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Tetracycline Inhibition of Cell-Free Protein Synthesis

I. Binding of Tetracycline to Components of the System

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ABSTRACT

DAY, L. E. (Chas. Pfizer & Co., Inc., Groton, Conn.). Tetracycline inhibition of cell-free protein synthesis. I. Binding of tetracycline to components of the system. J. Bacteriol. **91**:1917–1923. 1966.—Tetracycline, an inhibitor of cell-free protein synthesis, effected the dissociation of *Escherichia coli* 100*S* ribosomes to 70*S* particles in vivo and in vitro, but was not observed to mediate the further degradation of these particles. The antibiotic was bound by both 50*S* (Svedberg) and 30*S* subunits of 70*S* ribosomes and also by *E. coli* soluble RNA (sRNA), polyuridylic acid (poly U), and polyadenylic acid (poly A). The binding to ribosomal subunits was higher at 5×10^{-4} M Mg^{++} than at 10^{-2} M Mg^{++}. The binding to polynucleotide chains was highest when Mg^{++} was not added to the reaction mixture.

The tetracycline antibiotics have been observed to inhibit protein synthesis in *Staphylococcus aureus* (7, 9) and in *Escherichia coli* (20), whereas nucleic acid synthesis remained relatively unaffected. The ribonucleic acid synthesized during chlortetracycline inhibition of growing cells was contained in ribosomelike particles which sedimented more slowly than normal ribosomes (11). Effects of the tetracyclines have also been reported on cell-wall synthesis (2), oxidative phosphorylation in heart tissue (8), nitroreductase (23), reduced mammalian nicotinamide adenine dinucleotide-cytochrome oxidoreductase (3), and the enzymatic hydrolysis of poly-β-hydroxybutyrate (19). Many of these activities may be due to the chelation of essential cations by tetracycline. Morrison (12, 13) demonstrated three modes of action of tetracycline against *Aerobacter aerogenes*, one of which was the interference with protein synthesis. It was postulated (1) that all three modes were the result of inhibition of hydrogen transfer reactions. Shaposhnikov and Bakulev (24) support the view that the inhibition of protein synthesis is not the primary site of inhibition of growth of *S. aureus*, but that the tetracyclines inhibit the biosynthesis of flavin adenine dinucleotide and the cessation of protein synthesis is a secondary event.

Work with in vitro systems, both bacterial and mammalian (5, 6, 15, 21, 22), has established that these antibiotics inhibit cell-free protein synthesis. Hurwitz, Gold, and August (*personal communication*) could find no effect of tetracycline or oxytetracycline on ribonucleic acid (RNA)-dependent RNA polymerase (viral), deoxyribonucleic acid (DNA)-dependent RNA polymerase (*Escherichia coli*), DNA methylase, soluble RNA (sRNA) methylase, or ribosomal RNA methylase. Hartmann (*personal communication*) observed no effect of several tetracycline analogues on the DNA-dependent DNA polymerase. Hierowski (10) and Suarez and Nathans (25) observed that tetracycline antibiotics prevent the binding of aminoacyl-sRNA to ribosomes in the presence of polyuridylic acid (poly U), and this has led to speculation that tetracycline might occupy some sensitive site on the ribosome for the binding of sRNA. Izaki and Snell (16) could not detect the binding of tetracycline to ribosomes; however, Connamacher and Mandel (4) reported that tetracycline binds specifically to the 30*S* subunits and also to poly U. In a seldom-mentioned report, Yokota and Akiba (28) reported that tetracycline effects the breakdown of *E. coli* ribosomes to particles of less than 10*S* by chelation of Mg^{++} essential for ribosomal integrity.

The present work was initiated to investigate the mechanism of action of tetracycline in the cell-free protein-synthesizing system, and to determine the extent of binding, if any, of tetracycline to various nucleic acid components of the system.

MATERIALS AND METHODS

Culture and cultural methods. E. coli W was cultivated in 9-liter volumes of nutrient broth (Difco) with

added glucose (5 g/liter) in New Brunswick fermentors. A 50-ml amount of a 16-hr shake-flask culture was used to inoculate the fermentors, which were incubated at 30 C, stirred at 400 rev/min, and aerated at 1 v/v per min. Growth was stopped at about 10^8 cells per milliliter by adding cracked ice to the fermentors, and the cells were removed from the growth medium by centrifugation. The cells were washed twice with 0.02 M tris(hydroxymethyl)aminomethane (Tris)-HCl buffer (*p*H 7.9) containing 0.2 M NH_4Cl, 0.01 M $MgCl_2$, and 0.005 M mercaptoethylamine. The cells were suspended in 2 volumes of the same buffer.

