Proc. Nat. Acad. Sci. USA
Vol. 70, No. 8, pp. 2224–2228, August 1973

Identification of the Chloramphenicol-Binding Protein in *Escherichia coli* Ribosomes by Partial Reconstitution*

(LiCl 50S cores/ribosomal split proteins/equilibrium dialysis/chloramphenicol action)

DAGMAR NIERHAUS AND KNUD H. NIERHAUS

Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Berlin-Dahlem, Germany

Communicated by F. Lynen, April 30, 1973

ABSTRACT **50S-derived cores were prepared by treatment of 50S subunits with 0.4 M LiCl (0.4c core) and 0.8 M LiCl (0.8c core), respectively. 0.4c cores bind chloramphenicol whereas 0.8c cores do not. The split proteins obtained during the transitions 0.4c → 0.8c were separated by DEAE-cellulose chromatography and Sephadex G-100 gel filtration. Reconstitution experiments with the fractionated proteins demonstrated that protein L16 is involved in chloramphenicol binding.**

In contrast to chloramphenicol, the CACCA-(*N*-acetyl-leucyl) fragment is bound by the 0.8c core, i.e., this core contains the intact *p*-site moiety of the peptidyltransferase center.

Puromycin can inhibit chloramphenicol binding completely. In the concentration range tested (up to 20 mM) the trinucleotide CCA inhibits chloramphenicol binding as effectively as puromycin, whereas an amino-acid mixture shows no inhibition. It is concluded that chloramphenicol acts exclusively on the *a*-site part of the peptidyltransferase center interfering with the binding of the last two or three nucleotides (3′ end) of aminoacyl-tRNA.

Chloramphenicol, a well-known inhibitor of protein synthesis in prokaryotic cells, binds to ribosomes (1). *In vitro* (2, 3) and *in vivo* (4, 5), the binding occurs in a 1:1 stoichiometry. Only the large ribosomal subunit (50 S) is involved (6).

The drug action is related to peptide-bond formation. This is demonstrated by chloramphenicol inhibition of the "fragment reaction" (7), which is a model reaction for peptide-bond formation. The fragment reaction uses 50S subunits, a peptidyl-tRNA fragment [CACCA-(*N*-acetyl-leucyl)], puromycin as an analogue of an aminoacyl-tRNA fragment, K^+ and Mg^{++} ions, and the unusual requirement of 33% alcohol. Although evidence has accumulated that chloramphenicol inhibits the binding of the aminoacyl terminus of the aminoacyl-tRNA (8–12), interference with the peptidyl recognition center of the peptidyltransferase has been considered (13–16).

In this paper, we show that the ribosome contains one binding site for chloramphenicol that is located exclusively at the *a*-site moiety of the peptidyltransferase center.

Furthermore, we demonstrate that the ribosomal protein L16 is involved in chloramphenicol binding. Simultaneously, the same result was obtained by completely different techniques (O. Pongs, R. Bald, and V. A. Erdmann, the following paper, pages 2229–2233).

Abbreviations: CAM, D(−)-*threo* chloramphenicol; CACCA-(Ac[^{3}H]Leu), CACCA-(*N*-acetyl-[^{3}H]leucyl); EF-G, elongation factor G.

* Paper no. 50 on *Ribosomal Proteins*. Preceding paper is in preparation by R. S. Yu and H. G. Whittmann.

MATERIALS AND METHODS

70S, 50S, and 30S ribosomes were isolated from *Escherichia coli* strain A19 (17) as described elsewhere (K. H. Nierhaus and V. Montejo, manuscript in preparation). ^{14}C-Labeled L-phenylalanine (466 Ci/mol) and L-[^{3}H]proline (1.86 Ci/mmol) were obtained from New England Nuclear Corp., Boston, Mass., [^{14}C]chloramphenicol (4.91 Ci/mol) from Amersham/Searle Corp., England, and puromycin-dihydrochloride from Nutritional Biochemicals Corp., Cleveland, Ohio. Elongation factor G (EF-G) was kindly provided by Dr. A. Parmeggiani, Institut für Molekularbiologie, Stöckheim bei Braunschweig. The oligonucleotides CpCpA, CpA, ApC, and UpU were a generous gift from Dr. R. Bald, Max-Planck-Institut in Berlin-Dahlem.

