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A Kinetic Study of the Inhibition of Protein Synthesis by Several Antibiotics*

M. Protić and Ž. Kućan

Department of Organic Chemistry and Biochemistry »Ruđer Bošković« Institute, Bijenička 54, 41000 Zagreb, Croatia, Yugoslavia

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The effects of several antibiotics on the initial reaction rates of the polyphenylalanine and polylysine syntheses were measured in cell-free systems from *Escherichia coli MRE600*, stimulated by polyuridilic and polyadenylic acid, respectively. Michaelis-Menten-type of dependence on the tRNA concentration, and a sigmoidal (possibly cooperative) dependence on the polynucleotide concentration was obtained. Puromycin and chloramphenicol were competitive inhibitors with respect to tRNA, while erythromycin, streptomycin and chlorotetracycline were non-competitive inhibitors. On the other hand, erythromycin was apparently a competitive inhibitor with respect to the synthetic polynucleotide, while other above-mentioned antibiotics showed either uncompetitive, non-competitive or mixed type of inhibition. This kinetic approach enabled the calculation of inhibition constants for the examined antibiotics; they ranged from 2.5×10^{-7} mol dm^{-3} for erythromycin and streptomycin, to over $1 \cdot 10^{-4}$ mol dm^{-3} for puromycin.

INTRODUCTION

Since many antibiotics act as specific inhibitors of procaryotic protein synthesis in vivo and in vitro, they have been widely used in elucidation of the individual steps involved (for recent review, see ref. 1). As a by-product of this work, it became feasible to use various cell-free systems, capable of performing either the amino acid polymerization, or the individual steps involved, as suitable assay systems for comparing the activities of various antibiotics and their derivatives. Some of these experiments, like competition of drug derivatives with the parent compound for the binding to ribosomes[1,2] are based on measurements of equilibrium constants and yield accurate results, but, unfortunately, a radioactive parent compound is required. In many instances, it is much simpler to measure the inhibition of amino acid polymerization in cell-free systems programmed by synthetic polynucleotides. There are two important drawbacks in such experiments: (1) natural initiation and termination never occur in most of these systems, due to the absence of corresponding codons, and only the inhibition of the elongation step is actually measured; and (2) the extent of inhibition by most antibiotics depends on the

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composition of the synthetic polynucleotide used[3–6]. Quantitative interpretation of these experiments and their mutual comparison has been almost impossible, since in most of them one measured the degree of inhibition at some arbitrary time-point, often at the cessation of the amino acid polymerization. Determining the true rates of polymerization should obviously yield more reliable and meaningful data.

In the present paper we describe our attempts to apply simple kinetic formalism to the complex amino acid polymerization system from *Escherichia coli*. Some typical inhibitors of protein synthesis were studied with respect to two variable components of the system, i. e. the tRNA and synthetic polynucleotide. Rather simple Michaelis-Menten type kinetics was obtained in the former, but not in the latter case. This insight into the kinetics of amino acid polymerization in the presence of several typical inhibitors of protein synthesis should prevent oversimplification in the comparison of individual inhibitors, which is possible in the arbitrary-end-point-type of experiments. More importantly, our kinetic data enable the calculation of inhibition constants for the examined antibiotics.

MATERIALS AND METHODS

Escherichia coli MRE600 was grown to late exponential phase as described earlier,[7] washed, and broken using ultrasonic desintegrator at 20 KHz with cooling. Pre-incubated 30 000 × *g* supernatant, prepared by the usual procedure[3] had $A_{260\,nm}$ = = 380. It was frozen in small batches and kept at — 80 °C.

