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Antibiotic Effects on the Photoinduced Affinity Labeling of *Escherichia coli* Ribosomes by Puromycin†

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ABSTRACT: The effect of ribosomal antibiotics on the photoinduced affinity labeling of *Escherichia coli* ribosomes by puromycin [Cooperman, B. S., Jaynes, E. N., Brunswick, D. J., & Luddy, M. A. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2974; Jaynes, E. N., Jr., Grant, P. G., Giangrande, G., Wieder, R., & Cooperman, B. S. (1978) *Biochemistry 17*, 561] has been studied. Although blasticidin S, sparsomycin, lincomycin, and erythromycin are essentially without effect, major changes are seen on addition of either chloramphenicol or tetracycline. The products of photoincorporation have been characterized by one- and two-dimensional gel electrophoresis and by specific immunoprecipitation with antibodies to ribosomal proteins. In the presence of chloramphenicol, protein S14 becomes the major labeled protein. In the presence of tetracycline, L23 remains the major labeled protein, but the yield of labeled ribosomes is enormously increased, and the labeling is more specific for L23. These results are discussed in terms of the known modes of action of these antibiotics and the photoreactivity of tetracycline.

A current goal of research on *Escherichia coli* ribosomes is the development of a structure–function map permitting localization of given ribosomal functions to specific regions of the ribosome. We have been using the technique of photoaffinity labeling as an approach to this problem. In previous work, we showed that when ribosomes are irradiated in the presence of the antibiotic puromycin, the protein L23 is the major site of puromycin incorporation (Cooperman et al., 1975) and that such incorporation proceeds via a true affinity labeling process (Jaynes et al., 1978). Puromycin is unique among naturally occurring ribosomal antibiotics in being a substrate for the ribosome-catalyzed peptidyl transferase reaction. Many other ribosomal antibiotics have been shown to inhibit this reaction, with some acting as apparent competitive inhibitors toward puromycin. In addition, several antibiotics have been shown to induce significant confor-

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mational changes on binding to the ribosome (Miskin & Zamir, 1974). In this paper, we examine the effects of several ribosome-directed antibiotics on the photoincorporation of puromycin into ribosomes and, in the cases of chloramphenicol and tetracycline, where large changes are seen, characterize, by a combination of two-dimensional polyacrylamide gel electrophoresis (PAGE)[1] and specific immunoprecipitation, the major labeled proteins. In the following paper (Grant et al., 1979), the changes seen in the presence of chloramphenicol are subjected to more detailed scrutiny.

Experimental Section

Materials. Radioactive puromycin was obtained from Amersham ([8-^{3}H]puromycin; 3500 Ci/mol). Puromycin (Sigma or United States Biochemical), puromycin aminonucleoside (PANS) (Sigma), chloramphenicol (Calbiochem), and erythromycin (Aldrich) were obtained commercially. Tetracycline, lincomycin, blasticidin S, and sparsomycin were gifts from Dr. J. G. Flaks (University of Pennsylvania). *N*-Phenylalanylpuromycin aminonucleoside (PhePANS) was prepared as described (Harris et al., 1972). Cytidylyl-(3′–5′)-3′-*O*-phenylalanyladenosine (CAPhe) was a gift of Dr. S. Chladek (Michigan Cancer Foundation).

Preparation of Ribosomes and Ribosomal Subunits. Ribosomes were prepared from *E. coli* Q13 as previously described (Jaynes et al., 1978) by using a modification of the Traub procedure (Traub et al., 1971). Subunits were prepared from 70S ribosomes labeled with puromycin as previously described (Jaynes et al., 1978).

Photolytic Incorporation of Puromycin. Photolyses were performed as described (Cooperman et al., 1975; Jaynes et al., 1978) in standard buffer (50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, and 50 mM KCl) by using Rayonet lamps RPR-3500 Å. Prior heat activation of 70S ribosomes for 20–30 min at 37 °C had no obvious effect on the observed labeling patterns.

