97

ABSTRACTS

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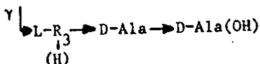
DD Carboxypeptidases and Mechanism of Action of Penicillin.

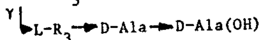
J.-M. GHUYSEN, M. LEYH-BOUILLE, M. NAKEL, J. DUSART, K. JOHNSON and J.-M. FRERE.
Service de Microbiologie, Département de Botanique, Université de Liège, 4000 SART TILMAN par Liège 1. Belgium.

Owing to the primary structures of the bacterial wall peptidoglycans for which four different chemotypes have been proposed (GHUYSEN, J.-M. Bact. Rev. 32, 425 (1968), the uncrosslinked peptide units as they are inserted into the nascent walls, have one of the following general sequences :



(R_3 being a diamino acid with its ω amino group either free or substituted by a short peptide),

or



(R_3 being either a neutral or a diamino acid residue; R_3' being a diamino acid residue or a diamino acid containing short peptide).

The crosslinking between the inserted peptide units is achieved by transpeptidation. The penultimate C-terminal D-alanine residue of a donor peptide is transferred to the amino group (either at the R_3 or at the R_3' position, depending upon the bacteria) of an acceptor peptide. Interpeptide bonds are formed and equivalent amounts of D-alanine residues are released from the donor peptides. Penicillin reduces or abolishes the efficiency of the membrane-bound transpeptidase involved in peptide crosslinking. Among a number of hypotheses, it has been proposed that penicillin acts as a structural analogue of the peptide unit and inactivates the transpeptidase through acylation of the active site. (STROMINGER, J.L. in Inhibitor Tools in Cell Research. Edit. Th. Bücher and H. Sies. Berlin-Heidelberg-New York : Springer 1969).

Streptomyces strains excrete D-alanyl-D-alanine carboxypeptidases which appear to be the exoforms of the transpeptidases. The difference in effective function of the enzymes would be that, after elimination of the C-terminal D-alanine residue, the peptide-enzyme complex reacts with water (carboxypeptidase) rather than with an amino acceptor (transpeptidase). That the DD carboxypeptidases are the transpeptidases and that inhibition of the DD carboxypeptidases by penicillin is actually related to the mechanism of killing by the antibiotic rest upon several pieces of evidence. (1) Strains of *Streptomyces* were selected on the basis of differences in penicillin sensitivity. Increasing resistance to penicillin estimated as the ability

to form single-cell colonies on plates in the presence of different concentrations of penicillin, was not related to the ability of the strains to produce penicillinase, but was paralleled by increasing penicillin resistance of the relevant isolated DD carboxypeptidases. Depending upon the strains, the dose levels of penicillin which reduced by 50 % the number of single-cell colonies, ranged from 1 to 20 μg of penicillin/ml. Concomitantly, the dose levels of antibiotic required to inhibit by 50 % the enzyme activity, ranged from 0.01 to 100,000 μg penicillin/ml. The conditions used for the *in vitro* tests are most probably far from being physiological. This may explain why for some strains, growth inhibition occurs at dose levels of penicillin much smaller than those required to inhibit the corresponding isolated enzymes. (2) Owing to the structure of the wall peptidoglycan in *Streptomyces* sp, the peptide units prior to transpeptidation must have the sequence L-alanyl-D-isoglutaminyl-(L_1); glycyl-(L_2); -LL-diaminopimelyl-(L_1)-D-alanyl-D-alanine; i.e. the amino group involved in transpeptidation is a glycyl residue substituting the side strain of LL diaminopimelic acid at the R_3 position. Regardless their extent of penicillin resistance, and despite individual variations, the *Streptomyces* DD carboxypeptidases exhibit substrate requirements compatible with those expected for the membrane-bound transpeptidases. There appeared to be a considerable specificity for C-terminal D-alanyl-D sequences. Increasing the length of the side chain at the L-R_3 position was paralleled by increasing enzyme efficiency. Presence of charged groups at the end of this side group exerted influences of prime importance.

For those DD carboxypeptidases sensitive to low concentrations of penicillin, Dixon plots of enzyme inhibition in the presence of penicillin gave straigth lines meeting at points from which K_i values were calculated. At the present, comparative studies of penicillin sensitive and resistant DD carboxypeptidases suggest models in which a structural analogy between penicillin and the substrate may not be necessarily involved in the molecular basis of penicillin action. Inactivation of the isolated DD carboxypeptidases through penicilloylation did not occur.

The "transpeptidase-DD carboxypeptidase" system appears to play important roles in the control of the size of the peptide moeity of the wall peptidoglycans. Several possible mechanisms are evoked.