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David Vázquez

Inhibitors of
Protein Biosynthesis



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List of Abbreviations

cAMP	Cyclic adenosin-monophosphate
RNA	Ribonucleic acid
mRNA	Messenger ribonucleic acid
MDMP	2-(4-methyl-2,6-dinitroanilino)-N-methyl-propionamide
-2'(3'),5'-ADP	2'(3'),5'-Adenosintriphosphate
PAP	<u>Phytolacca americana</u> protein
TPCK	1-Chloro-4-phenyl-3-tosylamido-2-butanone
PA toxin	<u>Pseudomonas aeruginosa</u> toxin
f-Met-tRNA _F or fMet-tRNA _F	Initiator formyl-methionyl-tRNA of prokaryotes
Met-tRNA _F	Initiator methionyl-tRNA of eukaryotes
GTP	Guanosintriphosphate
IF-1	Bacterial initiation factor 1
IF-2	Bacterial initiation factor 2
IF-3	Bacterial initiation factor 3
eIF-1	Eukaryotic initiation factor 1
eIF-2	Eukaryotic initiation factor 2
eIF-3	Eukaryotic initiation factor 3
eIF-4A	Eukaryotic initiation factor 4A
eIF-4B	Eukaryotic initiation factor 4B
eIF-4C	Eukaryotic initiation factor 4C
eIF-5	Eukaryotic initiation factor 5
m ⁷ G(5')ppp or m ⁷ G ^{5'} ppp	"cap" or 7-methylguanosine-5'-triphosphate
SV40	Simian virus 40
m ⁷ G ^{5'} p	7-Methylguanosine-5'-monophosphate
tRNA	Transfer ribonucleic acid
rRNA	Ribosomal ribonucleic acid
EF-2	Elongation factor 2

ATP	Adenosin-triphosphate
Ac-Phe	Acetyl-phenylalanine
EF-G	Elongation factor G
GTPase	GTPase
EF-Ts	Elongation factor Ts
Poly(U)	Polyuridylic acid
DNA	Deoxyribonucleic acid
m ⁷ G ^{5'} pp	7-Methylguanosine-5'-diphosphate
AMP	Adenosin-monophosphate
GMPPCP or GDPCP	Guanylyl methylenediphosphonate
GDPNP	Guanylyl imidodiphosphate
EF-T	Elongation factor T
RF-1	Release factor 1
RF-2	Release factor 2
RF-3	Release factor 3
m ⁷ G	7-Methylguanosine
5' ^{pm} ⁷ G	5'-Phosphate-7-methylguanosine
Sm ^D	Streptomycin dependent
EF-Tu	Elongation factor Tu
EF-1	Elongation factor 1
<u>C. diphtheriae</u>	<u>Corynebacterium diphtheriae</u>
NAD	Nicotinamide-adenine dinucleotide
PRT	<u>Penicillium roqueforti</u> toxin

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Chapter 1

Protein Synthesis and Translation Inhibitors

1. Introduction

Studies concerning selectivity, site, and mode of action of translation inhibitors have been widely developed and the subject has been reviewed repeatedly in the last fifteen years (Gale, 1963; Newton, 1965; Newton and Reynolds, 1966; Gottlieb and Shaw, 1967a; Vázquez and Monro, 1967; Weisblum and Davies, 1968; Pestka, 1971; Muñoz, García-Ferrandiz and Vázquez, 1972; Gale et al., 1972; Kaji, 1973; Vázquez, 1974; Corcoran and Hahn, 1975; Pestka, 1977; Vázquez, 1978a). This study will be concerned mainly with the present state of the problem. A complete survey of the literature will not be possible in this contribution and the above reviews should be very useful to readers interested in different aspects of the problem. Furthermore, there are a number of reviews concerning the chemistry, biosynthesis, toxicology, inhibitory spectra of the different inhibitors (Korzybski, Kowszyk-Gindifer and Kurylowicz, 1967a, 1967b; Gottlieb and Shaw, 1967a, 1967b; Glasby, 1976) and mechanism of drug resistance (Benveniste and Davies, 1973a; Mitsuhashi, Rosival and Krčmery, 1975; Mitsuhashi, 1977; Mitsuhashi and Hashimoto, 1977) which should be very useful to readers who are concerned with these topics.

