MECHANISM OF ACTION OF PHENOMYCIN, A TUMOR-INHIBITORY POLYPEPTIDE

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The mechanism of action of phenomycin, an antitumor antibiotic, was studied, using mammalian systems. Phenomycin was observed to inhibit protein synthesis, but not RNA or DNA synthesis in growing HeLa cells. Approximately 50 % inhibition of leucine incorporation into protein was demonstrated at a concentration of 100 mcg/ml of phenomycin when the cells were treated with the antibiotic for 24 hours. Phenomycin significantly inhibited protein synthesis in the lysate of EHRLICH carcinoma cells, although only a slight inhibition was observed with intact cells. Protein synthesis in ribosomal systems of rat liver and rabbit reticulocytes with native mRNA was markedly suppressed by the presence of phenomycin. In reticulocyte ribosomes, polyphenylalanine synthesis with poly U was rather resistant to the action of the antibiotic. The site of action of phenomycin in a protein-synthesizing system was studied, using rabbit reticulocyte system. It was demonstrated that phenomycin did not affect aminoacyl-sRNA formation but inhibited the amino acid transfer from aminoacyl-sRNA to polypeptide. The non-enzymatic binding of lysyl-sRNA to the ribosomes with poly A or with native mRNA was significantly inhibited by phenomycin. Contrary to the mammalian systems, protein synthesis was not significantly affected by the presence of phenomycin, in an *E. coli* system. The site of action and selective toxicity of phenomycin are discussed.

Phenomycin, a basic polypeptide antibiotic, exhibited significant tumor-inhibitory activity against EHRLICH carcinoma, sarcoma 180 and adenocarcinoma 755 in mice, but had no antimicrobial activity[1,2].

In order to elucidate the mechanism of action on a molecular basis, studies were carried out using intact cells and cell-free protein-synthesizing systems of HeLa cells, EHRLICH ascites carcinoma cells, rabbit reticulocytes and rat liver. The results are presented in this publication.

Materials and Methods

Chemicals:

The sample of phenomycin used in this experiment was a highly purified, as reported in the previous papers[1,2]. L-Leucine-^{14}C (U) (214 mc/mM), L-lysine-^{14}C (U) (214 mc/mM), L-proline-^{14}C (U) (179 mc/mM), and L-phenylalanine-^{14}C (U) (393 mc/mM) were purchased from Daiichi Chemical Co., Tokyo. Thymidine-6-^{3}H (n) (1850 mc/mM) and uridine-^{3}H (G) (2,700 mc/mM) were products of Radiochemical Centre, Amersham, England. Poly U and poly A were obtained from Calbiochem. Los Angeles, California; and poly C from Miles Chemical Co., Indiana.

Extracts of rat liver, EHRLICH carcinoma, and rabbit reticulocytes were prepared as follows:

The deoxycholate extracted ribosomes of liver were prepared from Wistar rats, weighing 100~180 g by the method of KIRSCH *et al.*[9] and the pH 5 fraction by the method of KELLER *et al.*[10], as described in the previous papers[3,4]. The lysates of EHRLICH carcinoma were prepared from the ascitic tumor cells of *ddD* mice inoculated with 2×10^6 carcinoma cells 7 days before. The cells were washed in saline, homogenized, and extracted in a buffer, consisting KCl 0.1 M, $MgCl_2$ 0.01 M, 2-mercaptoethanol 0.006 M and Tris 0.01 M, pH 7.8.

The extracts were centrifuged at 3,000×g for 10 minutes and the supernatant was used as a lysate. Reticulocytosis was produced by the injection of phenylhydrazine following the method of ALLEN and SCHWEET[5].

The lysate was prepared by freezing and thawing reticulocytes according to the method of WEINSTEIN[6]. The preparation of the ribosomes and pH 5 fraction of reticulocytes was prepared by separating the lysate (the packed cells : 0.0015 M $MgCl_2$=1 : 1) into a supernatant and a ribosomal fraction by centrifugation at 105,000×g for an hour, after the centrifugation at 15,000×g for 10 minutes.

Preparation of sRNA from *E. coli* B or rabbit reticulocytes :

The soluble fraction (105,000×g supernatant) of *E. coli* B or rabbit reticulocytes was extracted with phenol, and sRNA was precipitated by addition of ethanol from the water phase. The method principally followed the one described in the previous paper[3].