Polyacrylamide Gel Electrophoresis (PAGE). Protein was extracted from ribosomes by using the Mg^{2+}–acetic acid procedure (Hardy et al., 1969). The acetic acid supernatant was either lyophilized or the protein was precipitated by the addition of 5 volumes of acetone (Barritault et al., 1976). The proteins were then redissolved in 8 M urea and 10 mM 2-mercaptoethanol. One-dimensional PAGE was performed and the gels were analyzed as described (Jaynes et al., 1978). Two-dimensional PAGE was performed by using the Howard & Traut (1974) modification of the method of Kaltschmidt & Wittmann (1970). In some gels the sample was applied only to the anodic end of the first-dimension gel, allowing a longer first-dimension gel to be used and increasing the resolution of the basic proteins in the second dimension. Prior to running the second dimension, first-dimension gels were either dialyzed against 0.3 M HCl (20 min) and then briefly (5–10 min) rinsed with second-dimension starting buffer (Avital & Elson, 1974), procedure A, or dialyzed against starting buffer for 1–1.5 h (Kaltschmidt & Wittmann, 1970), procedure B. After completion of the run, the gel was stained and destained (Howard & Traut, 1974). Areas of the gel containing and surrounding the stained proteins as well as other areas of interest were cut out of the gel and radioactivity was determined as described for one-dimensional gels (Jaynes et al., 1978), except that 1 mL of the 30% hydrogen peroxide mixture was used per slice in a 20-mL vial, digestion proceeded for 12–16 h at 50 °C, and 10 mL of scintillation fluid was added prior to counting.

Procedure A gave consistently higher yields of recovered radioactivity than procedure B, although both procedures gave approximately the same relative amounts of radioactivity incorporated into ribosomal proteins. Using procedure A, we recovered 14 ± 4% of the radioactivity applied in the first dimension with the basic proteins in the second dimension. The results from two-dimensional gels were compared with one another by normalizing to a 14% recovery in the basic proteins and further correcting for differences in the amount of labeled protein added and in the specific radioactivity of puromycin. In general, absolute values for the larger peaks of radioactivity (S14 and L23) obtained in identical experiments by using a common ribosomal preparation varied ±20% when compared in this manner. Variability in absolute values in the smaller peaks of radioactivity, as well as in the larger peaks of radioactivity on comparing results obtained with different ribosomal preparations, was occasionally more pronounced, changes of the order of twofold sometimes being observed. The ratio of L23/S14 labeling was, however, fairly reproducible (±10–20%), even when different ribosomal preparations were used. Counts reported in Figures 1–4 are for 20% counting efficiency.

Purification of Ribosomal Proteins and Preparation of Ribosomal Protein Specific Antisera. The methods for the purification of *E. coli* 30S and 50S ribosomal proteins, as well as for the preparation of antisera to the individual proteins, are described elsewhere (Lindahl et al., 1977).

Immunoprecipitation. Antiglobulin immunoprecipitation (i.e., "sandwich") experiments were done similarly to those described by Roberts & Roberts (1975). The entire procedure was carried out at 0–4 °C. Ribosomal protein extracts (TP30, TP50, or TP70) were dissolved in minimal volumes of buffer A (10 mM KPO_4, 150 mM NaCl, and 6 M urea; adjusted to pH 6.5 with methylamine) and dialyzed overnight against buffer B (25 mM Tris-HCl, pH 7.4, 1 M KCl, and 1% Triton X-100). Protein concentrations were determined by the Lowry method (Lowry et al., 1951).

