[11]. Incubation mixtures are given in the legend of Table 1. Amino acid incorporation was measured by the paper disc technique of MANS and NOVELLI[12].

Preparation of rat liver ribosomes

Livers from starved Wistar male rats were homogenized at 0°C in 50 mM Tris-HCl buffer, pH 7.7, 25 mM KCl, 5 mM magnesium acetate, 7 mM 2-mercaptoethanol and 0.25 M sucrose (buffer 1). The homogenate was centrifuged at 30,000 *g* for 15 minutes and the supernatant centrifuged for 30 minutes. The supernatant was used as S-30 fraction. For preparation of ribosomes the S-30 fraction was centrifuged at 105,000 *g* for 3 hours. The 105,000 *g* supernatant was dialysed against buffer 1 before use in the amino acid incorporating system. The pellet was resuspended in buffer 1 with 1% Na-deoxycholate and after 30 minutes the suspension was pelleted overnight through two layers of 1.5 M and 2 M sucrose in 50 mM Tris-HCl, pH 7.6, 25 mM KCl and 10 mM magnesium acetate. The pellet was resuspended in buffer 1 without sucrose.

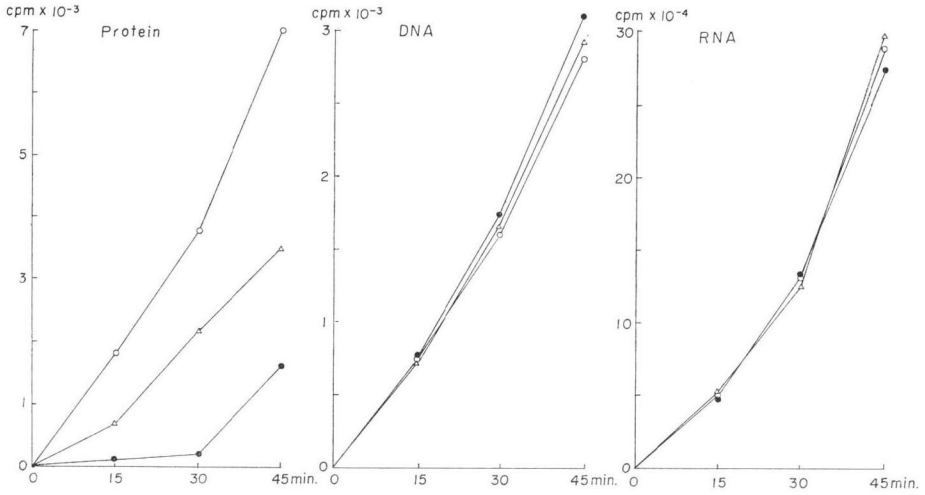
Incubation mixtures are given in the legend of Table 3. Amino acid incorporation was measured by the paper disc technique of MANS and NOVELLI[12].

Results

1. Effect of ST-F on macromolecular syntheses in intact cells of *E. coli* 15 TAU^-

The effect of ST-F on protein and nucleic acid syntheses of exponentially growing *E. coli* cells is shown in Fig. 2. It is seen that protein synthesis is the most strong inhibitor of the processes studied. ST-F at 0.03 mM inhibits protein synthesis almost completely within 15 minutes, while DNA and RNA syntheses are relatively unaffected.

Fig. 2. Effect of streptothricin F on DNA, RNA and protein syntheses in intact cells of *E. coli* 15 TAU⁻ (○) without antibiotic, (△) with 2×10^{-5} M streptothricin F, (●) with 3×10^{-5} M streptothricin F



2. Effect of ST-F on cell-free protein synthesis in *E. coli* and rat liver systems

Table 1 shows the influence of ST-F on cell-free protein synthesis from *E. coli* directed by the polynucleotides poly(U), poly(A) and poly(C). With poly(U) as messenger ST-F inhibits phenylalanine incorporation about 50% at 0.01 mM, while poly(A) directed lysine and poly(C) directed proline incorporation were stimulated by all ST-F concentrations tested.