Preparation of cell-free extracts and ribosomes. Washed cells were broken in a precooled French Pressure Cell at 18,000 psi. Ribosomes were prepared from cell extracts according to Lengyel et al. (17) as modified by Szer and Ochoa (26), except that Lubrol was not utilized in these preparations.

Ribosomal subunits (50*S* and 30*S*) were prepared from 70*S* ribosomes by dialysis for 18 to 24 hr at 3 C against two changes of 0.01 M Tris-HCl (*p*H 7.3) containing 5×10^{-4} M $MgCl_2$. The dissociated ribosomal suspension was layered over 54-ml sucrose density gradients (5 to 20%) containing 0.01 M Tris-HCl (*p*H 7.3) and 5×10^{-4} M $MgCl_2$. The gradients were centrifuged at 2.5×10^4 rev/min for 10 hr in the SW-25.2 rotor of the Spinco model L2-50 preparative ultracentrifuge. Gradients were fractionated at 3 C into 1.0-ml fractions with a Buchler piercing unit in conjunction with a Gilson LB-1 linear fraction collector. A 0.1-ml volume of each fraction was diluted to 3.0 ml for OD_{260} determinations. The fractions containing the 50*S* ribosomal subunits were pooled, as were those containing the 30*S* subunits. Pooled fractions were centrifuged in the 50 rotor of the L2-50 at 4.7×10^4 rev/min for 18 hr. The pellets were taken up in 0.01 M Tris-HCl (*p*H 7.3) and 5.0×10^{-4} M $MgCl_2$. The suspensions were frozen in liquid nitrogen until used.

Binding of tetracycline to ribosomes, ribosomal subunits, and other components of the cell-free system. A 2.0-ml suspension of 70*S* ribosomes (60 mg) was incubated with 16 μc of 7-H^3-tetracycline (sp. act.: 80 μc/μmole) at 37 C for 30 min, placed in 3/8-inch (0.95 cm) dialysis tubing, and dialyzed for 20 hr at 3 C against 4 liters of 0.01 M Tris-HCl (*p*H 7.3) with 0.01 M $MgCl_2$. A 100-μliter sample was removed from the reaction mixture, layered over a 4.6-ml sucrose density gradient, and centrifuged at 3.5×10^4 rev/min in the SW-39 rotor of the L2-50 ultracentrifuge for 2 hr. The gradient was fractionated into 5-drop fractions, and alternate fractions were used for OD_{260} determinations and radioactivity assays. The remainder of the reaction mixture was dialyzed against two changes of 0.01 M Tris-HCl (*p*H 7.3) containing 5×10^{-4} M $MgCl_2$ for 6 hr each at 3 C. This dialysate was placed on a 54-ml sucrose gradient (5 to 20%) in the same buffer and centrifuged at 2.5×10^4 rev/min for 10 hr in the SW-25.2 rotor. The gradient was fractionated into 1.0-ml fractions; 50 μliters was removed from each fraction for OD_{260} determinations and 50 μliters for radioactivity assay. The fractions containing the 50*S* subunits were pooled, as were those containing the 30*S* subunits. Both pools were then centrifuged in the SW-39 rotor at 3.7×10^4 rev/min for 19 hr. The ribosomal pellets were taken up in 100 μliters of 0.01 M Tris-HCl (*p*H 7.3) and 5×10^{-4} M $MgCl_2$. The suspensions were layered over 4.6-ml sucrose gradients containing 5×10^{-4} M $MgCl_2$ and centrifuged at 3.5×10^4 rev/min in the SW-39 rotor. The gradient containing the 50*S* ribosomes was centrifuged for 2 hr; the 30*S* gradient for 4 hr. The gradients were fractionated into 5-drop fractions and assayed for radioactivity and OD_{260} as described.

50S and 30S subunits. The binding of tetracycline to the separated subunits was studied as a function of the Mg^{++} concentration, and the reaction mixtures were set up as follows. Each reaction mixture contained approximately 1.0 mg of either 50*S* or 30*S* ribosomes, 0.5 μc of 7-H^3-tetracycline, and 0.01 M Tris-HCl (*p*H 7.3). The $MgCl_2$ concentration was made to 10^{-2} M or 5×10^{-4} M. The tubes were incubated at 37 C for 30 min, layered over 4.6-ml sucrose gradients containing the appropriate Mg^{++} concentration, and centrifuged in the SW-39 rotor at 3.5×10^4 rev/min. The tubes were fractionated 5 drops for OD_{260} and 5 drops for radioactivity.