Cell-free polypeptide synthesis was performed with low-molecular-weight components at the following concentrations (in mmol · dm^{-3}): 60 tris (hydroxymethyl) aminomethane, pH 7.8; 13 $MgCl_2$; 40 NH_4Cl; 60 2-mercaptoethanol; 1 ATP; 0.2 GTP; 6 phosphoenolpyruvate; 0.08 L-[U-^{14}C]phenylalanine, specific activity 10 Ci · mol^{-1}, or L-[U-^{14}C]lysine, specific activity 30 Ci · mol^{-1}. Pre-incubated 30 000 *g* supernatant was diluted to $A_{260\,nm}$ of 40 in the final incubation mixture and pyruvate kinase to 36 μg · cm^{-3}. If not otherwise stated, unfractionated *E. coli* tRNA was used at 2 mg/cm^3, poly(U) at 80 μg · cm^{-3} or poly (A) at 57 μg · cm^{-3}. Individual reaction mixtures had the volume of 0.125 cm^3. All components, containing the antibiotic as needed, but still lacking the 30 000 × *g* supernatant, were mixed at 0 °C in the total volume of 0.1 cm^3. Individual test-tubes were pre-incubated for 2 min at 37 °C (to reach the desired temperature) and the reaction was started by the addition of 25 mm^3 of preincubated 30 000 × *g* supernatant. Exactly 0.5 min before the end of the reaction (i. e. after 2.5 min of incubation in most experiments; cf. Results), 0.1 cm^3 of the reaction mixture was pipeted onto a disk of Whatman 3MM filter paper. At the end-point of incubation the disks were immersed into cold, 10% trichloroacetic acid (in the case of polyphenylalanine synthesis) or into the tungstate-trichloracetic acid[8] (in the case of polylysine synthesis), and then worked-up by the standard procedure[9].

Total incorporation of phenylalanine and lysine, as well as the reaction rates, were calculated so as to refer to the actual volume of reaction mixtures, i. e. 0.125 cm^3.

RESULTS AND DISCUSSION

Conditions for Kinetic Measurements

Polymerization of amino acids in rather crude cell extract supplemented with synthetic polynucleotide is undoubtedly a very complex set of reactions. In order to carry out simple kinetic measurements in such a system, it was necessary to find conditions fulfilling, at least formally, the basic requirements for enzyme kinetics. After determining optimum concentrations for all low molecular-weight components, the precise time-course of the polymerization of phenylalanine was measured in the presence of saturating concentration

of poly(U). As shown in Figure 1, the formation of acid-precipitable product proceeds with an initial lag of 0.8 min, and is linear for at least four additional minutes. The same linear course of reaction, following an initial lag, was found for the polymerization of lysine in the system with poly(A) as well as for both these reactions in the presence of inhibitors. In all experiments to be described in this paper, we chose the incubation time of 3 min as suitable to measure rates of reactions. This leads to an underestimation (by 25%) of the true reaction rates, but does not influence the calculation of inhibition constants.

The initial lag in the polymerization reaction does not represent the time needed for the reaction mixture to reach the incubation temperature, since it is not abolished by preincubation at 37 °C, as also shown in Figure 1. Instead, this initial lag may represent the time needed for the aminoacylation of a part of tRNA present in the reaction mixture. A steady state is then reached, maintaining a given concentration of Phe-tRNA^{Phe} or Lys-tRNA^{Lys}, which are the true substrates for polymerization reactions.

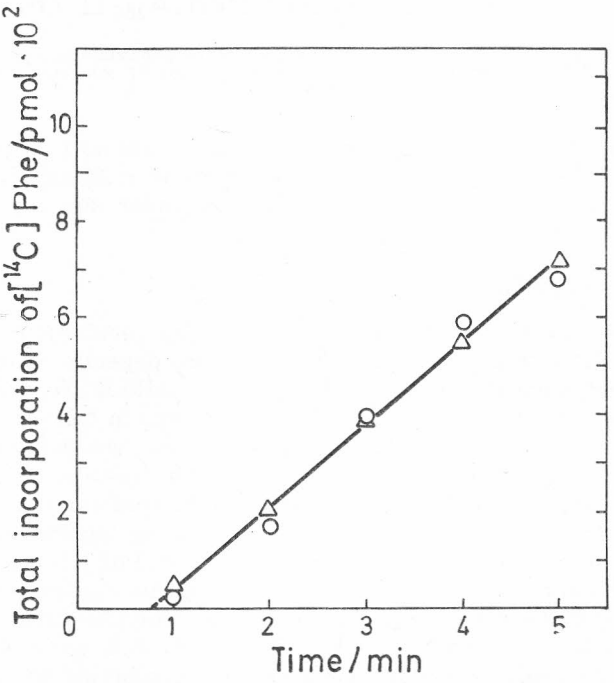

Figure 1. Time-course of the incorporation of phenylalanine into acid-insoluble product. The experiment was performed as described under MATERIALS AND METHODS, except that 0.1 cm³ samples were withdrawn from larger (0.6 cm³) reaction mixtures. The time shown on abscisa refers to the actual time of immersing the discs into trichloroacetic acid. The reaction was started at time 0 by the addition of cold 30.000 × *g* supernatant (○), or by the addition of the supernatant pre-incubated at 37 °C for 3 min (△).

