INHIBITION OF PEPTIDYL-sRNA BINDING TO RIBOSOMES BY PACTAMYCIN

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In recent years several antibiotic inhibitors of aminoacyl-sRNA binding to bacterial ribosomes have been described (Suarez and Nathans, 1965; Hierowski, 1965; Chang, Sih and Weisblum, 1966; Pestka et al., 1965; Kaji and Kaji, 1965; Ennis, 1966; Yamaguchi and Tanaka, 1967; Vazquez and Monro, 1967). Of these, only chlortetracycline (CTC) has been tested for its effect on the specific binding of peptidyl-sRNA to ribosomes (Rychlik, 1966; Goldberg and Mitsugi, 1967; Gottesman, personal communication, 1967). When ribosomes are in excess, poly A-dependent binding of polylysyl-sRNA to ribosomes is not affected by levels of CTC (10^{-4} M) that maximally inhibit both binding of lysyl-sRNA to ribosomes and polypeptide formation. Under these conditions polylysyl-sRNA is presumed to bind directly to the donor site on the ribosome and can interact with puromycin in the absence of added soluble factors (Rychlik, 1966; Goldberg and Mitsugi, 1967; Gottesman, personal communication, 1967). It would be of considerable interest to have an agent that inhibits the binding of peptidyl-sRNA to the ribosome and thus interferes with initiation of polypeptide synthesis. We now report on the antibiotic pactamycin, an inhibitor of protein synthesis (Colombo et al., 1966; Bhuyan, 1967), which interferes with the specific binding of (^{14}C) polylysyl-sRNA to E. coli ribosomes at concentrations that inhibit polylysine synthesis.

Materials and Methods

Salt (0.5 M ammonium chloride) washed E. coli B ribosomes, (^{14}C) polylysyl-sRNA and (^{14}C) lysyl-sRNA were prepared as previously described (Goldberg and Mitsugi, 1967). The binding of (^{14}C) polylysyl-sRNA and (^{14}C) lysyl-sRNA to ribosomes, and the puromycin-induced deacylation of polylysyl-sRNA were measured as reported (Goldberg and Mitsugi, 1967). Pactamycin and CTC were gifts from Dr. C. G. Smith of the Upjohn Co. and the Lederle Laboratories, respectively. Poly A was purchased from the Miles Chemical Co. Radioactivity was determined in a Packard scintillation counter.

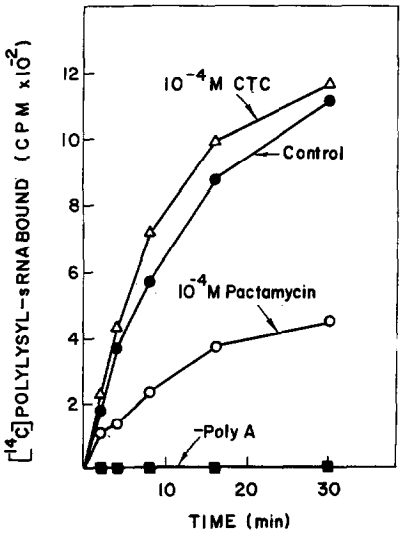

Fig. 1 Comparison of effects of pactamycin and chlortetracycline on binding of polylysyl-sRNA to ribosomes. The following were incubated at 25° with or without pactamycin (10^{-4}M) or chlortetracycline (10^{-4}M) in a volume of 0.35 ml: 0.1 M Tris-HCl (pH 7.2), 0.007 M magnesium acetate, 0.1 M ammonium acetate, 35 μg of poly A, 1085 μg of ribosomes and 57 μg of (^{14}C) polylysyl-sRNA (16,590 cpm) to initiate the reaction. At the indicated times, 50 μl samples were removed and assayed for ribosomal bound radioactivity as previously described (Goldberg and Mitsugi, 1967). Zero time values (control, 403 cpm, with pactamycin, 376 cpm, with chlortetracycline, 360 cpm, without poly A, 299 cpm) were subtracted at each time point.

Results and Discussion

At 7 mM Mg^{++} pactamycin (10^{-4} M) inhibits the poly A-dependent binding of (^{14}C) polylysyl-sRNA to ribosomes about 60 per cent, whether studied by the Millipore filter technique (Figure 1) or by sucrose density gradient centrifugation (Figure 2). The same concentration of CTC stimulates the binding slightly. The interference with peptidyl-sRNA binding to ribosomes by pactamycin is not secondary to an inhibition of the attachment of synthetic messenger polynucleotide to the ribosomes (Cohen and Goldberg, unpublished results, 1967). Maximal inhibition (50 to 60 per cent) of peptidyl-sRNA binding to ribosomes is reached at about 3×10^{-6} M pactamycin, at which concentration polylysine synthesis is almost completely inhibited. The incomplete effect of the highest levels of pactamycin on peptidyl-sRNA binding suggests that half or more of the polylysyl-sRNA binds to a ribosomal site susceptible to pactamycin and the remainder to a site resistant to the antibiotic. It should be noted that we have found a 30 per cent inhibition by pactamycin (10^{-4} M) of the specific binding of lysyl-sRNA to ribosomes at 7 mM Mg^{++} (Cohen and Goldberg, unpublished results, 1967). It will be of interest to learn whether this effect is on the binding site on the 30S ribosomal subunit or on the one created by the addition of the 50S subunit (Suzuka et al., 1966).