Specific inhibitory effects on bacterial protein synthesis by chloramphenicol and chlortetracycline, at their

minimal growth inhibitory concentrations, were first described by Gale and Paine (1950a, 1950b). At those concentrations the antibiotics did not affect respiration, fermentation, or amino acid accumulation (Gale and Paine, 1950b), but caused an immediate cessation of protein synthesis and an increase in the rate of nucleic acid accumulation in bacteria (Gale and Folkes, 1953). Similar effects were later observed in bacteria treated with a number of translation inhibitors (Gale, 1963; Newton, 1965; Newton and Reynolds, 1966; Gottlieb and Shaw, 1967a; reviews). The mechanism of protein synthesis remained obscure during the period 1950-1960, so that it was not possible to establish the site of action of the known translation inhibitors. A resolved cell-free system to study ribosomal amino acid incorporation directed by a synthetic polynucleotide such as mRNA was first described in 1961, and the specific inhibitory effect of chloramphenicol was confirmed in this system (Nirenberg and Matthaei, 1961). Model systems to study the individual reactions in protein synthesis were further developed in the following years and thus the specific steps blocked by different inhibitors of translation were elucidated.

Reports concerning inhibition of translation in higher cells did not appear until 1958, when the inhibitory effect of cycloheximide on protein synthesis in Saccharomyces carlsbergensis was described. Contrary to what was found in chloramphenicol-treated bacteria, the stringent control of nucleic acid synthesis in yeast was not abolished by cycloheximide and an inhibition of nucleic acid synthesis was also observed in the presence of the antibiotic (Kerridge, 1958). Similar results were later observed in higher cells treated with other translation

inhibitors (Newton and Reynolds, 1966; Gottlieb and Shaw, 1967a; reviews). Cell-free systems and model reactions to study protein synthesis have been developed in the last twenty years and the reactions blocked by the different inhibitors were elucidated.

For the sake of clarity we have adopted for the protein factors involved in translation the nomenclature developed at the International Symposia on Protein Synthesis held at the National Institutes of Health (Bethesda, USA) in 1971 and 1976 (Anderson et al., 1977). The nomenclature for the proteins of the bacterial and mammalian ribosomes is adopted from early studies on the subject (Kaltshmidt and Wittmann, 1970; Sherton and Wool, 1972).

2. Site of Action of Protein Synthesis Inhibitors

The process of protein synthesis can be arbitrarily divided into (a) steps taking place in early reactions in protein synthesis and (b) steps in the translation mechanisms taking place at the ribosome level. Following these criteria protein synthesis inhibitors of group (a) can be classified as indicated in Table 1. However, considering the specificity, selectivity, and permeability of the protein synthesis inhibitors, the most important compounds are undoubtedly those included within the ample group of translation inhibitors (see Tables 2, 3, and 4). Therefore we will now refer specifically to this group. The overall reactions inhibited by these compounds are indicated in Figures 1 and 2. However a number of inhibitors have pleiotropic effects on the ribosome and inhibit more than one reaction; in these cases we usually indicate the principal step(s) blocked by the inhibitors. A number of inhibitors are not presented in

Table 1. Inhibitors of protein synthesis acting on steps taking place prior to translation

Inhibitors of aminoacyl-tRNA formation		
Inhibitors of amino acid activation ^a	Inhibitors of amino acid transfer to RNA ^a	Inhibitors which are transferred to tRNA leading to synthesis of abnormal proteins ^a
7-Azatrptophan	Aminoalkyl-adenylates	Ethionine
Tryptazan	Borrelidin ^b	Norleucine
6-Fluorotryptophan	Furanomycin ^b	Alloisoleucine
5-Fluorotryptophan	Minosine ^b	Azetidine-carboxylic acid
Norvaline	4-Oxalysine	Canavanine
α -Amino- β -chlorobutyrate	2,6-Diamino-4-hexynoic acid	N-Ethylglycine
α -Aminobutyrate	Trans-4-dehydrolysine	O-Methyl threonine
Selenomethionine		2-Fluoro-L-histidine ^c
Ethionine		
Norleucine		
Methyl-ester of serine		
Ethyl-ester of serine		
Aminoalkyl-adenylates		
Tiramine		
L-Tyrosinol		
L-Tyrosine amide		
L-Tyrosine methyl ester		

^a Amino acid analogs specifically replace or compete with their corresponding amino acids.

^b Borrelidin, furanomycin, and minosine specifically inhibit threonyl-, isoleucyl-, and phenylalanyl-tRNA synthesis respectively.

^c Klein et al. (1977). Other data are taken from Vázquez (1974; review) and references therein.

