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Inhibition by Sparsomycin and Other Antibiotics of the Puromycin-Induced Release of Polypeptide from Ribosomes*

Irving H. Goldberg and Koji Mitsugi†

ABSTRACT: The antibiotic sparsomycin is a highly effective inhibitor of the puromycin-induced release of polypeptide (as polypeptidylpuromycin) from ribosomes. Sparsomycin blocks the puromycin-induced release of polyphenylalanine from prelabeled ribosomes, the initial rate being primarily affected. Similarly, sparsomycin, at very low concentrations (10^{-6}–10^{-7} M), prevents the puromycin (10^{-4} M) induced release of polylysine from polylysyl soluble ribonucleic acid (polylysyl-sRNA) bound to ribosomes in the presence of polyadenylic acid (poly A). The inhibition of the puromycin reaction by sparsomycin is not due to an effect of the antibiotic on the poly A dependent binding of polylysyl-sRNA to ribosomes. Gougerotin and chloramphenicol also failed to interfere with polylysyl-sRNA binding to ribosomes but inhibited the puromycin reaction at levels almost 2 logs greater than required with sparsomycin. Kinetic analysis reveals that sparsomycin is a competitive inhibitor of puromycin in this reaction, while gougerotin and chloramphenicol exhibit "mixed" types of inhibition kinetics. These data suggest that sparsomycin interferes with the peptide bond-forming step, either directly or by an allosteric mechanism.

As part of a study using antibiotics to help clarify the steps involved in nucleic acid (Goldberg and Reich, 1964) and protein synthesis, we have examined the mechanism of action of sparsomycin, a highly effective inhibitor of *in vitro* protein synthesis in *Escherichia coli* (Goldberg and Mitsugi, 1966, 1967). Sparsomycin resembles chloramphenicol in (1) being most effective against polypeptide synthesis promoted by synthetic polynucleotides with low uridylate content, and (2) blocking polypeptide formation at a point beyond the attachment of aminoacyl-sRNA to ribosomes. Sparsomycin, however, differs from chloramphenicol in being active at lower levels and being highly effective in mammalian systems *in vivo* (Goldberg and Mitsugi, 1966) and *in vitro* (Colombo *et al.*, 1966; T. L. Steck and I. H. Goldberg, 1966, unpublished data). Further, sparsomycin does not affect the binding of [^{14}C]chloramphenicol to ribosomes (Goldberg and Mitsugi, 1967). These results suggested that sparsomycin acts at or close to the peptide bond-forming reaction itself, but differs from chloramphenicol in precise interaction with the protein-synthesizing system.

Puromycin interferes with polypeptide chain elongation by acting as an analog of aminoacyl-sRNA and partaking in the peptide bond forming reaction, leading to the release of peptide as peptidylpuromycin from sRNA bound to ribosomes (Nathans, 1964). Accordingly, this reaction has proved to be a useful model for study of the formation of a single peptide bond (Nathans and Lipmann, 1961). We have followed peptide release due to puromycin using (1) zone centrifugation analysis of prelabeled ribosomes incubated with puromycin (Gilbert, 1963; Traut and Monro, 1964), and (2) the elegant, simplified procedure of Rychlik (1965a) employing polylysyl-sRNA, polyadenylic acid, and washed ribosomes. This paper will describe the effects of sparsomycin, gougerotin, and chloramphenicol on this reaction. Gougerotin, which is a dipeptidylpyrimidine nucleoside antibiotic (Fox *et al.*, 1964), also blocks protein synthesis beyond the stage of attachment of aminoacyl-sRNA (Clark and Chang, 1965), and has been reported to inhibit the puromycin reaction in a competitive fashion (Casjens and Morris, 1965). Like sparsomycin, it is effective against mammalian systems, but it is much less so, and is as potent an inhibitor of poly U promoted

* From the Department of Medicine, Harvard Medical School, and the Beth Israel Hospital, Boston, Massachusetts 02215. *Received September 27, 1966.* This work was supported by grants from the National Insitutes of Health, U. S. Public Health Service (GM 12573), and the American Cancer Society.

† Trainee, supported by Grant T1 CA 5167 of the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

polyphenylalanine formation as of poly A promoted polylysine formation (Goldberg and Mitsugi, 1967).

Materials and Methods

E. coli strain B crude extracts (S30), ribosomes, and high-speed supernatant (S105) were prepared as described by Nirenberg (1964). NH_4Cl-washed ribosomes used in the [^{14}C]polylysyl-sRNA release experiments were prepared by a modification of the method of Nishizuku and Lipmann (1966). The S30 fraction was preincubated for 45 min at 35° in buffer I (0.01 M Tris–HCl (pH 7.8), 0.014 M magnesium acetate, 0.06 M KCl, and 0.006 M β-mercaptoethanol), and then centrifuged at 50,000 rpm for 3 hr. The ribosomal pellet was resuspended in buffer I, cleared of aggregates by centrifugation at 32,000*g* for 40 min, and repelleted at 50,000 rpm for 3 hr. Following resuspension of the resulting pellet in buffer II (0.01 M Tris–HCl (pH 7.4), 0.0001 M $MgCl_2$, 0.5 M NH_4Cl, and 0.006 M β-mercaptoethanol), aggregates were removed as before, and the ribosomes were collected by centrifugation as above. The ribosomal pellet was resuspended in buffer III (same composition as buffer II except containing 0.01 M $MgCl_2$) and resedimented as above. This process was repeated once more and the final ribosomal pellet was resuspended in a small volume of 0.01 M Tris–HCl (pH 7.4) containing 0.01 M $MgCl_2$, subdivided into 0.2-ml aliquots, and stored at −96°. The ribosome concentration (based on OD_{260} of 14.4 = 1 mg/ml) was 29 mg/ml.

