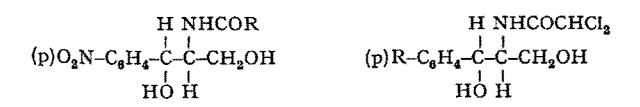
The Effect of Chloramphenicol Analogs on Protein Biosynthesis in a Cell-free *Escherichia coli* B System

A comparison of the structure-activity relations of chloramphenicol and several of its analogs that we had previously synthesized had led us to a number of inferences regarding the significance of the various fragments of the chloramphenicol molecule (aminopropanediol chain, nitrophenyl residue and dichloroacetyl grouping) in the manifestation of its antibiotic action[1,2].

The molecular mechanism by which chloramphenicol, a specific inhibitor of protein synthesis, acts is still not very clear[3–5]. However it has been established that the antibiotic does not inhibit the synthesis of aminoacyl S-RNA, apparently acting on subsequent stages of protein synthesis involving formation of the peptide chain[6]. Chloramphenicol has been shown to bind to the S-50 ribosome subunit[7,8]. The attachment is stereospecific like the antimicrobial activity of chloramphenicol and its inhibition of protein synthesis in a cell-free system. The stereoisomers of chloramphenicol are incapable of any such effects.

The objective of the present work was to investigate the effect of chloramphenicol and its analogs, differing in antimicrobial activity, on the incorporation of amino acids into protein in a cell-free system, thereby bringing the structure-activity study closer to the molecular level.

The study was carried out on chloramphenicol and its D-threo analogs of the types (I) and (II).



(I)

(II)

(Ia) R = $CHCl_2$ (chloramphenicol)
(Ib) R = $CHBr_2$
(Ic) R = CH_2Cl
(Id) R = CCl_3
(Ie) R = $CH{:}CHCHCl_2$
(If) R = CH_3

(IIa) R = CH_3SO_2
(IIb) R = NH_2SO_2
(IIc) R = CH_3CONH

The antibacterial activity of these compounds was determined by the serial dilution method in liquid synthetic nutrient medium[9]. The protein synthesis inhibiting activity of the antibiotic and its analogs in the cell-free system was estimated by comparing the amount of labelled amino acid incorporation into the protein with incorporation in the absence of the substance undergoing the test[10]. All analogs were investigated in concentrations of 0.048, 0.39, 3.13, 25 and $400M \cdot 10^{-6}$, chosen on the basis of the fact that at the lowest concentration chloramphenicol did not inhibit and even stimulated protein synthesis, and at the highest, caused 95% inhibition. In every case a control run was carried out with an equimolar concentration of chloramphenicol. The results of the experiments are given in the Table.

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[9] All the analogs were synthesized by Dr. M. G. Karapetyan and the antibacterial activity was determined by Dr. I. D. Ryabova, to both of whom the authors express their grateful acknowledgment.
[10] The bacterial mass was prepared from 6 h *E. coli* B cultures in a medium of the following composition: Hottinger's hydrolysate 250 ml, glucose 10 g, NH_4Cl 1 g, yeast autolysate 25 ml, H_2O to 1 l, pH 7.5–7.8. Separation of the subcellular fractions was carried out according to Nirenberg[11]. Protein was determined according to Lowry[12]. The washed ribosomes and the S-100 fraction (supernatant) each containing 1.5–2.0 mg/ml protein were incubated at 37 °C for 60 min in medium of the following composition ($M \cdot 10^{-3}$): *Tris*. HCl 100, $MgCl_2$ 10, KCl 50, 20 amino acids, each 0.05, GTP 0.2, ATP 1, PEP (K salt) 5, rabbit muscle PEPK[13] 20 γ/ml, pH 7.8. D,L-phenylalanine-1-C^{14} with an activity of 9.1 mcu/mmole and uniformly labelled L-leucine-C^{14} with an activity of 141 mcu/mmole were used as the tracer acids. After incubation the protein precipitate was freed of non-protein radioactive products according to Siekevitz[14], dissolved in 85% formic acid and applied to Whatman 3MM paper discs, the activities of the sample being determined in a liquid scintillation counter.

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[15] The 50 and 100% inhibition of growth of *E. coli* B by chloramphenicol is caused by concentrations of 0.155 and 0.261 μM/l, respectively. At concentrations of 0.048, 0.39, 3.13, 25 and 400 μM/l chloramphenicol inhibits amino acid incorporation into protein, in the cell-free system by -4.12 ± 1.8, 15.1 ± 3.2, 61.8 ± 5.1, 82.6 ± 3.1, 92.5 ± 1.4%, respectively.
[16] The positive sign indicates inhibition, the negative sign stimulation of incorporation.

