SHORT COMMUNICATION

Peptide Transport in *Staphylococcus aureus*

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Using the agar diffusion test of Zähler *et al.* (1960), it was demonstrated that bacilysin and a variety of di- and tri-L-peptides compete with each other for uptake into *Staphylococcus aureus*. Several di- and tripeptides containing L-phenylalanine produced transient growth inhibition of *S. aureus* NCTC 6571, but not of bacilysin-resistant mutants. The results confirm that the major peptide transport system in *S. aureus* recognizes both di- and tripeptides as substrates.

INTRODUCTION

Bacilysin is a dipeptide antibiotic produced by *Bacillus subtilis* A14 whose structure was determined by Walker & Abraham (1970). Its C-terminal amino acid, anticapsin, is a potent inhibitor of L-glutamine:D-fructose-6-phosphate aminotransferase ('glucosamine synthase', EC 5.3.1.19) in both *Staphylococcus aureus* NCTC 6571 and *Escherichia coli* (Kenig *et al.*, 1976; Whitney & Funderburk, 1970). It was suggested (Kenig & Abraham, 1976; Kenig *et al.*, 1976) that the antimicrobial action of bacilysin depends on its transport via a peptide transport system similar to those described for *E. coli* (reviewed by Payne & Gilvarg, 1978), followed by rapid intracellular hydrolysis to alanine and anticapsin, the latter then inhibiting 'glucosamine synthase' activity. It was also suggested that spontaneously occurring bacilysin-resistant mutants of *S. aureus* lacked the transport system (Kenig *et al.*, 1976).

Perry & Abraham (1979) used chloramphenicol-treated cell suspensions of *S. aureus* NCTC 6571 to show that bacilysin competed with ¹⁴C-labelled Ala-Tyr and Gly-Phe for uptake, as did a variety of di- and tripeptides, and that a bacilysin-resistant mutant had lost the ability to transport the ¹⁴C-labelled dipeptides and also di- and trialanine. Uptake of label from ¹⁴C-labelled dipeptides was very rapid, and the kinetics of uptake were complicated by the excretion of the aromatic amino acid residues and degradation of glycine to carbon dioxide. I therefore decided to examine peptide transport in growing cells, using a technique based on the reversal of bacilysin action, to extend the data previously obtained by a similar method (Kenig & Abraham, 1976), and to examine the conclusions reached using ¹⁴C-labelled dipeptides with cell suspensions (Perry & Abraham, 1979). During the course of these experiments an unexpected result was found, namely that several phenylalanine-containing peptides were growth inhibitory in their own right. This finding enabled the reversal of action of such a tripeptide by a dipeptide to be demonstrated, and their action on bacilysin-resistant mutants to be tested.

METHODS

Organisms. *Staphylococcus aureus* NCTC 6571 (Oxford) and a bacilysin-resistant mutant of this organism were as described by Kenig *et al.* (1976). Three other bacilysin-resistant strains were isolated independently from

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colonies growing within zones of inhibition produced by solutions of bacilysin (150 μM) in seeded Lab-Lemco agar plates. *Staphylococcus aureus* strains Little Falls and Evans were from Dr L. Sabath, University of Minnesota Medical School, U.S.A., and strain 209P from Dr A. Imada, Takeda Chemical Industries, Osaka, Japan.

Media. Kloos agar medium was that described by Kloos & Pattee (1965). Brain-heart infusion broth and Lab-Lemco agar were described by Kenig & Abraham (1976).

Reagents. The sample of bacilysin was that described by Perry & Abraham (1979). Di- and tripeptides were from Sigma. Unless otherwise specified all peptides contained amino acids of the L-configuration. Gly-[U- ^{14}C]Phe (21 mCi mmol $^{-1}$; 777 MBq mmol $^{-1}$) was from The Radiochemical Centre, Amersham, and was diluted with unlabelled peptide to 2.0 mCi mmol $^{-1}$ (74 MBq mmol $^{-1}$).

Agar plate diffusion and peptide antagonism tests. These were carried out according to Zähler *et al.* (1960). Strips of Whatman no. 1 paper (7 $\times$ 0.6 cm) were soaked in the peptide solution and excess was removed by blotting on sheets of the same paper. Appropriate strips were then laid on the surface of Kloos agar plates seeded with *S. aureus* as described by Kenig & Abraham (1976), and after 30 min at room temperature, the plates were incubated at 37 °C. Some of the more concentrated peptide solutions had to be warmed gently to effect solution.

Paper electrophoresis, ninhydrin staining and scintillation counting were performed as described (Perry & Abraham, 1979).

RESULTS AND DISCUSSION

Zähler *et al.* (1960) described an agar plate diffusion test by which antagonism of antibiotic activity by competitive and non-competitive inhibitors can be demonstrated. Diddens *et al.* (1976) used this method to show that various tripeptide inhibitors of glutamine synthetase in *E. coli* use the oligopeptide transport system to obtain entry into the cell. All results referred to here as competitive inhibition of the action of a peptide growth inhibitor showed the zone of inhibition pattern illustrated by Diddens *et al.* (1976) using this test with *E. coli*.

Table 1 shows the results of agar diffusion tests using a variety of di- and tripeptides as antagonists of bacilysin action. The relative effectiveness of Ala-Tyr and Gly₂ in this test correlates well with their respective K_m values for uptake found previously using ^{14}C -labelled substrates with washed cell suspensions (77 μM and 1.2 mM, respectively; Perry & Abraham, 1979). To obtain antagonism of bacilysin action by Gly₂ comparable to that seen with 12 mM-Ala-Tyr it was necessary to use a concentration approaching 1 M. Peptides found to be good competitors of the uptake of label from ^{14}C -labelled dipeptides in resting cells (Ala₂, Ala₃, Ala-Tyr, Tyr₃; Perry & Abraham, 1979) were also good antagonists of bacilysin action in this test, whilst poor competitors (Gly₂, Gly₂-Phe; Perry & Abraham, 1979) were found to be poor antagonists. Identical results were found using three other strains of *S. aureus* (Little Falls, Evans and 209P) and Ala₂ and Ala₃ as antagonists.