Table 1. The effect of streptothricin F on polypeptide syntheses of *E. coli* directed by synthetic homopolynucleotides

Streptothricin F (mM)	Incorporation					
	Phe with poly (U)		Lys with poly (A)		Pro with poly (C)	
	cpm	%	cpm	%	cpm	%
—	6,874	100	2,359	100	2,300	100
0.001	5,843	85	2,652	112	2,612	113
0.01	2,956	43	2,987	126	2,978	129
0.1	2,069	30	3,067	130	3,398	147

Reaction mixtures contained in 100 μl: 10 μMol Tris-HCl (pH 7.8), 1.4 μMol magnesium acetate, 5 μMol NH_4Cl, 0.1 μMol adenosine triphosphate, 0.003 μMol guanosine triphosphate, 0.6 μMol β-mercaptoethanol, 0.6 μMol phosphoenolpyruvate, 1.6 μg pyruvate kinase, 2.0 mμMol [^{14}C]-phenylalanine (66 μCi/μMol), 2.1 mμMol [^{14}C]-proline (47 μCi/μMol) or 4.5 mμMol [^{14}C]-lysine (82 μCi/μMol), 2 mμMol each of the other 19 non-radioactive amino acids, poly (U) (10 μg), poly (A) (10 μg) or poly (C) (40 μg), 300 μg of protein of the S-100 supernatant and 133 μg ribosomes.
Incubation: 20 minutes at 37°C.

ST-F inhibited poly(U) directed polyphenylalanine synthesis only at optimal and higher Mg^{++}-concentrations, whereas at less-than-optimal Mg^{++}-concentrations the drug has no effect (Table 2).

In contrast to results with *E. coli* extracts, cell-free protein synthesis in rat liver extracts directed by poly(U) or endogenous mRNA was not inhibited up to 1 mM ST-F (Table 3). At 1 mM ST-F a slight stimulation of protein synthesis was observed. The sensitivity of the rat liver extracts was tested with puromycin for comparison.

3. Miscoding activity of ST-F in polypeptide synthesis from *E. coli*

In polypeptide synthesis directed by homopolynucleotides ST-F causes a strong stimulation of the incorporation of amino acids which are normally not coded by the polymer (Table 4). With poly(U) misreading was observed for isoleucine, leucine, serine, and tyrosine. Furthermore misreading was demonstrated with poly(A) for glutamic acid and with poly(C) for serine. As is shown in Fig. 3, the extent of miscoding of poly(U) with different amino acids was increased with increasing ST-F concentrations, while miscoding induced by streptomycin is almost unaffected with increasing drug concentrations.

Table 2. Effect of magnesium ions on the inhibition of poly(U) directed polyphenylalanine synthesis in *E. coli* extracts by streptothricin F

Mg^{++} (mM)	Incorporation			
	−Streptothricin F		+Streptothricin F (0.01 mM)	
	cpm	%	cpm	%
10	8,975	100	8,960	100
15	9,150	100	3,843	42
20	7,557	100	2,644	35

Experimental conditions were as described in Table 1.

Table 3. The effect of streptothricin F on polypeptide synthesis of rat liver directed by poly(U) and endogenous mRNA

Antibiotic (mM)	Incorporation of			
	Phe with poly(U)		Phe with endogenous mRNA	
	cpm	%	cpm	%
None	2,221	100	3,010	100
Streptothricin F 0.01	2,330	104	3,110	103
0.10	2,360	106	3,171	105
1.00	2,665	120	3,532	117
Puromycin 0.01	1,625	54	1,861	61

Reaction mixtures of the poly(U) directed polypeptide synthesis contained in 100 μl: 5 μMol Tris-HCl (pH 7.8), 1.25 μMol magnesium acetate, 6 μMol KCl, 0.6 μMol β-mercaptoethanol, 0.1 μMol adenosine triphosphate, 0.02 μMol guanosine triphosphate, 0.75 μMol phosphoenolpyruvate, 3 μg pyruvate kinase, 0.3 mμMol [^{14}C]-phenylalanine (225 μCi/μMol), 20 μg tRNA, 12.5 μg poly(U), 94 μg ribosomes and 170 μg of protein of the S-100 supernatant. Incubation: 30 minutes at 35°C.

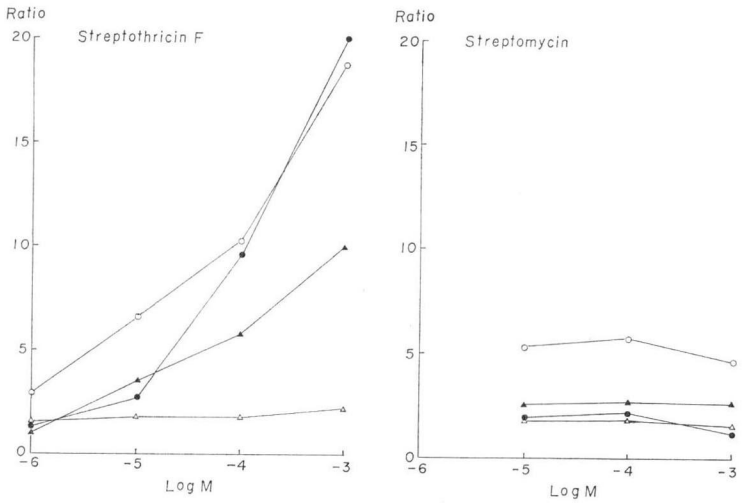
The experimental conditions for endogenous mRNA directed polypeptide synthesis were the same as those of poly(U) directed polypeptide synthesis except that poly(U) was omitted and 0.7 μMl magnesium acetate and 200 μg protein equivalent of S-30 fraction were used.

