References

Biswas, D. K., and Gorini, L. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 2141.
Caskey, C. T., Scolnick, E., Tompkins, R., Milman, G., and Goldstein, J. (1971), *Methods Enzymol. 20*, 367.
Dubnoff, J. S., and Maitra, U. (1971), *Methods Enzymol. 20*, 248.
Gordon, J. (1969), *J. Biol. Chem. 244*, 5680.
LeLong, J. C., Cousin, M. A., and Gros, F. (1972), *Eur. J. Biochem. 27*, 174.
Meyer, C. E. (1965), *Antimicrob. Ag. Chemother.*, 91.
Miskin, R., Zamir, A., and Elson, D. (1970), *J. Mol. Biol. 54*, 355.
Modolell, J., and Davis, B. D. (1970), *Proc. Nat. Acad. Sci. U. S. 67*, 1148.
Nirenberg, M. W., and Leder, P. (1964), *Science 145*, 1399.
Okuyama, A., and Tanaka, N. (1972), *Biochem. Biophys. Res. Commun. 49*, 951.
Reusser, F. (1969), *Biochemistry 8*, 3303.
Reusser, F. (1973), *Biochemistry 12*, 1136.

Misreading, a Fundamental Aspect of the Mechanism of Action of Several Aminoglycosides†

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ABSTRACT: The effect of various aminoglycoside antibiotics on the cell-free synthesis of total protein and of late T4 phage mRNA-induced lysozyme has been studied with ribosomes from streptomycin sensitive and resistant *Escherichia coli*. Lysozyme biosynthesis is more sensitive to aminoglycosides than total protein synthesis in both systems, indicating a high level of misreading, except in the case of kasugamycin and spectinomycin for which both effects are identical. The Mg^{2+} concentration significantly influences the effect of all the misreading inducing aminoglycosides. The effect of these amino glycosides on the initiation step was also investigated. On the basis of the results obtained, the antibiotics may be classified into three groups: (1) those inducing no misreading, *i.e.*, kasugamycin and spectinomycin, (2) an antibiotic acting both on initiation and fidelity of translation, namely, streptomycin, (3) those inducing a high level of misreading, *i.e.*, with a deoxystreptamine moiety.

Aminoglycoside antibiotics inhibit protein synthesis in sensitive bacteria (Erdos and Ullman, 1959), their site of action being located in the ribosomes (Spotts and Stanier, 1959; Jacoby and Gorini, 1967; Weisblum and Davies, 1968; Traub and Nomura, 1968; Ozaki *et al.*, 1969). In the study of their effect on translation mechanism in subcellular ribosomal systems, two fundamental observations have been made. (1) For blockage of the initiation of polypeptide chains, Modolell and Davis (1970) and Lelong *et al.* (1971) have shown that streptomycin inhibits the initial steps of mRNA translation *in vitro*, leading to the release of formylmethionyl-tRNA (fMet-tRNA) from the initiation complex without chain elongation; Okuyama *et al.* (1971) have established that initiation complex formation is inhibited on 30S ribosomes by kasugamycin, on 70S ribosomes by kanamycin A and gentamicin C. (2) Infidelity in the translation of the genetic code ("misreading effect"): this effect was primarily observed in studies on cell-free extracts programmed with synthetic polynucleotides (Davies *et al.*, 1965, 1966; Davies and Davis, 1968; Davies, 1970). Moreover, studies with viral mRNA have shown inhibited or stimulated polypeptide synthesis as measured by radioactive amino acid incorporation (van Knippenberg *et al.*, 1965). In these experiments, however, no specific enzyme activity was measured and thus the importance of misreading under conditions approximating biological conditions could not be clearly demonstrated. To pursue the study of the mechanism of action of aminoglycosides, we therefore selected the mRNA induced by late T4 phage infected *Escherichia coli* which codes for the translation of a specific enzyme, lysozyme, and studied the misreading effect of aminoglycosides by measuring not only the incorporation of a radioactive amino acid into protein, but also lysozyme biosynthesis. Preliminary accounts of these experiments have already been presented (Cousin *et al.*, 1971; Lando, 1972). Results for streptomycin-sensitive ribosomes were compared with those for streptomycin-resistant ribosomes, which according to Jacoby and Gorini (1967), are less sensitive to misreading. The effects on fidelity of translation were compared to those on the initiation step.