Poly U and poly A, and sRNA. E. coli sRNA was prepared according to Zubay (29). A reaction mixture of 1.0 mg of sRNA, 20 μg of 7-H^3-tetracycline (3.2 μc), and 0.08 μmole of Tris-HCl (*p*H 7.3) in a final volume of 200 μliters was incubated at 37 C for 30 min and placed on a Sephadex column (1.5 × 30 cm) containing Sephadex G-25 equilibrated with 0.01 M Tris-HCl (*p*H 7.3). The elution was carried out with the same buffer. One-ml fractions were collected, and 200 μliters was used for radioactivity determination and 500 μliters was diluted to 3.0 ml for OD_{260} assay. The reaction was also carried out in 10^{-2} M and 5×10^{-4} M $MgCl_2$, with poly U and poly A as the RNA component.

Effect of tetracycline on growing cells and intact ribosomes. Two Fernbach flasks containing 300 ml of the medium described were inoculated with 15 ml of a 16-hr culture of *E. coli* W and incubated on a rotary shaker at 37 C until the cell population was 10^8 per milliliter. To one flask was added 15 mg of tetracycline, and incubation was continued for another 2 hr. The other flask was removed from the shaker and refrigerated. Both cultures were then centrifuged to remove the cells, washed twice in 0.01 M Tris-HCl (*p*H 7.4) and 0.01 M $MgCl_2$. The cells were suspended in two volumes of the same buffer and broken in the French Pressure Cell at 18,000 psi. Both suspensions were centrifuged at 30,000 × *g* for 30 min. A portion of each supernatant fraction was then layered on a 4.6-ml sucrose density gradient, and centrifuged at 3.5×10^4 rev/min for 2 hr in the SW-39 rotor. The tubes were fractionated in 6-drop fractions, diluted to 3.0 ml, and the OD_{260} determined.

A suspension of crude ribosomes from cell-extracts which contained a high per cent of 100*S* particles was used to study the effect of tetracycline on these particles. A reaction mixture containing 7.5 mg of ribosomal protein and 200 μg of tetracycline in 0.01 M Tris-HCl (*p*H 7.3) and 0.01 M $MgCl_2$ was incubated at 37 C for 60 min. The control contained no antibiotic. The suspensions were placed on 4.6-ml sucrose

density gradients and centrifuged, fractionated, and assayed as described in the preceding paragraph.

Assays. Radioactivity of density-gradient fractions containing 7-H^3-tetracycline was assayed in scintillation vials containing 15 ml of toluene and ethyl alcohol (2:1) with 0.3% of 2,5-diphenyloxazole and 0.01% of *p*-bis-12-(5-phenyloxazolyl)-1-benzene. Counting was carried out in a TriCarb Liquid Scintillation Spectrometer, and all counts were corrected for background. OD_{260} determinations were made with 3.0-ml volumes in the Beckman DU spectrophotometer at 260 mμ. Protein was determined by the Lowry (18) method.

Materials. Poly U and poly A were purchased from Miles Laboratories, Inc., Elkhart, Ind. The 7-H^3-tetracycline was obtained from the New England Nuclear Corp., Boston, Mass., in the solid form at 80 mc/mmole, and was stored in 0.01 N HCl at 5 C. The tetracycline HCl used in these experiments is a working standard of Chas. Pfizer & Co., Inc., Brooklyn, N.Y.

RESULTS

The 100*S* ribosomes of *E. coli* is a dimer of the 70*S* particle and is formed as a function of the Mg^{++} concentrations of the environment. Tissieres et al. (27) demonstrated that when *E. coli* cells are ruptured in a buffer containing 0.01 M Mg^{++}, the main ribosomal component has the 100*S* characteristic; and when the Mg^{++} concentration is lowered to 0.001 M, the major component consists of 70*S* ribosomes. Fig. 1A is the sedimentation profile of a crude extract of log-phase *E. coli* prepared in 0.01 M Mg^{++} showing the 100*S* and 70*S* peaks. The effect of 50 μg/ml of tetracycline incubated for 2 hr with a log-phase culture is shown in Fig. 1B. There is a pronounced shift of the 100*S* ribosomes to the slower sedimenting 70*S* particles and an increase in UV-absorbing material remaining near the top of the gradient. A similar experiment was carried out with the extract prepared in 5×10^{-4} M Mg^{++} to obtain the sedimentation profile of the 50*S* and 30*S* subunits. The tetracycline-treated cells contained ribosomal subunits that sedimented slightly more slowly than 50*S* and 30*S* particles, and there was an increase in the low molecular weight UV-absorbing material at the top of the gradient. This observation (not shown here) is in agreement with that recently reported by Holmes and Wild (11).

The effect of tetracycline on ribosomes isolated from exponentially growing cultures is illustrated in Fig. 2. The 100*S* ribosomes are effectively dissociated to single 70*S* units in the presence of tetracycline. Higher levels of tetracycline (400 μg) did not result in further dissociation of the 70*S* ribosomes. This is not in agreement with the report (28) that tetracycline at 50 μg/ml caused the breakdown of *E. coli* 70*S* ribosomes to particles of 10*S* or less, although the ratio of tetracycline to ribosomes used in those experiments was not stated.

