SHORT COMMUNICATIONS

Effect of Piperacillin on D-Alanine Carboxypeptidase Activities from *Pseudomonas aeruginosa*

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Membrane-bound D-alanine carboxypeptidase activity from *Pseudomonas aeruginosa* is very sensitive to inhibition by piperacillin.

INTRODUCTION

Piperacillin, a new semi-synthetic penicillin, is reported to have a broad spectrum of anti-bacterial activity against Gram-positive and Gram-negative bacteria, and to have stronger *in vitro* and *in vivo* activities against *Pseudomonas aeruginosa* than both carbenicillin and sulbenicillin (Ueo *et al.*, 1977).

Suginaka *et al.* (1974) demonstrated that the membrane fraction from *P. aeruginosa* contained both D-alanine carboxypeptidase and transpeptidase activities, whereas the soluble fraction contained only the D-alanine carboxypeptidase. These enzymes were inhibited by penicillins (Suginaka *et al.*, 1974) at concentrations comparable to those inhibiting similar activities from *Escherichia coli* (Izaki & Strominger, 1968; Izaki *et al.*, 1968), although most strains of *P. aeruginosa* are highly resistant to penicillins.

We have investigated the effect of piperacillin on the membrane-bound D-alanine carboxypeptidase activity of the cytoplasmic membrane and compared it with the effect on the soluble D-alanine carboxypeptidase in the cytoplasm and/or periplasm of *P. aeruginosa*.

METHODS

Antibiotic. Piperacillin is 6-[D(-)- α -(4-ethyl-2,3-dioxo-1-piperziny)carbonylamino]- α -phenylacetamido]-penicillanate (sodium salt) and was supplied by Toyama Chemical Co. (Tokyo, Japan).

Organism. The organism used was *Pseudomonas aeruginosa* KM338, derived from ATCC 17653; this was the same strain as used in our previous study (Suginaka *et al.*, 1975) of penicillin-resistant mechanisms. As estimated by a serial tube dilution method in Trypticase Soy Broth (BBL) with an inoculum of about 10^4 bacteria ml⁻¹, the minimal inhibitory concentrations of piperacillin, carbenicillin and benzylpenicillin were 12.5, 125 and 30000 μ g ml⁻¹, respectively.

Preparation of enzyme sources. Organisms were grown in Trypticase Soy Broth at 37 °C with shaking and harvested at the late-exponential phase. Membrane (particulate) and soluble fractions were prepared from the washed organisms after alumina grinding and differential centrifugation as reported previously (Izaki *et al.*, 1968). These fractions were used as the sources of membrane-bound and soluble D-alanine carboxypeptidase.

Assay for D-alanine carboxypeptidase activity. The method used was that of Suginaka *et al.* (1974) with the following modifications. The assay mixture contained (in a total volume of 25 μ l): 1 M-Tris/HCl buffer pH 7.5, 5 μ l; 1 M-MgCl₂, 1 μ l; 1.84 μ M-uridine 5'-diphosphate-*N*-acetylmuramyl-L-alanyl-D-glutamyl-meso-

Fig. 1. Effect of piperacillin on the D-alanine carboxypeptidase activities of membrane (●) and soluble (○) fractions from *Pseudomonas aeruginosa* KM338. Assays were carried out as described in Methods. Results are expressed as percentage inhibition of release of D-[¹⁴C]alanine by piperacillin.

diaminopimelyl-D-[¹⁴C]alanyl-D-[¹⁴C]alanine (UDP-MurNAc-pentapeptide, 66 mCi mmol⁻¹), 4 μ l; membrane fraction (22 mg protein ml⁻¹) or soluble fraction (18 mg protein ml⁻¹), 10 μ l; piperacillin at various concentrations, 5 μ l. After incubation at 37 °C for 2 h, 20 μ l of the reaction mixture was spotted on Whatman 3MM filter paper. The paper was developed by the ascending system with 64 % (v/v) propanol for 3 to 4 h in a 'thin-layer' tank, and then redeveloped three times in the same direction with the same solvent. An autoradiogram was then prepared, and the area of the paper corresponding to alanine was cut out and counted in a liquid scintillation spectrometer.

RESULTS AND DISCUSSION

Membrane and soluble fractions from *P. aeruginosa* KM338 both contained high activities of D-alanine carboxypeptidase (Suginaka *et al.*, 1974). They could remove both D-alanine residues from the carboxyl terminus of UDP-MurNAc-pentapeptide, a peptidoglycan precursor. A part of this activity in the membrane fraction could be contributed by transpeptidase activity, which catalyses the cross-linking reaction of peptidoglycan synthesis.

The release of D-alanine was inhibited by low concentrations of piperacillin, as found previously with benzylpenicillin or carbenicillin (Suginaka *et al.*, 1974). However, the concentration of piperacillin required for inhibition of the membrane-bound enzyme activity of *P. aeruginosa* was very much lower than that of benzylpenicillin or carbenicillin. Thus, the concentrations of benzylpenicillin, carbenicillin and piperacillin giving complete inhibition were approximately 10, 10 and 0.05 μ g ml⁻¹, respectively.

The membrane-bound D-alanine carboxypeptidase was found to be much more sensitive to piperacillin than was the soluble enzyme (Fig. 1). In contrast, D-alanine carboxypeptidase activity of the soluble fraction from this organism was more susceptible to inhibition by benzylpenicillin than was that of the membrane fraction (Suginaka *et al.*, 1974). A similar result was reported for the membrane and soluble fractions from *Escherichia coli* (Izaki & Strominger, 1968).

At present, the specific mechanism responsible for the antibacterial activity of piperacillin against *P. aeruginosa* remains unclear. The highly sensitive inhibition of membrane-bound D-alanine carboxypeptidase activity demonstrated here might be responsible, although further investigation is needed to clarify this point.

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