Ribosomes were prelabeled with [^{14}C]polyphenylalanine (poly U stimulated) and the puromycin-induced release followed by zone centrifugation as described by Traut and Monro (1964). The ribosomes used in these experiments were not preincubated but had a low endogenous activity when assayed for polypeptide synthesis. The ribosomes charged with [^{14}C]polyphenylalanine were resuspended at 6.5 mg/ml (1.2×10^6 cpm/ml, 330 μc/μmole of [^{14}C]phenylalanine) in 0.01 M Tris–HCl (pH 7.4)–0.01 M $MgCl_2$, and used directly in the puromycin reaction.

[^{14}C]Polylysyl-sRNA was prepared by slight modification of the procedure of Rychlik (1965b). The incubation mixture contained the following in a total volume of 6 ml: 20 mg of stripped *E. coli* sRNA, 0.1 M Tris–HCl (pH 7.8), 0.05 M ammonium acetate, 0.015 M magnesium acetate, 0.006 M β-mercaptoethanol, 0.001 M ATP,[1] 0.0001 M GTP, 0.008 M phosphoenolpyruvate, 24 μg of pyruvate kinase, 600 μg of poly A, 50 μc of [^{14}C]lysine (50 μc/μmole), and 1.2 ml of preincubated S30. After incubation for 10 min at 35°, the reaction mixture was treated with phenol and the polylysyl-sRNA was isolated by ethanol precipitation, solution in 1 M NaCl, pH 7.0, extensive dialysis against distilled H_2O, and lyophilization. The dried powder was stored at −96° until just prior to use when an aqueous solution containing 5 mg/ml was prepared. An aliquot of this solution was incubated for 20 min at 35° in 0.1 M Tris (pH 7.22) immediately before use in the puromycin reaction. This was done to eliminate any contaminating [^{14}C]lysyl-sRNA in the polylysyl-sRNA preparation and resulted in a 10% decrease in cold TCA-precipitable radioactivity (final corrected specific activity of 8.8×10^4 cpm/mg of [^{14}C]polylysyl-sRNA). Carboxymethylcellulose chromatography of the peptides released by alkaline treatment of such [^{14}C]polylysyl-sRNA preparations showed a distribution of peptides having a peak at the heptamer with peptides up to 13 units long.

The incubation conditions used in the puromycin-release experiments are described individually in the legends of each figure and table. Release of [^{14}C]-polylysine (as polylysylpuromycin) from sRNA attached to ribosomes was measured as a loss of cold TCA-precipitable radioactivity following incubation. Remaining [^{14}C]polylysyl-sRNA was precipitated by addition to the reaction mixture of 2 ml of cold 5% TCA. After standing in ice for 15 min, the resulting precipitate was poured onto a Millipore filter (0.45 μ) and washed four times with 4 ml of cold 5% TCA. The filter was glued to a stainless-steel planchet, dried, and counted in a gas-flow counter (Beckman low β) with an efficiency of 20%.

Results

Effect of Sparsomycin on the Release of Polyphenylalanine from Prelabeled Ribosomes. As shown by Gilbert (1963) and Traut and Monro (1964), incubation with puromycin of ribosomes prelabeled with [^{14}C]phenylalanine leads to the release of polyphenylalanine from polyphenylalanyl-sRNA attached to the ribosomes. This release can be followed by zone centrifugation. Polyphenylalanine still attached to sRNA sediments more rapidly than released polyphenylalanylpuromycin. As shown in Figure 1 and Table I, sparsomycin blocks the release of [^{14}C]polyphenylalanine from sRNA induced by puromycin (10^{-3} M) and does not lead to the release of label when puromycin is absent. Further,

TABLE I: Sparsomycin Inhibition of Polyphenylalanine Release by Puromycin.[a]

Sparsomycin (M)	% Inhibn of Release at 5 min	1 min
8.8×10^{-5}	38	52
2.6×10^{-4}	53	—
7.9×10^{-4}	80	—

[a] These data are derived from Figures 1 and 2 by integrating the areas under the curves associated with polyphenylalanyl-sRNA and polyphenylalanylpuromycin and calculating the sparsomycin inhibition of release of polyphenylalanine due to puromycin (10^{-3} M).

[1] Abbreviations used: ATP, adenosine triphosphate; GTP, guanosine triphosphate; TCA, trichloroacetic acid.