Experimental Section

Materials

Streptomycin-sensitive (Sm^S)[1] and streptomycin-resistant (Sm^R) *E. coli* MRE 600 were used to prepare ribosomes and crude translation factors; *E. coli* D_{10} was used to prepare wild T4 phage mRNA.

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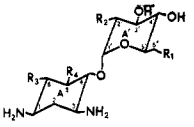
[1] Abbreviations used are: A-U-G, adenylyl-3′,5′-uridylyl-3′,5′-guanosine 3′-phosphate; Gm, gentamicin; Kg, kasugamycin; Km, kanamycin; Kn, kanendomycin; Nea, neamine; Nm, neomycin; Pa, paromamine; Pm, paromomycin; Sm, streptomycin; Sp, spectinomycin; ID_{50}, inhibition dose 50%.



	R_1	R_2	R_3	R_4
Neamine	NH_2	NH_2	OH	OH
Paromamine	OH	NH_2	OH	OH
Pseudoparomamine	NH_2	OH	OH	OH
Kanamycin A	NH_2	OH		OH
Kanendomycin	NH_2	NH_2		OH
Kanamycin C	OH	NH_2		OH
Gentamicin A	OH	NH_2		OH

	R_1	R_2	R_3	X_1	X_2
Neomycin B	NH_2	NH_2	OH	H	CH_2NH_2
Neomycin C	NH_2	NH_2	OH	CH_2NH_2	H
Paromomycin I	OH	NH_2	OH	H	CH_2NH_2
Paromomycin II	OH	NH_2	OH	CH_2NH_2	H

FIGURE 1: Aminoglycosides with a deoxystreptamine moiety. The A fragment (R_3 and R_4 = OH) corresponds to deoxystreptamine.

L-[^{3}H]Valine (1 Ci/mmol) and L-[^{3}H]methionine (0.6 Ci/mmol) were purchased from the Commissariat à l'Energie Atomique, France; A-U-G from Miles Chemical Co. Neomycin B and C, neamine, paromomycin (95% PmI, 5% PmII), and paromamine, as well as chloramphenicol, puromycin, and tetracycline, were produced by Roussel-Uclaf. Streptomycin was purchased from Rhône-Poulenc; kanamycin A from Mann Research Laboratories. Kanendomycin was donated by Meiji-Seika, kasugamycin by Banyu, and spectinomycin by Upjohn Co. Gentamicin A, C_1, C_{1A}, and C_2 were gifts from Schering Laboratories. The structures of the main aminoglycoside antibiotics used in these studies are represented in Figures 1 and 2.

Methods

Preparation of Ribosomes and Crude Factors (Revel *et al.*, 1968a). The high-speed supernatant fraction S_{150} containing elongation factors was dialyzed 5 hr against a 10 mM Tris-HCl–7 mM 2-mercaptoethanol buffer (pH 7.5). Ribosomes were washed free of initiation factors by treatment with 1 M NH_4Cl, 30 mM Tris-HCl, 10 mM magnesium acetate, and 12 mM 2-mercaptoethanol buffer (pH 7.6). Crude initiation factors were concentrated by precipitation with ammonium sulfate at 80% saturation, dissolved and dialyzed in the first-mentioned buffer.

Preparation of mRNA. The 25-min T4-infected bacteria RNA used to program *in vitro* lysozyme synthesis was prepared as described by Salser *et al.* (1967).

In Vitro Synthesis of Lysozyme. A total of 200 μl of reaction mixture (pH 7.8), containing 62.5 mM Tris-HCl, 62 mM NH_4Cl, 2 mM dithiothreitol, 8 mM magnesium acetate (unless otherwise indicated), 1.5 mM ATP, 0.25 mM GTP, 6 mM phosphoenolpyruvate, 10 μg/ml of pyruvate kinase, 3% (w/v) Polyethylene Glycol 6000 (omitted in certain experiments), 0.13 mM each of 19 amino acids, 0.02 mM tritiated valine (100 Ci/mol), 1.2 mg/ml of S_{150} supernatant, 500 μg/ml of crude initiation factors, 0.24 μM washed 70S ribosomes, and 750 μg/ml of 25-min T4 phage mRNA was incubated at 37° for 30 min. In some experiments, 40 mM KCl was added. Amino acid incorporation was measured by the radioactivity recovered in material precipitated by hot trichloroacetic acid (Mans and Novelli, 1961).

