

Inhibition of protein synthesis by streptogramins and related antibiotics

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The streptogramins and related antibiotics (the lincosamides and macrolides) (MLS) are important inhibitors of bacterial protein synthesis. The key reaction in this process is the formation of a peptide bond between the growing peptide chain (peptidyl-tRNA) linked to the P-site of the 50S ribosome and aminoacyl-tRNA linked to the A site. This reaction is catalysed by the peptidyl transferase catalytic centre of the 50S ribosome. Type A and B streptogramins in particular have been shown to block this reaction through the inhibition of substrate attachment to the A and P sites and inhibition of peptide chain elongation. Synergy between type A and B components results from conformational changes imposed upon the peptidyl transferase centre by type A compounds and by inhibition of both early and late stages of protein synthesis. The conformational change increases ribosomal affinity for type B streptogramins. Microbial resistance to the MLS_B antibiotics is largely attributable to mutations of rRNA bases, producing conformational changes in the peptidyl transferase centre. This can result in resistance to a single inhibitor or to a group of antibiotics (MLS_B). The activity of type A streptogramin is retained thus explaining the improved inhibitory action of the combined streptogramins against macrolide and lincosamide-resistant strains. However, the development of resistance to the streptogramins may be less of a problem because of the synergic effect of type A and B compounds which has also been demonstrated in strains resistant to MLS_B i.e., high level resistance to the combined streptogramins is only likely when type A streptogramin resistance determinants are present along with type B streptogramin resistance determinants.

Introduction

The pathway of bacterial protein synthesis is directed by ribosomes in conjunction with cytoplasmic factors which transiently bind to particles during the initiation phase (initiation factors IF1, IF2, IF3), elongation phase (elongation factor EF-Tu, EF-Ts and EF-G) and termination (RF1, RF2, RF3) phases. Bacterial ribosomes are 70S particles comprising two subunits, of 50S and 30S, which join at the initiation step of protein synthesis and separate at the termination step. Each subunit comprises RNA (one 5S and one 23S rRNA in 50S subunits and a 16S species in the 30S subunit) and ribosomal proteins.

Elongation involves the positioning of two RNA derivatives at two sites on the 50S ribosomal subunit: an aminoacyl-tRNA molecule (AA-tRNA) positioned at the acceptor A site, and the peptidyl-tRNA (pep-tRNA) precursor linked to the donor P site (Figure). The positioning

of AA-tRNA is promoted by the elongation factor EF-Tu. A peptide bond is then formed between the carboxyl terminus of the peptide chain at the P site and the NH₂ group of the amino terminus of AA-tRNA at the A site. This is catalysed by the peptidyl transferase catalytic centre (PTC) of the 50S ribosome. The third step in the elongation process, which is dependent upon the elongation factor EF-G and upon energy derived from GTP hydrolysis, involves the translocation of pep-tRNA (carrying a peptide chain one unit longer) from the A site to the P site.

Antibiotics may block different steps in bacterial protein synthesis by interfering with the function of either the cytoplasmic factors or the ribosomes. Inhibitors that bind to the 30S ribosomal subunit interfere primarily with initiation, although some may interfere with pairing of the mRNA codon with the AA-tRNA anticodon, so impairing elongation. Inhibitors that bind to the 50S ribosomal

