MECHANISM OF ACTION OF O-CARBAMYL-D-SERINE, A NEW MEMBER OF CELL WALL SYNTHESIS INHIBITORS

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O-carbamyl-D-serine, NH_2-CO-O-CH_2-CH(NH_2)-COOH, was first isolated from a Streptomyces by Hagemann et al (1955). Skinner et al (1956) synthesized the L-isomer, which was reported to inhibit purine biosynthesis as a glutamine antagonist. In the course of screening for new antibiotics in our laboratory, O-carbamyl-D-serine was obtained from a culture filtrate of a Streptomyces and a reversal of the activity by D-alanine was observed. Further studies revealed that it inhibits the biosynthesis of bacterial cell wall as described with penicillin, D-4-amino-3-isoxazolidone, bacitracin, novobiocin and vancomycin.

Reversal of the antibacterial activity of O-carbamyl-D-serine by D-alanine: Amino acids and glutamine were tested for the effects of antagonizing the inhibitory activity of O-carbamyl-D-serine by a cylinder plate method, using Bacillus subtilis PCI 219 as a test organism. The agar plate, containing the bacterial seed, was added with amino acid or glutamine to give the concentration of 0.25 or 0.5 mg/ml. The solution of O-carbamyl-D-serine at the concentration of 0.25 or 1 mg/ml was placed in the cylinder. The diameters of the inhibitory zones were compared with those in the plate, free of amino acid or glutamine. The medium employed was a glucose-mineral agar.

The antimicrobial activity of O-carbamyl-D-serine was markedly reversed by D-alanine, but was not significantly affected by glycine, L-alanine, L-serine, L-glutamic acid, L-lysine or L-glutamine. Zones of partial inhibition were observed in D-alanine-containing agar plates. The results are presented in Table 1.

Table 1. Effects of amino acids and glutamine on the antibacterial activity of O-carbamyl-D-serine.

Metabolite (in the agar plate)	O-carbamyl-D-serine (in the cylinder)	
	1 mg/ml	0.25 mg/ml
-	35.5 mm	23.5 mm
glycine 0.25 mg/ml	34.5	22.5
L-alanine "	34.5	23.0
L-serine "	36.5	23.5
L-glutamic acid "	36.0	24.5
L-lysine "	35.0	25.0
L-glutamine "	36.0	23.5
D-alanine "	15.0 (31.5)	0 (18.5)
" 0.5 mg/ml	0 (22.0)	0

The number represents the diameter (mm) of the inhibitory zone. The number in the bracket shows the zone of partial inhibition. Test organism: Bacillus subtilis PCI 219. Medium: a glucose-mineral agar.

Inhibition of incorporation of DL-glutamate-3,4- ^{14}C into the cell wall fraction of B. subtilis: A culture of B. subtilis PCI 219 grown in a glucose-glutamate-mineral medium was harvested while in the logarithmic phase of growth, washed and resuspended in a similar fresh medium. The bacterial suspensions were incubated at 30° for 30 minutes with DL-glutamate-3,4- ^{14}C (0.5 μc/ml) in the presence or absence of O-carbamyl-D-serine (0.4 mg/ml) or benzylpenicillin (2 u/ml). DL-glutamate-3,4- ^{14}C with specific activity of 3.1 mc/mM was obtained from N. V. Philips-Duphar, Amsterdam, Holland. After fractionation by Schneider's procedure, the hot acid-insoluble fraction was digested by trypsin. The radioactivity of the trypsin-digestible (protein) fraction or the trypsin-indigestible (cell wall) fraction was determined in a gas-flow counter and corrected for self-absorption.

The incorporation of ^{14}C-glutamate into the cell wall fraction was definitely inhibited by the presence of O-carbamyl-D-serine but that into the protein fraction was slightly affected. The same tendency was observed with benzylpenicillin. A similar result was obtained in the same sort of experiments using Staphylococcus aureus 209P. The results are summarized in Table 2.

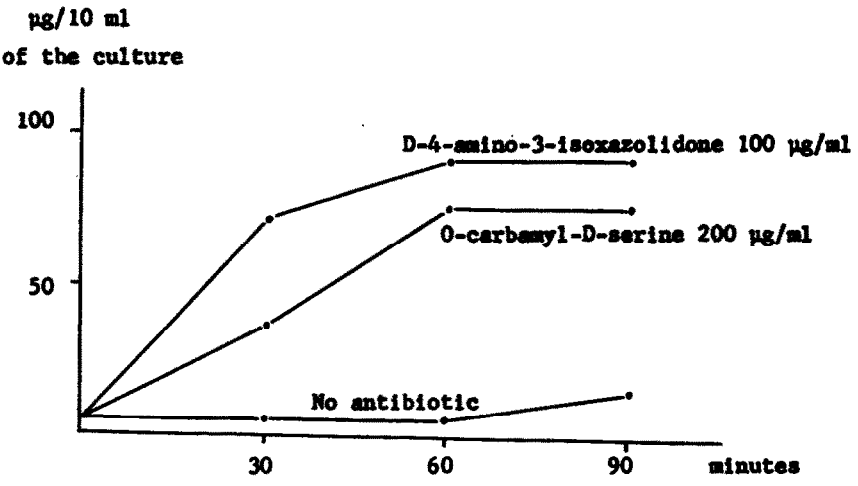
Table 2. Effects of O-carbamyl-D-serine on incorporation of DL-glutamate-3, 4- ^{14}C into cell wall or into protein in Bacillus subtilis and in Staphylococcus aureus.

Antibiotics	B. subtilis		S. aureus	
	Cell wall	Protein	Cell wall	Protein
-	705	6780	1020	3270
O-carbamyl-D-serine 0.4 mg/ml	297(58)	6030(11)	660(35)	2970(9)
Benzylpenicillin 2 u/ml	324(54)	5580(17)	50(95)	2960(6)

The number represents cpm/ml of the culture. The number in the bracket shows % inhibition.

Accumulation of intrabacterial N-acylamino sugar in the presence of O-carbamyl-D-serine: When O-carbamyl-D-serine was added to early logarithmic cultures of B. subtilis PCI 219 at the concentration of 200 µg/ml, analysis of the acid-soluble fraction of the bacteria revealed an intracellular accumulation of N-acylamino sugar over a period of 90 minutes. N-acylamino sugar was determined by the method of Reissig et al (1955). Parallel experiments with D-4-amino-3-isoxazolidone were carried out for comparison, and similar results were observed. The results are illustrated in Figure 1.

Figure 1. Intracellular accumulation of N-acylamino sugar in B. subtilis.



It is conspicuously demonstrated by the above experiments that O-carbamyl-D-serine inhibits the bacterial cell wall formation as a D-alanine antagonist. The effect appears to be attributed to the structural relationship of the antibiotic to D-alanine, a component of the bacterial cell wall.

Skinner et al (1956) reported that O-carbamyl-L-serine inhibits purine biosynthesis as a glutamine antagonist. However, the antibacterial activity of the D-isomer was not reversed by glutamine. It seems interesting that the biochemical basis for the activity of O-carbamyl-D-serine is completely different from that of the L-isomer, although both isomers exhibit antibacterial activity.

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