With saturating concentrations of tRNA and all other components, the rate of polymerization is proportional to the concentration of cell extract (Figure 2). Therefore, in the crude amino acid polymerization system we used, tRNA may be formally treated as substrate, and pre-incubated 30 000 x g supernatant as enzyme. This is so in spite of the facts that (1) aminoacyl-tRNA,

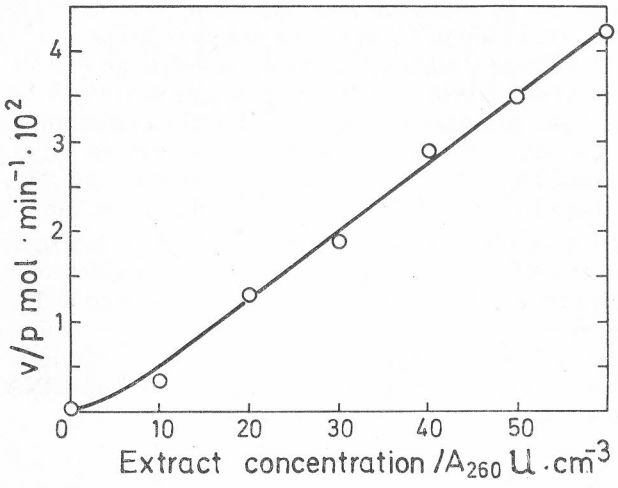

Figure 2. Dependence of initial rate of the polymerization of phenylalanine on the concentration of preincubated 30,000 g supernatant. 1 A_{260} U is the amount of material which, dissolved in 1 cm³ gives $A_{260nm} = 1$.

and not tRNA, is the true substrate, and (2) there are several protein factors, as well as the ribosomes, playing the true catalytic role, and it is not known which one controls the rate of polymerization under our experimental conditions.

Relative Efficiency of some Inhibitors

It has been previously reported that, in long incubations, inhibition by chloramphenicol[3], tetracycline[4], and erythromycin[6] depends on the composition of synthetic polynucleotide used as a model for mRNA. To demonstrate that this effect was not fortuitously caused by differences in cell extract or polynucleotide preparations used in various laboratories, we assayed these three antibiotics with five different polynucleotides. The results, obtained in collaboration with Dr. Š. Šimaga of our Department, confirm that the efficiency of inhibition of amino acid polymerization, in a long incubation, does depend on the nature of the synthetic polynucleotide used. Poly(U)-directed synthesis of polyphenylalanine was found rather resistant to erythromycin and chloramphenicol, and sensitive to chlorotetracycline. Poly(A)-directed synthesis of polylysine and poly(C)-directed synthesis of polyproline were in general more sensitive than the poly(U) system, erythromycin displaying the strongest inhibitory activity. Poly(U,A) (5.1:1), and poly(C,U) (1.25:1)-directed polypeptide synthesis displayed intermediate sensitivity, and the efficiency of inhibition decreased in the order: chlorotetracycline, erythromycin, chloramphenicol.

Based on these data, we chose two homopolymers, poly(U) and poly(A) for our kinetic analysis. Two more inhibitors were included: streptomycin, because of its strong activity in the poly(U) system (cf. Flaks et al.[10], but contradicting results were also reported[11]), and puromycin, because of its well-understood mechanism of action as an analogue of aminoacyl-tRNA[12]. There is very little structural similarity among these five compounds. Their concen-

trations, needed to cause 50% inhibition of the rate of polymerization under our standard conditions, are shown in Table I. Three cases of strong resistance to the antibiotics could be noticed: streptomycin is a poor inhibitor of the poly(A)/lysine system, while the poly(U)-directed synthesis of polyphenylalanine was only slightly inhibited by chloramphenicol and erythromycin. These three cases are omitted from further consideration to avoid misinterpretations based on small effects.

TABLE I

Antibiotic Concentrations Causing 50% Inhibition of Cell-free Polypeptide Synthesis

Antibiotic	50% inhibitory concentration/μmol · dm^{-3}	
	for polyphenylalanine synthesis	for polylysine synthesis
Puromycin	500	140
Chloramphenicol	800*	40
Streptomycin	0.3	~ 400
Erythromycin	30†	0.6
Chlorotetracycline	10	6

* 30% inhibition and †15% inhibition was obtained in these cases; testing higher concentrations was not practical because of solubility problems.