Labelling of sRNA with ^{14}C-lysine :

The sRNA was charged with ^{14}C-lysine according to the method of NATHANS and LIPMANN[11].

Chemical procedures and radioactivity measurement :

Unless otherwise stated, the reaction was terminated by adding an equal volume of 10 % TCA. The polylysine synthesis in the poly A systems was stopped by addition of an equal volume of a mixture of 0.5 % sodium tungstate and 10 % TCA, adjusted to pH 2.0.

The resultant precipitates were washed twice with 5 % TCA, extracted with 5 % TCA at 90°C for 20 minutes, washed with 5 % TCA, ethanol, and ethanol-ether (3 : 1) successively. The protein fraction, thus obtained, was suspended in 2 N NH_4OH ; and assayed for protein content by the method of LOWRY and for radioactivity in a windowless gas flow counter[8].

Results

Effects of phenomycin on growing HeLa cells :

Phenomycin was observed to inhibit the growth of HeLa cells, when it was added at the logarithmic phase of growth. At a concentration of 100 mcg/ml, the number of cells was markedly reduced. The growth was significantly inhibited at the concentration of 10 mcg/ml and slightly at 1 mcg/ml. The results are illustrated in Fig. 1.

Effects of phenomycin on the incorporation of ^{14}C-leucine, ^{3}H-uridine and ^{3}H-thymidine into protein and nucleic acids of HeLa cells at the logarithmic phase of growth :

Leucine incorporation into protein was significantly inhibited, but uridine incorporation into RNA and thymidine incorporation into DNA were not significantly affected by the presence of phenomycin. The inhibition of leucine incorporation was not very great but increased with preincubation of the cells in a phenomycin-containing medium. Approximately 50 % inhibition was observed at a concentration of 100 mcg/ml, when the cells were treated with phenomycin for 24 hours before the

Table 1. Effect of phenomycin on protein and nucleic acids syntheses in growing HeLa cells (Phenomycin : 24-hour treatment)

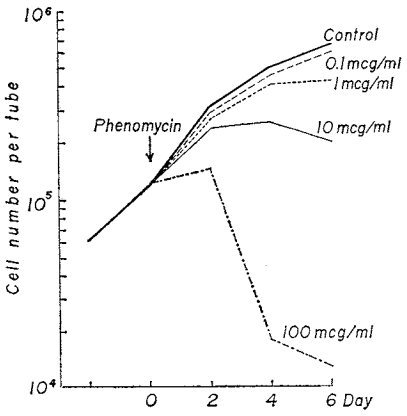
Phenomycin μg/ml	Cell number 10^5	^{14}C-Leucine		^{3}H-TdR		^{3}H-Uridine	
		cpm	%	cpm	%	cpm	%
0	4.36	518		1,980		2,207	
10	4.00	481	7	2,000	0	2,581	0
100	3.44	245	53	2,082	0	2,675	0

cmp : counts per minute per 10^5 cells
% : inhibition %

HeLa S-3 cells were grown on cover slips in EAGLE's minimal essential medium, containing 10% calf serum[7]. The media were replaced by antibiotic-containing media 48 hours after the inoculation, and the cells were incubated for additional 24 hours. Then radioactive precursors were added to the media : ^{14}C-leucine 0.1 μc/ml, ^{3}H-thymidine 0.25 μc/ml and ^{3}H-uridine 0.1 μc/ml. After one hour incubation, the cells on the cover slips were chilled in cold saline, fixed in methanol, immersed in 2% perchloric acid for 40 minutes to remove acid-soluble materials, rinsed in water, and dried. Radioactivity of the cells on each cover slip was determined in a windowless gas flow counter. The cell number was counted by citric acid-crystal violet staining method.

Fig. 1. Effect of phenomycin on the growth of HeLa cells

HeLa S-3 cells were grown in tubes at angle of 5° at 37°C in EAGLE's minimal essential medium containing 10 % calf serum[7]. On 0 day the media were changed for antibiotic-containing media. On 2, 4, and 6 days, 3 tubes of a group were enumerated of the nucleus number by citric acid-crystal violet staining method and the average number of each group was shown in the figure.



introduction of ^{14}C-leucine. The results are presented in Table 1. As described below, phenomycin inhibited protein synthesis in cell-free systems. The inhibition of protein synthesis by phenomycin was not marked, probably because the high molecular weight of the antibiotic permitted only slow penetration into the cells. The results established that phenomycin inhibits protein synthesis without significantly affecting nucleic acid synthesis.