Lysozyme Assay (Salzer *et al.*, 1969). Activity was measured with a Zeiss PMQII spectrophotometer on 50 μl of reaction mixture added to 1 ml of a suspension of chloroform-treated *E. coli* B^E and calculated from the maximum slope of a plot

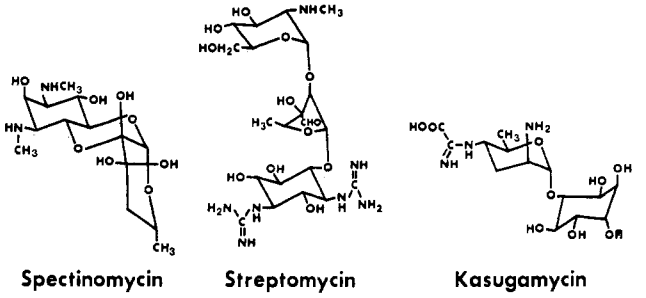


FIGURE 2: Aminoglycosides lacking the deoxystreptamine moiety.

of turbidity at 450 nm *vs.* time. A variation of transmission of 0.1/min corresponds to 1.4×10^{-4} μg of purified T4 phage lysozyme. The initial optical transmission of the substrate suspension was 0.1. In order to follow cell lysis, the increase in transmission over 10 min was measured. The slope was linear until 5–10 min according to the lysozyme activity.

Thermal denaturation of lysozyme was measured by incubating reaction mixture at 51.5° for different time periods.

Preparation and binding of [^{3}H]formylmethionyl-tRNA were according to Revel *et al.* (1968b).

Results

Effect of Various Antibiotics on in Vitro Biosynthesis of Total Protein and of a Protein Specific of T4 Phage, Lysozyme. EFFECT ON ACELLULAR PROTEIN SYNTHESIS. Figure 3 shows the effect of various concentrations of eight aminoglycosides with a common 2-deoxystreptamine residue (Figures 1 and 2) on lysozyme and total protein biosynthesis in cell-free systems from Sm^S and Sm^R *E. coli* strains. The left-hand column gives results for four antibiotics with a common neamine residue (Nm B, Kn, Nm C, and Nea), the right-hand column for three antibiotics of the paromamine series (Pm, Gm A, and Pa) and one antibiotic which could be denoted a "pseudoparomamine" derivative (Km A). All eight antibiotics inhibit total protein and lysozyme biosynthesis in both systems, but lysozyme activity is more sensitive to antibiotic action than total protein synthesis. The dissociation is a reflection of the degree of misreading. It is more pronounced in the cell-free Sm^R than Sm^S system, indicating a higher level of misreading. This result is not in agreement with Jacoby and Gorini's earlier observation (1967) that Sm^R ribosomes are less sensitive to misreading than Sm^S ribosomes.

In general, both total protein and lysozyme biosynthesis appear to be less sensitive to antibiotic action in the Sm^R than Sm^S system. In the Sm^S system total protein synthesis is little inhibited by the three antibiotics of the paromamine and pseudoparomamine series. Concentrations as high as 100 μM do not give rise to more than 40–50% inhibition. In the Sm^R system, this lack of inhibition is even more pronounced. Paromamine in fact stimulates total protein synthesis at high concentrations, but inhibits lysozyme biosynthesis. Paromamine derivatives are less active than neamine derivatives both on total protein and lysozyme biosynthesis.

Of the two structurally very similar antibiotics, kanamycin A and kanendomycin (Figure 1), kanendomycin inhibits total protein synthesis, lysozyme activity and also the *in vivo* growth of *E. coli* bacteria (unpublished data) more markedly than kanamycin. This correlation between *in vitro* and *in vivo* activities, also observed with the other aminoglycosides, suggests that inhibition of the synthesis of "functional" enzymes such as lysozyme, probably largely accounts for antibiotic