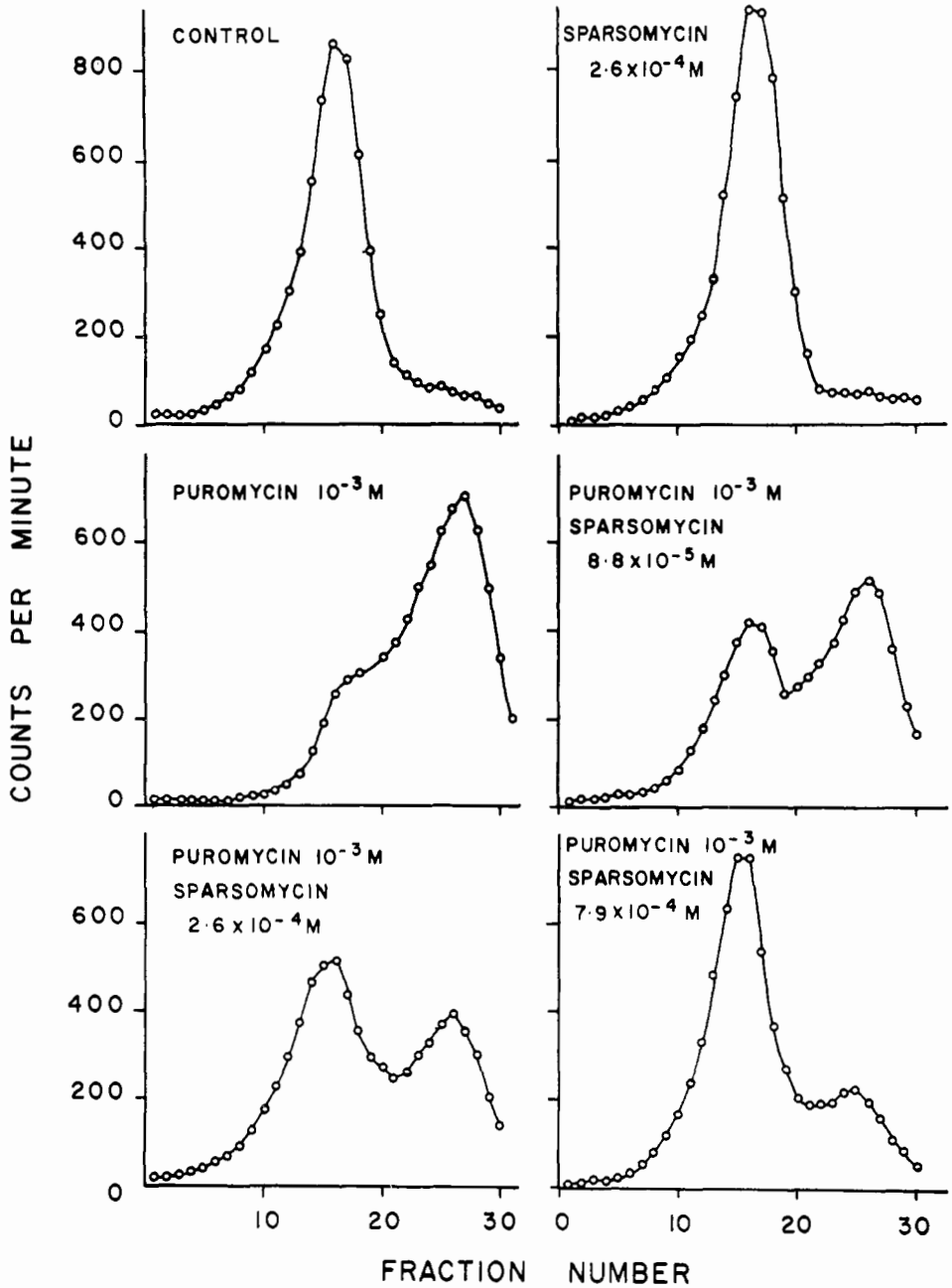

FIGURE 1: Effect of sparsomycin on the puromycin-induced release of [^{14}C]polyphenylalanine from ribosomes. Prelabeled ribosomes (65 μg, 1.2 $\times$ 10^4 cpm of [^{14}C]polyphenylalanine) prepared as described in Methods were incubated in 0.15 ml containing 0.02 M Tris (pH 7.4), 0.0175 M magnesium acetate, 0.16 M NH_4Cl, 0.006 M β-mercaptoethanol, 0.006 M phosphoenolpyruvate, 40 μg/ml of pyruvate kinase, 0.0004 M GTP, 15 μl of dialyzed S100, and puromycin and sparsomycin as indicated. After incubation at 30° for 5 min, the reaction was stopped by the addition of EDTA (0.015 M) and sodium dodecyl sulfate (0.5%), and 0.1-ml aliquots were layered on 4.8 ml of a linear sucrose density gradient (5–12.5%) containing 0.5% sodium dodecyl sulfate, 0.1 M LiCl, and 0.01 M Tris–HCl, pH 7.4. Following centrifugation in the SW39 rotor at 39,000 rpm for 12 hr at 10°, drops were collected from the bottom of the tube into plastic vials and assayed for radioactivity in the liquid scintillation counter after mixing with 10 ml of Bray's solution.

the sparsomycin inhibition is greater early (1 min) in the reaction than later (5 min) (Figures 1 and 2 and Table I). These findings are similar to those reported by Traut and Monro (1964) for chloramphenicol. Since this assay is a complicated one and does not lend itself readily to a detailed kinetic analysis and because the assay depends, at least in part, on GTP and soluble factors (Traut and Monro, 1964), a simpler system for studying this reaction was sought. The report of Rychlik (1965a) on the binding of added polyly-