Binding of tetracycline to ribosomes and ribosomal subunits. The recent report of Connamacher and Mandel (4) that tetracycline binds specifically to the 30*S* subunits of *E. coli* ribosomes had also been observed in this laboratory. Studies of the binding of the antibiotic to the 70*S* ribosomes were of necessity carried out in 10^{-2} M Mg^{++} to maintain the integrity of this structure.

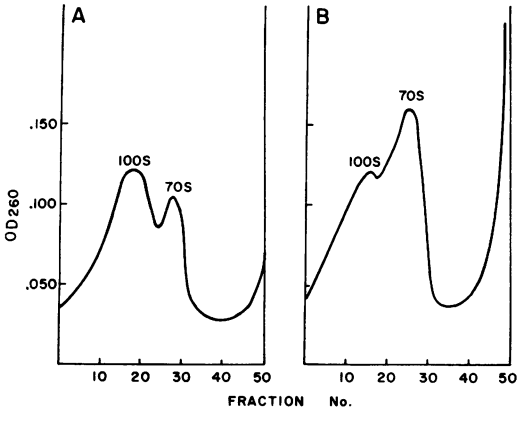

FIG. 1. *Effect of tetracycline on the ribosomal sedimentation profile of Escherichia coli. (A) Control (no tetracycline). (B) Culture incubated for 2 hr with tetracycline (50 μg/ml).*

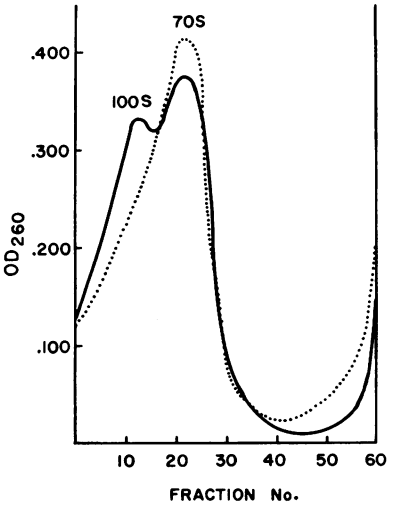

FIG. 2. *Effect of tetracycline (26.6 μg/mg of ribosomal protein) on a suspension of Escherichia coli ribosomes. Solid line, control; broken line, tetracycline-treated.*

The binding of 7-H^3-tetracycline to 70*S* ribosomes in this Mg^{++} concentration was not so extensive that it was readily apparent when analyzed by the technique of sucrose density-gradient centrifugation (Fig. 3). Therefore, the reaction mixture was dialyzed against 5×10^{-4} M Mg^{++} to dissociate the 70*S* ribosomes. Figure 4 depicts the OD_{260} sedimentation profile of the 50*S* and 30*S* ribosomal subunits of this reaction mixture, and the radioactive peak indicates that the tetracycline does bind to the 30*S* ribosome. It is also apparent that a significant amount of the radioactivity sediments to the lower regions of the gradient. To analyze the reaction further, the fractions containing the 50*S* subunits were isolated, pooled, centrifuged, and again subjected to sucrose density-gradient analysis. This was also carried out with the 30*S* fractions. The results (Fig. 5A and B) indicate that the antibiotic is bound by both of the ribosomal subunits. The reason for the double radioactive peak with the 30*S* particles is not clear, although only a single peak was observed when a similar experiment was carried out.

The binding of the radioactive tetracycline was also investigated by use of the separated individual subunits. Figure 6 shows the binding of the antibiotic to 50*S* ribosomes in 5×10^{-4} M Mg^{++} (Fig. 6A) and 10^{-2} M Mg^{++} (Fig. 6B). The binding is less in the presence of the higher concentration of Mg^{++}. The same result was observed with separated 30*S* subunits (Fig. 7A and B).

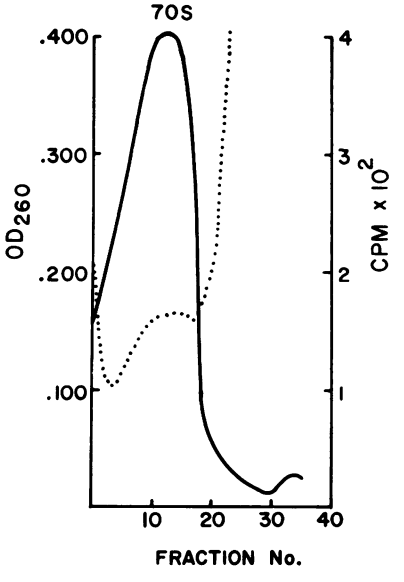

FIG. 3. *Binding of 7-H^3-tetracycline to 70S ribosomes of Escherichia coli. Solid line, OD_{260}; broken line, radioactivity.*