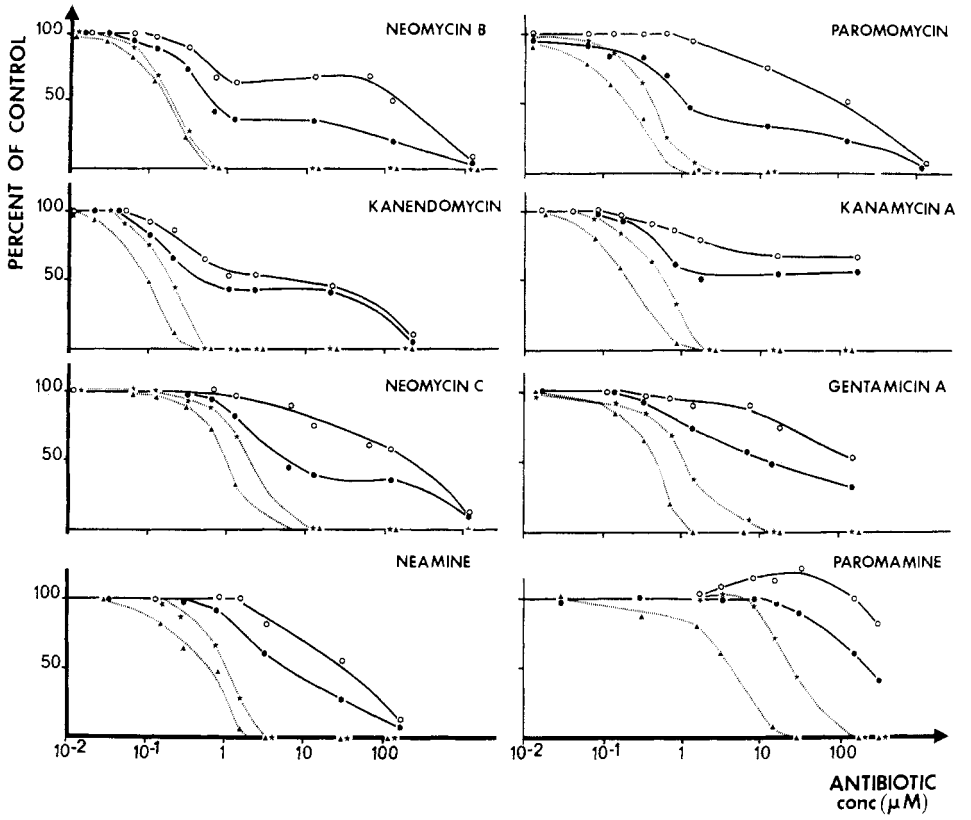


FIGURE 3: Effect of aminoglycoside antibiotics with a deoxystreptamine moiety on total protein and lysozyme biosynthesis. Results are expressed as a percentage of the activity recorded for control samples with no antibiotic. The experimental procedure is described under Materials and Methods. After incubation for 30 min at 37°, 50 μl was withdrawn from the incubation mixture. The control values for total protein synthesis were from 12,000 to 15,000 dpm in 50 μl (efficiency of counting = 45%), for media without mRNA, from 300 to 500 dpm. The Whatman paper filters containing hot trichloroacetic acid precipitable proteins were counted in 10 ml of toluene-based scintillation fluid in an Intertechnique liquid scintillation spectrometer. The amount of lysozyme in 50 μl was 2.5×10^{-2} μg. The reaction mixture contained 8 mM Mg^{2+} and 0.24 μM washed 70S ribosomes (0.5 A_{260} unit in 50 μl). Lysozyme represents about 1% of total proteins. *E. coli* streptomycin-sensitive acellular system: (●) total protein synthesis; (▲) lysozyme activity. *E. coli* streptomycin resistant acellular system: (○) total protein synthesis; (★) lysozyme activity.

activity. Some of these compounds are very effective antibiotics, yet never completely inhibit total protein synthesis.

Dissociation is less marked with streptomycin (Figure 4) than with deoxystreptamines, implying less misreading. Total inhibition of protein biosynthesis is achieved with 5 μM streptomycin as compared to at least 100 μM deoxystreptamine derivative. No dissociation between inhibition curves is observed with kasugamycin and spectinomycin (Figure 4) which both lack streptamine–deoxystreptamine residues.

Table I summarizes the results. The antibiotic concentrations needed for 50% inhibition of [^{3}H]valine uptake into total protein vary from 0.06 μM (Nm B) to 220 μM (Pa) n the Sm^S system, from 1 μM (Kn) to above 300 μM (Km, Pa) in the Sm^R system. The ratio $ID_{50}^{(1)}$ (total protein) over $ID_{50}^{(2)}$ (lysozyme) may be taken to express the level of misreading; it is equal to 1 for kasugamycin and spectinomycin (no misreading) and varies for the other antibiotics from 2 to 55 (Sm^S ribosomes), from 5.5 to over 500 (Sm^R ribosomes). Another measure of misreading is the level of total protein synthesis when lysozyme activity is zero (Table II). This level is equal to at least 38% of control activity (absence of antibiotic) for error-inducing aminoglycosides, to virtually zero for nonerror-inducing aminoglycosides.

