J. Dusart (1), M. Leyh-Bouille, M. Nguyen-Distèche, J. M. Ghuysen and P. E. Reynolds (2) (Service de Microbiologie, Faculté de Médecine, (1) Institut de Botanique, Université de Liège, Sart Tilman, B-4000 Liège and (2) Sub-Department of Chemical Microbiology, Department of Chemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 IQW, Great Britain).

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: $\text{Ac}_2\text{Lys}-\text{d-Ala-d-Ala} + \text{H}_2\text{O} \rightarrow \text{d-Ala} + \text{Ac}_2\text{Lys-d-Ala}$) exhibit a low transpeptidase activity, and conversely, the DD-transpeptidase (standard reaction: $\text{Ac}_2\text{Lys-d-Ala-d-Ala} + \text{Gly-Gly} \rightarrow \text{d-Ala} + \text{Ac}_2\text{Lys-d-Ala-Gly-Gly}$) performs the hydrolysis of the tripeptide donor with a very low efficiency. All these activities are inhibited by $\beta$-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 (Lehy-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 $\beta$-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.

This work was supported by the National Institutes of Health, Washington, D.C. (contract n° 1 RO1 AI 13364-03 MBC to J.M.G.) and by the Science Research Council to P.E.R. J.D. is Chercheur qualifié of the FNRS.
REFERENCES