Effects of phenomycin on protein synthesis in the lysate of EHRLICH carcinoma cells :

Phenomycin was observed to inhibit incorporation of ^{14}C-leucine into protein in the cell homogenate of EHRLICH carcinoma. The results are presented in Table 2. At concentrations of 1 and 10 mcg/ml of phenomycin, 63 % and 73 % inhibition was demonstrated by the method employed. Thus, protein synthesis was inhibited markedly in the cell lysate and slightly in intact cells, suggesting a requirement for penetration of the

Table 2. Effects of phenomycin on the ^{14}C-leucine incorporation into protein in the cell lysate of EHRLICH mouse carcinoma.

Series		Leucine incorporation	
		cpm/mg protein	% inhibition
Complete		5,010	
+Phenomycin	10 mcg/ml	1,370	73
	1	1,870	63
	0.1	4,200	16
	0.01	4,800	4
+Blasticidin S	10 mcg/ml	2,950	41
+Puromycin	20	715	86
+Chloramphenicol	32	5,020	0

The complete reaction medium contained (per ml) : 2.5 mg protein of EHRLICH carcinoma cell lysate, 1 μmole of ATP, 5 μmoles of creatine phosphate, 100 mcg of creatine kinase, 0.03 μmoles of GTP, 0.03 μmoles each of 20 amino acids minus leucine, 0.2 μc of ^{14}C-leucine, 10 μmoles of Tris, pH 7.8, 10 μmoles of $MgCl_2$, 100 μmoles of KCl, and 6 μmoles of 2-mercaptoethanol, in a total volume of 0.2 ml. Incubation was carried out for 60 minutes at 37°C.

antibiotic into the cells.

Effects of phenomycin on protein systhesis in the ribosomal system with native mRNA obtained from rat liver:

As presented in Table 3, incorporation of ^{14}C-leucine into protein was significantly inhibited by phenomycin in the ribosomal system of rat liver with endogenous mRNA as a template. At concentrations of 1, 10 and 100 mcg/ml, 57 %, 70 % and 75 % inhibition was observed respectively.

Table 3. Effects of phenomycin on protein synthesis in the ribosomal system with native mRNA obtained from rat liver.

Series	Incorporation of ^{14}C-leucine	
	cpm/mg protein	% inhibition
Complete	1,618	
0 time	12	
−ATP, GTP & generator	95	
−ribosomes	51	
−pH 5 fraction	83	
+Phenomycin 100 mcg/ml	411	75
10	490	70
1	695	57
+Puromycin 100 mcg/ml	356	78

The complete reaction medium contained (per ml): ribosomes 2.4 mg protein, pH 5 fraction 1.0 mg protein, ATP 1 μmole, creatine phosphate 3 μmoles, creatine kinase 30 mcg, GTP 0.03 μmoles, ^{14}C-leucine 0.2 μc, $MgCl_2$ 5 μmoles, KCl 50 μmoles, and Tris 50 μmoles, pH 7.6, in a total volume of 0.2 ml. Incubation was performed for 60 minutes at 37°C.

Table 4. Effects of phenomycin on protein synthesis in cell-free systems from rabbit reticulocytes with native mRNA.

Series		Leucine incorporation	
		Lysates	Ribosomes
Complete		1,060*	4,060
−ribosomes			92
−pH 5 fraction			400
−ATP, GTP & generator		67	11
+Phenomycin	10 mcg/ml	69(94)**	1,430(67)
	1	69(94)	2,800(33)
	0.1	325(69)	3,860 (8)
+Puromycin	100 mcg/ml	48(96)	
	20		438(90)
+Blasticidin S	100	69(95)	
+Chloramphenicol	32	825(22)	3,830 (9)

* cpm/mg protein ** % inhibition

The complete reaction mixtures (per ml); In the experiment with the lysates: 10,000×g supernatant of reticulocyte extracts 1.2 mg protein, ATP 1 μmole, creatine phosphate 5 μmoles, creatine kinase 100 mcg, GTP 0.03 μmoles, 20 kinds of amino acids minus leucine 0.03 μmoles each, ^{14}C-leucine 0.2 μc, KCl 60 μmoles, Tris 10 μmoles, pH 7.8, Mg acetate 5 μmoles, 2-mercaptoethanol 6 μmoles.

