Inhibitors of the Transfer of Amino Acids from Aminoacyl Soluble Ribonucleic Acid to Proteins*

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(Received for publication, September 24, 1964)

In recent years, puromycin (1-4), gougerotin (5), chloramphenicol (3, 6-8), neomycin (9), cycloheximide (10, 11), and the tetracyclines (12, 13) have been identified as inhibitors of the transfer of amino acids from aminoacyl soluble ribonucleic acid to proteins (“transfer reaction”). The diversity of structures of these compounds suggests they have different sites of inhibitor action. This paper reports investigations on the mode of action of these specific inhibitors of protein biosynthesis.

EXPERIMENTAL PROCEDURE

Amino Acid-incorporating System of Escherichia coli—H4C-L-Phenylalanyl S-RNA or H4C-L-lysyl S-RNA were prepared with the use of the E. coli 100,000 × g supernatant of Nirenberg, Matthaei, and Jones (14) and E. coli S-RNA (General Biochemical Industries) under the conditions of Holley et al. (15). The resultant H4C-L-phenylalanyl S-RNA or H4C-L-lysyl S-RNA was isolated by phenol extraction (specific activity, 3.6 to 4.4 nmoles of H4C-L-phenylalanine and 5.2 nmoles of H4C-L-lysine per mg) H4C-Polyphenylalanine or H4C-polylysine, or both, were synthesized from their respective “charged” H4C-aminoacyl S-RNA with the cell-free system of Nirenberg et al. (14) with the following modification. Unless stated otherwise, reaction mixtures, containing 50 μg of poly U or 5 μg of poly A and with 50 μmoles of NH4Cl replacing P-enolpyruvate and pyruvic kinase, supernatant enzyme, followed by addition of enzyme and 10 min tureres, containing 50 pg of poly U or 5 pg of poly A and with 50 mmoles of NH4Cl replacing P-enolpyruvate and pyruvic kinase, were preincubated at 37°C for 3 min in the absence of 100,000 × g supernatant enzyme, followed by addition of enzyme and 10 min of further incubation at 37°C. Reactions were stopped, and amino acid polymers were washed on membrane filters by the method of Clark and Gunther (5) with the modification that in cases of 14C-Laerylalanine synthesis, 50 pmoles of 14C-L-phenylalanine and 5.2 mpmoles of 14C-L-lysine were added in place of 14C-L-leucine, 19 other 14C-amino acids, and once washed ribosomes. Incubation mixtures were maintained at 37°C for 20 min before termination of the reactions and isolation of the precipitates by the method of Clark and Gunther (6).

Assay of Binding of 14C-Poly U by E. coli Ribosomes—The assays of effect of inhibitors upon the binding of 14C-poly U by ribosomes is modeled after that of Suarez and Nathans (17). Aliquots (0.1 ml each) of E. coli ribosomes (14), washed 3 times by suspension and sedimentation in 0.01 M Tris-Cl, pH 7.5; 0.01 M magnesium acetate; 0.06 M KCl; and 0.006 M β-mercaptoethanol (final concentration, 0.6 mg of protein per ml), were incubated for 10 min at 30°C with 0.08 ml of the same buffer containing or lacking 7 × 10⁻⁴ M tetracycline or 2 × 10⁻⁴ M neomycin B. The samples were then cooled to 0°C and mixed with 0.02 ml of a 1 mg per ml solution of 14C-poly U (300,000 per mg) in the above buffer. The samples were incubated for 20 min at 0°C and then layered onto 4.4 ml of a cold, linear 5 to 20% gradient of sucrose in the above buffer, containing where necessary 7 × 10⁻⁴ M tetracycline or 2 × 10⁻⁴ M neomycin B. These preparations were centrifuged at 20,000 rpm in the Spinco SW 39 rotor (35,000 rpm, 90 min) before collection of separate 10-drop fractions through a 20-gauge needle inserted into the bottom of the tube. Odd numbered fractions were diluted with 1 ml of water and examined for 14C material with the use of appropriate blanks. Even numbered tubes were treated with 3 ml of cold 10% trichloroacetic acid. The resultant precipitates were collected on membrane filters, dried, and then counted by liquid scintillation.