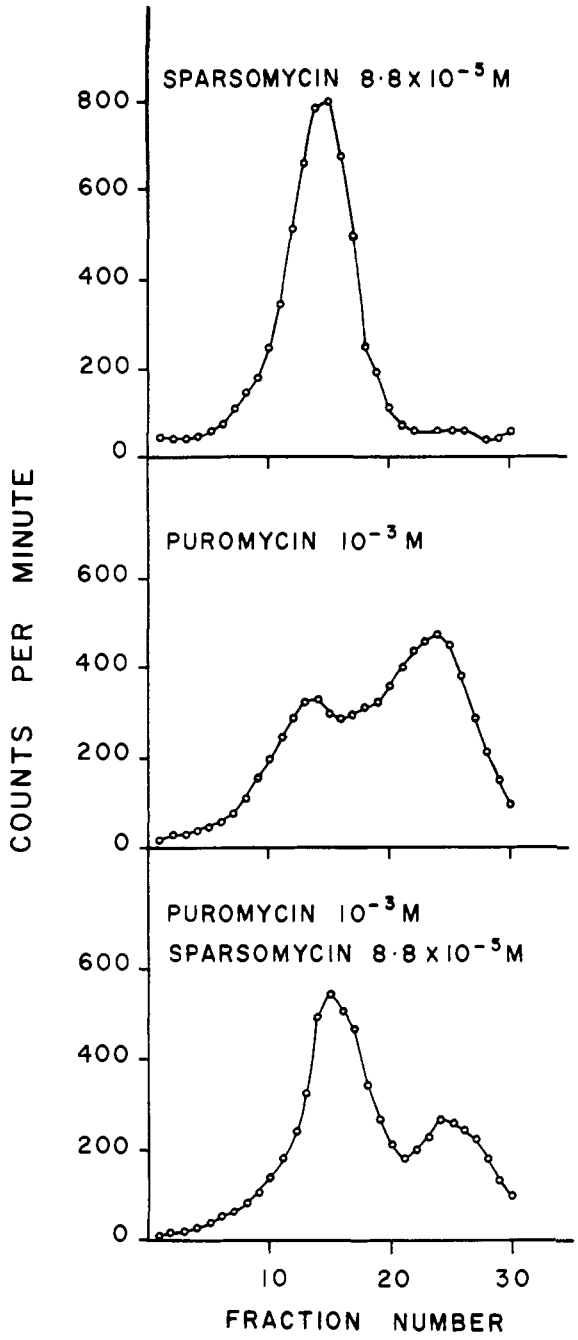

FIGURE 2: Effect of sparsomycin on the puromycin-induced release of [^{14}C]polyphenylalanine from ribosomes in a 1-min incubation. This experiment was performed exactly as described for Figure 1 except that incubation was for 1 min.

syl-sRNA to ribosomes and the release of lysyl peptides upon incubation with puromycin provided such a system.

Effect of Sparsomycin on the Release of Polylysine from sRNA. As found by Rychlik (1965a), the release of ribosome-bound polylysine depends on the presence of poly A and puromycin (Table II). Sparsomycin

TABLE II: Effect of Antibiotics on the Puromycin-Induced Release of Lysine Peptides.[a]

	Cpm TCA Precipitable	Cpm Released	% Inhibn of Release
Complete	600	790	—
−Puromycin	1390	—	100
−Poly A	1384	6	99
−Puromycin + sparsomycin	1376	14	—
+Sparsomycin	1324	66	92
−Puromycin + gougerotin	1330	60	—
+Gourgerotin	820	570	28
−Puromycin + chloramphenicol	1382	9	—
+Chloramphenicol	900	490	38

[a] The following were incubated in a volume of 0.1 ml: 0.1 M Tris–HCl (pH 7.2, 0.01 M magnesium acetate, 0.1 M ammonium acetate), 15.9 μg of [^{14}C]polylysyl-sRNA (1395 cpm), 10 μg of poly A, puromycin (10^{-4} M), and 145 μg of ribosomes. Sparsomycin (10^{-4} M), gougerotin (10^{-4} M), and chloramphenicol (10^{-4} M) were added as indicated. After incubation for 30 min at 35°, the reaction was stopped with 2 ml of cold 5% TCA and the precipitate was washed and assayed as described in Methods. A zero-time control had 1400 cpm.

inhibits this reaction and does not lead to release in the absence of puromycin. It should be noted that in these experiments the dependency of this reaction on poly A is more pronounced and the extent of the puromycin-induced release is greater (60 *vs.* 40%) than that reported by Rychlik (1965a).

Comparison of Sparsomycin, Gougerotin, and Chloramphenicol. We have found sparsomycin to be about 200-fold more effective than gougerotin and chloramphenicol in blocking puromycin-promoted polylysine release (Figure 3). The release produced by 10^{-4} M puromycin in a 30-min reaction is inhibited 50% by 10^{-6} M sparsomycin, 1.7×10^{-4} M chloramphenicol, or 1.8×10^{-4} M gougerotin. Neither gougerotin nor chloramphenicol cause significant release when present without puromycin. Our results differ from those reported by Rychlik (1965a) in that much higher concentrations of chloramphenicol (about 2 logs greater) were required in our experiments to obtain comparable degrees of inhibition. Results similar to those reported here with chloramphenicol and gougerotin have also been found by M. E. Gottesman (personal communication). The explanation for this marked difference in chloramphenicol sensitivities is not clear.

