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NECESSITY OF THE SULFOXIDE MOIETY FOR THE BIOCHEMICAL AND BIOLOGICAL PROPERTIES OF AN ANALOG OF SPARSOMYCIN

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SUMMARY: An analog of the peptidyl transferase inhibitor sparsomycin was a competitive inhibitor (K_i = 1.8 μM) of peptidyl-puromycin synthesis on E. coli polysomes. Preincubation of polysomes with the compound enhanced the degree of inhibition of peptide bond formation. A model for the involvement of a histidine residue in peptidyl transferase activity is presented as a result of our observations which include direct association of [^{3}H] labelled analog with 70S ribosomes. The correct oxidation state of sulfur in the compound was necessary for the "preincubation effect" and entry of the compound into bacterial cells.

INTRODUCTION: Sparsomycin is a sulfoxide-containing antibiotic isolated from the culture filtrate of Streptomyces sparsogenes (1). Since the elucidation of its structure in 1970 (2), sparsomycin has captured interest as a signficant synthetic challenge (3-7) and as a novel "A" site inhibitor of protein synthesis (8-11). The limited number of structural analogs of sparsomycin reported to date have shed little light on the exact mode of action of this compound (11, 13-14). No mechanistic rationale has been proposed for the role of the sulfoxide moiety in the inhibition of peptidyl transferase by sparsomycin, although Lee and Vince (10) suggested that it may be involved in irreversible reactions at ribosomal sites.

Our interest in sparsomycin analogs resulted from the intent to utilize the Pummerer reaction (15) as a means of effecting enzyme-activated irreversible inhibition of peptidyl transferase. Glick has implicated a histidine residue in the functioning of ribosomal peptidyl transferase (16). In principle, an acylimidazole intermediate to peptide bond formation could possess sufficient chemical energy to acylate a suitably situated sulfoxide, thus initiating the Pummerer process.

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The ribosomal model depicted in Figure 1 summarizes how sparsomycin may act as an enzyme-activated inhibitor of protein synthesis. Stage 1 of this model shows a charged ribosome whose P-site is occupied by peptidyl t-RNA and whose A-site is occupied by sparsomycin. Comparison of sparsomycin, puromycin, and the adenosine terminus of aminoacyl-t-RNA shows some striking spatial similarities. During the elongation step, when peptide bond formation occurs, the proposed acylhistidine intermediate is formed (Stage 2) which could acylate the sulfoxide moiety of sparsomycin. Rearrangement to an electrophilic sulfenium ion (Stage 3) would then occur, affording an opportunity for capture of an enzyme-bound nucleophile such as the histidine residue or the liberated carboxy terminus of the peptide chain. Such a process would be consistent with the observed preincubation effect of sparsomycin-like compounds (19, 11). This report describes the preparation and activity of a readily available sparsomycin analog, MDL 19,152. The biological significance of the sulfoxide moiety of this compound is discussed.

MATERIALS AND METHODS:

BACTERIAL STRAINS: Two *E. coli* strains were used. *E. coli* W (ATCC 9637, designated as Es39) and an *E. coli* mutant supersensitive to beta-lactam antibiotics, Es31 of Aoki *et al* (17).

PEPTIDYL-PUROMYCIN SYNTHESIS: This enzyme assay, used as a measure of peptidyl transferase activity, was that described by Pestka (18) using *E. coli* Es39 polysomes prepared according to Godson and Sinsheimer (19). For inhibition studies reactions were terminated after 1 min at 21°C.

SYNTHESIS: The synthesis of MDL 19,152 outlined in Scheme I followed closely that of Lee and Vince (10) except that d,l-cysteine 1 was S-benzylated in absolute ethyl alcohol using three equivalents of triethylamine. After stirring at 25°C for 8 hr the reaction mixture was diluted with water until homogeneous and the pH was adjusted to 4. The amino acid 2 was isolated in 75% yield by filtration and reduced with lithium aluminum hydride (10). The resulting amino alcohol 3 was coupled to 6-methyluracilacrylic acid 4 to give sulfide, MDL 19,117, which was identical in all respects to that prepared by Lee and Vince (10). Oxidation of this sulfide with sodium metaperiodate in aqueous dioxane gave the sulfoxide, MDL 19,152, in good yield with a small amount of the sulfone, MDL 19,568, as a minor impurity. These compounds were separable by chromatography (20% ethanol/$CHCl_3$). Similarly, a tritiated sample of MDL 19,152 was prepared by reducing amino acid 2 with lithium aluminum triteride. The two racemic diastereomers of MDL 19,152 were separated by HPLC to 95% purity using 20% ethanol/$CHCl_3$ and recycling techniques.