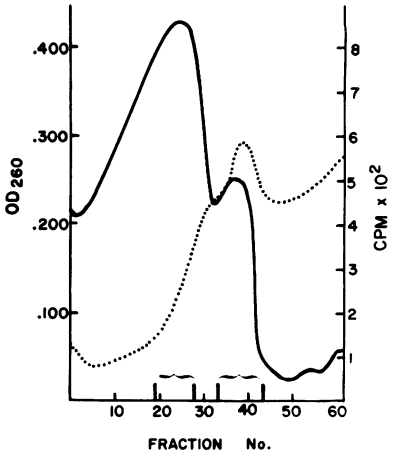

FIG. 4. *Binding of 7-H^3-tetracycline to 50S and 30S subunits of Escherichia coli 70S ribosomes. Solid line, OD_{260}; broken line, radioactivity. The 70S ribosomes contained in the reaction mixture shown in Fig. 2 were dissociated and fractionated by sucrose density-gradient centrifugation. Fractions 19 to 28 were pooled and centrifuged to resediment the 50S subunits. This was also carried out with fractions 33 to 43 to isolate 30S subunits. Both types were then analyzed on sucrose density gradients (Fig. 5).*

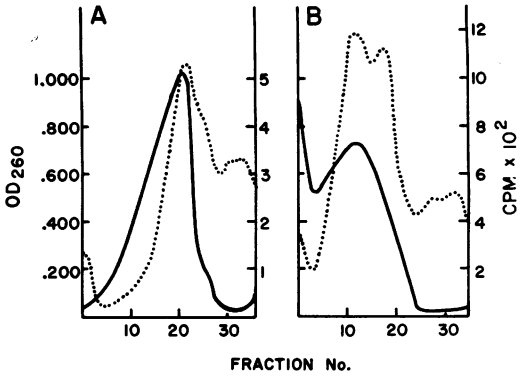

FIG. 5. *Sucrose density-gradient profiles of ribosomal subunits isolated from the reaction mixture, as described in Fig. 3 and 4. (A) 50S subunits. (B) 30S subunits. Solid line, OD_{260}; broken line, radioactivity.*

Binding of tetracycline to polynucleotides. Kohn (14) reported that tetracyclines bind to macromolecules through metal chelates, and he studied specifically the interaction between sperm DNA and human serum albumin and the antibiotic. The binding of tetracycline to poly U was observed by Connamacher and Mandel (4) by use of a technique of dialysis binding. A reaction

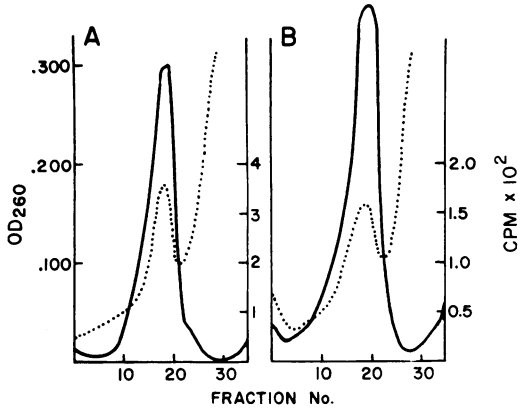

FIG. 6. *Binding of 7-H³-tetracycline to 50S ribosomal subunits. (A) Reaction carried out in 5 × 10⁻⁴ M Mg⁺⁺, and the gradient centrifuged in SW-39 rotor for 120 min at 3.5 × 10⁴ rev/min. (B) Reaction carried out in 10⁻² M Mg⁺⁺, and the gradient centrifuged for 105 min at 3.5 × 10⁴ rev/min. Solid line, OD₂₆₀; broken line, radioactivity.*

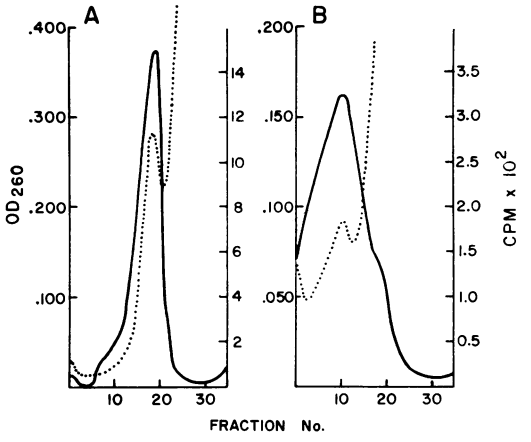

FIG. 7. *Binding of 7-H³-tetracycline to 30S ribosomal subunits. (A) Reaction carried out in 5 × 10⁻⁴ M Mg⁺⁺, and the gradient centrifuged in SW-39 rotor for 210 min at 3.5 × 10⁴ rev/min. (B) Reaction carried out in 10⁻² M Mg⁺⁺ and the gradient centrifuged for 120 min at 3.5 × 10⁴ rev/min. Solid line, OD₂₆₀; broken line, radioactivity.*