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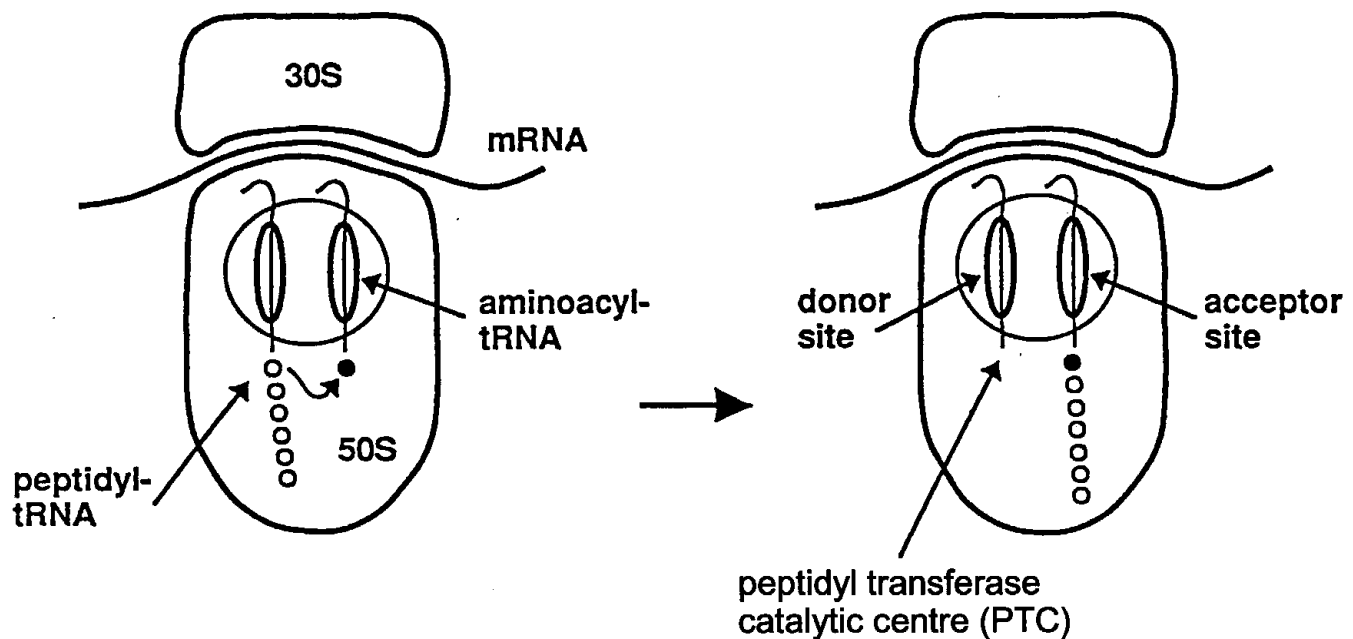


Figure. Peptide bond synthesis catalysed by the peptidyl transferase centre of the 50S ribosome subunit.

subunit or to the elongation factors, which are transiently linked to ribosomes at certain steps of the cycle,¹ interfere with steps involved in the elongation process. This review is essentially focused on the MLS group of antibiotics, which inhibit PTC function.

Biological effects of streptogramins

Streptogramins are produced by the genus *Streptomyces* and are of two types, A and B. Although otherwise chemically unrelated, both A and B streptogramins are macrocyclic lactone rings. However, type B compounds are cyclic hexadepsipeptides and type A compounds are highly modified cyclopeptides with multiple conjugated double bonds.^{2,3}

Streptogramins A and B synergically inhibit bacterial cell growth;^{2,4-7} individually, A and B components are bacteriostatic, whereas in combination they are bactericidal. Consequently, protein synthesis is halted temporarily by type A or B compounds, and permanently by the combination of A and B.^{3,8} Multiplication of most Gram-positive bacteria is rapidly stopped on incubation with single streptogramin components. Proliferation is resumed, however, when cells are transferred to antibiotic-free media, although resumption in the growth of cells treated with type A compounds occurs only after a prolonged lag (bacteriopause). When bacteria are incubated with a mixture of components A and B, the viable count decreases by several logs within a single generation time.⁹

Most Gram-negative bacteria are resistant to strep-

togramins, owing to the impermeability of the bacterial cell wall. However, permeability mutants of Gram-negative bacteria may be susceptible to streptogramins and related antibiotics. It is noteworthy that the synergic effect induced by type A compounds (increased growth inhibition and decreased viability) still occurs in mutants resistant to type B streptogramins.^{9,10}

Mechanism of action of type A streptogramins

In the presence of type A streptogramins such as virginiamycin M, ribosomal initiation complexes are assembled in an apparently normal fashion but are functionally inactive, suggesting an inhibition of the elongation phase.¹¹ Moreover, type B streptogramins have been shown to inhibit the first two of the three elongation steps (AA-tRNA binding to the A site and peptidyl transfer from site P), and do not affect the third step, namely translocation from the A site to the P site.¹¹⁻¹³

Studies have shown that type A streptogramins block substrate attachment to both the acceptor site and the donor site of the PTC.¹⁴ This binding has been shown to be tight but reversible, and to increase ribosomal affinity for the type A streptogramin, virginiamycin S, which persists after virginiamycin M removal.¹⁵

The question of inhibition of both PTC sites by type A streptogramins, when it is known that each virginiamycin M molecule has only one binding site, has been addressed by a new model, whereby the acceptor and donor substrate binding sites of the PTC exchange position, conformation and function at each elongation step.^{16,17}

Interestingly, type A streptogramins can bind to both

50S subunits and free 70S ribosomal particles, but not to ribosomes engaged in protein synthesis or to polysomes. In fact, translating particles carrying peptidyl-tRNA or AA-tRNA are protected against these antibiotics.¹⁷ In agreement with the proposed model of interchangeable peptidyl transferase sites, particles are protected by AA-tRNA bound to site A as well as by pep-tRNA bound to site P, or by AA-tRNA derivatives translocated from the A site to the P site. These observations imply that type A streptogramins can bind only to the free arms of peptidyl transferase, and thus to particles at the termination-initiation phase (run-off ribosomes).