Table 4. Miscoding activity of streptothricin F in polypeptide syntheses (*E. coli*) directed by synthetic homopolynucleotides

Polynucleotide and amino acid		cpm incorporated		Ratio B/A
		A −Streptothricin	B +Streptothricin (0.1 mM)	
Poly(U)	Ileu	122	1,256	10.3
	Leu	4,715	7,983	1.7
	Ser	247	1,425	5.8
	Tyr	380	3,672	9.7
Poly(A)	Glu	281	636	2.3
Poly(C)	Ser	368	877	2.4

Experimental conditions were the same as described in Table 1 with the exception that the 19 nonradioactive amino acids were omitted. The concentrations of the radioactive amino acids were: 0.6 mμMol isoleucine (210 mCi/mMol), 0.8mμMol leucine (185 mCi/mMol), 0.95 mμMol serine (105 mCi/mMol), 0.31 mμMol tyrosine (315 mCi/mMol), 0.57 mμMol glutamic acid (175 mCi/mMol).

Fig. 3. Miscoding activity by streptothricin F and streptomycin in poly (U) directed polypeptide synthesis as a function of drug concentration.
Conditions for incorporation as in Table 1.
Ratio: incorporation with antibiotic/incorporation without antibiotic.
(○) with ^{14}C-isoleucine, (●) with ^{14}C-tyrosine, (▲) with ^{14}C-serine, (△) with ^{14}C-leucine



Discussion

Our experiments have shown that ST-F is a specific inhibitor of protein synthesis in intact cells and cell-free systems of *E. coli*; the antibiotic does not inhibit cell-free protein synthesis in rat liver extracts directed by poly(U) or endogenous mRNA. The results further indicate that ST-F induces misreading of synthetic homopolynucleotides in cell-free *E. coli* systems. This mode of action is a characteristic feature of aminoglycoside antibiotics with a streptamine- or deoxystreptamine moiety, such as streptomycin or kanamycin[13]. Miscoding activity has also been demonstrated for negamycin, a peptide-like antibiotic consisting of δ-hydroxy-β-lysine linked to methylhydrazinoacetic acid[14,15]. Since negamycin does not have an aminoglycoside structure, its miscoding effect appears to be an exception. However, UEHARA *et al.*[15] showed that there is similarity in the three-dimensional structure of negamycin and the aminoglycoside antibiotics with miscoding activity. They demonstrated that the hydrazide and the β-amino group of the δ-hydroxy-β-lysine moiety of negamycin can be superimposed upon the 1- and 3-amino groups in the 2-deoxystreptamine moiety of aminoglycoside antibiotics and that the ε-amino group of negamycin has a configuration similar to the 2′-hydroxyl group in kanamycin.

Thus, it is not surprising that ST-F also induces miscoding of homopolynucleotides, since it also contains a β-lysine moiety, which is linked to gulosamine *via* a peptide-like bond. The β-lysine moiety seems not to be the only prerequisite for the miscoding activity of ST-F and negamycin, since viomycin, another β-lysine containing antibiotic, does not induce miscoding, although it inhibits protein synthesis[16].

With regard to inhibition of polypeptide synthesis and miscoding activity, ST-F induces similar but also different effects in comparison to those of other miscoding antibiotics. In poly(A) and poly(C) directed polypeptide synthesis ST-F was found to stimulate not only the incorporation of the incorrect amino acids, but also the incorporation of the correct amino acids lysine and proline, thus resembling streptomycin, kanamycin and negamycin[17,15]. The pattern of miscoding induced by ST-F was the same as that caused by streptomycin, kanamycin and negamycin[17,14]. However, the miscoding effects induced by ST-F are dose-dependent and increase with increasing ST-F concentrations. In this respect ST-F resembled kanamycin and negamycin more closely than streptomycin[15]. Concentration-depend-

ent miscoding, demonstrated for a variety of aminoglycoside antibiotics, has been ascribed to multiple binding sites for the drug on the ribosome[18]. This could also be the case for ST-F.

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