In the experiment with the ribosomal system: ribosomes 0.3 mg protein, pH 5 fraction 0.3 mg protein, ATP 1.2 μmoles, creatine phosphate 5 μmoles, creatine kinase 100 mcg, GTP 0.3 μmoles, ^{14}C-leucine 0.1 μc, $MgCl_2$ 4 μmoles, CH_3COONH_4 100 μmoles, 2-mercaptoethanol 6 μmoles, Tris 10 μmoles, pH 7.6. The volume of the reaction medium was 0.2 ml, and incubation was carried out at 37°C for 40 minutes.

Effects of phenomycin on protein synthesis in cell-free systems from rabbit reticulocytes with native mRNA:

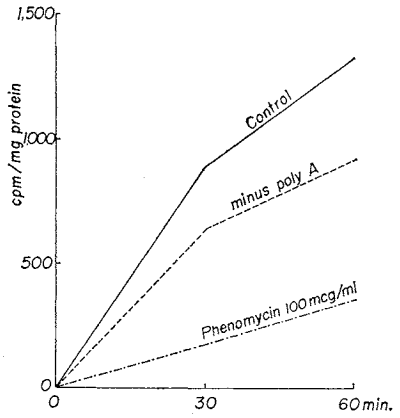
The incorporation of ^{14}C-leucine into protein was markedly inhibited by the presence of phenomycin in lysates and ribosomes of rabbit reticulocytes with endogenous mRNA and soluble cell components. In the lysate system, approximately 90 % inhibition was observed at concentrations of 1 and 10 mcg/ml and 70 % inhibition at 0.1 mcg/ml. In the ribosomal system, approximately 70, 30 and 10 % inhibitions were obtained at 10, 1 and 0.1 mcg/ml, respectively. The results are presented in Table 4. The reaction medium used in the experiment with the lysate was a modification of WEINSTEIN[6] and the medium used with the ribosomes was a modification of COLOMBO *et al.*[7]

Effects of phenomycin on lysine incorporation into polypeptide in a rabbit liver ribosome system with poly A:

Phenomycin was observed to inhibit the incorporation of ^{14}C-lysine into polypeptide in a rabbit ribosome system in the presence of poly A. Approximately 70 % inhibition was demonstrated at a concentration of 100 mcg/ml. By the method employed, the stimulation of lysine incorporation by poly A was not so marked. The results are illustrated in Fig. 2.

Fig. 2. Effects of phenomycin on ^{14}C-lysine incorporation into polypeptide in the rabbit liver ribosome system with poly A.

The complete reaction medium contained (per ml): ribosomes 0.71 mg protein, pH 5 fraction 0.1 mg protein, ATP 2 μmoles, creatine phosphate 2 μmoles, creatine kinase 50 mcg, GTP 0.03 μmoles, 20 kinds of amino acids minus lysine 0.01 μmole each, ^{14}C-lysine 0.4 μc/ml, poly A 100 mcg, KCl 50 μmoles, $MgCl_2$ 5 μmoles, Tris 50 μmoles, pH 7.6, in a total volume of 0.2 ml. The ribosomes, pH 5 fraction, energy sources and amino acids, were first incubated at 37°C for 20 minutes. Then the antibiotic, ^{14}C-lysine and poly A were added, and further incubated for 30, and 60 minutes.



Effects of phenomycin on polyribonucleotide-dependent polypeptide synthesis in the ribosomal system obtained from rabbit reticulocytes:

The incorporation of ^{14}C-phenylalanine into polypeptide with poly U, of ^{14}C-lysine with poly A and of ^{14}C-proline with poly C was performed in the ribosomal system obtained from rabbit reticulocytes. Phenomycin was observed to inhibit lysine incorporation with poly A and proline incorporation with poly C,

Table 5. Effects of phenomycin on polyribonucleotide-dependent polypeptide synthesis in the ribosomal system obtained from rabbit reticulocytes.