Antisera to individual ribosomal proteins were assumed to contain approximately 10 mg/mL γ-globulin, of which 5–10% was assumed to be ribosomal protein specific. Using an average molecular weight of 15000 for all ribosomal proteins, we mixed 5–15-μg samples of ribosomal proteins with 10- to 15-fold molar excesses of the appropriate antisera. Samples were incubated for 15–18 h at 0 °C to form the primary complex. To form the secondary complex (sandwich precipitate), goat antibody to rabbit γ-globulin (GARGG, Calbiochem Cat. No. 539844) was then added at a level of 1 unit/40 μg of γ-globulin, and samples were incubated for an additional 90 min at 0 °C. The precipitates were pelleted by centrifugation at 20000*g* for 30 min; the supernatants were saved, and the precipitates were dispersed in 0.5 mL of buffer C (50 mM Tris-HCl, pH 7.4, 1.2 M KCl, and 1.2% Triton X-100). The precipitates were pelleted 3 more times in this manner and dispersed in 0.5 mL of buffer D (50 mM Tris-HCl, pH 7.4, and 100 mM NaCl). The precipitates were again pelleted, washed once more with buffer D, repelleted, and lyophilized. For each sample, all supernatants were combined and likewise lyophilized.

Lyophilized supernatant and precipitate fractions were suspended in 1.5 mL of 67% acetic acid, 15-mL portions of Aquasol (New England Nuclear Corporation) were added, and the samples were counted in a Packard Tri-Carb liquid

[1] Abbreviations used: PANS, puromycin aminonucleoside; PhePANS, *N*-phenylalanylpuromycin aminonucleoside; CAPhe, cytidylyl-(3′–5′)-3′-*O*-phenylalanyladenosine; PAGE, polyacrylamide gel electrophoresis.

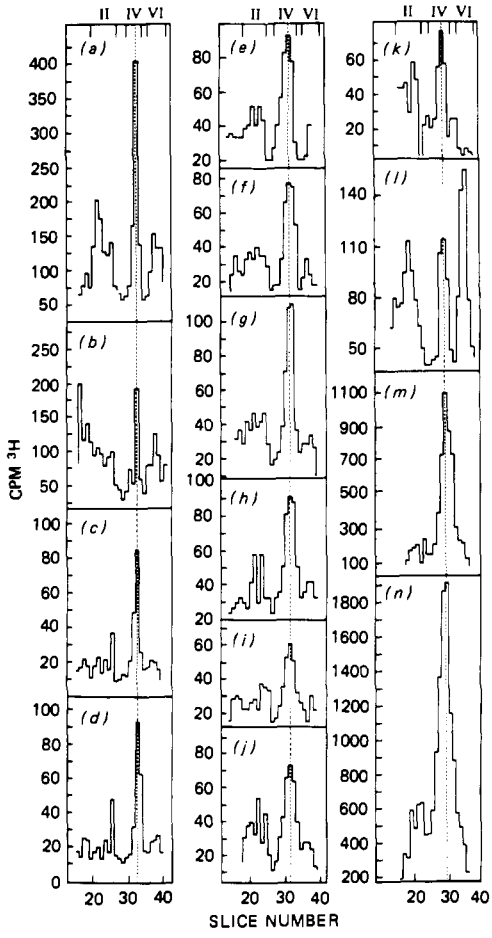

FIGURE 1: One-dimensional polyacrylamide gel patterns of proteins from 70S ribosomes labeled with puromycin in the presence of other substances: (a) puromycin alone, (b) plus 0.25 mM CAPhe, (c) puromycin alone, (d) plus 1 mM lincomycin, (e) puromycin alone, (f) plus 0.05 mM sparsomycin, (g) plus 0.50 mM sparsomycin, (h) plus 0.05 mM blasticidin S, (i) plus 0.50 mM blasticidin S, (j) plus 0.05 mM erythromycin, (k) plus 0.5 mM erythromycin, (l) plus 0.1 mM chloramphenicol, (m) plus 0.05 mM tetracycline, (n) plus 0.50 mM tetracycline. Experimental conditions: (a) and (b) 99 A_{260} units/mL ribosomes, 0.15 mM puromycin (926 Ci/mol), 120-min photolysis; (c) and (d) 99 A_{260} units/mL ribosomes, 0.044 mM puromycin (3820 Ci/mol), 8-min photolysis; (e)–(n) 100 A_{260} units/mL ribosomes, 0.1 mM puromycin (750 Ci/mol), 20-min photolysis. Counts reported are for protein from 7 A_{260} units of ribosomes. Note the change in scale in Figure 1m and 1n.

scintillation counter. Results were expressed as the percentage of the total radioactivity found in the sandwich precipitate after correction for nonspecific occlusion of labeled protein (by using a preimmune serum control sample).

