METABOLIC PRODUCTS OF MICROORGANISMS 176*. ON THE TRANSPORT OF SMALL PEPTIDE ANTIBIOTICS IN BACTERIA

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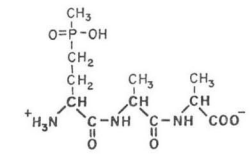
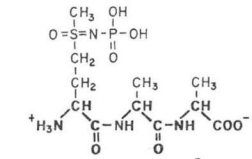
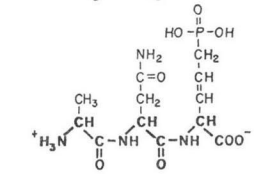
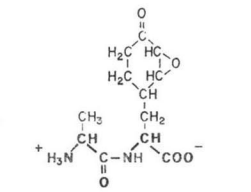
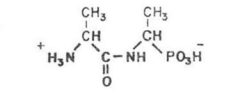
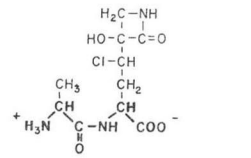
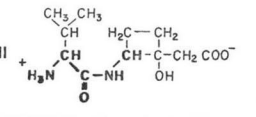
In a continuation of studies on antibiotics which can enter bacterial cells *via* the transport systems for peptide nutrients[1], the uptake of a tripeptide antibiotic and of some dipeptide antibiotics into cells of *Escherichia coli* K-12 was investigated. It was described previously[1] that the 2 tripeptide antibiotics, L-phosphinothricyl-alanyl-alanine (**I**, Fig. 1) and L-(N^5-phosphono)-methionine-(S)-sulfoximinyl-alanyl-alanine (**II**, Fig. 1), enter the cells of *E. coli* K-12 *via* the oligopeptide transport system (opp)[2]. This paper demonstrates that the tripeptide antibiotic plumbemycin B (**III**, Fig. 1), an antagonist of L-threonine isolated from *Streptomyces plumbeus* by PARK and coworkers[3], is also transported *via* the opp. In reference to the investigations on opp it can be demonstrated that the dipeptide inhibitors bacilysin (**IV**, Fig. 1), alaphosphin (**V**, Fig. 1), (S)-alanyl-3-[α-(S)-chloro-3-(S)-hydroxy-2-oxo-3-azetidinylmethyl]-(S)-alanine (**VI**, Fig. 1), and 1-(S)-hydroxy-2-(S,S)-valylamidocyclobutane-1-acetic acid (**VII**, Fig. 1) reach their target *via* peptide permeases.

KENIG and coworkers[4,5] found that **IV** is transported into cells of *E. coli* B and *Staphylococcus aureus via* the dipeptide transport system (dpp). Anticapsin, the C-terminal amino acid of **IV**, is a very poor antimicrobial agent[5] but is a powerful inhibitor of glucosamine synthetase in extracts of *S. aureus*. It was suggested that the antibacterial activity of **IV** depends on its transport into the organism, on its hydrolysis to anticapsin, and on inhibition of glucosamine synthetase by the latter[4]. The synthetic antimicrobial agent **V**[6], a phosphonate analogue of L-alanyl-L-alanine, inhibits selectively alanine racemase and to a lesser extent UDP-N-acetylmuramyl-L-alanine ligase[7,8]; both enzymes play a role in bacterial cell wall biosynthesis. **VI** is

Fig. 1. Structures of the tested peptides and the transport systems involved.

I, L-Phosphinothricyl-alanyl-alanine; **II**, L-(N^5-phosphono)-methionine-(S)-sulfoximinyl-alanyl-alanine; **III**, plumbemycin B; **IV**, bacilysin; **V**, alaphosphin; **VI**, (S)-alanyl-3-[α-(S)-chloro-3-(S)-hydroxy-2-oxo-3-azetidinylmethyl]-(S)-alanine; **VII**, 1-(S)-hydroxy-2-(S,S)-valylamidocyclobutane-1-acetic acid; opp, oligopeptide transport system; dpp, dipeptide transport system.