Equilibrium Dialysis. Equilibrium dialysis was performed in plastic dialysis cells (2 × 2 × 2 cm). Each cell contains two 50-μl chambers separated by a dialysis membrane (Visking, Serva, Heidelberg). The two cell halves are fixed by a metal clamp. In one chamber ribosomes (4 A_{260} units of 30S, 8 A_{260} units of 50S, or 12 A_{260} units of 70S) and in the other 0.5 nM [^{14}C]chloramphenicol and other compounds were injected by a Hamilton syringe. The buffer contained 25 mM Tris·HCl, 5 mM $MgCl_2$, 280 mM NH_4Cl, and 5 mM 2-mercaptoethanol. After incubation (20 hr at 4°) 10 μl were twice removed from each chamber and counted. The average difference between the two chambers was taken as a measure of antibiotic bound to ribosomes. Each dialysis assay was performed with two cells. The statistical error was less than ±5%.

The binding data were plotted according to Scatchard (18) by the equation

$$\frac{\bar{\nu}}{(A)} = K_A (n - \bar{\nu})$$

where $\bar{\nu}$ is mol of chloramphenicol bound per mol of ribosome, (A) is mol of unbound chloramphenicol, K_A is the association constant, and n is the number of binding sites available on the ribosome.

Binding of the CACCA-(Ac[^{3}H]Leu) Fragment. The assay was a modification of the method described by Celma *et al.* (19). The incubation mixture contained 30 mM Tris·HCl (pH 7.4), 225 mM KCl, 100 mM NH_4Cl, 10 mM Mg acetate, 50% (v/v) ethanol, the CACCA-(Ac[^{3}H]Leu) fragment with about 20,000 cpm per assay, and 10 A_{260} units of 50S subunits

or 50S derivatives, or an equimolar amount of 5 A_{260} units of 30S subunits. 180-μl Aliquots were incubated at 0° for 15 min, and the aggregated ribosomes were pelleted at 7000 × g for 5 min. 100 μl of the supernatant or from the control assay (without ribosomes) were mixed with 0.5 ml of Soluene before addition of 5 ml of scintillation fluid. The amount of bound substrate was calculated by the difference between the assay containing ribosomes and the control assay.

Other Methods. Cores and split proteins from the 50S subunit were prepared, and partial reconstitution and the fragment assay were performed as described elsewhere (K. H. Nierhaus and V. Montejo, manuscript in preparation).

The EF-G-dependent GTPase test followed the method described by Parmeggiani (20).

RESULTS

Identification of the protein involved in chloramphenicol binding

Table 1, *Exp. 1*, characterizes the equilibrium dialysis used for chloramphenicol binding. 70S ribosomes and 50S subunits bound chloramphenicol nearly to the same extent, whereas 30S subunits showed only background binding.

The binding of cores derived from 50S subunits by LiCl splitting is demonstrated in Table 1, *Exp. 2*. 0.4c core (treated with 0.4 M LiCl) bound chloramphenicol almost as well as 50S subunits, and the 0.8c core showed very little binding. Binding activity of the 0.8c core can be restored to the 0.4c core level by reconstitution of the 0.8c core with the difference split protein $SP_{0.4-0.8}$ (Table 1, *Exp. 2*). The split protein fraction contained some RNA (23 S and 5 S, as seen by RNA gel electrophoresis). After removal of most of the RNA the split protein $SP_{0.4-0.8}$ (containing the proteins L1, L2, L5, L6, L7, L8/9, L10, L11, L12, L15, L16, L18, L25, L27, L28, and L30) were fractionated by DEAE-cellulose chromatography and Sephadex G-100 gel filtration (Fig. 1; for detailed description see K. H. Nierhaus and V. Montejo, manuscript in preparation). Only the third peak of the elution profile after gel filtration (Fig. 1) showed binding activity after reconstitution with 0.8c cores. This peak contained the proteins L6, L11, and L16. To decide which of these three proteins was responsible for binding, we tested fractions 39 and 43. The reconstituted particles (0.8c + fraction 39) and (0.8c + fraction 43) were tested for chloramphenicol binding, and their protein patterns were displayed by 2-dimensional electrophoresis (Fig. 2 and Table 2). L6 is present only in (0.8c + fraction 39) and not in (0.8c + fraction 43), whereas L16 is found in (0.8c + fraction 39) in traces and in (0.8c + fraction 43) in much greater amounts. L11 is present in both particles in about the same amount. In the chloramphenicol binding test, (0.8c + fraction 43) bound more than twice the amount of chloramphenicol bound by (0.8c + fraction 39) (Table 2). The binding by the two particles was correlated to the amount of L16 (Table 2). From another preparation of cores and split proteins, we prepared a particle containing L6 and L11 but not L16. This particle had the same background binding as the 0.8c core. Therefore, it can be concluded that L16 is responsible for the chloramphenicol binding.