Results

One-Dimensional PAGE Analysis of the Effect of Antibiotics on Puromycin Photoincorporation into Ribosomes. Photoincorporation of puromycin into protein L23 can be monitored by one-dimensional PAGE analysis of total ribosomal protein (Jaynes et al., 1978). Although L23 is incompletely resolved from several other ribosomal proteins by such PAGE analysis, this procedure is valid because of the much higher labeling of L23 as compared with that found in these other proteins. Figure 1 shows a limited region of a typical one-dimensional PAGE of ribosomal proteins extracted from ribosomes photolyzed in the presence of radioactive puromycin. The large peak in region IV corresponds to labeled L23 (dotted line) (Jaynes et al., 1978). In the presence of CAPhe, a structural analogue of puromycin which is also active as a peptidyl transferase acceptor, L23 labeling is specifically

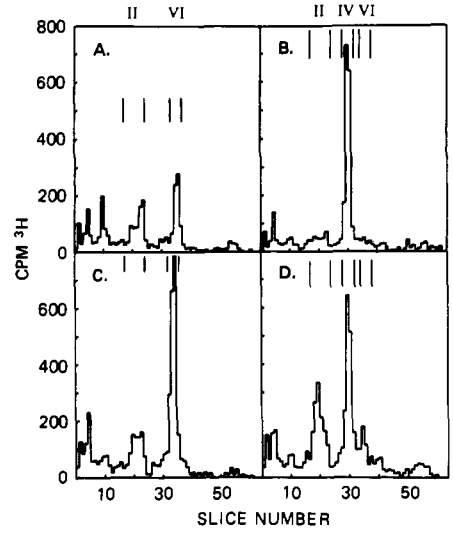

FIGURE 2: One-dimensional polyacrylamide gel patterns of proteins from ribosomal subunits derived from 70S ribosomes labeled with puromycin in the presence and absence of chloramphenicol: (A) 30S proteins, puromycin alone; (B) 50S proteins, puromycin alone; (C) 30S proteins, puromycin plus chloramphenicol; (D) 50S proteins, puromycin plus chloramphenicol. Experimental conditions: 100 A_{260} units/mL ribosomes; 0.10 mM puromycin (1500 Ci/mol); (A) and (B) 30-min photolysis; (C) and (D) plus 0.10 mM chloramphenicol, 20-min photolysis. Counts reported are for protein from 4 A_{260} units of 30S subunits and 8 A_{260} units of 50S subunits.

decreased (Figure 1b). Similar results have been obtained with PhePANS, another structural and functional analogue of puromycin (Jaynes et al., 1978). This approach has now been extended to study the effects of other ribosomal antibiotics on puromycin photoincorporation. As can be seen, lincomycin (1 mM; Figure 1c,d), sparsomycin (0.05 and 0.5 mM; Figure 1e–g), and moderate concentrations of blasticidin S (0.05 mM; Figure 1e,h) or erythromycin (0.05 mM; Figure 1e,j) have little or no effect no puromycin incorporation. At a higher concentration, blasticidin S (0.5 mM; Figure 1e,i) causes a generalized decrease in incorporation while erythromycin (0.5 mM; Figure 1e,k) may slightly reduce the relative labeling in region IV. On the other hand, the presence of either chloramphenicol or tetracycline induces large changes in the labeling results. In the presence of chloramphenicol (0.1 mM), overall incorporation increases by 70 ± 10% and a large new peak appears in region VI (Figure 1e,l). The labeling of region II relative to region IV also increases but not as a single sharp peak. In the presence of tetracycline (0.05 mM), overall incorporation increases four- to fivefold, and there is a dramatic increase in the selectivity of region IV labeling (Figure 1e,m). Qualitatively similar results are obtained at a higher tetracycline concentration (0.5 mM) with the overall incorporation being increased 11- to 12-fold (Figure 1e,n). In the presence of puromycin alone, photoincorporation proceeds predominantly into ribosomal protein (≥70%) (Jaynes et al., 1978). None of the added antibiotics significantly altered the percentage of RNA labeling.