Type of Inhibition with Respect to tRNA

Typical kinetic data for three inhibitors active in the poly(U)/phenylalanine system and four inhibitors active in the poly(A)/lysine system are shown in Figures 3 and 4, respectively. Two inhibitors, puromycin (Figures 3a and 4a) and chloramphenicol (Figure 4b), are competitive with respect to tRNA. This type of inhibition by puromycin can be expected from its action as an analogue of aminoacyl-tRNA[12]. It is interesting, however, that chloramphenicol is also a competitive inhibitor, though the structural similarity with the substrate is less apparent. The competitive type of inhibition by chloramphenicol (at low concentrations) with respect to puromycin has been reported for peptidyl--puromycin formation on native polysomes[13].

On the other hand, chlorotetracycline (Figures 3c and 4d), streptomycin (Figure 3b) and erythromycin (Figure 4c) are non-competitive inhibitors with respect to tRNA. The same three types of antibiotics were found very poor inhibitors of the formation of peptidyl-puromycin on native polysomes, where only the transpeptidation step is assayed[13].

Comparison of Figures 3 and 4 shows that the same type of inhibition is obtained in both the poly(U) and the poly(A) system, whenever these two systems are sensitive to a certain antibiotic.

Type of Inhibition with Respect to Synthetic Polynucleotide

A synthetic polynucleotide has to combine functionally with ribosomes in order to direct the amino acid polymerization. We have hoped to detect, by kinetic measurements, whether some inhibitors of protein synthesis compete with polynucleotides for the formation of such functional complexes. To our surprise, we found that the rate of amino acid polymerization with an excess of other components, but with variable concentrations of poly(U) or poly(A), does not follow simple saturation kinetics. Sigmoidal curves are obtained

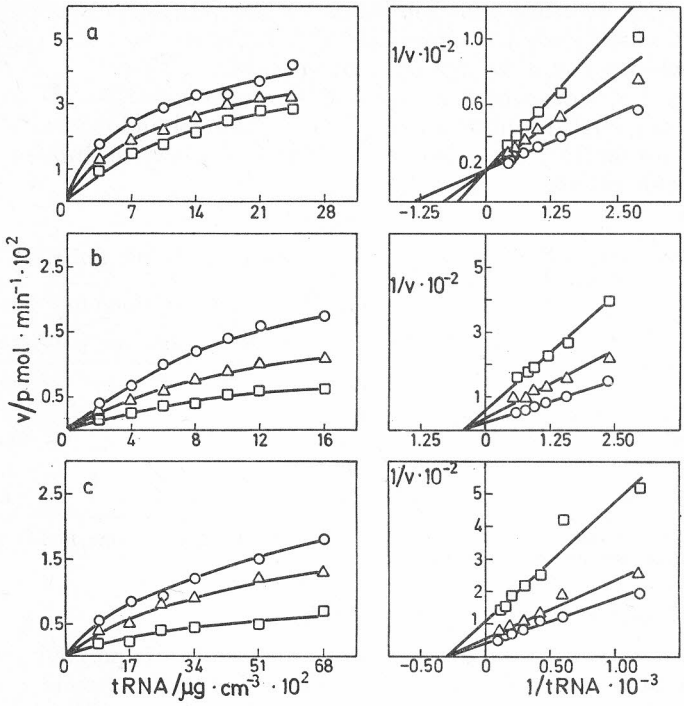

Figure 3. Dependence of initial rates of the polymerization of phenylalanine on the tRNA concentration in the absence of inhibitors (○), and in the presence of: (a) puromycin (200 µmol · · dm⁻³, △; 400 µmol · dm⁻³, □); (b) streptomycin (0.2 µmol · dm⁻³, △; 0.4 µmol · dm⁻³, □); (c) chlorotetracycline (6 µmol · dm⁻³, △ 20 µmol · dm⁻³, □).

instead (»controls« in Figures 5 and 6), pointing to some kind of cooperative effect. Formally, these curves could be represented by the Hill equation, $v = V \cdot s^h/(s^h + K_H{}^h)$, where v is the reaction rate, V is the maximum rate of reaction, and s, the usual substrate concentration, is taken to mean the polynucleotide concentration. The least square minimalization method for h (the Hill coefficient), K_H (Hill constant) and V gave h values around 2.0. We would like to suggest that the observed quasi-cooperativity is caused by the difference in polymerizing activity between artificially formed polysomes (present at low polynucleotide:ribosome ratio, presumably representing jamed and rigid, rather inactive complexes) and the artificial oligosomes and monosomes (formed at higher polynucleotide: ribosome ratios, presumably representing flexible complexes, similar to native structures).