TABLE 1. *Chloramphenicol binding measured by equilibrium dialysis*

Exp.	Particle	[^{14}C]Chloramphenicol bound (pM/pM particle)
1	30S	0.02
	50S	0.80
	70S	0.87
2	50S	0.64
	0.4c	0.54
	0.8c	0.049
	$SP_{0.4-0.8}$	0.03
	(0.8c + $SP_{0.4-0.8}$)	0.41

0.4c and 0.8c are derived cores obtained by LiCl treatment with 0.4 M and 0.8 M LiCl, respectively. $SP_{0.4-0.8}$ is the protein fraction split off from the 0.4c cores by incubation with 0.8 M LiCl.

TABLE 2. *Chloramphenicol binding of 0.8c core and two reconstituted particles*

Particle	Relative concentrations of protein			[^{14}C]-Chloramphenicol bound (pM/pM particle)
	L6	L11	L16	
0.8c	−	−	−	0.03
(0.8c + fraction 39)	++	+	(±)	0.07
(0.8c + fraction 43)	−	+	++	0.18

The relative concentrations of the proteins L6, L11, and L16 in the particles (0.8c + fraction 39) and (0.8c + fraction 43) were estimated from the 2-dimensional electrophoresis pattern (Fig. 2).

Characterization of the chloramphenicol binding

A Scatchard plot (Fig. 3) of the data for chloramphenicol binding revealed one binding site for chloramphenicol (K_A = $9.0 \times 10^5\ M^{-1}$).

Table 3 demonstrates that 0.8c core binds the CACCA-(AcLeu) fragment nearly as well as the 50S subunit. Therefore the 0.8c core contains the intact p-site part of the peptidyltransferase center. On the other hand, the 0.8c core does not

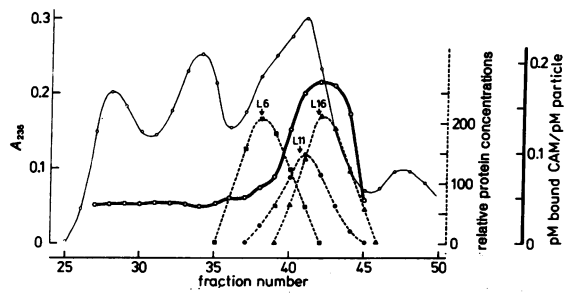


FIG. 1. The gel filtration profile (Sephadex G-100) from basic protein fraction of the $SP_{0.4-0.8}$ proteins (O——O). An aliquot of each fraction was analyzed by acrylamide-gel electrophoresis. Proteins stained by amido black were scanned with a Joyce–Loebl scanner. Relative concentrations of proteins were determined from the areas of protein peaks (- - -). Furthermore, a reconstitution was carried out with 0.8c cores and each fraction. The chloramphenicol binding of the reconstituted particles was measured with the equilibrium dialysis technique (O——O).