Assay of Transfer Reactions with Purified Transfer Enzymes of Rabbit Reticulocytes—“Shock-treated” rabbit reticulocyte ribosomes were prepared according to the method of Arlinghaus, Schaeffer, and Schweet (18). H4C-L-Phenylalanyl S-RNA was prepared with the use of E. coli S-RNA and E. coli 100,000 × g supernatant as described above (specific activity, 3.9 nmoles of L-phenylalanine per mg of S-RNA). Binding enzyme and peptide synthetase were purified according to the method of Arlinghaus, Bishop, and Schweet (19).

Aminoacyl S-RNA binding to shock-treated ribosomes of rabbit reticulocytes was assayed with the use of the messenger RNA and GTP-requiring binding enzyme of Arlinghaus et al. (18, 19) and an adsorption assay of Nirenberg and Leder (20). The 1.4-ml reaction mixture, containing 50 μmoles of Tris-Cl buffer, pH 7.5; 100 μmoles of KCl; 10 μmoles of MgCl₂; 0.1 μmole of GTP; 0.1 μmole of H4C-L-phenylalanine; 250 μg of H4C-L-phenylalanyl S-RNA; 100 μg of poly U; 1.5 mg of shock-treated ribosomes; 100
µg of binding enzyme; and inhibitors where indicated, was incubated for 30 min at 37° and then chilled to 0°. Cold 0.01 M Tris-Cl buffer, 3.5 ml, pH 7.5, containing 0.002 M MgCl₂ was added, and the mixtures were immediately poured through chilled membrane filters. The filters were then rapidly washed three times with 5-ml washes of the same cold buffer (total time interval, 15 min), transferred to counting vials, dried at 90° for 15 min, and counted to within 2% of correct values by liquid scintillation.

The assay mixture for the synthesis of ³H-polypheenylaniline, with the use of both purified binding enzyme and peptide synthetase, contained the ingredients of the above binding reaction plus 15 µmoles of GSH (adjusted to pH 7.5 with KOH), 100 µg of peptide synthetase, 120 µg of creatine kinase, and 10 µmoles of phosphocreatine in a final volume of 1.4 ml. The reaction mixtures were incubated for 30 min at 37° before termination of the reactions and isolation of the precipitates by the method of Clark and Gunther (5).

Release of Radioactive Materials from Prelabelled Ribosomes—Ribosome complexes prelabelled with ³H-L-leucine (2 × 10⁶ cpm per µmole) were prepared by the method of Morris et al. (2). The release of labeled material from prelabelled ribosome complexes, with or without inhibitors, was studied by modification of the method of Morris et al. (2). The complete reaction mixture contained 1 mg of prelabelled ribosome complexes; 1.3 mg of 70% ammonium sulfate enzyme fraction; 100 µg of S-RNA; 2 µg of pyruvic kinase; 1.0 µmole of ATP adjusted to pH 7.5 with KOH; 50 µmoles of Tris-Cl buffer, pH 7.5; 2.5 µmoles of P-enolpyruvate; 20 µmoles of GSH (adjusted to pH 7.5 with KOH); 50 µmoles of KCl; 5 µmoles of MgCl₂; 0.05 µmole of GTP; 0.05 µmole of a mixture of 20 amino acids (each 0.001 M); and inhibitors, in a final volume of 1.4 ml. After incubation at 37° for 10 min, the assays were chilled and transferred to 7-ml centrifuge tubes with the aid of 5 ml of 0.25 M sucrose. The ribosomes from each assay were isolated by centrifugation at 100,000 × g for 60 min. The ribosome pellets were then rinsed with 2 ml of 0.25 M sucrose solution and homogenized in 1 ml of 0.1 M Tris-Cl buffer, pH 7.5. The ribosome suspensions were then transferred to a tube containing 2% casein solution in an amount calculated to give 15 mg of total protein per tube. The centrifuge tubes were washed once with 1 ml of 0.1 M Tris-Cl buffer, pH 7.5, and the washings were added to the ribosome suspensions. These ribosome preparations were precipitated with 0.1 volume of cold 50% trichloroacetic acid. The precipitates were washed twice with 5 ml of 5% trichloroacetic acid; dissolved in 0.5 ml of 1 M NaOH; and, after 2 min, reprecipitated with 5 ml of 5% trichloroacetic acid. The precipitates containing the acid-insoluble ribosomal materials were washed twice with 5 ml of 5% trichloroacetic acid and finally collected on membrane filters for counting by the method of Clark and Gunther (5).