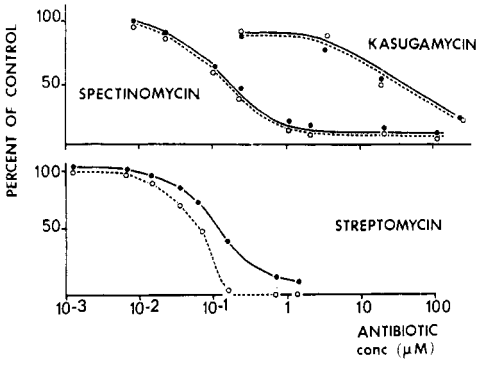


FIGURE 4: Effect of streptomycin, spectinomycin, and kasugamycin on total protein synthesis and lysozyme activity in the streptomycin-sensitive acellular system described in the legend of Figure 3: (●) total protein synthesis; (○) lysozyme activity.

The aminoglycosides studied were compared to three antibiotics not known to induce misreading, namely, chloramphenicol, puromycin, and tetracycline. The $ID_{50}^{(1)}/ID_{50}^{(2)}$ ratios for these last two compounds are low, 1.6 and 1.7, respectively, compared to the values obtained for misreading-inducing aminoglycosides. Moreover, as shown in Table II, for these three antibiotics, when lysozyme activity falls to zero, total protein synthesis is also totally inhibited, whereas for antibiotics inducing misreading, it remains at 38–90% of control activity. A surprising observation in the case of chloramphenicol is that lysozyme synthesis is less inhibited than total protein synthesis (mean of five experiments); this was the only case when an $ID_{50}^{(1)}/ID_{50}^{(2)}$ ratio below 1, namely 0.5, was recorded.

HEAT-LABILE T4 PHAGE LYSOZYME SYNTHESIZED IN THE

TABLE I: Antibiotic Concentrations in Micromoles Giving Rise to 50% Inhibition of Total Protein Synthesis ($ID_{50}^{(1)}$) and Lysozyme Activity ($ID_{50}^{(2)}$).[a]

	Sm^S Ribosomes			Sm^R Ribosomes		
	$ID_{50}^{(1)}$	$ID_{50}^{(2)}$	$ID_{50}^{(1)}/ID_{50}^{(2)}$	$ID_{50}^{(1)}$	$ID_{50}^{(2)}$	$ID_{50}^{(1)}/ID_{50}^{(2)}$
Neomycin B	0.06	0.02	3.0	10	0.02	500
Kanendomycin	0.45	0.10	4.5	1.0	0.18	5.5
Neomycin C	0.50	0.10	5.0	20	0.17	117
Neamine	5.00	0.60	8.3	30	1.0	30
Paromomycin	0.10	0.02	5.0	10	0.04	250
Kanamycin A	1.50	0.20	7.5	>300	0.50	
Gentamicin A	14	0.50	28	140	1.0	140
Paromamine	220	4.0	55	>300	24	
Gentamicin C_1	0.24	0.10	2.4			
Gentamicin C_{1A}	0.10	0.03	3.3			
Gentamicin C_2	0.10	0.04	2.5			
Streptomycin	0.15	0.07	2.1	100	5.0	20
Kasugamycin	50	50	1.0	200	200	1
Spectinomycin	0.18	0.15	1.2			
Chloramphenicol	4.0	8.0	0.5			
Puromycin	1.10	0.70	1.6			
Tetracycline	3.50	2.10	1.7			

[a] The data are the means of at least five experiments. The ratio $ID_{50}^{(1)}/ID_{50}^{(2)}$ is an indication of the level of misreading; it is less than 2, virtually equal to 1, for no misreading. Even very high concentrations of paromamine and kanamycin A (Sm^R ribosomes) did not give 50% inhibition of protein synthesis.

PRESENCE OF LOW INHIBITORY DOSES OF ANTIBIOTIC. It has been shown that random amino acid substitutions frequently render an enzyme more subject to denaturation by heat (Holliday and Tarrant, 1972). When lysozyme biosynthesis is only partially inhibited by antibiotics with a deoxystreptamine residue, the thermal stability of the lysozyme decreases proportionally to the percentage inhibition of enzyme activity as shown in Figure 5 for paromomycin. No difference in thermal stability was observed, however, with lysozyme synthesized in the presence of kasugamycin. These results confirm our first conclusion on misreading effects. None of the test compounds affects lysozyme activity directly, at least at the concentration used in the assay.