In addition to interfering with binding when present from the start, pactamycin also induces the release of previously bound polylysyl-sRNA without its cleavage (Table I). Again, at 3×10^{-6} M pactamycin the extent of release levels off at about 60 per cent of that initially bound. At higher Mg^{++} concentrations (10 mM or 14 mM) the pactamycin effect decreases, while the puromycin-induced release of peptide bound to ribosomes is greater than at 7 mM Mg^{++} (Cohen and Goldberg, unpublished results, 1967). While

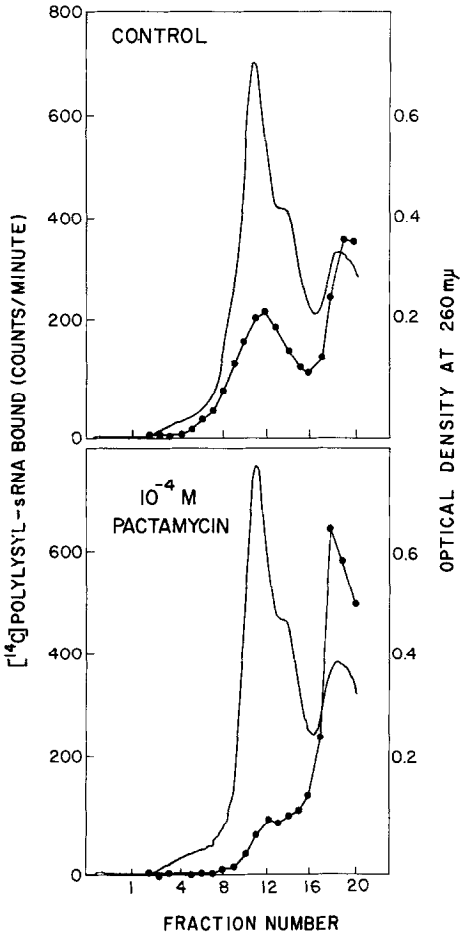

Fig. 2 Sucrose density gradient analysis of polylysyl-sRNA binding to ribosomes in presence or absence of pactamycin. The following were incubated at 35° with or without pactamycin (10^{-4}M) in a volume of 0.1 ml: 0.1 M Tris-HCl (pH 7.2), 0.007 M magnesium acetate, 0.1 M ammonium acetate, 10 μg of poly A and 247 μg of ribosomes. After 10 minutes, 15.6 μg of (^{14}C) polylysyl-sRNA (4540 cpm) was added and incubation was continued for 15 minutes. A 75 μl aliquot of the reaction mixture was placed on a 5 ml linear sucrose gradient (5-20%) containing 0.1 M Tris-HCl (pH 7.2), 0.01 M magnesium acetate and 0.1 M ammonium acetate. After centrifugation in a SW 39 rotor at 39,000 rpm for 2 hr at 4°, three drop fractions were collected and assayed for radioactivity as previously described (Goldberg and Mitsugi, 1967). Optical density (——) was recorded automatically in the Gilford absorbance recorder. ●——● counts/minute.

these results suggest that pactamycin may exert a greater effect on ribosome-

bound peptidyl-sRNA which does not interact with puromycin; i. e., is not bound to the donor site, further experimentation is required to settle this point. Since complete inhibition of polylysine synthesis by pactamycin (10^{-4} M) is observed at 14 mM Mg^{++}, where the effect on binding of both polylysyl-sRNA and lysyl-sRNA to ribosomes is relatively small, it is not clear how these actions of the antibiotic are related.

Table I

Comparison of Effect of Pactamycin on the Release of Bound Polylysyl-sRNA

	Binding		TCA-Insoluble	
	CPM Bound	% Released	CPM	% Deacylation
Control	2943	-	4257	-
+ Pacta	1256	57	4071	4

The following were incubated at 35° in a volume of 0.1 ml: 0.1 M Tris-HCl (pH 7.2), 0.007 M magnesium acetate, 0.1 M ammonium acetate, 10 μg of poly A, 247 μg of ribosomes, and 14.2 μg of (^{14}C) polylysyl-sRNA (4140 cpm) to initiate the reaction. After 15 minutes, to allow for completion of binding of (^{14}C) polylysyl-sRNA to ribosomes, where indicated, pactamycin (10^{-4} M) was added and incubation was continued for 15 minutes. The reaction was stopped by addition of either cold buffer or 5% TCA and assayed for ribosomal bound or cold TCA-precipitable radioactivity, respectively, as previously described (Goldberg and Mitsugi, 1967). The extent of the deacylation of (^{14}C) polylysyl-sRNA was measured as a conversion of radioactivity from acid-insoluble to acid-soluble material (Rychlik, 1966).

These results are consistent with those of Felicetti et al. (1966) who found by sucrose gradient centrifugation that pactamycin does not interfere with the puromycin-induced release of labelled peptide from polyribosomes in reticulocytes. It would be of interest to know whether some of the released peptide in these experiments was still attached to sRNA. The finding of Colombo et al. (1966) that the polyribosomes are degraded to single ribosomes when reticulocytes are incubated with pactamycin may result from an effect

of the antibiotic on the initiation of polypeptide synthesis. Under these conditions physiological breakdown of polyribosomes would not be accompanied by their reformation.

Since it appears likely that the specific binding of peptidyl-sRNA to ribosomes is analogous to the specific binding of initiator sRNA's, pactamycin should interfere with the binding of the latter as well. Such studies with formylmethionyl-sRNA and N-acetyl-phenylalanyl-sRNA are being conducted.

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