Casein solution (2%) was added to the supernatants collected after centrifugation at 100,000 × g for 60 min, to give a total protein content of 15 mg per tube. These mixtures were then precipitated with 0.1 volume of cold 50% trichloroacetic acid. The protein precipitates, representing acid-precipitable proteins in the supernatant fraction, were washed, hydrolyzed in alkali, washed, and counted in the same manner as the ribosomes above.

The radioactivity (counts per min) released as acid-soluble supernatant materials was calculated as the difference between the total acid-insoluble counts per min present initially and the total acid-insoluble counts per min recovered from the ribosomal and supernatant fractions at the end of the 10-min incubation.

Sources of Materials—Cycloheximide, neomycin B trisulfate, and tetracycline hydrochloride were gifts from the Upjohn. Purvomycin dihydrochloride was obtained from Nutritional Biochemicals; gougerotin was kindly provided by Takeda Chemical Industries; chloramphenicol was obtained from Parke-Davis. Where necessary, inhibitors were titrated with KOH to pH 7 before use. ³H-C-L-Amino acids were obtained from Schwarz BioResearch; poly U, poly A, and ³H-poly U were obtained from the Upjohn.

RESULTS

Inhibition of Protein Synthesis in Cell-free Systems of E. coli and Rabbit Reticulocyte—Table I shows that several compounds inhibit the cell-free system of E. coli that catalyzes the poly U-dependent transfer of ³H-C-L-phenylalanine from ³H-C-L-phenylalanyl S-RNA to ³H-C-polypheenylaniline. This is as previously reported for puromycin (3), gougerotin (5), chloramphenicol (3), and tetracycline (13) in other cell-free systems of E. coli. Only cycloheximide fails to demonstrate high levels of inhibition in this bacterial system.

Similar levels of these same inhibitors also inhibit the formation of polyphenylaniline and hemoglobin in the cell-free system of rabbit reticulocytes (Tables I and II). These inhibitors function during the transfer of amino acids from aminoacyl S-RNA to protein, for they do not inhibit the formation of the aminoacyl S-RNA of reticulocytes, when assayed at levels known to inhibit

TABLE I

<table>
<thead>
<tr>
<th>Condition</th>
<th>E. coli Activity</th>
<th>Inhibition</th>
<th>Rabbit reticulocyte Activity</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm</td>
<td>%</td>
<td>cpm</td>
<td>%</td>
</tr>
<tr>
<td>Complete</td>
<td>507</td>
<td>0</td>
<td>421</td>
<td>0</td>
</tr>
<tr>
<td>Complete + 4 mM puromycin</td>
<td>499</td>
<td>99</td>
<td>36</td>
<td>99</td>
</tr>
<tr>
<td>Complete + 4 mM gougerotin</td>
<td>224</td>
<td>63</td>
<td>176</td>
<td>58</td>
</tr>
<tr>
<td>Complete + 8 mM chloramphenicol</td>
<td>404</td>
<td>0</td>
<td>352</td>
<td>17</td>
</tr>
<tr>
<td>Complete + 10 mM chloramphenicol</td>
<td>500</td>
<td>1</td>
<td>56</td>
<td>86</td>
</tr>
<tr>
<td>Complete + 4 mM cycloheximide</td>
<td>49</td>
<td>0</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Complete + 4 mM neomycin B</td>
<td>24</td>
<td>0</td>
<td>98</td>
<td>98</td>
</tr>
</tbody>
</table>
the transfer reaction. It should be noted that cycloheximide serves as an effective inhibitor for protein synthesis in the reticulocyte system while chloramphenicol is not an effective inhibitor of this system.