Typical experiments demonstrating the effect of antibiotics on the synthesis of polyphenylalanine and polylysine at various concentrations of poly(U) and poly(A) are shown in Figures 5 and 6, respectively. Each experiment is also represented after linearization of the Hill equation, i. e. as a plot of $\lg [v/(V - v)]$ versus lg polynucleotide concentration. In the discussion to follow, it is assumed that the effect of inhibitors on K_H and V, as calculated by the least square minimalization method of sigmoidal curves, has the same meaning here as in the simple case of Michaelis-Menten kinetics. Depending on the inhibitors used, several types of inhibition are observed:

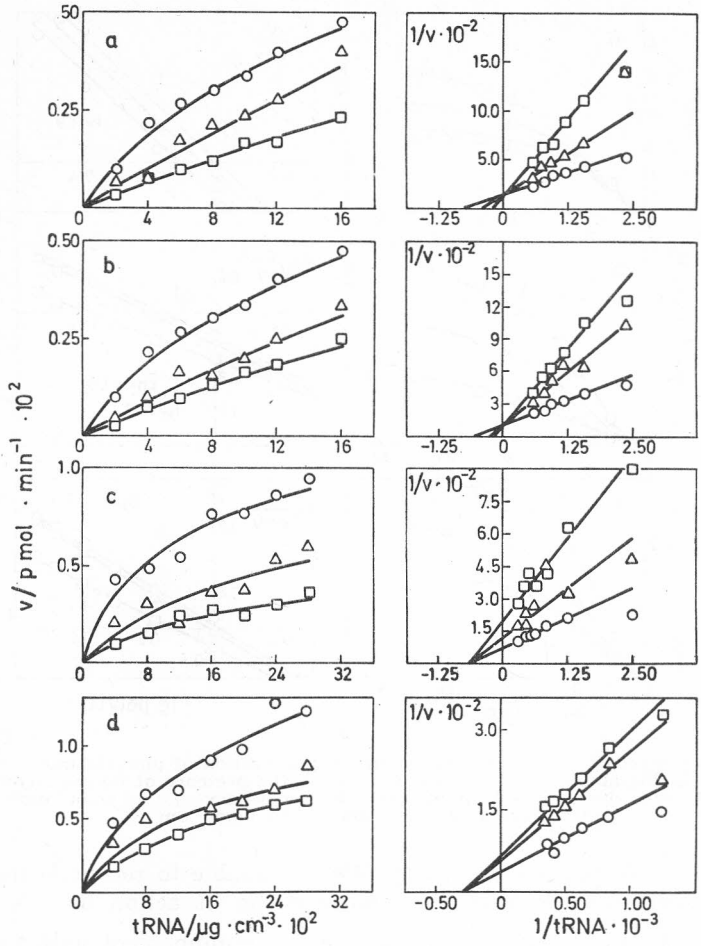

Figure 4. Dependence of the initial rates of the polymerization of lysine on the tRNA concentration in the absence of inhibitors (○) and in the presence of: (a) puromycin (60 µmol · dm⁻³, △; 120 µmol · dm⁻³, □); (b) chloramphenicol (20 µmol · dm⁻³, △; 50 µmol · dm⁻³ □); (c) erythromycin (0.4 µmol · dm⁻³, △; 1.2 µmol · dm⁻³, □); (d) chlorotetracycline (2 µmol · dm⁻³, △; 6 µmol · · dm⁻³, □).

(i) Puromycin lowers V and K_H by the same factor indicating uncompetitive type of inhibition in both polymerization systems (Figures 5a and 6a). The quasi-cooperativity is not affected, i. e. there is no change in h. Since puromycin actually causes the formation of peptidyl-puromycin and free ribosome-polynucleotide complex, which is presumably ready to reenter the production of polypeptide chains, the uncompetitive type of inhibition with respect to synthetic polynucleotides is not surprising.

