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 antibacterial 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-(-)-a-(4-ethyl-2,3-dioxo-1-piperazinylcarbonylamino)-a-phenylacetamidol]-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 µg 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 µl): 1 M-Tris/HCl buffer pH 7.5, 5 µl; 1 M-MgCl₂, 1 µl; 1.84 μM-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]alanyl (UDP-MurNAc-pentapeptide, 66 mCi mmol⁻¹), 4 µl; membrane fraction (22 mg protein ml⁻¹) or soluble fraction (18 mg protein ml⁻¹), 10 µl; piperacillin at various concentrations, 5 µl. After incubation at 37 °C for 2 h, 20 µl 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 μg 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.
REFERENCES