Table 1 (continued)

Inhibitors of f-Met-tRNA _F formation		
Inhibitors of N ¹⁰ -formyl-H ₄ folate synthesis	Inhibitors depleting the pool of N ¹⁰ -formyl-H ₄ folate	Analogs of N ¹⁰ -formyl-H ₄ folate
Aminopterin	Hydroxylamine	Pyrimidine analogs
Amethopterin		Pteridine analogs:
(synonym		tetrahydropteroate
methotrexate)		N ⁵ -formyl-H ₄ folate
Pteroylaspartic acid		N ⁵ -methyl-H ₄ folate
Trimethoprim		tetrahydrohomofolate
6-Chloro-8-aza-9- cyclopentylpurine		tetrahydrohomopteroate

Table 2. Inhibitors of translation acting on prokaryotic systems

Althiomycin	Micrococcin
Avilamycin	Negamycin
Berninamycin	Rubradirin
Bottromycin A ₂	Spectinomycin
Chloramphenicol group:	Streptogramin A group:
Chloramphenicol	Ostreogrycin G
D-AMP-3	Streptogramin A
D-Thiomycetin	Streptogramin B group:
D-Win-5094	Staphylomycin S
Cloacin DF13	Streptogramin B
Colicin E3	Viridogrisein
Griseoviridin	Streptomycin group:
Kasugamycin	Amikamycin
Lincomycin group:	Gentamicin
Celesticetin	Kanamycin
Clindamycin	Neomycin
Lincomycin	Paromomycin
Macrolide antibiotics:	Sisomicin
Carbomycin group:	Streptomycin
Carbomycins	Tobramycin
Josamycin	Streptothricins
Leucomycins	Thermorubin
Niddamycins	Thiostrepton group:
Erythromycin group:	Siomycin
Erythromycins	Sporangiomycin
Neospiramycins	Thiopeptin
Oleandomycin	Thiostrepton
Lancamycin group:	Viomycin group:
Chalcomycin	Capreomycin
Kujimycin A	Viomycin
Lancamycin	
Methymycin group:	
Forocidins	
Methymycin	
Narbomycin	
Neomethymycin	
Picromycin	

Table 2 (continued)

Spiramycin group:

Angolamycin

Relomycin

Spiramycins

Tylosin

Table 3. Inhibitors of translation acting on eukaryotic systems

Abrin

Alpha sarcin

Anisomycin

5-Azacytidine

Bruceantin

Crotins

Curcins

Diphtheria toxin

Emetine group:

Emetine

Tubulosine

Enomycin

Glutarimide group:

Actiphenol

Cycloheximide

Streptimidone

Streptovitacin A

Harringtonine group:

Harringtonine

Homoharringtonine

Isoharringtonine

Lycorine group:

Lycorine

Pseudolycorine

MDMP

Narciclasine group:

Haemanthamine

Narciclasine

Pretazettine

PAP

Pederine

Phenomycin

Ricin

Sodium fluoride

Tenuazonic acid

Trichotecene antibiotics:

Trichodermin group:

Fusarenon-X

Trichodermin

Trichodermol

Trichothecin

Verrucarin A group:

Deacetoxyscirpenol

Nivalenol

Toxin T-2

Verrucarin A

Tylophora alkaloids:

Cryptopleurine

Tylocrebrine

Tylophorine

Table 4. Inhibitors of translation acting on prokaryotic and eukaryotic systems

Actinobolin	Hygromycin B
Adrenochrome	Nucleocidin
AHR-1911	Pactamycin
Amicetin group:	Polydextran sulphate
Amicetin	Polyvinyl sulphate
Bamicetin	Puromycin
Plicamicetin	Pyrochatechol violet
Anthemycin	Showdomycin
Aurintricarboxylic acid	Sparsomycin
Blasticidin S	Tetracycline group:
Chartreusin	Chlortetracycline
Edeine A ₁	Doxycycline
Fusidic acid	Oxytetracycline
Gougerotin	Tetracycline
Guanylyl-methylene-diphosphate	Tosylphenylalanylchloromethane
Guanylyl-imido-diphosphate	

Fig. 1. Translation process in bacteria. Site of action of translation inhibitors. ►

* Do not interact with polysomes. Therefore bind to free ribosome subunits and prevent only the first few rounds of peptide bond formation. ** Is an inhibitor of aminoacyl-tRNA binding in intact cells or in integrated systems in which elongation is proceeding in the presence of EF-Tu and EF-G. Does not inhibit aminoacyl-tRNA binding in resolved systems in the absence of EF-G. Can inhibit translocation in cell-free systems. *** Can also inhibit translocation in cell-free systems. **** Does not inhibit peptide bond formation in resolved assays. However, it blocks this step in intact cells and integrated systems by preventing the release of EF-Tu-GDP bound to the ribosome. ***** Do not interact with polysomes. Do not inhibit translocation in many model systems. Bind to free ribosome subunits and prevent elongation of the nascent polypeptide chain when it reaches a certain size, before polysome formation