(ii) Erythromycin, which inhibits only the poly(A)/lysine system, increases K_H, and does not affect V (Figure 6c). Hence, a conclusion about competitive inhibition emerges. Our results also show that h for poly(A) is greatly changed by erythromycin. Moderate concentrations of the antibiotic lead to complete

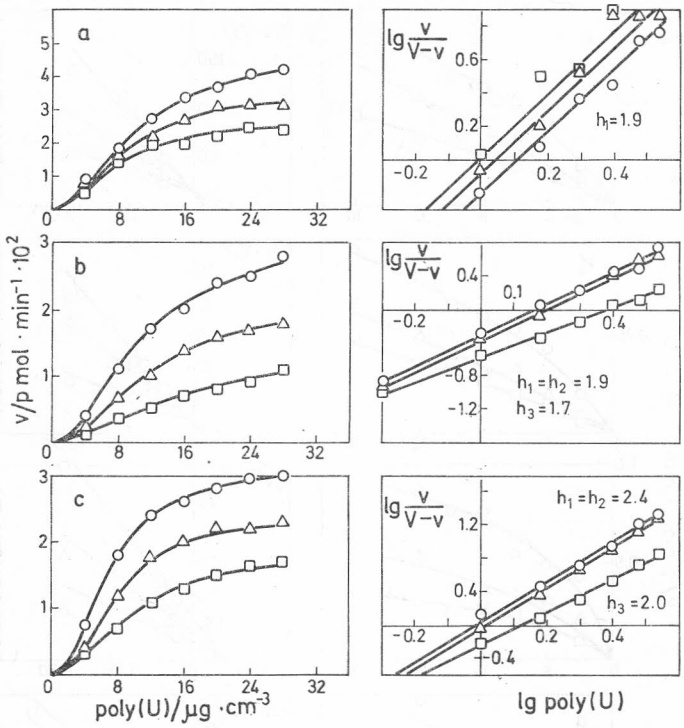

Figure 5. Dependence of the initial rates of the polymerization of phenylalanine on the poly(U) concentration in the absence of inhibitors (○) and in the presence of (a) puromycin (200 μmol · · dm⁻³, △; 400 μmol · dm⁻³, □); (b) streptomycin (0.2 μmol · dm⁻³, △; 0.4 μmol · dm⁻³, □); (c) chlorotetracycline (4 μmol · dm⁻³, △; 8 μmol · dm⁻³, □).

disappearance of quasi-cooperativity. We are unable to reconcile these kinetic effects of erythromycin with the known mode of action of this antibiotic.

(iii) In all other cases examined, i. e. chloramphenicol with poly(A) (Fifure 6b), streptomycin with poly(U) (Figure 5b), and chlorotetracycline with both poly(U) and poly(A) (Figures 5c and 6d), V is lowered by the presence of inhibitors, and K_H is either unchanged or increased, suggesting either non-competitive or mixed type of inhibition. In some of these cases the Hill coefficient, h, seems also to be changed, especially at higher concentrations of inhibitors, as can be seen from changes in the slopes of linear plots. Non-competitive inhibition with respect to the synthetic polynucleotide should be expected, indeed, whenever the presence of the antibiotic does not interfere with the formation of functional ribosome: polynucleotide complex.

Determination of Inhibition Constants

The inhibition constants, K_i, derived from experiments in which either polynucleotide or tRNA concentrations were varied, are shown in Table II. Clearly, for any inhibitor, K_i is not the same for poly(U) and poly(A) systems. This can be seen by comparing the data for puromycin and chlorotetracycline in the two systems, and noticing that the data for chloramphenicol and erythro-