Series	Phenylalanine incorporation with poly U	Lysine incorporation with poly A	Proline incorporation with poly C
Complete	166,000*	7,640	8,450
— ribosome	375	401	226
— pH 5 fraction		1,800	762
— ATP, GTP & generator	158	272	247
— polynucleotide	3,550	6,920	7,500
+ Phenomycin 100 mcg/ml	144,000 (18)**	658 (91)	4,800 (42)
10	164,000 (2)	629 (91)	5,350 (37)
1	168,000 (0)	1,460 (81)	7,220 (5)
0.1		5,700 (26)	8,560 (0)
+ Puromycin 100 mcg/ml		3,810 (50)	2,770 (67)
+ Blasticidin S 100	54,700 (67)	3,720 (51)	452 (95)
+ Chloramphenicol 32 mcg/ml	160,000 (4)	7,610 (1)	8,340 (1)

* cpm/mg protein ** % inhibition

The incorporation was performed in the reaction medium (per ml) : ribosomes 1.65 mg protein, pH 5 fraction 1.65 mg protein, ATP 1 μmole, creatine phosphate 5 μmoles, creatine kinase 100 μg, GTP 0.03 μmoles, 20 kinds of amino acids minus phenylalanine, lysine or proline 0.03 μmoles each, ^{14}C-phenylalanine, ^{14}C-proline or ^{14}C-lysine 0.2 μc, poly U 200 mcg, poly A 100 mcg or poly C 100 mcg, KCl 60 μmoles, Mg acetate 5 μmoles, 2-mercaptoethanol 6 μmoles, and Tris 10 μmoles, pH 7.8, in a volume of 0.3 ml.

The ribosomes, pH 5 fraction, and energy source were first incubated at 37°C for 20 minutes; and then antibiotic, ^{14}C-amino acid and polyribonucleotide were added and further incubated for 40 minutes.

in which the stimulation by polyribonucleotide was not significant and the incorporation seemed to be due to native mRNA. The poly U-dependent polyphenylalanine synthesis was slightly affected by the presence of phenomycin. The results are summarized in Table 5. In the lysine incorporation, approximately 90 % inhibition was observed at concentrations of 100 and 10 mcg/ml and 80 % inhibition at 1 mcg/ml. In the proline incorporation, approximately 40 % inhibition was demonstrated at 10 and 100 mcg/ml. The incorporation of lysine and proline seemed to be dependent upon the presence of native mRNA. In the poly U-dependent polyphenylalanine synthesis, approximately 20 % inhibition was observed at 100 mcg/ml.

As described above, phenomycin was observed to inhibit polypeptide synthesis directed by endogenous mRNA and added synthetic polyribonucleotides in cell-free systems obtained from various mammalian cells. For the purpose of establishing the site of action of phenomycin in the protein-synthesizing system, the following experiments were performed.

Table 6. Effects of phenomycin on labeling of sRNA with ^{14}C-amino acid by the cell extracts of rabbit reticulocytes.

Series		^{14}C-Leucine	^{14}C-Lysine
Control		3,830*	92,700
+ Phenomycin	100 mcg/ml	3,860	84,700
	10	4,180	102,000
	1	4,000	
+ Puromycin	20 mcg/ml	4,200	

* cpm/mg protein

The reaction mixture contained (per ml) : pH 5 fraction 1~1.56 mg, ATP 5 μmoles, ^{14}C-lysine or ^{14}C-leucine 0.2 μc, KCl 60 μmoles, Mg acetate 5 μmoles, 2-mercaptoethanol 6 μmoles, and Tris 10 μmoles, pH 7.8 in a volume of 0.3 ml. It was incubated at 37°C for 10 minutes. The RNA fraction was extracted and RNA content estimated by UV absorption at 260 mμ. Radioactivity was determined in a windowless gas flow counter, after removing TCA with ether.

Effects of phenomycin on labeling of sRNA with amino acids by the cell extracts of rabbit reticulocytes :

Aminoacylation of sRNA in the pH 5 fraction of rabbit reticulocytes, using ^{14}C-leucine or lysine, was not significantly affected by the presence of phenomycin even at a high concentration of 100 mcg/ml. The results are presented in Table 6.

Effects of phenomycin on the transfer of ^{14}C-lysine from lysyl-sRNA to protein on ribosomes with native mRNA obtained from rabbit reticulocytes :

The transfer of ^{14}C-lysine from lysyl-sRNA to protein on ribosomes with endogenous mRNA was significantly inhibited by phenomycin. In the experiments using *E. coli* sRNA as a source of lysyl-sRNA, approximately 80 % inhibition was observed at a concentration of 1 mcg/ml and 60 % inhibition at 0.1 mcg/ml. Greater inhibition was obtained at 10 and 100 mcg/ml.