		Structures	Transport systems
Tripeptides	I		opp
	II		opp
	III		opp
Dipeptides	IV		opp, dpp
	V		opp, dpp
	VI		opp, dpp
	VII		opp, dpp

* 175. W. WEBER, H. ZÄHNER, J. SIEBERS, K. SCHRÖDER and A. ZEECK: Tetracenomycin C. Arch. Microbiol. in press

conspicuously similar to tabtoxin[9], an inhibitor of the glutamine synthetase[10]. The toxic action of both **VI** and tabtoxin can be reversed by glutamine[11]. The antibacterial action of **VII** is antagonised by L-cysteine and L-methionine. It is thought that the C-terminal γ-amino acid is the active part of the molecule, but there has been no direct evidence for this hypothesis[12].

E. coli K-12 served as the test organism. The isolation of the opp-deficient mutant of *E. coli* K-12 (henceforth called mutant A) and the evaluation of the agar plate diffusion tests and antagonism tests were performed as described previously[1]. Mutant B, defective in both the opp and the dpp, is a spontaneous mutant derived from mutant A and was isolated from agar plates containing 20 μM of **V**. The rate of mutation was about $1:10^5$. Mutant B was characterized according to its sensitivity to the following: **V**, the toxic dipeptide glycyl-L-leucine[13], **I**, **II**, and the toxic tripeptide tri-L-ornithine. Mutant B exhibited full cross-resistance to all these toxic di- and tripeptides (data not shown). The mutants A and B retained complete sensitivity to the following antibiotics: albomycin, chloramphenicol, D-cycloserine, erythromycin, flavomycin, ketomycin, neomycin, phleomycin, rifampicin, tetracycline, and viomycin.

The uptake of the peptide antibiotics into the wild type cells of *E. coli* K-12 and into mutant A *via* peptide transport systems was investigated by antagonism tests with various peptides. The results are summarized in Table 1. The data of agar plate diffusion tests, using the wild type strain, mutant A, and mutant B are shown in Fig. 2. All peptides which were accepted by the opp abolished competitively the inhibitory effect of **III**; however there is no effect on the antibacterial action of **III** by peptides which used different transport systems. The mutants A and B exhibited full cross-resistance to all the tripeptide antibiotics **I**, **II**, and **III** (data not shown). The results clearly show that **III** is transported *via* the opp as shown previously for **I** and **II**[1]. In reference to these results the data presented here demonstrate that the dipeptides **IV**, **V**, **VI**, and also with some reservation **VII** are transported *via* dpp. Mutant B exhibits full cross-resistance to all dipeptide antibiotics. The difference between the mutation rates from the wild type and mutant A to mutant B was about 10^3. This indicates that the dipeptide antibiotics also can enter the cell of *E. coli* K-12 *via* the opp, but the efficiency of the transport by dpp is sufficient to kill the cells. Since **VII** contains a C-terminal γ-carboxyl group, its molecular structure does not fulfill the specific requirement of the dipeptide permease[16]. Lowered sensitivity of mutant A, and total resistance of mutant B to **VII** can be explained by a low affinity of **VII**

Table 1. Antagonism tests with inhibitors **III**, **IV**, **V**, **VI**, and **VII** and various peptides.
Test strains *E. coli* K-12 wild type (wt; opp^+ dpp^+) and mutant A (opp^- dpp^+).
Assay method: antagonism test according to ZÄHNER and coworkers[14]; medium: DAVIS & MINGIOLI's minimal medium[15] with the addition of 15 g/liter agar; concentration of the peptides: 10 mM; (+), reversion of inhibition; (—), no reversion; (n.t.), not tested.