TABLE 3. *Binding of CACCA-(Ac[^{3}H]Leu) fragment*

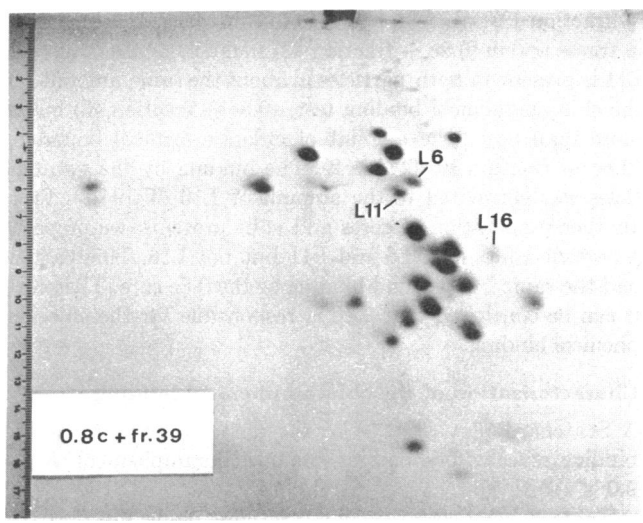
Particle	Cpm of bound fragment
50S	1355
0.8c	1295
30S	322

Incubation of particles and the fragment was performed in the presence of 50% ethanol at 4° for 10 min. After the particles were pelletted, an aliquot of the supernatant was removed and counted. The difference from a control without ribosomes gave the cpm of bound fragment. The method was a modification of Celma *et al.* (19).

bind chloramphenicol. Thus, chloramphenicol does not act on the *p*-site part of the peptidyltransferase center.

As chloramphenicol inhibits binding of aminoacyl oligonucleotides (for example CCA-Phe) to ribosomes (8, 10), we checked a mixture of 20 amino acids and oligonucleotides for inhibition of chloramphenicol binding to ribosomes. As is seen in Fig. 4, a mixture of 20 amino acids does not inhibit even with the highest concentration tested (20 mM). In contrast, puromycin as an analogue of an aminoacyl oligonucleotide inhibits chloramphenicol binding. About 85% inhibition is seen with 40 mM puromycin. At least up to a concentration of 20 mM the trinucleotide CpCpA inhibited the chloramphenicol binding nearly to the same extent as puromycin did. At 20 mM CpA shows a slight but significant inhibition, whereas ApC and UpU have no effect (Table 4).

As chloramphenicol acts mainly on the *a*-site part of the peptidyltransferase center and the EF-G-dependent GTPase center seems to be located in the *a*-site (ref. 21; R. Werner, K. Bordasch, and K. H. Nierhaus, manuscript in preparation), an interaction of chloramphenicol and this GTPase activity could be possible. However, chloramphenicol in concentrations up to 25 mM did not significantly alter the EF-G-dependent GTPase activity (Table 5).



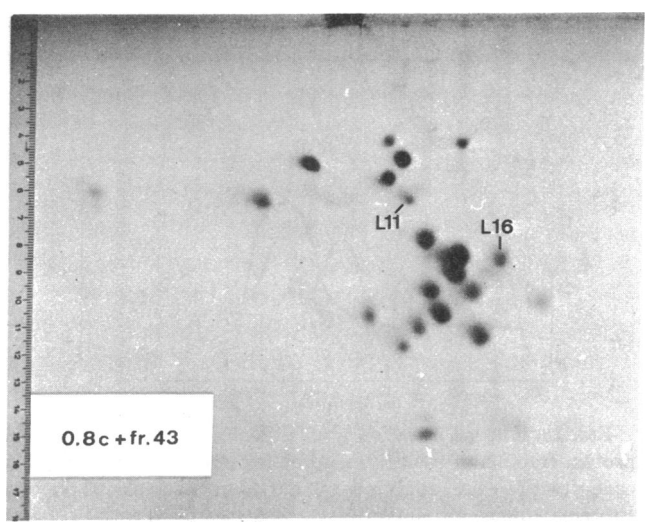


FIG. 2. 2-dimensional electrophoresis pattern of the particles reconstituted with fractions 39 and 43 of the gel-filtered basic protein fraction of $SP_{0.4-0.8}$ (see Fig 1). The (0.8c + fraction 39) particle bound 0.07 pM and the (0.8c + fraction 43) particle bound 0.18 pM chloramphenicol per pM particle.