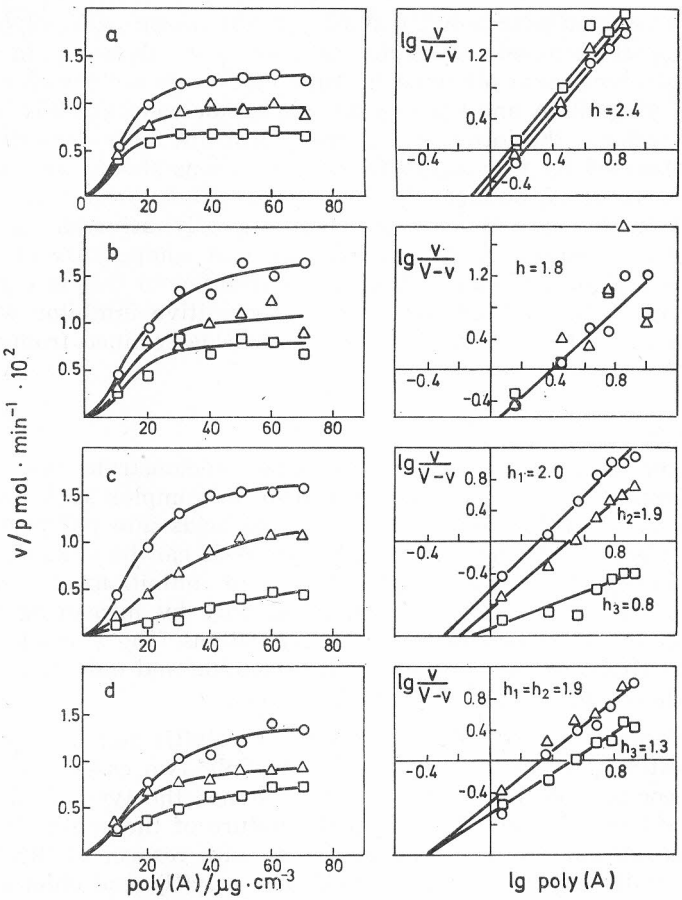

Figure 6. Dependence of the initial rates of the polymerization of lysine on the poly(A) concentration in the absence of inhibitors (○) and in the presence of (a) puromycin (60 µmol · dm⁻³, △; 120 µmol · dm⁻³, □); (b) chloramphenicol (40 µmol · dm⁻³, △; 80 µmol · dm⁻³, □); (c) erythromycin (0.4 µmol · dm⁻³, △; 0.8 µmol · dm⁻³, □) and (d) chlorotetracycline (2 µmol · dm⁻³, △; 4 µmol · dm⁻³, □).

TABLE II

Inhibition Constants, K_i, for Five Inhibitors of Protein Synthesis

Cell-free system	Variable Component	$10^6 \times K_i$/mol · dm⁻³				
		Pur	CAM	SM	E	CTC
Poly(U)/phenylalanine	Poly(U)	480 ± 30[b]		0.4 ± 0.1[b]		13 ± 4[b]
	tRNA	260 ± 70[a]		0.25 ± 0.05[b]		14 ± 3[b]
Poly(A)/lysine	Poly(A)	140 ± 40[b]	70 ± 10[b]		0.25 ± 0.05[a]	6 ± 2[b]
	tRNA	60 ± 20[a]	31 ± 8[a]		0.6 ± 0.1[b]	4 ± 2[b]

[a] and [b]: K_i was calculated from K_m (a) or V (b) in the presence of inhibitor, assuming that the changes in these values can be attributed to a simple factor $[1 + (i/K_i)]$, where i is the inhibitor concentration. With synthetic polynucleotides as variable components, resulting in sigmoidal rate-dependence curves, the computer values for K_H and V, obtained by least square minimalization method were used. When tRNA was varied, the values obtained graphically from Lineweaver-Burk plots were also included. Values are represented as means of several estimates, all experimental values falling within the range shown.

mycin were ommitted with poly(U), and for streptomycin with poly(A), because of uncertainty of obtained values due to very poor inhibition. In addition, a significant difference was obtained in some cases between apparent K_i determined by varying tRNA and varying polynucleotide concentrations. Since only the former, and not the latter, is the true substrate for polymerization, only the values obtained by varying tRNA concentrations should be taken as true inhibition constants. K_i obtained in this way is either lower than or equal to K_i derived from the experiments with varying polynucleotide concentration. The difference is especially pronounced for two competitors of tRNA, i. e. puromycin and chloramphenicol. The only exception to this is erythromycin, which appears to be, at least formally, a competitive inhibitor with respect to poly(A) and not tRNA: here K_i is lower when determined from the data of varying poly(A) concentrations.

General comments

The experiments described in this paper demonstrate that the simple Michaelis-Menten formalism can be applied to the complex sequence of reactions which lead to the polymerization of amino acids into polypeptide chains. Hence, the effect of inhibitors of protein synthesis can be studied in cell-free systems, yielding information about the type of inhibition and enabling the calculation of K_i. This can be accomplished only by measuring true initial rates of reactions at known inhibitor concentrations. The variable component can be either tRNA or the synthetic polynucleotide and only relative concentrations of these components ought to be known.