Compound	Relative growth inhibition of *E. coli* B by		Relative inhibition of amino acid incorporation into protein in the cell-free system of *E. coli* B concentration (μM/l)[16]				
	50%	100%	0.048	0.039	3.13	25	400
(Ia) R = $CHCl_2$ Chloramphenicol[15]	100	100	− 100	100	100	100	100
(Ib) R = $CHBr_2$	88	30	+	352	106	104	100
(Ic) R = CH_2Cl	25	25	+	119	80	97	97
(Id) R = CCl_3	18	11	− 184	−	57	80	99
(Ie) R = $CH{:}CHCHCl_2$	9	10	− 86	100	43	73	96
(If) R = CH_3	0.6	0.5	− 258	71	97	100	100
(IIa) R = CH_3SO_2	4	3	− 900	−	8	74	100
(IIb) R = NH_2SO_2	0.3	0.3	− 139	−	2	19	86
(IIc) R = CH_3CONH	0.2	0.2	− 190	56	24	37	54

As one can see from the Table, all the analogs clearly inhibit amino acid incorporation into protein in the cell-free system. However modification of the various parts of the chloramphenicol molecule affects differently the relation between the antibacterial activity of the antibiotic and its inhibition of protein biosynthesis in ribosomes. In type (I) compounds the difference in antibacterial activity observed between chloramphenicol and its analogs is much less strongly expressed with respect to protein synthesis in a cell-free system. Thus, compounds (Ic) and (If) which display only $^1/_4$ and $^1/_{170}$, respectively, of the antibacterial potency of chloramphenicol, show almost no difference from the antibiotic in the ability to inhibit protein synthesis (see Table). The analog (Ib) approaching chloramphenicol in antibacterial properties is somewhat more active in the cell-free system than compounds (Ia), (Ic) and (If). The analogs (Id) and (Ie), possessing antibacterial activity of the same order of magnitude as (Ic), and much inferior to chloramphenicol in this respect, inhibit protein synthesis somewhat less than the former compounds.

In the series of analogs of type (II) all 3 of the compounds studied are less active in the cell-free system than chloramphenicol. Here, too, as in the type (I) analogs, the differences between the effects on protein synthesis in the ribosomes are much less than the differences in the antibacterial activity. However, the type (II) compounds do fall into the same order with respect to both relative antibacterial activity and protein synthesis inhibition.

These findings lead to the conclusion that the acyl residue of chloramphenicol plays a more important role in the penetration of the antibiotic into the bacterial cell, or in the transformation of the antibiotic on its path towards the ribosomes, than in the actual interaction with the protein synthesizing systems. On the contrary, substitution in the aromatic part of the chloramphenicol molecule affects its interaction with these systems. This is indirectly supported by evidence from the recent paper of VASQUEZ[17], who showed that Ar-substituted D-threo-analogs of chloramphenicol, including (IIa) and (IIb) investigated by us, compete with the antibiotic for the site of attachment to the ribosomes. However, the more equalized effects of type (II) analogs on the cell-free system than the differences in their antibiotic activities indicates that the nitrophenyl part of the molecule also plays a certain role in the penetration of the antibiotic into the microbial cell[18].

Выводы. Сравнительное изучение антибактериальной активности аналогов хлорамфеникола и их влияния на биосинтез белка в бесклеточной системе *E. coli* показало, что ацильный радикал этого антибиотика имеет значение для его проникновения в микробную клетку, а арильный радикал - для взаимодействия с белоксинтезирующей системой.

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[19] Laboratory of Biological Investigations.
[20] Laboratory for Chemistry of Antibiotics.

Mass Spectrometric Determination of the Amino Acid Sequence in Arginine-Containing Peptides

Earlier[1-3] we showed that the mass spectrometric method can be used for direct determination of the amino acid sequence in peptides containing residues of all the ordinary amino acids except arginine, which causes complications due to the specific behaviour of its guanidine grouping under the mass spectrometric conditions. Because arginine is a component part of many naturally occurring polypeptides and proteins, in order for the method to assume a universal character, its extension to include also the sequential analysis of arginine-containing peptides was highly desirable.

Attempts to overcome the unfavourable effects of the guanidine grouping on the fragmentation of arginine-containing peptides by its diacylation did not lead to positive results, but two other possibilities proved to be quite promising. The first involves conversion of the arginine residue into an ornithine residue in the peptides, since the esters of N^{α}, N^{δ}-diacylated ornithine-containing peptides undergo the normal amino acid type of mass spectrometric fragmentation[1]. For this purpose the peptides could not be incubated with arginase or subjected to alkaline hydrolysis, because this causes not only conversion of the guanidine residue but also intensive cleavage of the amide bonds. On the contrary, refluxing of the arginine-containing peptides with 20% aqueous hydrazine for $^1/_2$–1 h leads to their practically complete transformation into the corresponding ornithine-containing peptides. Although here also one may sometimes encounter partial cleavage of the peptide bonds, the degree of cleavage is usually not very great and can be controlled chromatographically. This is illustrated in Figure 1, lower, by the mass spectrum of N^{α}, N^{δ}-didecanoylornithylleucine methyl ester (1) prepared from arginylleucine by treatment with hydrazine under the above

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