Lack of Effect of Antibiotics on Polylysyl-sRNA Binding to Ribosomes. Using the system characterized by Rychlik (1965a), we have failed to find any inhibition

TABLE III: [^{14}C]Polylysyl-sRNA Binding to Ribosomes.[a]

	[^{14}C]Polylysyl-sRNA Bound to Ribosomes	
	Cpm	% Inhibn
Experiment I		
Complete	1119	—
−Poly A	111	90
+Sparsomycin (10^{-4} M)	1078	4
+Chlortetracycline (10^{-4} M)	946	15
Experiment II		
Complete	477	—
+Chloramphenicol (2×10^{-3} M)	479	—
+Gougerotin (2×10^{-3} M)	512	—

[a] Experiment I: The reaction (containing the following in 0.2 ml: 0.1 M Tris–HCl (pH 7.22), 0.01 M magnesium acetate, 0.1 M ammonium acetate, 20 μg of poly A, and 145 μg of ribosomes) was started by the addition of 31.8 μg of [^{14}C]polylysyl-sRNA (3035 cpm). After incubation for 30 min at 25°, 4 ml of cold buffer containing 0.1 M Tris–HCl (pH 7.22), 0.01 M magnesium acetate, and 0.1 M ammonium acetate was added and the entire contents poured over a Millipore filter (0.45 μ). The filters were washed three times with 4 ml of cold buffer, placed on planchets, and counted as described in Methods. Experiment II: Same as expt I but incubation volume was 0.1 ml.

by sparsomycin, chloramphenicol, or gougerotin of the binding of added [^{14}C]polylysyl-sRNA to washed ribosomes (Table III). Actually, gougerotin appears to stimulate binding slightly. M. E. Gottesman (personal communication) has had a similar experience with gougerotin. Rychlik (1965a) has reported similar results with chloramphenicol and erythromycin, and, as we confirm, only a slight inhibition by chlortetracycline (10^{-4} M), which prevents polynucleotide-promoted aminoacyl-sRNA binding to ribosomes (Suarez and Nathans, 1965; Hierowski, 1965). The binding of polylysyl-sRNA to ribosomes is more poly A dependent than reported by Rychlik (1965a). In our experiments binding in the absence of poly A was 10% that in its presence, while Rychlik (1965a) found that the poly A independent reaction amounted to 30–50% of that with poly A.

Relation between Time Course and Degree of Inhibition by Sparsomycin. As seen in Figure 4, the sparsomycin inhibition of release of polylysine from sRNA is greatest early in the course of the reaction. This finding is similar to that noted above with ribosomes prelabeled with polyphenylalanine. Similarly, when incubation is carried out with a constant concentration of sparsomycin (5×10^{-7} M) but varying puromycin concentration, the inhibition found at 8 min is greater than that found at 30 min for all levels of puromycin (Figure 5). Figure 5 also shows that there is a competition between puromycin and sparsomycin. In other experiments chlortetracycline (10^{-4} M) did not alter the sparsomycin inhibition of the puromycin reaction.

Competition between Sparsomycin and Puromycin. A Lineweaver–Burk plot of an experiment similar to that shown in Figure 5 but incubated for 15 min with two different concentrations of sparsomycin is shown in Figure 6A. The kinetics suggest that sparsomycin is a competitive inhibitor of puromycin in the peptide-release reaction. Similar plots of the 8- and 30-min incubation lead to the same conclusion. Figure 6B, C shows Lineweaver–Burk plots of similar experiments using gougerotin or chloramphenicol instead of sparsomycin. In each case competition between the antibiotics and puromycin is found but the kinetics of the gougerotin and chloramphenicol effects are more complex and below to the "mixed" type of inhibitors, as described by Dixon and Webb (1958). The degree of inhibition of the puromycin reaction caused by sparsomycin, gougerotin, and chloramphenicol is not decreased by increasing the concentration of polylysyl-sRNA (Table

TABLE IV: Relation of [^{14}C]Polylysyl-sRNA Concentration and the Effect of Antibiotics on the Puromycin Reaction.[a]

Concentration of [^{14}C]-Polylysyl-sRNA (μg/tube)	[^{14}C]Polylysine Released (cpm)			
	Complete	+Chloramphenicol	+Gougerotin	+Sparsomycin
3.2	196	109 (44.4)[b]	147 (25.0)	134 (31.5)
7.9	467	274 (41.3)	325 (30.5)	333 (28.9)
15.8	727	396 (45.5)	418 (42.5)	506 (30.5)

[a] The following were incubated with or without sparsomycin (5×10^{-7} M), chloramphenicol (10^{-4} M), or gougerotin (10^{-4} M) in a final volume of 0.05 ml: 0.1 M Tris–HCl (pH 7.22), 0.01 M magnesium acetate, 0.1 M ammonium acetate, 5 μg of poly A, indicated amounts of [^{14}C]polylysyl-sRNA, 10^{-4} M puromycin, and 116 μg of ribosomes. After incubation for 15 min at 35°, the reaction was stopped and assayed for cold TCA-precipitable radioactivity as described in Methods. There were 286, 723, and 1399 cpm, respectively, in control incubations lacking puromycin and containing increasing amounts of [^{14}C]polylysyl-sRNA. [b] Per cent inhibition of release.