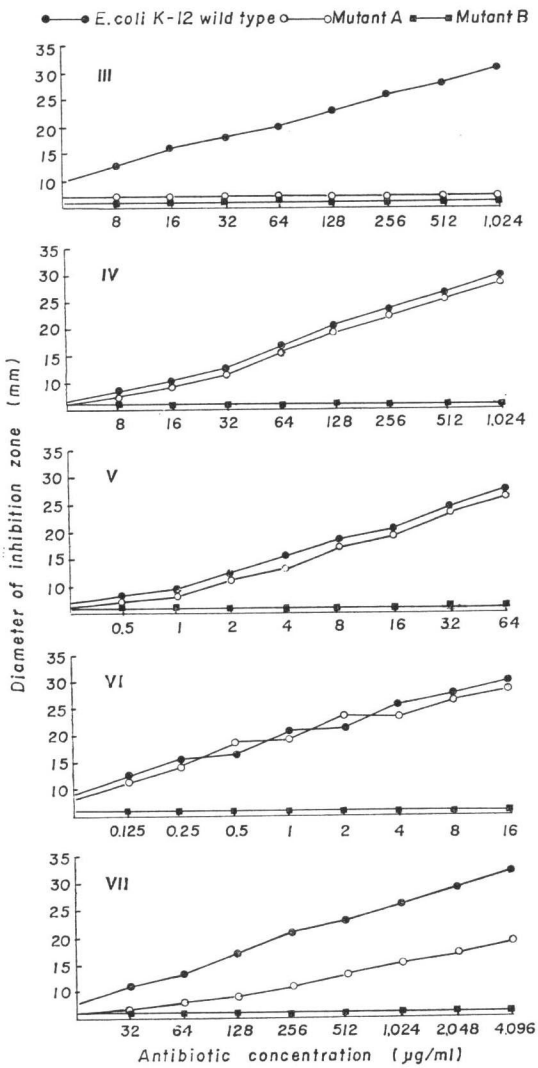
Peptide	Growth response								
	III (2.0 mM)	**IV** (0.02 mM)		**V** (2.0 mM)		**VI** (0.2 mM)		**VII** (4.0 mM)	
	wt	wt	mut.A	wt	mut.A	wt	mut.A	wt	mut.A
$(Ala)_2$	—	+	+	+	+	+	+	+	+
Ala-Gly	—	+	+	+	+	+	+	—	—
Gly-Ala	—	+	+	+	+	+	+	—	—
$(Ala)_3$	+	—	—	—	—	—	—	+	+
$(Lys)_3$	+	—	—	—	—	—	—	—	—
Glutathione	—	—	—	—	—	—	—	—	—
$(Ala)_4$	+	—	—	—	—	—	—	—	—
$(Gly)_3$	+	—	—	—	—	—	—	—	—
$(Gly)_2$-Ala	+	—	—	—	—	—	—	—	—
$(Met)_3$	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	+	+

for dpp. In the case of mutant A, the rate of transport of **VII** is the limiting factor for the effectiveness of the antibacterial action of the antibiotic. It appeared that more than one peptide transport system exists in *E. coli* K-12 and that the system mediating peptide transport is complex. Recently, other peptide transport systems were described[17,18,19] which are distinct from opp and which have more restricted side-chain specificity. At present it is not possible to determine whether there exists a more specific peptide permease for **VII** besides the poorly transporting opp and dpp. On the premise that **VII** may interfere with the biosynthesis of sulfur-containing amino acids[12], one can interpret the competition with tri-L-methionine either as a reversion of the action at the level of the target, or as an antagonism at the binding site of a specific peptide permease.

Fig. 2. Agar diffusion test.

Sensitivities of *E. coli* K-12 (wild type), mutant A, and mutant B to the inhibitors **III**, **IV**, **V**, **VI**, and **VII**.

Assay method: agar diffusion test (diameter of filter paper discs: 6 mm), medium: DAVIS & MINGIOLI's minimal medium[15] with the addition of 15 g/liter agar.



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