**Inhibition of Transfer Reactions with Purified Transfer Enzyme Fractions of Rabbit Reticulocyte**—The transfer of amino acids from aminoacyl S-RNA to protein involves at least two distinct steps, i.e. the binding reaction to form the ribosome, messenger RNA, aminoacyl S-RNA complexes (ribosome complexes), and the peptide synthesis reaction (18). The overall transfer reaction is envisioned as an alternation of binding reactions and peptide syntheses with the resultant development of peptidyl S-RNA-messenger RNA-ribosome complexes during the travel of ribosomes along the messenger RNA (18, 21). Completed protein chains are then released from the ribosome complexes to yield supernatant or soluble protein.

The well defined cell-free system of rabbit reticulocytes (18, 19) offers a chance to study the action of inhibitors on either the binding reactions or the peptide synthesis reactions. The availability of both purified binding enzyme (18, 19) and an adsorption assay for ribosome complex formation (20) provides an easy assay of inhibitor action on the first or binding reaction as it occurs in rabbit reticulocytes. Comparison of these findings with the results obtained with this system during the complete transfer of amino acids from aminoacyl S-RNA to protein (i.e. in the presence of \(^{14}C\)-L-phenylalanyl S-RNA, poly U, ribosomes, GTP, and both binding enzyme and the enzyme peptide synthesis [18]) allows assignment of the relative site of action of inhibitors.

Table III reveals that only tetracycline appreciably inhibits the binding reaction while tetracycline, puromycin, gaurgerotin, neomycin B and, to a lesser extent, cycloheximide all express inhibitions when assayed in the complete transfer reaction.

A possible explanation for this tetracycline-dependent loss in aminoacyl S-RNA-binding ability (Table III) is that tetracycline decreases the binding of messenger RNA (poly U) to ribosomes and thus lowers the binding of aminoacyl S-RNA. This possibility is eliminated by the data of Fig. 1 which show that preincubation of E. coli ribosomes with tetracycline or neomycin B does not reduce the overall binding of \(^{14}C\)-poly U. These data are in agreement with those of Suarez and Nathans (17) and those of Hierowski (22). Similar preincubation of messenger-free ribosome of E. coli with tetracycline or neomycin B (at the concentrations of Fig. 1) does not alter the sucrose density patterns obtained from the messenger-free ribosomes. Thus, the slight aggregation of ribosomes that occurs upon pretreatment with tetracycline and subsequent treatment with \(^{14}C\)-poly U requires poly U for expression.

An additional control experiment shows that tetracycline and neomycin B, at the concentrations of Table III, do not change the adsorption properties of membrane filters towards E. coli ribosomes. Thus, the membrane filter assay is a valid assay of aminoacyl S-RNA, messenger RNA, ribosome complex formation. It then follows that the lowered complex formation observed in the presence of tetracycline must represent an interference by tetracycline with the association of aminoacyl S-RNA upon messenger RNA-containing ribosomes.

It should be noted (Table III) that neomycin B causes an apparent two-fold increase in aminoacyl S-RNA binding. Control experiments show that this neomycin B-dependent increase in aminoacyl S-RNA binding requires the presence of both ribosome and poly U.

