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Penicillin-binding proteins in the membranes of *Streptomyces sp.*

The peptidoglycan-crosslinking enzyme system of *Streptomyces* strains R61, K15 and K11 (LEYH-BOUILLE *et al.*, 1977) consists of : (1) a set of DD-carboxypeptidase activities (*a*) exocellular (*b*) cell-bound, but releasable by lysozyme treatment and (*c*) membrane-

bound, respectively; and (2) a membrane-bound DD-transpeptidase. The DD-carboxypeptidases (standard reaction : Ac_2-L-Lys-D-Ala-D-Ala + $H_2O \rightarrow$ D-Ala + Ac_2-L-Lys-D-Ala) exhibit a low transpeptidase activity, and conversely, the DD-transpeptidase (standard reaction : Ac_2-L-Lys-D-Ala-D-Ala + Gly-Gly $\rightarrow$ D-Ala + Ac_2-L-Lys-D-Ala-Gly-Gly) performs the hydrolysis of the tripeptide donor with a very low efficiency. All these activities are inhibited by β-lactam antibiotics (DUSART *et al.*, 1977) which immobilize the enzymes in the form of rather stable acyl-enzyme complexes. By using [^{14}C] benzylpenicillin, penicillin-binding proteins [PBPs] (known to be synonymous to penicillin-sensitive enzymes, at least in some cases) can be detected by fluorography after polyacrylamide gel electrophoresis in the presence of SDS.

The isolated membranes of *Streptomyces* R61 and K11 contain at least four PBPs, with apparent molecular weights of 20 000-25 000, 49 000, 60 000 and 95 000, respectively, the 20 000-25 000 daltons protein being the major component. *Streptomyces* K15 possesses three major PBPs exhibiting apparent molecular weights of 26 000, 40 000 and 60 000, respectively.

The identification of the DD-transpeptidase as the 20 000-25 000 daltons PBP rests upon the following observations :

(1) Membrane-bound transpeptidases of the three strains under consideration can be solubilized by the use of cationic detergents (LEYH-BOUILLE *et al.*, 1977) and then, partially purified by molecular-sieve filtration, with in parallel to this, a corresponding specific enrichment of the 20 000-25 000 daltons PBP. However, the R61 DD-transpeptidase thus obtained, still possesses an additional 50 000-55 000 daltons PBP (probably a triplet).

(2) With a series of β-lactam antibiotics, there is a good parallelism between the concentrations needed to inhibit the membrane-bound DD-transpeptidase activity and those needed for the detection of the 20 000-25 000 daltons protein as a PBP.

(3) The stability at 37 °C of the 20 000-25 000 daltons protein also indicates that it is the DD-transpeptidase.

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