INFLUENCE OF Mg^{2+} CONCENTRATION. Szer and Ochoa (1964) showed that at high Mg^{2+} concentrations, ambiguities in translation were observed with artificial mRNA The experiments described so far have been carried out with an optimal magnesium ion concentration, 8 mM (Figure 6), the control preparation containing no antibiotic being very sensitive to even slight variations in this concentration. In the presence of antibiotic, the sensitivity of the subcellular system is also Mg^{2+}-dependent except in the case of kasugamycin and tetracycline, neither of which induce misreadings. Neomycin B and paromomycin concentrations above those necessary to completely inhibit lysozyme synthesis seem to enhance total protein synthesis as compared to the control with the same Mg^{2+} concentration; it would appear that in the case of these compounds the combination magnesium–aminoglycoside is particularly conducive to misreading effects.

INFLUENCE OF POLYETHYLENE GLYCOL AND POTASSIUM IONS. Experiments without polyethylene glycol or with potassium ions have revealed no differences in the relative action of the aminoglycosides. Total protein and lysozyme synthesis are virtually halved in the absence of polyethylene glycol, but enhanced in the presence of potassium ions. Under the outlined conditions, the optimum potassium concentration is 40 mM for the controls.

Effect of Various Antibiotics on Initiation of Protein Synthesis. Experiments were carried out to determine the influence of the above aminoglycosides on the initiation step of cell-free protein synthesis by observing their effect on binding of fMet-tRNA to 70S ribosomes in the presence of GTP and initiation factors. Binding of fMet-tRNA in the presence of the triplet A-U-G or natural mRNA from 25-min T4 phage infected bacteria gave identical results (Table III). Streptomycin whether at a concentration of 1, 10, or 100 μM inhibits

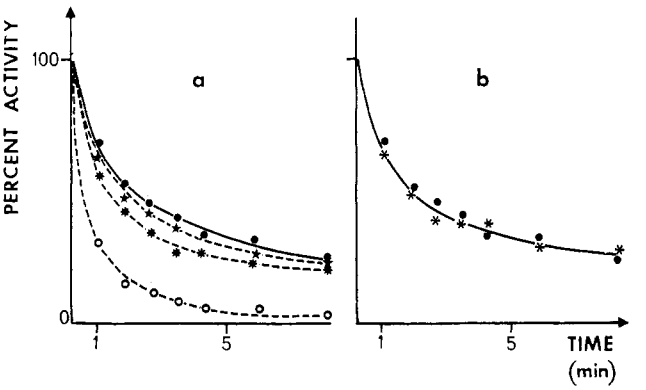


FIGURE 5: Inactivation at 51.5° of *in vitro* synthesized T4 phage lysozyme. 50 μl of each reaction mixture was incubated for different time periods. Lysozyme activity was measured as indicated in Materials and Methods. The data are expressed as a percentage of the activity of a reaction mixture without heating as a function of incubation time. (a) Effect of paromomycin: (●) control; (*) 66% of control activity; (*) 46%; (○) 21%; (b) effect of kasugamycin: (●) control; (*) 50% of control activity.

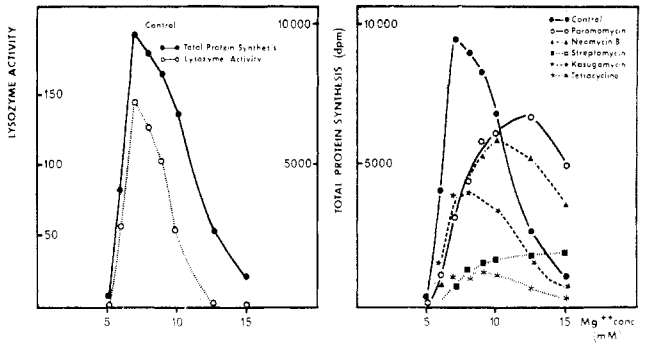


FIGURE 6: Effect of antibiotics on total protein synthesis as a function of magnesium concentration. Lysozyme activity was measured in arbitrary units according to the method described under Materials and Methods: paromomycin, 1.2 μM; streptomycin, 0.7 μM; tetracycline, 21 μM; neomycin, 1.2 μM; kasugamycin, 22 μM.

fMet-tRNA binding to only 40%. An inhibition of the same order is obtained with 10 μM neomycin B or paromomycin, the other aminoglycosides inhibiting initiation even less at this concentration. Inhibition as marked as 80% is only obtained with 100 μM neomycin B or paromomycin.