**Effect of Inhibitors upon Release of Radioactive Materials from Predesigned Ribosomes**—Inhibitor studies with the use of the rabbit reticulocyte system, \(^{14}C\)-amino acids, and \(^{14}C\)-prelabeled ribosome complexes (i.e. ribosome complexes containing messenger RNA and \(^{14}C\)-peptidyl S-RNAs) after the method of Morris et al. (2) offer a second tool to define the relative sites of action of the inhibitors of the transfer reaction in protein synthesis. As seen in Table IV, all the inhibitors of hemoglobin synthesis in Table II also inhibit the reactions necessary for the completion of

**TABLE III**

**Effect of inhibitors on the binding reactions with use of purified transfer enzyme fractions of rabbit reticulocyte**

Assay conditions as described in "Experimental Procedure." The binding reaction assay is free of peptide synthesis activity, for no radioactivity is recovered in precipitates after assaying for polyphenylalanine synthesis as described in "Experimental Procedure."

<table>
<thead>
<tr>
<th>Condition</th>
<th>Binding reaction</th>
<th>Polyphenylalanine synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete, zero time</td>
<td>114</td>
<td>7</td>
</tr>
<tr>
<td>Complete, 30 min</td>
<td>3000, 2900</td>
<td>4994, 4790</td>
</tr>
<tr>
<td>Complete, - poly U; 30 min</td>
<td>221</td>
<td>143</td>
</tr>
<tr>
<td>Complete, - ribosome; 30 min</td>
<td>96</td>
<td>7</td>
</tr>
<tr>
<td>Complete, + 1.0 (\mu) mole of puromycin; 30 min</td>
<td>3200</td>
<td>1087</td>
</tr>
<tr>
<td>Complete, + 4.0 (\mu) moles of gaurgerotin; 30 min</td>
<td>2741</td>
<td>933</td>
</tr>
<tr>
<td>Complete, + 0.25 (\mu) mole of neomycin B; 30 min</td>
<td>5957</td>
<td>265</td>
</tr>
<tr>
<td>Complete, + 1.0 (\mu) mole of tetracycline; 30 min</td>
<td>1671</td>
<td>2508</td>
</tr>
<tr>
<td>Complete, + 1.0 (\mu) mole of cycloheximide; 30 min</td>
<td>3103</td>
<td>3349</td>
</tr>
</tbody>
</table>
partially formed protein and therefore retard the release of acid-insoluble completed protein from ribosomes. In addition, puromycin demonstrates the previously reported (2, 4) release of acid-soluble peptides. The other inhibitors act differently than puromycin in that they do not enhance the release of acid-soluble peptides from ribosomes.

Puromycin has the most clearly defined mechanism of action of the inhibitors used in these studies. Puromycin apparently substitutes for aminoacyl S-RNA during the peptide transfer from peptidyl S-RNA to the amino acid of an adjacent aminoacyl S-RNA (2, 4, 23). Such substitution results in acid-soluble peptides terminating with puromycin at the COOH-terminal end (puromycin peptides). This mechanism provides a third point of reference for determining the relative sites of action of inhibitors of the transfer reaction. If an inhibitor acts at a site before the formation of aminoacyl or peptidyl S-RNA, messenger RNA, ribosome complexes yet before or identical with that of puromycin action, then addition of the inhibitor to 14C-peptidyl S-RNA-containing ribosome complexes in the presence of puromycin and transfer enzymes will result in a decrease in puromycin-dependent release of 14C-labeled acid-soluble peptides from the prelabeled ribosome complexes. If an inhibitor acts before the formation of ribosome complexes or at some point in the transfer reaction after that of puromycin action, addition of the inhibitor to a prelabeled ribosome complex system containing puromycin would not alter the expected response to puromycin.

An excerpt in Table IV, gougerotin inhibits the puromycin-dependent release of 14C-materials from prelabeled ribosome complexes. Gougerotin must therefore act after the formation of prelabeled ribosome complexes. If an inhibitor acts before the formation of ribosome complexes or at some point in the transfer reaction after that of puromycin action, addition of the inhibitor to a prelabeled ribosome complex system containing puromycin would not alter the expected response to puromycin.