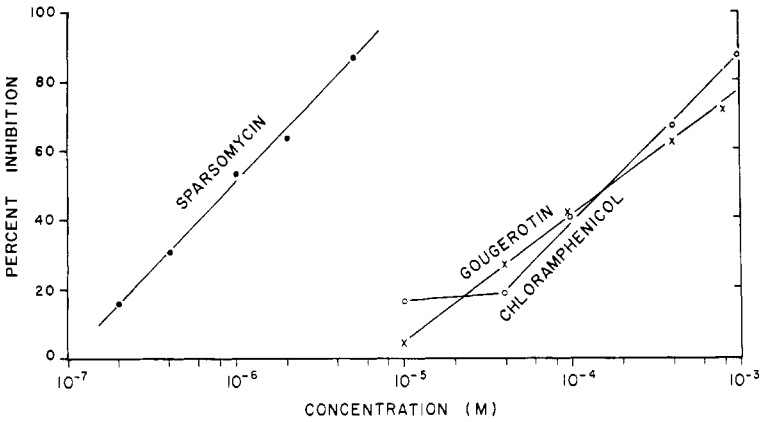

FIGURE 3: Relation of concentration of antibiotic to inhibition of the puromycin-induced release of polylysine from sRNA. Incubation conditions were the same as described in Table II. The concentrations of sparsomycin (●—●), gougerotin (×—×), or chloramphenicol (○—○) were varied as indicated. Except for incubated controls, all reactions contained 10^{-4} M puromycin. After incubation for 30 min at 35°, the reaction was stopped with 2 ml of cold 5% TCA, and the precipitate was washed and assayed as described in methods. On the ordinate is plotted the per cent inhibition by the antibiotic of the release due to 10^{-4} M puromycin. In the absence of other antibiotics 727 cpm was released by 10^{-4} M puromycin.

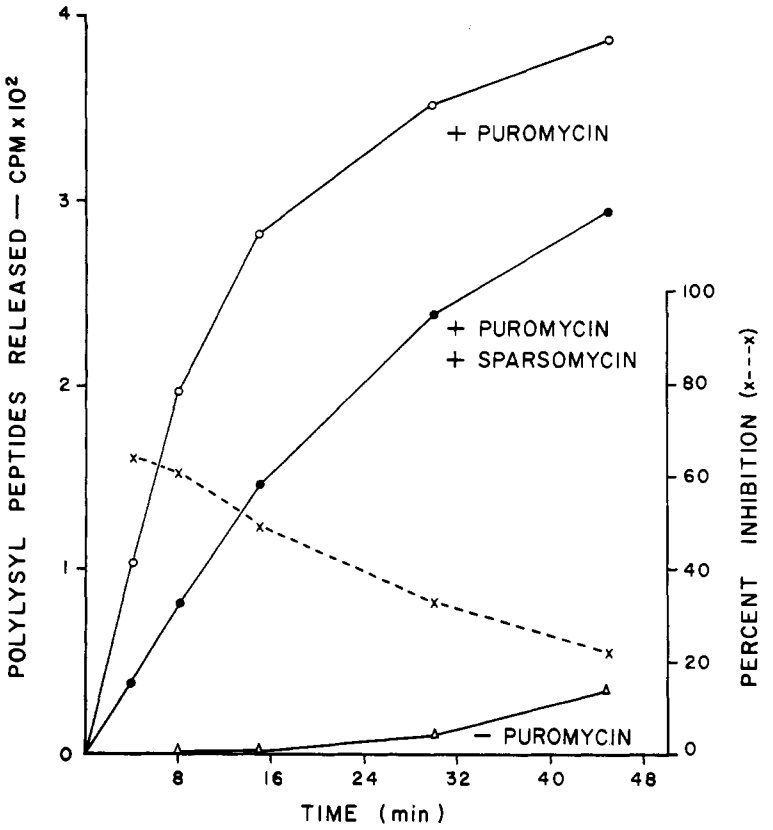

FIGURE 4: Time course of inhibition of the puromycin reaction by sparsomycin. The following were incubated without (○—○) or with (●—●) 5×10^{-7} M sparsomycin in a final volume of 0.3 ml: 0.1 M Tris-HCl (pH 7.22), 0.01 M magnesium acetate, 0.1 M ammonium acetate, 30 μg of poly A, 47.5 μg of [^{14}C]polylysyl-sRNA (4185 cpm), 10^{-4} M puromycin, and 435 μg of ribosomes. A similar reaction lacking both sparsomycin and puromycin (Δ—Δ) served as the control. After incubation for the indicated time at 35°, a 50-μl aliquot of the reaction mixture was precipitated with cold 5% TCA and assayed as described in Methods. Release in the presence of puromycin has been corrected for the nonpuromycin-promoted release at each time period. The per cent inhibition of the puromycin reaction by sparsomycin (×—×) is also shown.

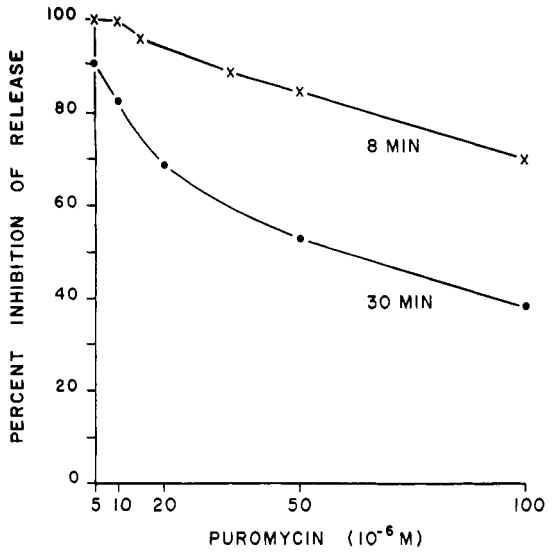

FIGURE 5 (above): Relation of incubation time and puromycin concentration to the sparsomycin inhibition of peptide release. Incubation conditions and assay were the same as described in Table II except that the puromycin concentration was varied as indicated and the incubation time was 8 (×—×) or 30 min (●—●). The per cent inhibition of puromycin-induced release by sparsomycin (5×10^{-7} M) was determined for each concentration of puromycin.