Mechanism of action of type B streptogramins

Type B streptogramins have been shown to block peptide bond synthesis *in vitro*, preventing the extension of polypeptide chains and inducing the detachment of incomplete protein chains.^{18,19}

Interestingly, unlike type A streptogramins, which interact exclusively with naked particles, type B compounds also interact with ribosomes engaged in protein synthesis and with polysomes.⁶ Such a dichotomous effect of the type A and type B streptogramins can be explained by the fact that the former bind only to the free arms of peptidyltransferase (which are thus protected by bound AA-tRNA derivatives), whereas the latter interact with a portion of the PTC domain distinct from both the catalytic and the substrate binding sites of the enzyme.¹⁷

Molecular basis of synergy between type A and B streptogramins

The synergic inhibitory activity of type A and B streptogramins is attributable to conformational changes in the 50S ribosomal subunit induced by the attachment of type A compounds.²⁰⁻²² In addition, type A and B streptogramins inhibit early and late stages of the protein synthesis cycle, respectively.

Antagonism has been demonstrated between erythromycin and type B streptogramins for binding to the 50S subunit.^{23,24} The two antibiotics have similar mechanisms of action,¹⁸ with overlapping binding sites making their ribosome fixation mutually exclusive. As ribosomal affinity is greatest for erythromycin, this macrolide is capable of displacing type B streptogramins from the ribosome complex. However, in the presence of type A streptogramins such a displacement does not occur, because of conformational changes in the 50S subunit.²³

A breakthrough in the analysis of the synergic effect of type A and B streptogramins was the observation that in a cell-free system from the *Escherichia coli* strain EM4/pERY (resistant to type B streptogramins only), ribosomal affinity for the type B compounds, in the presence of type A components, reached the highest registered value.¹⁰

Microbial resistance to MLS antibiotics

Culture of microorganisms in the presence of inhibitors favours development of antibiotic-resistant phenotypes and sub-inhibitory concentrations of antibiotics are particularly effective in inducing the expression of certain resistant phenotypes such as MLS_B^R, and mutant selection. Such altered phenotypes are due either to mutations of chromosomal genes or to the acquisition of new genes carried by plasmids or transposons.

Three major mechanisms of resistance to antibiotics have been identified: (i) impaired permeability (production of altered permeases or presence of efflux pumps), (ii) antibiotic inactivation by intracellular or extracellular enzymes, and (iii) changes in the target structure (receptor alteration). In the case of MLS antibiotics, the latter type of resistance can be produced by mutations of the genes coding for the two ribosomal components, rRNA and ribosomal proteins.

Impaired permeability

The presence of a plasmid-mediated gene, *vga*, encoding for a putative ATP-binding protein has been associated with an active efflux of streptogramin A group compounds.²⁵ This gene has been found chiefly in coagulase-negative staphylococci.

Antibiotic inactivation

Lactonases capable of cleaving the macrocyclic lactone ring structure of type B streptogramins have been identified in the genera *Actinoplanes*,²⁶ and *Streptomyces*²⁷ and in staphylococci (*vgb* gene).²⁸ The absence of such lactonases active against type A compounds suggests the existence of an alternative catabolic pathway for the degradation of such compounds in streptogramin-producing microorganisms. Recently, two staphylococcal related determinants, *vat* and *vat_B*, encoding an acetyltransferase which inactivates type A streptogramins have been characterized.^{29,30} However, the absence of any of the genes in some streptogramin A-resistant strains suggests the presence of one or more unknown genetic resistance determinants.³¹

Target modification

Target modification appears to be a more common mechanism of resistance to MLS antibiotics than antibiotic inactivation. In principle, alterations of the MLS binding site may concern either ribosomal proteins or rRNA. However, recent investigations suggest that the involvement of rRNA is more probable.

Two types of resistance towards type B streptogramins and related antibiotics (MLS_B) have been described: the dissociated type, whereby resistance is restricted to a

Table. Base changes in 23S rRNA conferring resistance to different antibiotics

Microorganisms ^a	Mutations ^b			Phenotype ^{c,d}
	2057	2058	2611	
<i>Saccharomyces cerevisiae</i>				
Wild type	G	A	C	sensitive
Mutant	G	A	U	Spm ^R
Mutant	G	A	G	Spm ^R , Ery ^R
Mutant	G	G	C	Ery ^R
<i>E. coli</i>				
Wild type	G	A	C	sensitive
pE194	G	m ₂ A	C	MLS _B ^R
pERY	G	U	C	MLS _B ^R
pDVE	G	G	C	MLS _B ^R
pCAM	A	A	C	Cam ^R , Ery ^R
pBFL1	G	A	U	Ery ^R

^a *E. coli* strains with wild type and mutated plasmids. *S. cerevisiae* with wild type and mutated mitochondrial DNA.