Table 1. *Antagonism tests with bacilysin and various peptides*

Tests were performed as described in Methods and Diddens *et al.* (1976), using various peptide concentrations. Competitive inhibition is scored as +, no effect as −, and a small effect on zone pattern (but insufficient to score +) as − (slight).

Peptide	Concn (mM) : competitive effect
Ala ₂	12 : +
Ala ₃	12 : +
Gly ₂	12 : − 200 : − 1000 : +
Gly ₂ Phe	20 : −
Ala-Tyr	12 : +
Tyr ₃	20 : +
N-AcAla ₃	20 : −
Ala ₃ OMe	20 : − (slight)
Lys ₂	200 : − (slight)
Gly-D-Phe	100 : −

Blocking either terminus of trialanine resulted in a large reduction in, if not complete loss of, antagonism of bacilysin action compared to trialanine itself. Thus, like the oligopeptide transport system of *E. coli*, the peptide transport system of *S. aureus* would seem to require free carboxyl and amino functions for good binding of its substrate. Gly-D-Phe (at 100 mM) had no effect on bacilysin action, suggesting that some requirement for stereospecificity exists, at least in dipeptides, a feature commonly found for peptide transport by other micro-organisms (Payne & Gilvarg, 1978).

A series of phenylalanine di- and tripeptides were examined for antagonism of bacilysin action, but the interpretation of the inhibition zone patterns was complicated by the fact that several of these peptides were themselves growth inhibitory. For instance, a filter paper strip soaked in 100 mM-Gly-Phe produced a zone of inhibition after 16–17 h incubation at 37 °C, but after 24–40 h, the colonies within this zone had grown to normal size. Phe-Gly, Ala-Phe and Phe-Gly₂ (at 100 mM) also produced this slowing of growth, but Gly₂-Phe did not.

The uptake of Ala-[¹⁴C]Tyr and Gly-[¹⁴C]Phe by washed cell suspensions has been shown to be followed by excretion of the appropriate aromatic amino acid residue (Perry & Abraham, 1979), so it seemed possible that this growth inhibition might be in some way connected with this effect. However, neither Gly-Tyr nor Ala-Tyr produced growth inhibition. That the excretion of phenylalanine following uptake of Gly-Phe found with washed cell suspensions also occurs with growing cells was shown by adding Gly-[U-¹⁴C]Phe (50 µM) to a culture in the exponential phase of growth in brain–heart infusion broth, and examining the culture filtrate after further incubation by paper electrophoresis. After 1–5 h incubation, the peak corresponding to Gly-[U-¹⁴C]Phe had disappeared and a substantial proportion of the added label now appeared at a position corresponding to free phenylalanine. Phenylalanine at 100 mM produced no effect in the present test, however, so that the growth inhibition is not due to inhibition of uptake of essential amino acids from the medium by excreted phenylalanine. This conclusion is supported by the fact that the tyrosine analogues of these inhibitory peptides had no growth inhibitory effect. The effect may be connected with an imbalance in the cell pool arising from continued uptake of large amounts of phenylalanine, or alternatively, the intact peptides themselves may be inhibitory, as found for leucine peptides in *E. coli*. Vonder Haar & Umbarger (1972) suggested that Gly-Leu inhibited the growth of *E. coli* by acting on threonine deaminase, and such inhibition of an essential enzyme may be responsible for the effects observed here.

When tested against four independently isolated bacilysin-resistant mutants of *S. aureus* NCTC 6571, neither Gly-Phe nor Phe-Gly₂ produced any growth inhibition. Using *S. aureus* NCTC 6571, it was possible to show that Ala₂ competes for uptake with Phe-Gly₂ in the agar diffusion antagonism test, although the inhibition zone pattern was difficult to photograph [the phenolphthalein phosphate stain of Barber & Kuper (1951) could be used to increase contrast].

The evidence presented in this and previous papers (Kenig & Abraham, 1976; Perry & Abraham, 1979) shows that the major peptide transport system in *S. aureus* recognizes both di- and tripeptides as substrates with similar affinities, a situation different from that in *E. coli*, where separate di- and oligopeptide transport systems exist (Payne & Gilvarg, 1978). Dipeptides can use the oligopeptide transport system of *E. coli*, but seem to have lower affinities than oligopeptides (for instance, the K_m for Gly₃ transport is 1–3 µM, and K_i for Gly₂ as competitor for Gly₃ transport is 6 mM; C. Gilvarg, personal communication). Bacilysin has recently been shown to use both di- and oligopeptide transport systems in *E. coli* (Diddens *et al.*, 1979), and this accounts for the inability to obtain permeability mutants of *E. coli* resistant to bacilysin (Kenig & Abraham, 1976). In contrast, in *S. aureus*, mutants resistant to bacilysin arise readily by loss of a single transport system. It remains to be seen if *S. aureus* possesses specialized peptide transport systems such as those found in *E. coli* (Barak & Gilvarg, 1975; Naider & Becker, 1975). Recently, it has been shown that the major transport system for peptides in *Saccharomyces cerevisiae* also recognizes both di- and tripeptides (Nisbet & Payne, 1979 *a, b*; Marder *et al.*, 1978).

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