Identification of the Major Proteins Labeled by Puromycin in the Presence of Chloramphenicol or Tetracycline. The one-dimensional PAGE prompted us to seek to identify the major proteins labeled in the presence of chloramphenicol and tetracycline, by using both one- and two-dimensional polyacrylamide gel electrophoresis and specific immunoprecipitation. When ribosomes labeled in the presence of chloramphenicol were separated into subunits and the proteins from each of the subunits were analyzed on one-dimensional gels, the large, new peak in region VI (Figure 1l) is found to be due

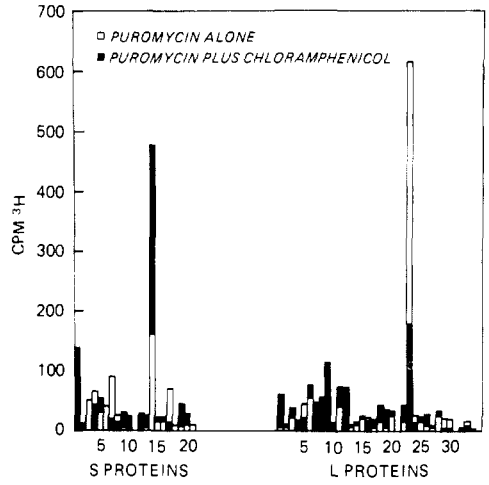

FIGURE 3: Pattern of ribosomal protein labeling with puromycin in the presence and absence of chloramphenicol as determined by two-dimensional polyacrylamide gel electrophoresis. The values for proteins labeled with puromycin alone are the average of two gels, and those for proteins labeled in the presence of chloramphenicol are the average of seven gels. In displaying the data, the lower radioactivity value is superimposed on the higher one. Experimental conditions: puromycin alone (□), same as described in Figure 2A,B; puromycin plus chloramphenicol (■), same as described in Figure 2C,D. Counts reported are for protein from 20 A_{260} units of ribosomes.

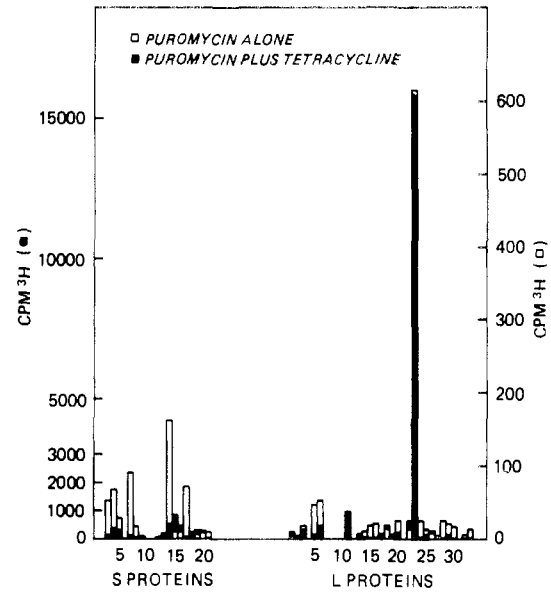

FIGURE 4: Two-dimensional gel pattern of ribosomal protein labeled by puromycin in the presence and absence of tetracycline. Gels were analyzed as described under Experimental Section. The results have been plotted with the heights of the L23 peaks equal to emphasize the relative suppression of the labeling in proteins other than L23 in the presence of tetracycline. However, the differences in the actual magnitude of the peaks should be noted. In displaying the data, the lower value is superimposed on the higher one. Experimental conditions: puromycin alone (□), same as described in Figure 2A,B; plus tetracycline (■), 100 A_{260} units/mL ribosomes, 0.10 mM puromycin (1500 Ci/mol), 0.50 mM tetracycline, 20-min photolysis.