**TABLE IV**

**Effect of inhibitors upon release of radioactive materials from prelabeled ribosomes**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Activity</th>
<th>Ribosomal insoluble material</th>
<th>Supernatant insoluble protein</th>
<th>Supernatant acid-soluble peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero time</td>
<td></td>
<td>453</td>
<td>43</td>
<td>0</td>
</tr>
<tr>
<td>Complete</td>
<td></td>
<td>263, 301</td>
<td>164, 167</td>
<td>68, 38</td>
</tr>
<tr>
<td>Complete, + 0.1 μmole of puromycin</td>
<td>115</td>
<td>88</td>
<td>293</td>
<td></td>
</tr>
<tr>
<td>Complete, + 2.0 μmole of gougerotin</td>
<td>423</td>
<td>55</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Complete, + 1.0 μmole of neomycin B</td>
<td>378</td>
<td>51</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Complete, + 2.0 μmole of tetracycline</td>
<td>491</td>
<td>55</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Complete, + 2.0 μmole of cycloheximide</td>
<td>394</td>
<td>57</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Complete, + 0.1 μmole of puromycin and 2.0 μmole of gougerotin</td>
<td>339</td>
<td>63</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Complete, + 0.1 μmole of puromycin and 1.0 μmole of neomycin B</td>
<td>263</td>
<td>91</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Complete, + 0.1 μmole of puromycin and 2.0 μmole of tetracycline</td>
<td>210</td>
<td>76</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>Complete, + 0.1 μmole of puromycin and 2.0 μmole of cycloheximide</td>
<td>120</td>
<td>89</td>
<td>287</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 2.** Inhibition of 14C-polyphenylalanine synthesis by chloramphenicol. Assay conditions as described in "Experimental Procedure" but with varied amounts of poly U. ■■■■, counts per min incorporated in control; □□□□□□, counts per min incorporated in the presence of 2 μmoles of chloramphenicol; ○○○○○○, percentage of inhibition.
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Fig. 3. Inhibition of ¹⁴C-polylysine synthesis by chloramphenicol. Assay conditions as described in Fig. 2 but with varied amounts of poly A. ■■■■ counts per min incorporated in control; ○—○, counts per min incorporated in the presence of 0.02 \( \mu \) mole of chloramphenicol; ◊—◊, percentage of inhibition.

Third, puromycin does not inhibit the binding of aminoacyl S-RNA into ribosome complexes (Table III). This finding, along with the verification of the findings (2, 4) that puromycin releases acid soluble \(^{14}C\) materials from \(^{14}C\)-peptidyl S-RNA, messenger RNA, ribosome complexes (Table IV), suggests the concept that puromycin acts as an analogue of aminoacyl-S-RNA during the peptide synthesis reaction, resulting in the release of peptides terminating in puromycin.

Fourth, the aminoacyl nucleoside gougerotin acts differently than the related aminoacyl nucleoside puromycin in that gougerotin does not enhance the enzymatic release of acid-soluble \(^{14}C\)-materials from \(^{14}C\)-peptidyl S-RNA, messenger RNA, ribosome complexes (Table IV). The findings that gougerotin does not inhibit the binding reaction (Table III) and does inhibit puromycin peptide formation (Table IV) suggest that gougerotin influences the peptide synthesis reaction. It is likely that peptide synthetase accepts the aminoacyl nucleoside puromycin and completes the transfer of the amino acid or peptide chain to the puromycin molecule (2, 4). A related theory, in light of our data, is that peptide synthetase similarly accepts the aminoacyl nucleoside, gougerotin, but cannot complete the amino acid or peptide transfer step, with the result that gougerotin acts as an inhibitor of peptide synthetase.