The results are presented in Table 7. In the experiments using rabbit reticulocyte sRNA as a source of lysyl-sRNA, a similar but lesser degree of inhibition was observed, and approximately 50 % inhibition was demonstrated at a concentration of 1 mcg/ml of phenomycin. In both cases, the inhibition was sufficiently strong to consider that the transfer of amino acid from aminoacyl-sRNA to protein may be the site of action of phenomycin.

Effects of phenomycin on the non-enzymatic binding of ^{14}C-lysyl-sRNA to the ribosomes :

The non-enzymatic binding of ^{14}C-lysyl-sRNA to ribosomes with native mRNA

Table 7. Effects of phenomycin on the transfer of ^{14}C-lysine from lysyl-sRNA to protein on the ribosomes with native mRNA obtained from rabbit reticulocytes.

Series	Lysine incorp. cpm/mg protein	Inhibition %
Complete	9,550	
— ribosome	24	
— pH 5 fraction	67	
+ Phenomycin 100 mcg/ml	203	98
10	433	96
1	2,360	79
0.1	3,880	60
+ Puromycin 100	572	94
10	1,630	83

The complete reaction mixture contained (per ml) : ribosomes 0.3 mg protein, pH 5 fraction 0.3 mg protein, ATP 1 μmole, creatine phosphate 5 μmoles, creatine kinase 100 mcg, GTP 0.03 μmoles, ^{14}C-lysyl-sRNA of *E. coli* 0.15 mg (3,300 cpm), 20 kinds of amino acids minus lysine 0.03 μmoles each, Mg acetate 5 μmoles, KCl 60 μmoles, 2-mercaptoethanol 6 μmoles, and Tris 10 μmoles, pH 7.8 in a volume of 0.3 ml. Incubation was performed at 37°C for 40 minutes.

Table 8. Effects of phenomycin on the transfer of ^{14}C-lysine from lysyl-sRNA to polypeptide on the ribosomes, obtained from rabbit reticulocytes.

Series	Incorporation of ^{14}C-lysine	
	cpm/mg protein	% inhibition
Complete	1,130	
— ribosomes	13	
— pH 5 fraction	290	
— ATP, GTP & generator	190	
+ phenomycin 10 mcg/ml	410	64
+ 〃 1	530	53
+ 〃 0.1	925	18
+ 〃 0.01	1,030	8
+ puromycin 20 mcg/ml	135	84

The reaction mixture contained (per ml) : ribosomes 0.67 mg protein, pH 5 fraction 0.48 mg protein, ATP 1 μmole, creatine phosphate 5 μmoles, creatine kinase 50 mcg, GTP 0.03 μmoles, ^{14}C-lysyl-sRNA of rabbit reticulocytes 0.375 mg (4,200 cpm), Mg acetate 5 μmoles, KCl 60 μmoles, 2-mercaptoethanol 6 μmoles, and Tris 10 μmoles, pH 7.8, in a volume of 0.3 ml. Incubation was carried out at 37°C for 45 minutes.

was studied with rabbit reticulocyte system, because the site of action of phenomycin was located in the process of transfer of amino acid from aminoacyl-sRNA to protein on the ribosomes. Phenomycin was observed to inhibit the binding of ^{14}C-lysyl-sRNA to ribosomes with native messengers or poly A by the Millipore filter method. At a concentration of 100 mcg/ml, 28 % or 21 % inhibition was demonstrated. The results are presented in Table 9.

Table 9. Effects of phenomycin on the non-enzymatic binding of ^{14}C-lysyl-sRNA to the ribosomes with native mRNA or poly A, obtained from rabbit reticulocytes.