to a 30S subunit protein (Figure 2). A two-dimensional PAGE analysis of proteins from 70S ribosomes labeled in the presence of chloramphenicol showed the major peak of radioactivity to be virtually coincident with the stained spot for protein S14. The second largest peak was found slightly to the upper left of L23 in the same position as had been observed previously for labeling by puromycin alone (Jaynes et al., 1978). Full analyses of two-dimensional gels of proteins from 70S ribosomes labeled in the presence of either chloramphenicol (0.1 mM) or tetracycline (0.5 mM) are presented in Figures 3 and 4, respectively. The results obtained on labeling with puromycin alone are also presented in these figures for purposes of comparison. In addition to the large increase in S14 labeling, the presence of chloramphenicol also leads to modest increases in the labeling of several other proteins, thus accounting for the observed rise in region II labeling in one-dimensional gels (Figure 2). In the presence of tetracycline, the major peak seen was again found just to the upper left of protein L23. The coincidence of this position of labeled protein with that obtained in the absence of tetracycline is an indication that the modified L23 which results from puromycin incorporation is the same or similar whether or not tetracycline is present. It is clear from Figure 4 that the major effect of tetracycline is to vastly increase the stoichiometric extent of L23 labeling (some 26 times over that found with puromycin alone).

The results of specific immunoprecipitation experiments, summarized in Table I, provide the necessary confirmation that L23 and, secondarily, S14 are the major proteins labeled on irradiation in the presence of puromycin alone, that S14 and, secondarily, L23 are the major proteins labeled on addition of chloramphenicol, and, finally, that L23 is the major protein labeled on addition of tetracycline. The immunoprecipitation and two-dimensional gel results are also in good quantitative accord with respect to the relative amounts of puromycin incorporation into L23 and S14. Thus, the PAGE results (Figures 3 and 4) give L23 incorporation/S14 incorporation ratios of 3.8 in the absence of chloramphenicol, of 0.38 in the presence of chloramphenicol, and of 30 in the presence of tetracycline, while the corresponding values determined by immunoprecipitation (Table I) are 5.1, 0.42, and 16. However, the percentages of total radioactivity in proteins S14 and L23 determined by immunoprecipitation are quite generally lower (by a factor of 1.5–2.0) compared with those estimated from PAGE analysis. Although we are not sure of the reason(s) for these differences, it should be kept in mind that estimates derived from immunoprecipitation results are based on total radioactivity extracted from a ribosomal subunit

Table I: Immunoprecipitation Results[a]

antibody[b] to protein	% labeled 30S or 50S protein precipitated		% labeled 70S protein precipitated		
	puromycin alone[c]	plus chloramphenicol[d]	puromycin alone[e]	plus chloramphenicol[f]	plus tetracycline[g]
S1					8
S12	7	10	2.0	6	
S13	3	9	1.0	5	
S14	16.5	40	5.5	25	2
S18	8	6	3	3	
S19	0	3	0	1.5	
L18 + L22	0	0	0	0	
L19	0	1	0	0.5	
L21	0	1	0	0.5	
L23	43	25	28	10.5	32
L25	0	0	0	0	

[a] Photolyses are for 30 min (puromycin alone) or 20 min (puromycin plus chloramphenicol or plus tetracycline). Puromycin concentration, 0.1 mM; chloramphenicol concentration, ±0.1 mM; tetracycline concentration, ±0.5 mM. [b] S12, S13, S18, and S19 are the closest 30S proteins to S14 in the two-dimensional PAGE system. Likewise, L18, L19, L21, L22, and L25 are the closest 50S proteins to L23. [c] Measured. [d] Measured. [e] Calculated from the column entitled "puromycin alone" (30S or 50S protein) and measured ratio of radioactivity in 50S protein compared to 30S protein. [f] Calculated from the column entitled "plus chloramphenicol" (30S or 50S protein) and measured ratio of radioactivity in 50S protein compared to 30S protein. [g] Measured.

or 70S ribosome and that such extracts may include small amounts of strongly adsorbed noncovalently bound puromycin, as well as puromycin incorporated into ribosomal protein which may have lost its antigenicity as a result of covalent labeling, proteolysis, or light-induced denaturation. By contrast, estimates derived from PAGE results are based on total radioactivity comigrating with ribosomal proteins.