Fifth, the data presented do not define a specific inhibitor action for the polycation (31) neomycin B. This inhibitor does not influence messenger RNA binding to ribosomes (Fig. 1), yet it does cause a messenger RNA, ribosome-dependent increased binding of aminoacyl S-RNA when in the presence of messenger RNA (Table III). Such increased binding suggests that neomycin B influences or alters the attachment of aminoacyl S-RNA into messenger RNA-containing ribosomes. The observed changes in messenger RNA “coding” potential in the presence of neomycin (9) support this hypothesis. Continuation of the above reasoning dictates that neomycin would not alter puromycin peptide formation. This is observed when one employs the higher levels of neomycin known to enhance aminoacyl S-RNA binding into ribosome complexes (Table IV).

Sixth, Fig. 2 suggests that chloramphenicol competes in the transfer reaction with poly U. This supports the evidence of others (24-26) that chloramphenicol interferes with the utilization of messenger RNAs by ribosomes. Yet this competition cannot be a simple competition between messenger RNA and chloramphenicol for a site upon ribosomes for excess levels of messenger RNA (poly U) do not further decrease the percentage of inhibition characteristic of a fixed level of chloramphenicol (Fig. 2). The extent of polyphenylalanine synthesis in Fig. 2 is ribosome-limiting once high nonlimiting levels of poly U are employed. One explanation for the failure of high levels of poly U to decrease the percentage of a chloramphenicol-dependent inhibition is that there is more than one ribosomal site involved in poly U binding. Our data fit the theory that there is a competition for a second ribosomal site between chloramphenicol and a ribosomal bound poly U.

Further support for theories that chloramphenicol competes with messenger RNA on ribosomes comes from the report (29) that ribosomes of E. coli have a higher affinity for poly U than poly A. If chloramphenicol interferes equally with such binding, then less chloramphenicol is needed for inhibition of poly A-dependent systems than for those dependent upon poly U. The observation (8, 30) that chloramphenicol is a better inhibitor of
poly A-dependent polylysine synthesis than it is of poly U-dependent polyphenylalanine synthesis is in agreement with this hypothesis. Final proof of this theory of chloramphenicol action must await explanation of why chloramphenicol cannot be shown to compete with poly A during protein synthesis (Fig. 3) (30).

Seventh, the inhibitor cycloheximide is shown to have no effect upon the binding reaction (Table III) and puromycin peptide formation (Table IV) in the rabbit reticulocyte system. These data are in agreement with findings of Williamson and Schweet (32) that cycloheximide extensively aggregates ribosomes. The different inhibitory potentials expressed by cycloheximide and chloramphenicol towards protein synthesis in different species (Tables I to III) suggest that these two inhibitors affect different ribosomal functions since species-dependent differences are known for the properties of ribosomes towards protein synthesis (3).

SUMMARY

Puromycin, gougerotin, neomycin B, tetracycline, chloramphenicol, and cycloheximide inhibit protein synthesis in various cell-free systems. Assays designed to determine the mechanism of action of these inhibitors indicate (a) that tetracycline inhibits the binding of aminoacyl soluble ribonucleic acid to messenger ribonucleic acid-containing ribosomes; (b) that the aminoacyl nucleoside, puromycin, releases peptide material from peptidy1 soluble ribonucleic acid, messenger ribonucleic acid, ribosome complexes while the related aminoacyl nucleoside, gougerotin, blocks this release; (c) that neomycin B alters the binding of aminoacyl soluble ribonucleic acid to messenger ribonucleic acid, ribosome complexes; (d) that chloramphenicol can compete with a messenger ribonucleic acid for sites on bacterial ribosomes; and (e) that cycloheximide does not influence the binding of aminoacyl soluble ribonucleic acid to ribosomes or the puromycin-dependent release of peptides from peptidy1 soluble ribonucleic acid, messenger ribonucleic acid, ribosome complexes.

Acknowledgments—We wish to thank Dr. Richard Schweet for his encouragement of this work and Mrs. Ronnie Barker for invaluable technical assistance.

REFERENCES

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