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DD-Carboxypeptidases/Transpeptidases and Penicillin Action

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It is widely accepted that transpeptidases responsible for cross-linking in bacterial peptidoglycans are the target for penicillin action. Whether penicilloylation of the enzyme is an essential part of this process remains an open question. Some strains of Streptomyces produce soluble DD-carboxypeptidases that have been shown to perform transpeptidation to suitable amino compounds with the simultaneous elimination of a C-terminal D-alanine residue. For this purpose the donor substrate was Ac_2-L-Lys-D-Ala-D-Ala (usually ^{14}C-labelled in the acetyl groups) and suitable acceptors were, for strain R61, glycine, D-alanine, diaminopimelic acid or diaminoadipic acid with D-centres present, peptides with glycine or D-alanine as N-terminus, ω-amino acids, aminohexuronic acids, D-cycloserine, 6-aminopenicillanic acid but not L-amino acids or peptides with an L-centre at the N-terminus. Peptides, such as α-glycyl-α'-acetyl-LL-diaminopimelic acid, that were analogues of the acceptor site for cross-linking transpeptidation in the parent Streptomycete, were also acceptors. The transpeptidation, like the carboxypeptidase action, was reversibly sensitive to penicillin, there being no evidence for the involvement of a penicilloylation reaction. Membranes prepared from Streptomyces R61 also contain an enzyme that can perform transpeptidation from the donor Ac_2-L-Lys-D-Ala-D-Ala to a range of amino compounds as acceptors similar to those utilized by the soluble enzyme. The membrane-bound enzyme, however, does not behave as a carboxypeptidase. It is inhibited by penicillin, and its relative sensitivity to a large variety of β-lactam antibiotics is closely similar to the relative sensitivity of the growing

organism to the same antibiotics. The purified soluble enzyme from strain R61 has a mol. wt. of about 38,000 in a single polypeptide chain. It forms a 1:1 complex with penicillin, as shown by depression of fluorescence and by changes in circular dichroism. The association constant calculated from these measurements is close to that observed as a K_i in kinetic experiments on the inhibition of carboxypeptidase action. Kinetic studies of transpeptidation by the soluble enzyme suggest that the reaction proceeds through an ordered mechanism in which the acceptor molecule binds first to the enzyme. Acceptors behave as non-competitive inhibitors of the hydrolysis pathway.

The penicillin-sensitive carboxypeptidase from strain R39 also performs transpeptidation in vitro, but requires as acceptors glycine, D-amino acids or peptides that, like the "natural" acceptor in the parent organism, contain the D-centre of diaminopimelic acid with both amino and carboxyl groups free. Certain peptides of this type inhibit both carboxypeptidase and transpeptidase function of the enzyme when present at higher concentrations. It seems possible that these peptides may exert their action through a control site on the enzyme.