mixture of sRNA and 7-H³-tetracycline was passed through a G-25 Sephadex column, and the elution profile of UV absorbance and radioactivity indicated that the tetracycline was bound by the sRNA (Fig. 8). This mixture contained no added Mg^{++}. The technique of Sephadex filtration was used to study further the binding of the antibiotic to sRNA, poly U, and poly A under various Mg^{++} concentrations. These results are tabulated in Table 1. The specific activity (count/min of tetracycline per OD_{260} polynucleotide)

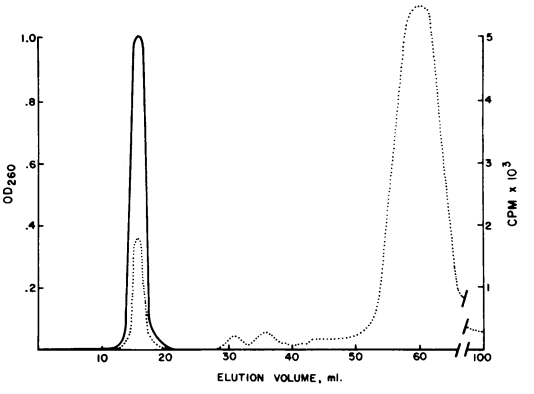

FIG. 8. *Binding of 7-H³-tetracycline to sRNA in Tris-HCl buffer (pH 7.3). Reaction mixture applied to column containing Sephadex G-25 and eluted with 0.01 M Tris-HCl (pH 7.3). V_0 (void volume) of column was 15 ml. Solid line, OD_{260}; broken line, radioactivity.*

TABLE 1. *Effect of Mg^{++} concentration on the binding of 7-H³-tetracycline to polynucleotides*

Polynucleotide	Mg^{++} concn		
	0	5×10^{-4} M	10^{-2} M
sRNA	4,204*	1,468	356
Poly U	243	119	174
Poly A	518	235	—†

* Values tabulated are expressed as the radioactivity per OD unit at 260 mμ (counts/min per OD_{260}) at the polynucleotide peak eluted from a Sephadex G-25 column.

† Poly A precipitated from solution in the presence of 10^{-2} M $MgCl_2$ and tetracycline.

was found to decrease as the Mg^{++} concentration was increased, although the binding to poly U was much less than to sRNA at all Mg^{++} concentrations.

DISCUSSION

The chelation properties of tetracycline are sufficient to cause the dissociation of 100*S* ribosomes to the two constituent 70*S* monomers. However, the bonds holding the 50*S* and 30*S* subunits together appear to be stronger, since the antibiotic has no effect on the intact 70*S* ribosomes. The observation of Yokota and Akiba (28) that tetracycline brings about an almost complete breakdown of the 70*S* ribosomes was not demonstrated during this study. The organism that they have used may be a mutant type which shows altered characteristics of association between ribosomal subunits and allows easier

separation of the two by chelating agents. However, they pointed out that 50 μg/ml of tetracycline was much more effective in this regard than ethylenediaminetetraacetic acid (EDTA) at 2 mg/ml, indicating that tetracycline exerts an effect more specific than chelation. It is also significant that the resulting particles observed by Yokota and Akiba were less than 10*S*, which indicates that the RNA chains of the ribosomes have been digested to molecules of a size less than that normally encountered in the ribosome, i.e., 23*S* and 16*S*. This breakdown of ribosomes was not observed by others whose studies with tetracycline antibiotics in cell-free systems would make this phenomenon apparent (10, 11, 25).

From the data accumulated in these experiments, calculations were made to determine the extent of binding of the antibiotic to the ribosomal subunits. These calculations were based on the following assumptions and facts: (i) 1.0 mg of ribosomes = 16 OD_{260} units; (ii) 50*S* ribosomal subunit molecular weight = 1.8×10^6; (iii) 30*S* ribosomal subunit molecular weight = $.9 \times 10^6$; (iv) specific activity of 7-H^3-tetracycline = 80 μc/μmole; (v) efficiency of the liquid scintillation counter = 6%; (vi) 1.0 μc of radioactivity = 2.2×10^6 dpm. A sample calculation is presented here with use of the data from Fig. 6A. At the coincident radioactive peak and OD_{260} peak, there is a ratio of 350 count/min per .300 OD_{260}. To convert the radioactivity to micromoles of tetracycline:

$$\frac{350 \text{ counts/min}}{.06 \text{ (efficiency)}} = 5833 \text{ dpm}$$

$$\frac{5833 \text{ dpm}}{2.2 \times 10^6 \text{ dpm}/\mu\text{c}} = 2.7 \times 10^{-3} \ \mu\text{c}$$

$$\frac{2.7 \times 10^{-3} \ \mu\text{c}}{80 \ \mu\text{c}/\mu\text{mole}} = 3.4 \times 10^{-5} \ \mu\text{moles of tetracycline}$$