Discussion

The inhibition of the biosynthesis of a specific enzyme, lysozyme, coded for by a natural mRNA, was judged to be a more valid criterion of the efficiency of various aminoglycoside antibiotics, than just the inhibition of total protein synthesis as measured by the incorporation of labeled amino acid. The data obtained in the foregoing experiments in fact suggest strongly that the effects on lysozyme synthesis are due to errors in translation.

TABLE II: Level of Protein Synthesis (Per Cent of Control) When Lysozyme Activity Is Zero.[a]

	Sm^S *E. coli*	Sm^R *E. coli*
Neomycin B	46	70
Kanendomycin	48	64
Neomycin C	50	88
Neamine	59	80
Paromomycin	46	90
Kanamycin A	60	78
Gentamicin A	74	89
Paromamine	90	100
Gentamicin C_1	38	
Gentamicin C_{1A}	39	
Gentamicin C_2	45	
Streptomycin	40	
Kasugamycin	0	
Spectinomycin	0	
Chloramphenicol	0	
Puromycin	7	
Tetracycline	0	

[a] Zero lysozyme activity. The substrate, *i.e.*, chloroform-treated *E. coli*, is not lyzed after 10-min incubation at 27°. This implies that the level of lysozyme activity is less than 1% of that of the control without antibiotic. (The activity of the control was 2.5×10^{-2} μg of T4 lysozyme in 50 μl of incubation medium.)

TABLE III: Effect on fMet-tRNA Binding.[a]

	Antibiotic Concentration		
	1 μM	10 μM	100 μM
Neomycin B	75	50	20
Kanendomycin	90	75	60
Neamine	100	85	40
Paromomycin	90	55	23
Kanamycin A	100	80	75
Gentamicin A	100	75	55
Streptomycin	65	60	60
Kasugamycin	100	80	50

[a] The reaction mixture contained in 50 μl: 50 mM Tris-HCl (pH 7.5), 80 mM NH_4Cl, 7 mM 2-mercaptoethanol, 0.7 μM washed ribosomes, 1 mg/ml of crude initiation factors, 2 mg/ml of total tRNA charged with [^{3}H]fMet (805,000 dpm/mg), 1 mM GTP, 100 μg/ml of A-U-G triplet in the presence of 5 mM Mg^{2+} or 1 mg/ml of 25-min late T4 phage mRNA in the presence of 8 mM Mg^{2+}. Incubation was at 25° for 12 min with A-U-G or 37° for 12 min with natural mRNA. The radioactivity, collected on Millipore filter, was measured in an Intertechnique SL30 scintillation spectrometer. Results are expressed as per cent control radioactivity. A value of 100 indicates no inhibition of initiation step.

The conclusions of various teams working with artificial messengers (Davies *et al.*, 1966; Tanaka *et al.*, 1967; Anderson *et al.*, 1967; Davies and Davis, 1968; Davies, 1969) were confirmed. Derivatives with a streptamine or deoxystreptamine moiety gave rise to misreading; misreading was more pronounced with neomycin than streptomycin; kasugamycin and spectinomycin did not induce misreading.

As already noted by van Knippenberg *et al.* (1965) for streptomycin, total protein synthesis in the presence of aminoglycosides is affected by the magnesium ion concentration. The degree of stimulation of protein synthesis in the presence of magnesium observed in these studies depends upon the capacity of the aminoglycoside to induce misreading.

The thermal stability of the T4 phage lysozyme synthesized *in vitro* is a function of antibiotic concentration only in the case of misreading inducing aminoglycosides. This result suggests that, for low doses of antibiotic, residual lysozyme activity was due to a protein with errors in the primary sequence which affect thermal stability by also altering secondary structure.

It therefore appears that inhibition of lysozyme is not due to a selective inhibitory effect on certain genes as suggested by Kozak and Nathans (1972) for some antibiotics. They showed that kasugamycin, tetracycline, chloramphenicol, unlike misreading inducing aminoglycosides such as neomycin, streptomycin, and kanamycin, inhibit more markedly the biosynthesis of maturation protein than that of coat protein, induced *in vitro* by RNA coliphage MS_2. In our experiments all misreading inducing aminoglycosides, but neither tetracycline nor kasugamycin, inhibit selectively lysozyme biosynthesis.