Series	Template	Binding of ^{14}C-lysyl-sRNA	
		cpm/ml	% inhibition
Control	Native mRNA	870	
—ribosomes		0	
+phenomycin 100 mcg/ml		628	28
Control	poly A	970	
—ribosomes		0	
—poly A		566	
+phenomycin 100 mcg/ml		766	21

The reaction mixture contained (per ml) : ribosomes 1.6 mg protein, ^{14}C-lysyl-sRNA 1.1 mg (13,000 cpm), KCl 60 μmoles, Mg acetate 5 μmoles, 2-mercaptoethanol 6 μmoles, and Tris 10 μmoles, pH 7.8, in a volume of 0.5 ml. The incubation was performed at 0°C for 20 minutes. In the poly A system, the ribosomes were preincubated, and ^{14}C-lysyl-sRNA, 0.14 mg (12,000 cpm), was used.

The reaction mixture was filtered through a Milipore filter (HA 0.45 μ, Milipore Filter Corp., 25 mm). It was washed with a buffer consisting of Tris 0.01 M, pH 7.8, KCl 0.06 M, Mg acetate 0.005 M, and 2-mercaptoethanol 0.006 M, and dried. The radioactivity was determined in a windowless counter.

The inhibition of non-enzymatic binding of aminoacyl-sRNA to the ribosomes was significantly less than that of protein synthesis. Nevertheless, the former may be the cause of the latter, because the sequential binding of aminoacyl-sRNA to ribosomes is needed for protein synthesis, and a slight inhibition of the

binding of aminoacyl-sRNA may result in a marked inhibition of protein synthesis.

Effects of phenomycin on protein synthesis in the ribosomal system of *E. coli*:

The effects of phenomycin on the incorporation of ^{14}C-leucine, ^{14}C-lysine, ^{14}C-phenylalanine, or ^{14}C-proline into protein was investigated in a cell-free system of *E. coli* B with endogenous mRNA. Phenomycin exhibited a slight inhibition of ^{14}C-amino acid incorporation, in contrast to the marked inhibition in mammalian cell-free systems described above. The protein synthesizing system used was sensitive to chloramphenicol. The results are presented in Table 10.

Table 10. Effects of phenomycin on protein synthesis on ribosomes with native mRNA obtained from *E. coli* B.

Series		Incorporation of amino acid			
		^{14}C-Leu	^{14}C-Lys	^{14}C-Phe	^{14}C-Pro
Complete		4,920*	4,300	760	1,000
+ Phenomycin	100 mcg/ml	3,240 (34)**	2,570 (40)	560 (26)	900 (10)
	50		4,020 (6)	670 (12)	890 (11)
	10	4,370 (11)	4,180 (3)		
	1	4,570 (7)	4,120 (4)		
+Chloramphenicol	10 mcg/ml	1,920 (61)	850 (80)	160 (79)	330 (67)

* cpm/mg protein ** inhibition %

The complete reaction mixture contained (per ml): ribosomes 2.5~4 mg protein, 105,000×g supernatant 1.25~1.5 mg protein, ATP 2 μmoles, creatine phosphate 2 μmoles, creatine kinase 50 mcg, ^{14}C-amino acid 0.2 μc, KCl 100 μmoles, $MgCl_2$ 10 μmoles, 2-mercaptoethanol 6 μmoles, and Tris 50 μmoles, pH 7.8. Incubation was performed at 37°C for 20 minutes.

Discussion

Phenomycin was observed to inhibit protein synthesis, but not nucleic acid synthesis in growing HeLa cells. However, the grade of inhibition of protein synthesis was not very great. It seemed to be due to slow penetration of the antibiotic into the cells, because protein synthesis was markedly inhibited in cell-free systems. The high molecular weight of phenomycin may be a cause of the slow penetration.

In mammalian systems, the site of action of the antibiotic is located in the amino acid transfer from aminoacyl-sRNA to protein. The non-enzymatic binding of aminoacyl-sRNA to ribosomes was inhibited by the presence of phenomycin, and seemed to the primary site of action of antibiotic. However, the binding of aminoacyl-sRNA to ribosomes was inhibited less than protein synthesis. The discrepancy is not yet elucidated. However, it may be due to the fact that sequential binding of aminoacyl-sRNA to ribosomes is needed for protein synthesis, and a slight inhibition of the former may result in a marked inhibition of the latter.

Phenomycin exhibits significant antitumor activity but no antibacterial activity. The protein synthesizing systems of mammalian cells were sensitive to the antibiotic, but those of bacteria were resistant. It was parallel to the biological activity of phenomycin. The selective toxicity of phenomycin in protein-synthesizing systems was similar to those of enomycin[12,13] and diphtheria toxin[14].

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