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Isolation of the membrane-bound 26000-M_r penicillin-binding protein of *Streptomyces* strain K15 in the form of a penicillin-sensitive D-alanyl-D-alanine-cleaving transpeptidase

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The membrane-bound, 26000-M_r penicillin-binding protein of *Streptomyces* K15 has been isolated in the form of an effective, penicillin-sensitive D-alanyl-D-alanine-cleaving peptidase exhibiting high transpeptidase activity (>95%) and very low carboxypeptidase activity (<5%). The penicillin-binding protein/transpeptidase can be extracted directly from the mycelium with *N*-cetyl-*NNN*-trimethylammonium bromide (Cetavlon) and subsequently obtained at 90% purity and with an 8000-fold specific enrichment (when compared with the activity of the isolated membranes) by a two-step procedure involving Sephadex filtration and affinity chromatography on ampicillin-linked CH Sepharose 4B in the presence of detergent. At saturating concentrations of the co-substrates diacetyl-L-Lys-D-Ala-D-Ala and Gly-Gly, the catalytic-centre activity is about 0.3 s^{-1}.

Previous studies (Leyh-Bouille *et al.*, 1977) have shown that the plasma membranes of *Streptomyces* strains R61, K15 and *rimosus* exhibit D-alanyl-D-alanine-cleaving transpeptidase activity, catalysing the reaction:

$$Ac_2\text{-L-Lys-D-Ala-D-Ala} + \text{Gly-Gly} \rightarrow \text{D-Ala} + Ac_2\text{-L-Lys-D-Ala-Gly-Gly}$$

and carboxypeptidase activity, catalysing the reaction:

$$Ac_2\text{-L-Lys-D-Ala-D-Ala} + H_2O \rightarrow \text{D-Ala} + Ac_2\text{-L-Lys-D-Ala}$$

Both reactions are inhibited by penicillin and other β-lactam antibiotics. Disruption of the membrane bilayer by treatment with the cationic detergent *N*-cetyl-*NNN*-trimethylammonium bromide (Cetavlon) followed by chromatography on Sephadex G-100 in the presence of the detergent has been used as a means to obtain water-soluble preparations selectively enriched in transpeptidase activity. Other studies (Dusart *et al.*, 1981) have also shown that the plasma membranes of various *Streptomyces* strains have an atypical PBP pattern characterized by the presence of a predominating 26000-M_r PBP. Strong experimental evidence has suggested that, at least in *Streptomyces* R61, this 26000-M_r PBP is the penicillin-sensitive transpeptidase. The present paper describes the isolation to 90% purity of the membrane-bound, 26000-M_r PBP of *Streptomyces* K15 in the form of an effective D-alanyl-D-alanine-cleaving transpeptidase.

Abbreviations used: Ac, acetyl; PBP, penicillin-binding protein; A_2pm, 2,2′-diaminopimelic acid.

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Materials and methods

β-Lactam compounds

Benzylpenicillin was purchased from Rhône Poulenc, Paris, France, ampicillin from Bristol Benelux S.A., Brussels, Belgium, and [^{14}C]benzylpenicillin (sp. radioactivity 51 Ci/mol) from The Radiochemical Centre, Amersham, Bucks., U.K. β-Iodopenicillanic acid was a gift from Pfizer Central Research, Sandwich, Kent, U.K.

Protein estimation

The samples (membranes or protein solutions) were hydrolysed with 6 M-HCl at 100°C for 20 h and the free amino groups were estimated after dinitrophenylation (Frère *et al.*, 1973). All the data given below were obtained with this procedure except those concerning the fraction of K_D 0.45, in

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which case the protein concentrations were determined by measuring A_{260} and A_{280} values (Kalckar, 1947).

Protein and PBP patterns

Samples were incubated for 15 min at 37°C with [^{14}C]benzylpenicillin at the indicated concentrations, the reaction being terminated by the addition of a large excess of non-radioactive benzylpenicillin (0.15 M), and the PBPs were stabilized by boiling the preparations in the presence of 1% sodium dodecyl sulphate and 5% mercaptoethanol. After separation by polyacrylamide (10%)-slab-gel electrophoresis at pH 8.3 in the presence of sodium dodecyl sulphate, the gels were stained with Coomassie Brilliant Blue and then the PBPs were visually detected by fluorography (for more details, see Leyh-Bouille *et al.*, 1981). The relative proportions of the proteins and PBPs were determined by microdensitometry measurements. The standard proteins used were: catalase, M_r 60000; D-amino acid oxidase, 37000, and chymotrypsinogen, 25700.