The difference bewteen the sensitivity of poly(U) and poly(A) system to individual antibiotics is still puzzling. In the only two cases where we were able to get comparable inhibition in both systems, the type of inhibition for the given antibiotic did not depend on the nature of the synthetic polynucleotide, i. e. puromycin was always competitive with respect to tRNA and non-competitive with respect to both poly(A) and poly(U), and chlorotetracycline was always non-competitive with respect to tRNA and to both synthetic polynucleotides. The extent of inhibition, on the other hand, did depend on the nature of the synthetic polynucleotide, especially with chloramphenicol, erythromycin and, though with the reversed preference, with streptomycin. We are still not able to explain the difference bewteen the two systems. However, we find it difficult to accept the suggestion of Spirin et al.[14], based on the »factor-free translation« experiments, that the lack of secondary structure in poly(U) makes its reading easier, hence less sensitive to inhibitors. Our experiment with streptomycin ($K_i = 2.5 \times 10^{-7}$ mol · dm^{-3} in the poly(U)-system; cf. also Table I) and lack of strong preference in some other cases (Tables I and II) suggest that the true molecular basis for this effect must be more complicated and less clearly understood.

Acknowledgements. — We are greatly indebted to Dr. Elsa Reiner for many hours of useful discussion, which has straightened our view on enzyme kinetics (without straightening our sigmoidal curves). We would also like to acknowledge the collaboration of Dr. Š. Šimaga in the earliest stage of this project, when he helped us to confirm the published data from various laboratories concerning differential sensitivity of cell-free systems to chloramphenicol, erythromycin and chlorotetracycline. This work was supported, and a fellowship to M.P. was given, by Croatian Research Council (SIZ II).

REFERENCES

1. S. Pestka, *Insight into Protein Biosynthesis and Ribosome Function through Inhibitors,* in: W. E. Cohn (Ed.), *Progress in Nucleic Acid Research and Molecular Biology,* Vol. **17,** Academic Press, New York, San Francisco and London, 1976, pp. **217—245.**
2. S. Pestka, R. A. LeMahieu, and P. Miller, *Antimicrob. Ag. Chemother.* **6** (1974) 489—491.
3. Ž. Kućan and F. Lipmann, *J. Biol. Chem.* **239** (1964) 516—520.
4. M. Hierowski, *Proc. Nat. Acad. Sci. U.S.A.* **53** (1965) 594—599.
5. D. Vazquez, *Biochim. Biophys. Acta* **114** (1966) 289—295.
6. J. C. H. Mao and R. G. Wiegand, *Biochim. Biophys. Acta* **157** (1968) 404—413.
7. Ž. Kućan, J. N. Herak, and I. Pečevsky-Kućan, *Biophys. J.* **11** (1971) 237—251.
8. S. Nishimura and G. D. Novelli, *Proc. Nat. Acad. Sci. U.S.A.* **53** (1965) 178—184.
9. R. I. Mans and G. D. Novelli, *Arch. Biochem. Biophys.* **94** (1961) 48—53.
10. J. G. Flaks, E. C. Cox, and J. R. White, *Biochem. Biophys. Res. Commun.* **7** (1962) 385—389.
11. P. Anderson, J. Davies, and B. D. Davis, *J. Mol. Biol.* **29** (1967) 203—215.
12. M. B. Yarmolinsky and G. L. de la Haba, *Proc. Nat. Acad. Sci. U.S.A.* **45** (1959) 1721—1729.
13. S. Pestka, *J. Biol. Chem.* **247** (1972) 4669—4678.
14. A. S. Spirin, O. E. Kostiashkina, and J. Jonák, *J. Mol. Biol.* **101** (1976) 553—562.

SAŽETAK

Kinetički studij inhibicije sinteze proteina nekim antibioticima

M. Protić i Ž. Kućan

Utjecaj nekih antibiotika na početne brzine sinteze polifenilalanina i polilizina bio je mjeren u sistemu bez stanica, pripremljenom iz bakterije *Escherichia coli,* i stimuliranom dodatkom poliuridilne, odnosno poliadenilne kiseline. Dobivena je Michaelis-Mentenova ovisnost o koncentraciji tRNA, te sigmoidna (možda kooperativna) ovisnost o koncentraciji sintetskog polinukleotida. Puromicin i kloramfenikol pokazali su se kompetitivnim inhibitorima s obzirom na tRNA, dok su eritromicin, streptomicin i klortetraciklin bili nekompetitivni inhibitori. U odnosu na sintetski polinukleotid, eritromicin je bio prividno kompetitivan inhibitor, a ostali su se ispitani antibiotici pokazali kao akompetitivni, nekompetitivni ili miješani inhibitori. Taj je kinetički pristup omogućio izračunavanje konstanti inhibicije za ispitane antibiotike; dobivene vrijednosti bile su u rasponu od $2{,}5 \times 10^{-7}$ mol · dm^{-3} za eritromicin i streptomicin, do iznad 1×10^{-4} mol · dm^{-3} za puromicin.

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