Discussion

Blasticidin S, erythromycin, and lincomycin have all been found to bind to unique tight sites on washed ribosomes with dissociation constants respectively of 2 (Kinoshita et al., 1970), 0.01 (Pestka, 1974a), and 34 μM (Fernandez-Muñoz et al., 1971). The results shown in Figure 1 provide strong evidence that the tight-site binding of each of these antibiotics is not directly competitive with puromycin binding[2] nor do any possible conformational changes accompanying the binding of these antibiotics (Miskin & Zamir, 1974) result in any major change in the puromycin-labeling pattern. Sparsomycin is also without effect on puromycin incorporation, despite its being a strict competitive inhibitor for puromycin in the peptidyl transferase reaction, with a K_I below 1 μM (Goldberg & Mitsugi, 1967; Lee & Vince, 1978). This result is consistent with the earlier findings of Tada & Trakatellis (1970) and of Pestka (1974b) showing sparsomycin not to bind to washed ribosomes.

Chloramphenicol is also a competitive inhibitor for puromycin in the peptidyl transferase reaction, at least under certain conditions (Coutsogeorgopoulos, 1966; Pestka, 1970), but, unlike sparsomycin, binds directly to washed ribosomes (Vazquez, 1966) with a dissociation constant of approximately 1 μM (Fernandez-Muñoz et al., 1971; Lessard & Pestka, 1972). Although the results of both the two-dimensional PAGE and the immunoprecipitation analyses are in accord in showing that a saturating (100 μM) concentration of chloramphenicol does not completely block L23 labeling, there is an apparent quantitative difference between the two types of experiments regarding the precise effect of chloramphenicol on puromycin incorporation into L23. Added chloramphenicol (100 μM) results in an increase in overall puromycin labeling of 50–100%. Thus, the immunoprecipitation results in Table I, though showing a decrease in the percentage of puromycin incorporation into L23, show little, if any, decrease in the absolute amount of L23 labeling. By contrast, the two-dimensional PAGE results in Figure 3 indicate a twofold decrease in L23 labeling on addition of chloramphenicol. Since the immunoprecipitation results compare *relative* values of incorporation which are intrinsically more reliable than the *absolute* values obtained by two-dimensional PAGE (see Experimental Section), conclusions based on the immunoprecipitation results are more likely to be correct. We therefore conclude that L23 labeling is not decreased in any major way on chloramphenicol addition, so that, by this criterion, chloramphenicol, like blasticidin S, erythromycin, lincomycin, and sparsomycin, does not compete for the puromycin binding site involved in L23 labeling. Additional evidence in support of this conclusion may be found in the following paper (Grant et al., 1979). The lack of such competition is not unexpected given the considerable structural specificity that the peptidyl transferase reaction displays with respect to the puromycin site (Nathans & Neidle, 1963; Vince et al., 1975; Symons et al., 1978). That chloramphenicol is a competitive inhibitor for puromycin requires that chloramphenicol and functional puromycin binding be mutually exclusive under the conditions of the peptidyl transferase assay, but such exclusivity need not be true of washed ribosomes.

On the basis of the two-dimensional PAGE results (Figure 4), the effects of tetracycline are not only to greatly increase the extent of L23 labeling but also to increase the specificity of this labeling with respect to that of other ribosomal proteins. Tetracycline is known to bind to a large number of sites on the ribosome (Connamacher & Mandel, 1965; Day, 1966; Maxwell, 1968) and has also been reported to have a single strong interaction site (Strel'tsov et al., 1975; Tritton, 1977). It also is known to sensitize a number of light-induced reactions, such as viral particle inactivation (Esparza et al., 1976), and we have recently shown it to photoincorporate directly into ribosomes.[3] Furthermore, it has an absorption maximum at 350 nm, which coincides with the maximum output of the 3500-Å lamps used in this study. Taken together, these facts adequately account for the first tetracycline effect. The second effect is more problematic. In the first place, the immunoprecipitation results (Table I) show only a small increase in the specificity of L23 labeling on addition of tetracycline. This is another example where the PAGE and immunoprecipitation methods give somewhat different quantitative results. Second, tetracycline is not a peptidyl transferase inhibitor and is known to bind more tightly to the 30S than to the 50S subunit (Connamacher & Mandel, 1965; Maxwell, 1968), so its ability to increase the specificity of L23 labeling would be surprising. Further work is obviously required before a definitive conclusion can be reached regarding this effect. In any case, addition of tetracycline provides a method for the preparation of ribosomes in which L23 is labeled by puromycin to a stoichiometrically significant extent, which may be useful in a variety of future experiments.