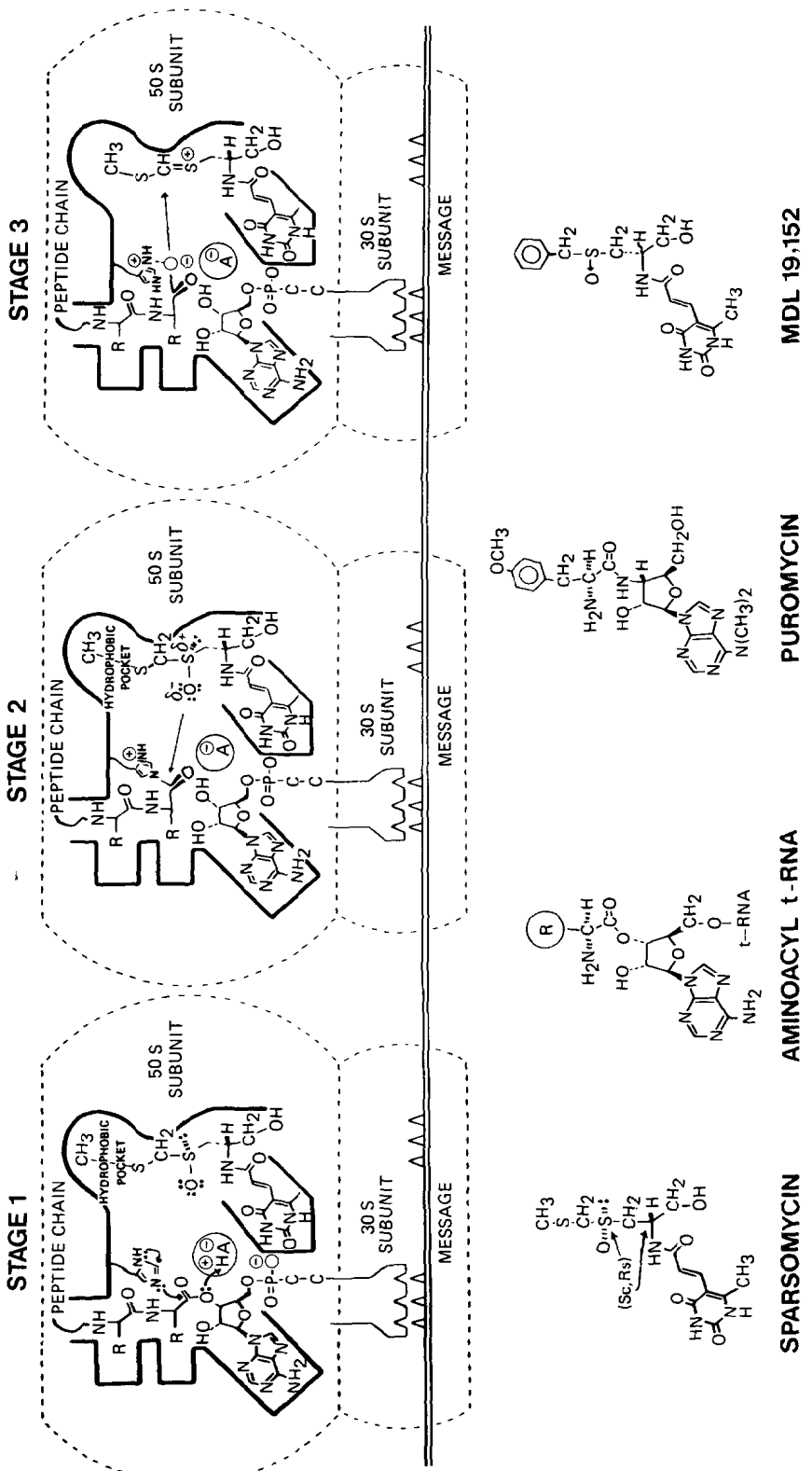
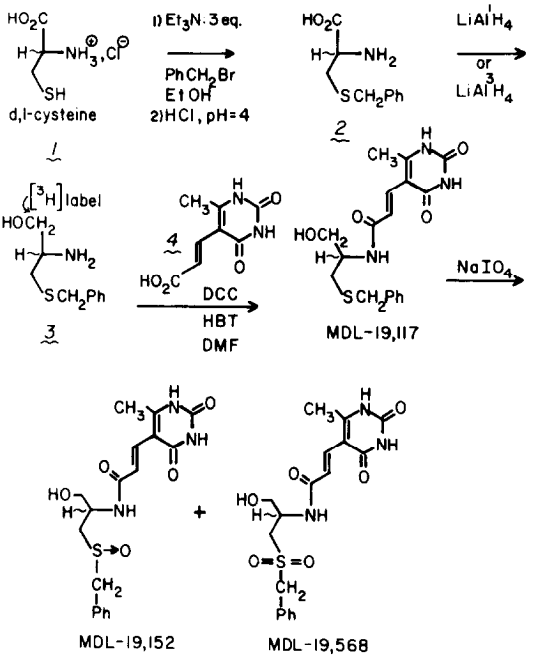