^b Base changes in the 23S rRNA gene.

^c Antibiotic-resistant phenotype: MLS_B (macrolide, lincosamide, streptogramin B group); Cam, chloramphenicol; Ery, erythromycin; Spm, spiramycin.

^d Superscript R denotes resistance.

A, adenine; C, cytosine; G, guanine; m₂A, dimethyladenine; U, uracil.

single antibiotic, and the undissociated type in which bacteria incubated with one inhibitor become resistant to all members of the group. Undissociated resistance may either be due to the mutation of a base within the 23S rRNA gene, or to a post-transcriptional modification of the rRNA *erm* gene, which encodes for a methylase which modifies the peptidyl transferase activity of the 23S rRNA base in the ribosome, resulting in resistance to macrolides, lincosamides and type B streptogramins, but not to type A streptogramins and the synergic mixture of A plus B compounds. The latter may also be responsible for dissociated resistance.^{32–36}

The phenomenon of inducible undissociated resistance to MLS_B antibiotics has been the subject of numerous publications, which have revealed the sophisticated regulatory mechanism of this peculiar phenotypic expression.^{37–41} This is attributable to the methylation of an rRNA base which greatly hinders attachment of the MLS_B antibiotics to the 50S subunit.^{42–46}

In theory, random mutation of microorganisms should provide ribosomal alterations responsible for both dissociated and undissociated MLS_B-resistant phenotypes. However, such an approach is feasible only with a small number of prokaryotes and with eukaryotic organelles (e.g. mitochondria from yeasts) carrying a single copy of rRNA genes. This approach does not apply to organisms such as *E. coli*, which carries seven copies of the rRNA genes, as the mutation of one such gene would produce a recessive mutation leading to an unselectable strain. This problem has been circumvented by the use of a multicopy

plasmid containing an *rrn* operon.^{47–51} In-vivo mutagenesis and growth under conditions allowing plasmid multiplication has led to the isolation of resistant strains. Several rRNA mutations leading to antibiotic resistance have been identified. In particular, it has been shown that adjacent rRNA base changes produce both dissociated and undissociated MLS_B resistance.^{48–50}

In staphylococci, resistance to a mixture of A plus B compounds is always associated with the presence of streptogramin A resistance determinants. When those determinants were combined with streptogramin B resistance determinants, i.e. *erm* (MLS_B resistance phenotype) or *vgb* (S_B phenotype), high level resistance to the synergic mixture was likely.

Peptidyl transferase and its inhibitors

The PTC is located at the base of the 50S subunit, at the interface of the two ribosomal subunits. Several large subunit (L) and small subunit (S) proteins have been identified within this centre and presumably participate in its function.^{52–57} Portions of the 23S rRNA, notably the central loop of domain V and adjacent stems and some segments of domain II, have been shown to be key constituents of the PTC.^{58–62}

Peptidyl transferase inhibitors supposedly bind to different sections of the PTC domain.⁶³ rRNA sequences protected by ribosome-bound antibiotics (macrolides, type B streptogramins and chloramphenicol) have been

located within the central loop of domain V,⁶⁴ while bases protected by type A streptogramins have been located in two sequential stems adjacent to this loop.^{62,65}

In a different approach, particles subjected to mild deproteinization procedures were tested for their ability to perform peptidyl transfer to puromycin ('fragment reaction'). Although L proteins were not removed completely, the amount of residual peptides was reduced, supporting the notion that 23S rRNA is an essential, if not the sole, active component of PTC.⁶⁶

While the studies of mutations to resistance and antibiotic-mediated protection have clearly indicated the involvement of 23S rRNA in peptidyl transferase activity, the evidence for nucleic acids being solely responsible for this function is still incomplete, though there is some evidence to suggest that this is the case.

Conclusion

Interference with the function of the PTC of the 50S ribosome is fundamental to the antibacterial activity of the MLS group of antibiotics. This is achieved through inhibition of substrate attachment to both the acceptor and donor sites of this subunit and through an inhibitory effect on the extension of polypeptide chains. Target modification resulting from mutations and modifications to rRNA bases is the most frequent mechanism of resistance to MLS antibiotics. However, activity of type A streptogramin is retained, thus accounting for improved activity of the combined streptogramins against macrolide- or lincosamide-resistant strains. In addition, resistance by other means may be less of a problem for the streptogramins than for other compounds because of the synergic effect of type A and B compounds and hence the need for resistance to develop to both type A and B components in order to achieve high level resistance.

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