DISCUSSION

Chloramphenicol inhibits the fragment reaction (7). This reaction can be divided into three steps, as pointed out by Pestka (8): (*a*) binding of the peptidyl-tRNA fragment to the *p*-site part of the peptidyltransferase center, (*b*) binding of the analogue (puromycin) of an aminoacyl-tRNA fragment to the *a*-site, and (*c*) the covalent linkage of the peptidyl residue to puromycin (peptidyltransferase reaction). There is no doubt that chloramphenicol acts at least on the *a*-site moiety for the following reasons:

(*1*) A phenylalanyl-tRNA fragment (CACCA-Phe) binds

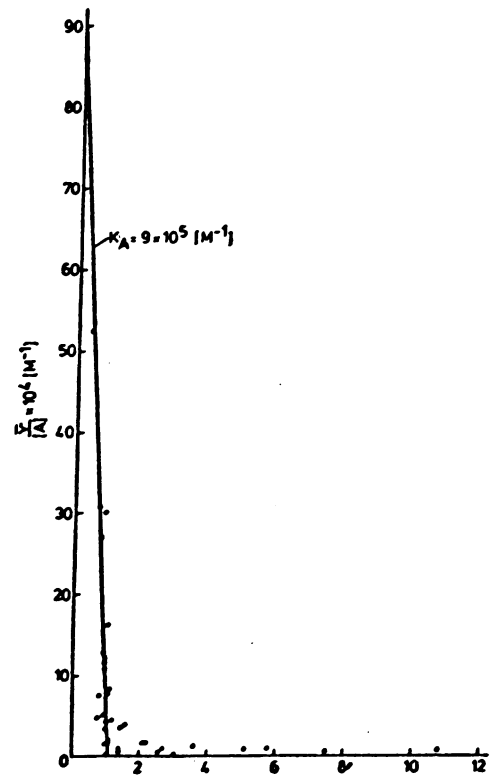


FIG. 3. A Scatchard plot of the equilibrium dialysis data from chloramphenicol binding to 50S subunits. Extrapolation to infinitely high concentration of unbound drug (*A*) indicates 1 binding site per 50S subunit. The association constant is $K_A = 9.0 \times 10^5\ (M^{-1})$.

to the *a*-site of 70S ribosomes since this binding occurs at a puromycin-insensitive site (i.e., phenylalanyl–puromycin formation does not take place, which is the definition of the *a*-site) and can be inhibited by puromycin, i.e., puromycin binds to the same or an overlapping site (8). Chloramphenicol inhibits this fragment binding (8).

(*2*) If chloramphenicol and puromycin both inhibit CACCA-Phe binding, chloramphenicol should inhibit puromycin binding and vice versa. In fact a partial inhibition of chloramphenicol binding by puromycin has been described (11, 12). Furthermore, Fig. 4 demonstrates that puromycin can completely inhibit chloramphenicol binding. Thus, chloramphenicol and puromycin seem to compete for the same site.

This conclusion has been confirmed by Celma *et al.* (10) who demonstrated a complete inhibition of UACCA-Leu binding under fragment reaction conditions by a chloramphenicol concentration sufficient to give nearly complete inhibition of the fragment reaction, whereas under the same conditions the CACCA-(AcLeu) binding was not affected. CACCA-(AcLeu) most probably binds to the *p*-site. Thus, it is evident that chloramphenicol does not interact with the *p*-site of the peptidyltransferase center. This is confirmed by the binding studies with the 0.8c core. Table 3 shows that the CACCA-(AcLeu) fragment is bound to the 0.8c core to the same extent as to the 50S subunit, i.e., the 0.8c core contains the intact *p*-site part of the peptidyltransferase center. However, 0.8c core does not bind chloramphenicol (Table 1, *Exp. 2*). This result shows that chloramphenicol does not bind to the *p*-site part of the peptidyltransferase center. Therefore, the chloramphenicol binding site belongs exclusively to the *a*-site part of the peptidyltransferase center.