IV). In fact, there appeared to be an increase in the gougerotin effect with increasing polylysyl-sRNA.

Cooperative Effects of Antibiotics in Inhibiting the Puromycin Reaction. As seen in Table V, the effects of any combination of two antibiotics (sparsomycin, gougerotin, or chloramphenicol) are partially but not completely additive, except for the combination of gougerotin and chloramphenicol, which effects are somewhat more than additive.

Discussion

According to current concepts, there are two sRNA binding sites on bacterial ribosomes, one for free aminoacyl-sRNA and the other for peptidyl-sRNA (or the chain-initiating formylmethionyl-sRNA). Puromycin either has access only to the aminoacyl-sRNA

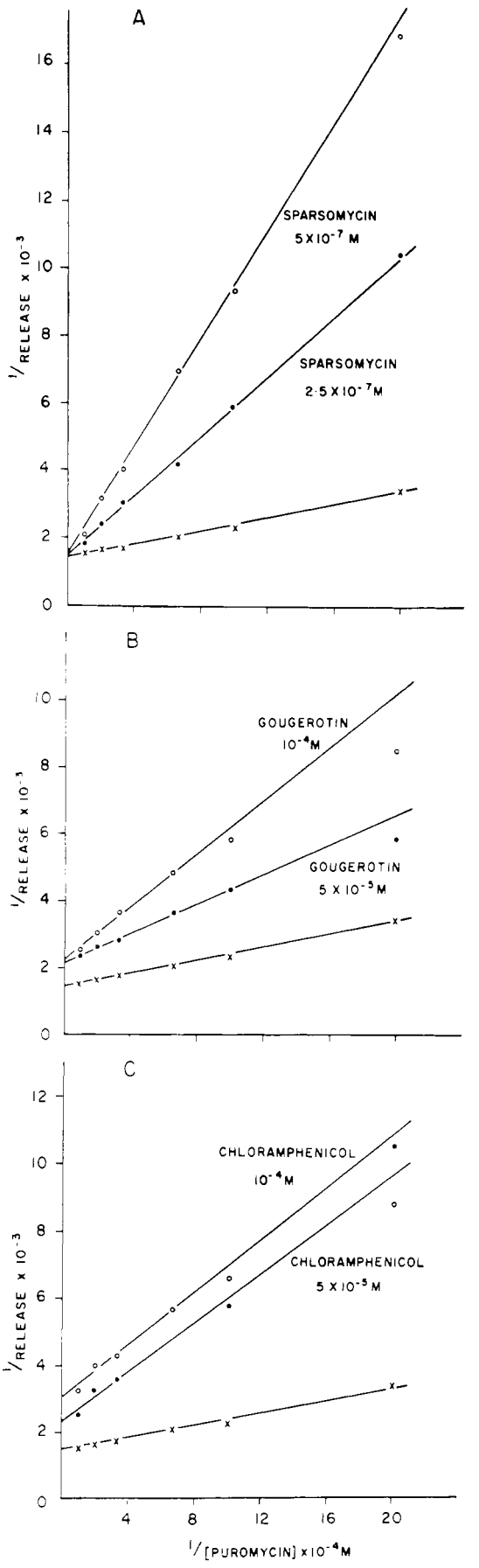

FIGURE 6 (right): Lineweaver–Burk plots of the effect of sparsomycin, gougerotin, and chloramphenicol on the puromycin-induced release of polylysine from sRNA bound to ribosomes. Incubation conditions and assay were the same as described in Table II except that the puromycin concentration was varied as indicated and incubation was for 15 min. (A) Sparsomycin, (B) gougerotin, and (C) chloramphenicol were included in the incubation as indicated. ×—× represents incubations containing puromycin but no other antibiotic.

TABLE V: Cooperative Effects of Sparsomycin, Chloramphenicol, and Gougerotin on Puromycin-Stimulated Release of Peptides.[a]

Antibiotic			^{14}C Peptides Released (cpm)	% Inhibn of Release
Sparsomycin	Gougerotin	Chloramphenicol		
−	−	−	413	−
+	−	−	253	38.8
−	+	−	296	28.3
−	−	+	264	36.1
+	+	−	163	60.5
+	−	+	141	65.9
−	+	+	101	75.6

[a] The following were incubated with or without sparsomycin (10^{-6} M), gougerotin (10^{-4} M), or chloramphenicol (10^{-4} M) in a final volume of 0.05 ml: 0.1 M Tris–HCl (pH 7.22), 0.01 M magnesium acetate, 0.1 M ammonium acetate, 5 μg of poly A, 7.95 μg of [^{14}C]polylysyl-sRNA, 10^{-4} M puromycin, and 73 μg of ribosomes. After incubating for 30 min at 35°, the reaction was assayed for cold TCA-precipitable radioactivity as described in Methods. Control incubations lacking puromycin had 570 cpm.