Figure 1: RIBOSOME MODEL



Scheme 1

RESULTS

MDL 19,152 was a competitive inhibitor of puromycin in the peptidyl transferase assay with *E. coli* polysomes (K_i = 1.8 x 10^{-6} M). Preincubation of polysomes with the compound resulted in an increase in the level of inhibition observed (Table 1). The sulfoxide group appears to be necessary for demonstration of this "preincubation effect" since sulfide- and sulfone-containing analogs failed to yield increased inhibition of peptidyl-puromycin formation after preincubation with polysomes. Isomers of MDL 19,152 separated by HPLC were tested for inhibition of peptidyl transferase with and without preincubation. The results (Table 1) suggest that isomer β has a greater inhibitory effect when incubated with polysomes prior to running the assay.

E. coli incubated in the presence of [^{3}H] labelled MDL 19,152 was found to incorporate the label into ribosomes (Figure 2). Warming of cellular extracts at 37°C prior to density gradient centrifugation failed to free the tritium label from the 70S ribosomal peak suggesting that at least a portion of the compound was associated with ribosomal structures

Table 1. Effect of Preincubation on the Effect of Sparsomycin Analogs on Peptidyl-Puromycin Synthesis

Compound	% Inhibition -Preincubation	% Inhibition + Preincubation[1]	+/-[2]
MDL 19,152 (SO)	29	59	2.03
MDL 19,152, α[3]	31	55	1.77
MDL 19,152, β	24	63	2.62
MDL 19,117 (S)	27	25	0.92
MDL 19,568 (SO_2)	22	14	0.64

1. All compounds at a final concentration of 1 μM were incubated with E. coli polysomes for 20' at 21°C prior to starting the reaction with ^3H puromycin. The peptidyl puromycin assay was for 1 min at 21°C.
2. Ratio of % inhibition + preincubation to % inhibition - preincubation.
3. α and β refer to diastereomers of MDL 19,152.

and not the nascent polypeptide chain. The failure to find increased label in the soluble fraction at the top of the gradient or decreased label in the 70S fraction after warming was consistent with this suggestion.