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[2] If the light fluences employed were such that puromycin incorporation reached a saturating value as a function of light fluence, the observed failure of these antibiotics to prevent puromycin labeling could conceal a true competitive effect on the rate, as opposed to the final extent, of puromycin incorporation. However, in the experiments shown in Figure 1, puromycin incorporation is essentially proportional to light fluence, so that the failure to prevent incorporation reflects a failure to compete for the puromycin site. The relatively small effects seen at high blasticidin S and erythromycin concentrations may arise from less specific binding of these antibiotics.

[3] R. A. Goldman and B. S. Cooperman, unpublished experiments.

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On the Mechanism of Chloramphenicol-Induced Changes in the Photoinduced Affinity Labeling of *Escherichia coli* Ribosomes by Puromycin. Evidence for Puromycin and Chloramphenicol Sites on the 30S Subunit†

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ABSTRACT: Chloramphenicol has been shown to cause a major change in the ribosomal protein labeling pattern when *Escherichia coli* ribosomes are photolyzed in the presence of radioactive puromycin. In the absence of chloramphenicol, the major labeled protein is L23, while in its presence S14 becomes the major labeled protein [Grant, P. G., Strycharz, W. A., Jaynes, E. N., Jr., & Cooperman, B. S. (1979) *Biochemistry* (preceding paper in this issue)]. This paper reports a detailed investigation of this change, which has allowed the following conclusions to be drawn. (1) The labeling of S14 by puromycin proceeds from a puromycin binding site. (2) The stimulation of S14 labeling by chloramphenicol requires a specific chloramphenicol binding site. (3) Both of the above binding sites are located on the 30S subunit. (4) The stimulation of S14 labeling occurs as a result of a chloramphenicol-dependent light-induced alteration of the 30S subunit. Overall, our results and those obtained in related studies provide evidence that there are binding sites for both chloramphenicol and puromycin on both the 50S and 30S subunits and that the sites on each of the subunits are close to one another. They also provide a clear demonstration of the importance of examining labeling patterns as a function of light fluence in photoincorporation experiments. The possible significance of the previously unsuspected puromycin and chloramphenicol sites on the 30S subunit is discussed.

We have been investigating the photoinduced affinity labeling of the *Escherichia coli* ribosome by puromycin. In previous work we have shown that L23 is the major protein labeled by puromycin, that S14 is labeled to a secondary extent, and that labeling of these proteins occurs from a puromycin-specific binding site or sites (Cooperman et al., 1975; Jaynes et al., 1978). In the preceding paper (Grant et al., 1979) we extended these studies to measure the effects of other ribosome-directed antibiotics on the labeling process. A dramatic change was noted in the presence of chloramphenicol, in the presence of which S14 becomes the major protein labeled. In this paper we explore this change in detail by addressing the following questions. (a) Does the labeling of S14 proceed from a puromycin binding site? (b) Does the effect of chloramphenicol arise from a specific chloramphenicol–ribosome interaction? (c) Are S14 and L23 labeled from a single puromycin binding site on the 70S ribosome or from separate 30S and 50S sites? (d) Are photoinduced alterations in the ribosome important for the observed change in the labeling pattern?

Experimental Section

Materials. L-*erythro*-2,2-Dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenyl]acetamide (LECAM)[1] was a gift

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