The minimal structure of aminoacyl-tRNA that can bind to the *a*-site part at the peptidyltransferase center consists of the 3′-terminal CpA and the aminoacyl residue (22, 23). Therefore we tested the interference of appropriate oligonucleotides (CpCpA, CpA, and as a control, ApC and UpU) and of an amino-acid mixture with chloramphenicol binding (Fig. 4 and Table 4). CpCpA inhibited the chloramphenicol binding as effectively as puromycin at least up to a concentration of 20 mM; CpA showed a slight inhibition, and ApC, UpU, and the amino-acid mixture showed no inhibition. We conclude that chloramphenicol interferes with the binding of the last two or three nucleotides (3′ end) of aminoacyl-tRNA. In contrast to the strong inhibition of binding of aminoacyl oligonucleotides by chloramphenicol, the drug only slightly affects aminoacyl-tRNA binding to the *a*-site (24, 25). Thus,

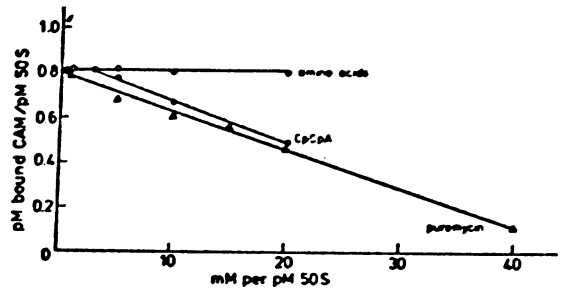


FIG. 4. Inhibition of chloramphenicol binding by puromycin, the trinucleotide CpCpA, and an amino-acid mixture. Puromycin-dihydrochloride was dissolved in 90 mM Tris (unbuffered)–10 mM MgAc, and the pH was adjusted to 6.5 with 1 N KOH (concentration of puromycin-dihydrochloride was 120 mM). The puromycin solution was diluted as indicated; final concentration of the buffer during equilibrium dialysis was 50 mM Tris·HCl (pH 7.5); other conditions were as described in *Methods*. The equilibrium dialysis mixture containing 40 mM puromycin showed a slight turbidity; all the other mixtures were clear. CpCpA was the triethylammonium salt of the trinucleotide. The amino-acid mixture contained the 20 amino acids in equimolar amounts. The indicated concentrations are for each of the amino acids.

the binding of the aminoacyl end of an aminoacyl-tRNA contributes a negligible part to the binding of the whole aminoacyl-tRNA molecule.

The Scatchard plot revealed one binding site of chloramphenicol per 50S subunit (Fig. 3). The plot for 70S ribosomes is similar to the data for the 50S subunits with $n = 1.2$ binding sites per 70S ribosome and the association constant $K_A = 1 \times 10^6$ (M^{-1}) (unpublished observation). Our data do not confirm the finding of Lessard and Pestka (12) who postulated a second binding site—a low affinity site—for chloramphenicol. We found with high chloramphenicol concentration (0.25–1 mM) several points with $n = 2$ up to $n = 11$, which can be expected by unspecific binding with low affinity (Fig. 3).

An analysis of the chloramphenicol-binding site was carried out with 50S-derived cores and fractionated split proteins. Reconstitution studies with 0.8c cores and fractionated proteins of the $SP_{0.4-0.8}$ split proteins showed that chloramphenicol binding depends on the presence of L16 on the 0.8c core (see results, Fig. 2, and Table 1, *Exp. 2*). Although only the L16-containing fraction showed significant chloramphenicol binding after reconstitution with 0.8c cores, the maximum yield of chloramphenicol binding was not more than half (0.18 pM/pM particle) of the activity obtained with total split proteins $SP_{0.4-0.8}$ [compare particle (0.8c + frac-

TABLE 4. *Inhibition of chloramphenicol binding*

Added compound (20 mM)	pM Bound CAM/pM 50S subunit	% Inhibition
Complete	0.73	0
+ puromycin	0.43	41
+ amino acids	0.72	2
+ CpCpA	0.47	36
+ CpA	0.59	19
+ ApC	0.69	5
+ UpU	0.70	4

Chloramphenicol binding was measured by equilibrium dialysis. *CAM*, chloramphenicol.