To convert the .300 OD_{260} units to micromoles of ribosomes:

$$\frac{.300 \ OD_{260}/\text{ml}}{16 \ OD_{260}/\text{mg}} = 18.8 \ \mu\text{g of ribosomes per ml}$$

Since total fraction was diluted to 3.0 ml for OD_{260} assay, multiply $\times$ 3 = 56.4 μg of total ribosomes.

$$\frac{56.4 \ \mu\text{g}}{1.8 \times 10^6 \ \mu\text{g}/\mu\text{moles}}$$

$$= 3.1 \times 10^{-5} \ \mu\text{moles of 50}S \text{ ribosomes}$$

Ratio of tetracycline to 50*S* ribosomes: 3.4 μmole/3.1 μmole. To both the 50*S* and 30*S* ribosomes, approximately 1 molecule of tetracycline was bound per subunit in 5×10^{-4} M Mg^{++}. At Mg^{++} concentrations of 10^{-2} M, the binding was about 50% less, or one tetracycline per two ribosomes. When the tetracycline was bound originally to the 70*S* ribosome, followed by dialysis to remove excess unbound antibiotic, dialysis to dissociate the ribosomes, and fractionation and separation to isolate the subunits, the binding was calculated to be 1 mole of tetracycline per 2 moles of ribosomal subunits. This binding is of the order of magnitude expected if a sensitive site of inhibition exists on either one or both of the ribosomal subunits.

The binding of tetracycline to the homopolymers poly A and poly U, to sRNA, and to ribosomal subunits decreased as Mg^{++} concentration increased. At 10^{-2} M Mg^{++}, the ratio of the cation to tetracycline in the reaction mixture was of the order of 100:1, and at this ratio an extensive complexing between the two takes place (Gordon, *personal communication*). This undoubtedly prevents much of the tetracycline from binding with the available sites on the polynucleotides and ribosomes. At 10^{-4} M Mg^{++}, the Mg^{++}:tetracycline ratio was more nearly 1:1 and the binding was more extensive, particularly with sRNA. Whether Mg^{++} is required for binding is not known, since it is difficult to ascertain that Mg^{++} is being completely excluded from nucleic acid preparations. With ribosomes, the binding studies must include Mg^{++} at concentrations of at least 10^{-4} M to preserve structural integrity of the subunits.

That tetracycline binds to several macromolecules through metal chelates has been reported before (14); however, the binding to ribosomes and other cellular nucleic acid species has not been investigated thoroughly. The only two reports dealing with ribosomal binding (4, 16) were in disagreement. Connamacher and Mandel (4) did indicate the binding of the antibiotic to poly U and to the 30*S* subunit of the ribosome, but they did not pursue the matter further. This report demonstrates that tetracycline does bind firmly to ribosomes, since the tritium label sediments with 50*S* and 30*S* subunits after three dialyses and three centrifugations. The role of this binding of the antibiotic to these structures and to other species of nucleic acid which serve a function in protein synthesis will be the subject of a second paper.