Inhibition of E. coli (Es39) growth was observed with the sulfoxide-containing analog (Figure 3, left). Sulfide- and sulfone-containing compounds failed to inhibit growth at 100 μM. Since the growth

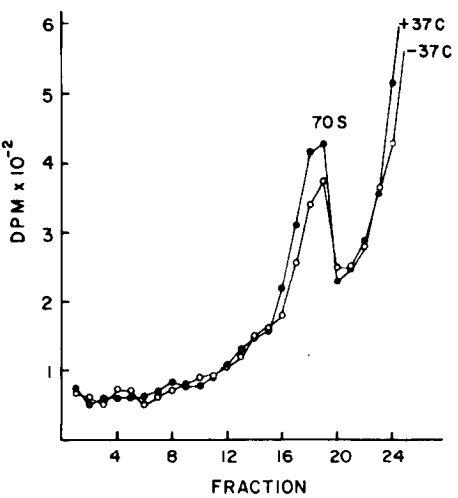


Figure 2. Sedimentation of E. coli ribosomes labelled with [^3H] MDL 19,152. Cell extracts were prepared according to Godson and Sinsheimer (19) from E. coli incubated with [^3H] MDL 19,152 (100 μM, specific activity 1.88 Ci/mmol) for 5 min at 37°C. Extracts were then incubated on ice or at 37°C for 1 min prior to centrifuging through a 5-20% sucrose gradient (SW25.1, 21,500 RPM, 3.5 hr, 4°C).

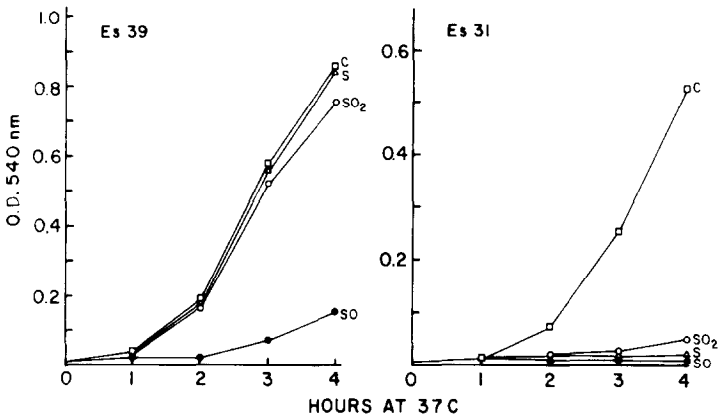


Figure 3. Growth of E. coli strains in the presence of MDL 19,152 analogs. Bacteria were grown in nutrient broth (+6% glucose) with each compound at a final concentration of 100 μM. SO = MDL 19,152, S = sulfide analog, SO_2 = sulfone analog, C = control, no compound.

of E. coli Es 31, (which is freely permeable to a variety of antibiotics) was inhibited by all three analogs (Figure 3, right), it would seem that the oxidation state of the sulfur atom is also important for entry of the compounds into bacteria with normal permeability barriers.

DISCUSSION

The importance of the sulfoxide moiety , which occurs in only one of its two possible configurations in sparsomycin, has been alluded to in the past (10) but little is known about the chemical and biological role of this group. Our studies indicate that, although the inhibition constants of the sulfoxide-, sulfide-, and sulfone-containing compounds are similar, only the sulfoxide analog shows a "preincubation effect" (i.e., increased inhibition of peptidyl transferase). The structural similarities between sparsomycin (R_S, S_C) and the adenine terminus of L-aminoacyl-tRNA depicted in Figure 1 makes consideration of the Pummerer reaction an attractive mechanistic rationale for the preincubation effect. Our hypothesis is supported by the observance of radiolabelled 70S ribosomes derived from E. coli incubated with tritium-labelled MDL 19,152.

While the role of a histidine residue at the site of peptidyl transferase was discussed as an alternative to mere aminolysis promoted by the proximity of the ester and amino groups (20), the active role of an acyl-

histidine intermediate can best account for the proposed Pummerer mechanism.

The natural occurrence of the R_s configuration at sulfur in sparsomycin is thought to be noncoincidental since this configuration is required to place the oxygen-sulfur bond in the same orientation as the nitrogen-carbon bond of the natural substrate, L-aminoacyl-tRNA. The observed difference in preincubation effect between the alpha and beta diastereomers of MDL 19,152 may be a result of selectivity for the R_s configuration during peptide chain elongation.

The sulfoxide group facilitates uptake of MDL 19,152 by the bacterial cell. The in vitro growth curves of E. coli treated with the sparsomycin analogs (i.e., sulfide, sulfoxide and sulfone) illustrate the effectiveness of the sulfoxide moiety in this regard.

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