site on the ribosome or, perhaps more likely, interacts directly with the analogous site of the peptide bond forming unit (? enzyme) itself. It is apparent from the work of Rychlik (1965a), Bretscher (1966), M. E. Gottesman (personal communication), and that reported here that ribosomes alone are self-sufficient in the aminoacylation of puromycin when synthetic polynucleotides are used as messengers. Formation of a single peptide bond, therefore, does not require soluble factors other than a source of peptidyl-sRNA and aminoacyl-sRNA or its substitute, puromycin. Since sparsomycin does not affect either aminoacyl-sRNA or peptidyl-sRNA binding to ribosomes, its inhibition of the puromycin reaction appears to involve the peptide bond forming system itself. The high affinity of sparsomycin for this system may indicate that sparsomycin bears a structural resemblance to a normal component of the peptide bond forming unit. Further speculation, however, must await additional data on the chemical structure of the antibiotic. Since sparsomycin acts as a competitive inhibitor of puromycin (and presumably of bound aminoacyl-sRNA), it is possible that the antibiotic interacts with the active site of the peptide bond forming unit, although an allosteric effect is also possible. It is not yet clear, however, how such an action might account for the different sensitivities to sparsomycin of various polynucleotide-promoted polypeptide synthesis (Goldberg and Mitsugi, 1966, 1967), especially when puromycin does not show this phenomenon (Speyer *et al.*, 1963).

Similar considerations also apply to the actions of gougerotin and chloramphenicol. The structural dissimilarity of these two antibiotics to the known components of the peptide bond forming system[2] and the mixed nature of their competition with puromycin suggest that these agents may function as allosteric inhibitors. It should be noted, however, that our results with gougerotin differ from those of Casjens and Morris (1965) who reported data suggesting that gougerotin was a strictly competitive inhibitor of puromycin in prelabeled reticulocytes.

Finally, it should be mentioned that kinetic analysis of inhibitor effects in any system as poorly defined and as complex as the ribosomal peptide bond forming system demands extreme caution in interpretation. While the kinetics reported here indicate that sparsomycin competes with puromycin in peptide bond synthesis, this may be fortuitous and not indicative of a direct interaction of these two agents at the same site. As an extension of the concept that a small molecule can change the configuration at the active site of an enzyme by interaction at some other site on the same protein, it is possible to envisage how such an interaction at one end of a ribosome might influence function at another end. Each of the three antibiotics studied here might interact at different sites on the ribosome and yet produce, as the end result, interference with the catalytic site concerned with formation of the peptide bond.

Acknowledgment

The technical assistance of Miss Victoria Weyler is gratefully acknowledged.

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[2] Gougerotin, which contains a cytosine aglycon and a dipeptide (sarcosyl-D-serine) in acylamino linkage to a 4-aminohexose (Fox *et al.*, 1964), differs radically in structure from the amino acid bearing end of sRNA. Further, although gougerotin contains a dipeptide, it does not prevent binding of peptidyl-sRNA to ribosomes nor compete with peptidyl-sRNA in the puromycin-release reaction.

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Studies on the Nature of the Chloroplast Lamella. I. Preparation and Some Properties of Two Chlorophyll–Protein Complexes*

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ABSTRACT: Chloroplasts have been treated with the anionic detergent sodium dodecylbenzene sulfate (SDBS) and two chlorophyll–protein complexes were obtained by electrophoresis in polyacrylamide gels. These components (complexes I and II) account for approximately 25 and 50%, respectively, of the lamellar protein. Complex I has a chlorophyll a/b ratio of 12:1 and complex II has a ratio of 1.2:1. Complex I is more readily extracted by successive SDBS treatments. Sublamellar particles, prepared using digitonin, depleted in photochemical system I, give higher proportions of complex II, and those deficient in system II yield more complex I. We conclude that complexes I and II are derived from systems I and II, respectively, and that complex I represents a more superficial particle in the lamellar matrix.

The lipids, pigments, and cations associated with the gross lamellar lipoprotein material of the chloroplast are well documented (Lichtenthaler and Park, 1963), but little is known of the number and composition of the macromolecules in this complex. This paucity of knowledge is due mainly to the difficulty encountered in rendering the lamellae soluble at physiological pH. Bailey *et al.* (1966) have reviewed recently the methods used to solubilize the lipoprotein complex and concluded that in their hands anionic detergents were the most useful reagents for the complete dissociation of the lamella into its components.

Solutions of the anionic detergents, SDS[1] and SDBS, have been used frequently to dissolve the insoluble material of chloroplasts (Smith and Pickels, 1941; Wolken, 1956; Chiba, 1960; Itoh *et al.*, 1963) and a single sedimenting boundary (2–5.5 S) has been observed in the ultracentrifuge with these extracts (a similar sedimentation coefficient has also been observed for the lipid-free total protein of lamellae dissolved in SDS solution (Biggins and Park, 1965)). However, the most recent work on the solubilization of the lamellae with anionic detergents (Bailey *et al.*, 1966; Ogawa *et al.*, 1966; Sironval *et al.*, 1966) has shown that several protein components are present, some of which contain chlorophyll and all of which presumably contain detergent. Characterization of the macromolecules in such extracts is essential to our understanding of the structure and function of the lamella.

In this communication a method is described for the isolation of two major chlorophyll-containing proteins and evidence adduced for their possible relation to photochemical systems I and II (Witt *et al.*, 1961; Duysens *et al.*, 1961). A subsequent communication will describe the composition of the two complexes. A preliminary note on the isolation and characterization of the two major chlorophyll–protein components has been reported (Thornber *et al.*, 1966).

Methods

Preparation of Chloroplasts. Chloroplasts were

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[1] Abbreviations used in this work: SDS, sodium dodecyl sulfate; SDBS, sodium dodecylbenzene sulfate; Chl, chlorophyll; NADP, nicotinamide–adenine dinucleotide phosphate.