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LITERATURE CITED

1. BENBOUGH, J., AND G. A. MORRISON. 1965. Bacteriostatic actions of some tetracyclines. J. Pharm. Pharmacol. **17**:409–422.
2. CHENG, L., AND J. F. SNELL. 1962. Studies in metabolic spectra. IV. Effects of tetracyclines, some of their derivatives, and chloramphenicol on the accumulation of glutamic acid in *Escherichia coli*. J. Bacteriol. **83**:711–719.
3. COLAIZZI, J. L., A. M. KNEVEL, AND A. N. MARTIN. 1965. Biophysical study of the mode of action of the tetracycline antibiotics. Inhibition of metalloflavoenzyme NADH cytochrome oxidoreductase. J. Pharm. Sci. **54**:1425–1436.
4. CONNAMACHER, R. H., AND H. G. MANDEL. 1965. Binding of tetracycline to the 30*S* ribosomes and to polyuridylic acid. Biochem. Biophys. Res. Commun. **20**:98–103.
5. FRANKLIN, T. J. 1963. The inhibition of incorporation of leucine into protein of cell-free systems from rat liver and *Escherichia coli* by chlortetracycline. Biochem. J. **87**:449–453.
6. FRANKLIN, T. J. 1964. The effect of chlortetracycline on the transfer of leucine and "transfer" ribonucleic acid to rat-liver ribosomes *in vitro*. Biochem. J. **90**:624–628.
7. GALE, E. F., AND J. P. FOLKES. 1953. The assimilation of amino acids by bacteria. Biochem. J. **53**:493–498.
8. GOMAZKOV, O. A. 1963. Mechanism of action of tetracycline antibiotics. Federation Proc. **23**: 876–878.
9. HASH, J. H., M. WISHNICK, AND P. A. MILLER. 1964. On the mode of action of the tetracycline antibiotics in *Staphylococcus aureus*. J. Biol. Chem. **239**:2070–2078.
10. HIEROWSKI, M. 1965. Inhibition of protein synthesis by chlortetracycline in the *E. coli* in vitro system. Proc. Natl. Acad. Sci. U.S. **53**:594–599.
11. HOLMES, I. A., AND D. G. WILD. 1965. The synthesis of ribonucleic acid during inhibition of *Escherichia coli* by chlortetracycline. Biochem. J. **97**:277–283.
12. JONES, J. G., AND G. A. MORRISON. 1962. The bacteriostatic actions of tetracycline and oxytetracycline. J. Pharm. Pharmacol. **14**:808–824.
13. JONES, J. G., AND G. A. MORRISON. 1963. Inhibition by tetracycline and oxytetracycline of the consumption of pyruvate by *Aerobacter aerogenes*. J. Pharm. Pharmacol. **15**:34–44.
14. KOHN, K. W. 1961. Mediation of divalent metal ions in the binding of tetracycline to macromolecules. Nature **191**:1156–1158.
15. LASKIN, A. I., AND W. M. CHAN. 1964. Inhibition by tetracyclines of polyuridylic acid directed phenylalanine incorporation in *Escherichia coli* cell-free systems. Biochem. Biophys. Res. Commun. **14**:137–142.
16. LAST, J. A., K. IZAKI, AND J. F. SNELL. 1965. The failure of tetracycline to bind to *Escherichia coli* ribosomes. Biochim. Biophys. Acta **103**:532–534.
17. LENGYEL, P., J. F. SPEYER, AND S. OCHOA. 1961. Synthetic polynucleotides and the amino acid code. Proc. Natl. Acad. Sci. U.S. **47**:1936–1942.
18. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. MERRICK, J. M. 1965. Effect of polymyxin B, tyrocidine, gramicidin D, and other antibiotics on the enzymatic hydrolysis of poly-β-hydroxybutyrate. J. Bacteriol. **90**:965–969.
20. NAKAYA, R., AND H. P. TREFFERS. 1959. The growth rates and adaptive enzyme activities of chloramphenicol- and oxytetracycline-resistant *Escherichia coli*. Antibiot. Ann. 1958–1959, p. 865–872.
21. OKAMOTO, S., AND D. MIZUNO. 1964. Mechanism of chloramphenicol and tetracycline resistance in *Escherichia coli*. J. Gen. Microbiol. **35**:125–133.
22. RENDI, R., AND S. OCHOA. 1962. Effect of chloramphenicol on protein synthesis in cell-free preparations of *Escherichia coli*. J. Biol. Chem. **237**:3711–3713.
23. SAZ, A. K., AND R. B. SLIE. 1953. Manganese reversal of aureomycin inhibition of bacterial cell-free nitroreductase. J. Am. Chem. Soc. **75**:4626–4627.
24. SHAPOSHNIKOV, V. N., AND V. K. BAKULEV. 1964. Interrelations between biosynthesis of proteins, of ribonucleic acid, and of certain coenzymes in the action of tetracyclines on *Staphylococcus aureus* 209P. Dokl. Akad. Nauk Uz. SSR **158**:987–989.
25. SUAREZ, G., AND D. NATHANS. 1965. Inhibition of aminoacyl-sRNA binding to ribosomes by tetracycline. Biochem. Biophys. Res. Commun. **18**:743–750.
26. SZER, W., AND S. OCHOA. 1964. Complexing ability and coding properties of synthetic polynucleotides. J. Mol. Biol. **8**:823–834.
27. TISSIERES, A., J. D. WATSON, D. SCHLESSINGER, AND B. R. HOLLINGWORTH. 1959. Ribonucleoprotein particles from *Escherichia coli*. J. Mol. Biol. **1**:222–233.
28. YOKOTA, T., AND T. AKIBA. 1962. Studies on the mechanism of transfer of drug-resistance in bacteria. 23. Mechanisms of the antibacterial action of tetracycline and the tetracycline resistance in the artificial, TC-resistant strain of *E. coli*. Med. Biol. Tokyo **64**(2):39–44.
29. ZUBAY, G. 1962. The isolation and fractionation of soluble ribonucleic acid. J. Mol. Biol. **4**:347–356.