TABLE 5. *EF-G-dependent GTPase: Effect of chloramphenicol*

Mixture	Mol of GTP hydrolyzed per mol 70S
Complete	53
+ CAM 5 mM	52
+ CAM 25 mM	46
+ fusidic acid 1 mM	12

EF-G-dependent GTPase was measured as described by Parmeggiani *et al.* (20).

tion 43) in Table 2 with particle (0.8c + $SP_{0.4-0.8}$) in Table 1, *Exp. 2*]. We cannot decide from our experiments whether this loss of binding activity was due to partial denaturation of L16 during fractionation or to missing proteins that can stimulate the L16-dependent binding. In any case, L16 is involved in chloramphenicol binding. This finding is supported by affinity labeling experiments showing that the amphenicol residue of iodoamphenicol can be covalently linked to protein L16 (O. Pongs, R. Bald, and V. A. Erdmann, the following paper).

From the discussion above it is clear that L16 is the chloramphenicol-binding protein and that this protein must be a part of the *a*-site moiety of the peptidyltransferase center. Furthermore, L16 should be a neighbor of the peptidyltransferase, which is probably protein L11 (K. H. Nierhaus and V. Montejo, manuscript in preparation).

Yukioka and Morisawa (15) showed that EF-G and GTP counteract the inhibition of poly(U)-dependent poly(phenylalanine) synthesis by chloramphenicol. As the EF-G-dependent GTPase center seems to be located in the *a*-site (ref. 21; R. Werner, K. Bordasch, and K. H. Nierhaus, manuscript in preparation), the chloramphenicol-binding site may overlap or may be allosterically linked to the EF-G-dependent GTPase center. Therefore it was of interest to test whether chloramphenicol affects the EF-G-dependent GTPase. Table 5 demonstrates that even the highest chloramphenicol concentration (25 mM) tested has no significant influence on the activity of the EF-G-dependent GTPase. We conclude, that the GTPase center is not localized in the *a*-site part of the peptidyltransferase center even if it is part of the complete *a*-site.

We thank Dr. H. G. Wittmann for many helpful discussions and criticisms and Dr. R. Crichton for critically reading the manuscript. The technical assistance of Miss F. Bittner and Mr. A. Franz is greatly appreciated.