On the basis of the results obtained, the aminoglycosides tested may be divided into three groups. (a) Streptomycin which contains the streptamine residue, induces a low level of

misreading and exerts low inhibition of initiation. (b) Aminoglycosides which contain the deoxystreptamine residue and which induce a high level of misreading as compared to streptomycin. The antibiotic concentrations necessary to significantly inhibit initiation are very much higher than those required to induce misreading, implying that action on initiation is not the primary mode of action and that misreading plays a fundamental role in the effect of these compounds. (c) Other aminoglycosides; only kasugamycin and spectinomycin inhibit concomitantly total protein and lysozyme biosynthesis. Both these antibiotics lack a streptamine residue and thus do not induce wrong proteins (Tanaka *et al.*, 1967). Dose–response studies suggest that the inhibitory action of kasugamycin on protein synthesis could be explained simply by action on initiation.

It now seems necessary to correlate the bactericidal effect of aminoglycoside antibiotics and their inhibitoroy effect on the cell-free synthesis of this enzyme and others.

Acknowledgments

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References

Anderson, P., Davies, J., and Davis, B. D. (1967), *J. Mol. Biol. 29*, 203.

Cousin, M. A., Privat de Garilhe, M., Gros, F., and Lando, D. (1971), *Advan. Antimicrob. Antineoplasic Chemother. Proc. 7th Int. Congr. Chemother.*, 807.

Davies, J. (1969), *Progr. Mol. Subcellular Biol. 1*, 47.

Davies, J. (1970), *Biochim. Biophys. Acta 222*, 674.

Davies, J., and Davis, B. D. (1968), *J. Biol. Chem. 243*, 3312.

Davies, J., Gorini, L., and Davis, B. D. (1965), *Mol. Pharmacol. 1*, 93.

Davies, J., Jones, D. S., and Khorana, H. G. (1966), *J. Mol. Biol. 18*, 48.

Erdos, T., and Ullman, A. (1959), *Nature (London) 183*, 618.

Holliday, R., and Tarrant, G. M. (1972), *Nature (London) 238*, 26.

Jacoby, G. A., and Gorini, L. (1967), *in* Antibiotics, Vol. 1, Gottlieb, D., and Shaw, P. D., Ed., New York, N. Y., Springer-Verlag, p 726.

Kozak, M., and Nathans, D. (1972), *J. Mol. Biol. 70*, 41.

Lando, D. (1972), *Euchem. Conf. Antibiot. Biosyn. Function.*

Lelong, J. C., Cousin, M. A., Gros, D., Grunberg-Manago, M., and Gros, F. (1971), *Biochem. Biophys. Res. Commun. 42*, 530.

Mans, R. J., and Novelli, G. D. (1961), *Arch. Biochem. Biophys. 94*, 48.

Modolell, J., and Davis, B. D. (1970), *Proc. Nat. Acad. Sci. U. S. 67*, 1148.

Okuyama, A., Machiyama, N., Kinoshita, T., and Tanaka, N. (1971), *Biochem. Biophys. Res. Commun. 43*, 196.

Ozaki, M., Mizushima, S., and Nomura, M. (1969), *Nature (London) 222*, 333.

Revel, M., Herzberg, M., Becarevic, A., and Gros, F. (1968a), *J. Mol. Biol. 33*, 231.

Revel, M., Lelong, J. C., Brawerman, G., and Gros, F. (1968b), *Nature (London) 219*, 1016.

Salser, W., Gesteland, R. F., and Bolle, A. (1967), *Nature (London) 215*, 588.

Salser, W., Gesteland, R. F., and Ricard, B. (1969), *Cold Spring Harbor Symp. Quant. Biol. 34*, 771.

Spotts, C. R., and Stanier, R. Y. (1961), *Nature (London) 192*, 633.

Szer, W., and Ochoa, S. (1964), *J. Mol. Biol. 8*, 823.

Tanaka, N., Masukawa, H., and Umezawa, H. (1967), *Biochem. Biophys. Res. Commun. 26*, 544.

Traub, P., and Nomura, M. (1968), *Science 160*, 198.

van Knippenberg, P. H., van Ravenswaay Claasen, J. C., Grijm-Vos, M., Veldstra, H., and Bosch, L. (1965), *Biochim. Biophys. Acta 95*, 461.

Weisblum, B., and Davies, J. (1968), *Bacteriol. Rev. 32*, 493.