1. Vazquez, D. (1963) "Antibiotics which effect protein synthesis: The uptake of ^{14}C-chloramphenicol by bacteria," *Biochem. Biophys. Res. Commun.* **12**, 409–413.
2. Wolfe, D. & F. E. Hahn (1965) "Effects of chloramphenicol upon a ribosomal amino acid polymerization system and its binding to bacterial ribosomes," *Biochim. Biophys. Acta* **95**, 146–155.
3. Fernandez-Muñoz, R., Monro, R. E. & Vazquez, D. (1971) "Ribosomal peptidyltransferase: Binding of inhibitors," *Methods in Enzymology* (Academic Press, New York and London), Vol. XX, part C, pp. 481–490.
4. Das, H. K., Goldstein, A. & Kanner, L. C. (1966) "Inhibition by chloramphenicol of the growth of nascent protein chains in *E. coli*," *Mol. Pharmacol.* **2**, 158–170.
5. Hurwitz, Ch. & Braun, C. B. (1967) "Measurement of binding of chloramphenicol by intact cells," *J. Bacteriol.* **93**, 1671–1676.
6. Vazquez, D. (1964) "The binding of chloramphenicol from ribosomes by *Bacillus megaterium*," *Biochem. Biophys. Res. Commun.* **15**, 464–468.
7. Monro, R. E., Staehelin, T., Celma, M. L. & Vazquez, D. (1969) "The peptidyl transferase activity of ribosomes," *Cold Spring Harbor Symp. Quant. Biol.* **34**, 357–368.
8. Pestka, S. (1969) "Studies on the formation of transfer ribonucleic acid-ribosome complexes XI. Antibiotic effects on phenylalanyl-oligonucleotide binding to ribosomes," *Proc. Nat. Acad. Sci. USA* **64**, 709–714.
9. Pestka, S. (1970) "Survey of the effect of antibiotics on *N*-acetyl-phenylalanylpuromycin formation: Possible mechanism of chloramphenicol action," *Arch. Biochem. Biophys.* **136**, 80–88.
10. Celma, M. L., Monro, R. E. & Vazquez, D. (1971) "Substrate and antibiotic binding sites at the peptidyl transferase centre of *E. coli* ribosomes: Binding of UACCA-Leu to 50S subunits," *FEBS Lett.* **13**, 247–251.
11. Fernandez-Muñoz, R., Monro, R. E., Torres-Pinedo, R. & Vazquez, D. (1971) "Studies on the chloramphenicol, lincomycin and erythromycin sites," *Eur. J. Biochem.* **23**, 185–193.
12. Lessard, J. L. & Pestka, S. (1972) "Chloramphenicol, aminoacyl-oligonucleotides and *E. coli* ribosomes," *J. Biol. Chem.* **247**, 6909–6912.
13. Coutsogeorgopoulos, C. (1966) "On the mechanism of action of chloramphenicol in protein synthesis," *Biochim. Biophys. Acta* **129**, 214–217.
14. Coutsogeorgopoulos, C. (1967) "Inhibition of the reaction between puromycin and polylysyl-RNA in the presence of ribosomes," *Biochem. Biophys. Res. Commun.* **27**, 46–52.
15. Yukioka, M. & Morisawa, S. (1970) "Reversibility of chloramphenicol inhibition of the poly U directed polyphenylalanine synthesis by G factor and GTP," *Biochem. Biophys. Res. Commun.* **40**, 1331–1339.
16. Yukioka, M. & Morisawa, S. (1971) "Enhancement of the phenylalanyl-oligonucleotide binding to the peptidyl recognition centre of ribosomal peptidyltransferase and inhibition of the chloramphenicol binding to ribosomes," *Biochim. Biophys. Acta* **254**, 304–315.
17. Gesteland, R. F. (1966) "Isolation and characterization of ribonuclease I mutants of *E. coli*," *J. Mol. Biol.* **16**, 67–84.
18. Scatchard, G. (1949) "The attractions of proteins for small molecules and ions," *Ann. N.Y. Acad. Sci.* **51**, 660–672.
19. Celma, M. L., Monro, R. E. & Vazquez, D. (1970) "Substrate and antibiotic binding sites at the peptidyltransferase centre of *E. coli* ribosomes," *FEBS Lett.* **6**, 273–277.
20. Parmeggiani, A., Singer, C., & Gottschalk, E. M. (1971) "Purification of the amino acid polymerization factors from *E. coli*," *Methods in Enzymology* (Academic Press, New York and London), Vol. XX, part C, pp. 291–302.
21. Celma, M. L., Vazquez, D. & Modolell, J. (1972) "Failure of fusidic acid and siomycin to block ribosomes in the pretranslocated state," *Biochem. Biophys. Res. Commun.* **48**, 1240–1246.
22. Rychlík, I., Chládek, S. & Žemlička, J. (1967) "Release of peptide chains from the polylysyl-tRNA-ribosome complex by cytidylyl-(3′ → 5′)-2′(3′)-*O*-glycyladenosine," *Biochim. Biophys. Acta* **138**, 640–642.
23. Symons, R. H., Harris, R. J., Clarke, L. P., Wheldrake, J. F. & Elliott, W. H. (1969) "Structural requirements for inhibition of polyphenylalanine synthesis by aminoacyl and nucleotidyl analogues of puromycin," *Biochim. Biophys. Acta* **179**, 248–250.
24. Vazquez, D. & Monro, R. E. (1967) "Effects of some inhibitors of protein synthesis on the binding of aminoacyl tRNA to ribosomal subunits," *Biochim. Biophys. Acta* **142**, 155–173.
25. Suarez, G. & Nathans, D. (1965) "Inhibition of aminoacyl-sRNA binding to ribosomes by tetracycline," *Biochem. Biophys. Res. Commun.* **18**, 743–750.