Membranes

Streptomyces K15 was grown in Merck peptone medium (batch 7213) with shaking at 28°C for 48 h (under which conditions, the isolated membranes had maximal carboxypeptidase and transpeptidase activities). The collected mycelium was transformed into protoplasts by lysozyme action (without supplemental Mg^{2+} ions) and the membranes isolated as described by Leyh-Bouille *et al.* (1977). The lysozyme treatment, however, lasted only 3 h at 4°C instead of 16 h as proposed in the initial procedure. This modification resulted in increased yields in membranes (70 mg of protein/ml of culture) and D-alanyl-D-alanine-cleaving transpeptidase and carboxypeptidase activities (see the Results section). The presence of phenylmethanesulphonyl fluoride (0.4 mg/ml) during the various steps of membrane preparation had no effect. The final membrane preparations (about 40 mg of protein/ml) were in 15 mM-potassium phosphate buffer, pH 7.5. They were stored at −20°C.

Fraction with K_D 0.45 after Cetavlon extract

The washed and homogenized mycelium (from 15 litres of culture) was treated, at 37°C and for 45 min, with 1 litre of a 2% Cetavlon solution made up in 30 mM-potassium phosphate buffer, pH 7.5. The extract (as obtained after removal of the cell debris) was maintained at 0°C for 16 h (during which time part of the detergent precipitated), and the supernatant was concentrated to 50 ml by ultrafiltration. The Cetavlon extract was then submitted to successive filtrations on Sephadex G-100 in 30 mM-potassium phosphate buffer, pH 7.5, containing 0.05% Cetavlon, as previously described (Leyh-Bouille *et al.*, 1977). The fraction with K_D 0.45 (specifically enriched in transpeptidase activity) was concentrated by ultrafiltration to a final concentration of 8.7 mg of protein/ml.

Purification of the transpeptidase of fraction K_D 0.45 by affinity chromatography on ampicillin-linked CH Sepharose 4B

The affinity chromatography reagent was prepared as described by Coyette *et al.* (1978); it contained about 10 μmol of linked ampicillin/ml of packed resin (hydroxamate assay). Fraction K_D 0.45 (20 mg of protein) was mixed with 2.5 ml (packed volume) of ampicillin-linked CH Sepharose 4B in 10 ml (final volume) of 7.5 mM-potassium phosphate buffer, pH 7.5, containing 0.0125% Cetavlon. The suspension was stirred gently for 15 min at 22°C, poured in a small column and the column was washed at 22°C with 20 ml of 30 mM-potassium phosphate buffer, pH 7.5, containing 0.05% Cetavlon. The effluent contained about 10% of the original transpeptidase activity. After further washing with 15 ml of the above phosphate buffer containing 0.05% Cetavlon and 1 M-NaCl, the column, with 90% of the original transpeptidase bound to it, was treated at 22°C, with 2.5 ml samples of 0.5 M-Tris/HCl buffer, pH 7.8, containing 0.05% Cetavlon and 1 M-hydroxylamine, successively for 1 min (eluent 1), 2 min (eluent 2), 4 min (eluent 3) and then three times for 10 min each time (eluents 4–6). The eluents were separately dialysed against 30 mM-potassium phosphate buffer, pH 7.5, containing 0.05% Cetavlon and concentrated by ultrafiltration to about 300 μl.

Measurements of carboxypeptidase and transpeptidase activities

All the reactions were carried out at 37°C in 30 μl (final volume) of 10 mM-potassium phosphate buffer, pH 7.5. In the standard procedure, the carboxypeptidase and transpeptidase activities of the enzyme preparations were estimated in separate experiments by measuring either the amount of [^{14}C]Ac_2-L-Lys-D-Ala liberated from 1.5 mM-[^{14}C]Ac_2-L-Lys-D-Ala-D-Ala (sp. radioactivity 0.8 Ci/mol) or the amount of Ac_2-L-Lys-D-Ala-[^{14}C]Gly-Gly formed from 1.5 mM-Ac_2-L-Lys-D-Ala-D-Ala and 1.5 mM-[^{14}C]Gly-Gly (sp. radioactivity 1.9 Ci/mol) (for more details, see Leyh-Bouille *et al.*, 1977). The transpeptidation/carboxypeptidation ratio expresses the relative efficacy of the enzyme preparations to perform transpeptidase and carboxypeptidase activities. With the purified transpeptidase, the two activities were also estimated by using the co-substrate system 3.6 mM-[^{14}C]Ac_2-L-Lys-D-Ala-D-Ala (i.e. at a concentration equivalent to the K_m value) and 3.6 mM-*meso*-A_2pm and measuring the amounts of [^{14}C]Ac_2-L-Lys-D-Ala-(D)-*meso*-A_2pm

(transpeptidation) and [^{14}C]Ac_2-L-Lys-D-Ala (carboxypeptidation) formed concomitantly during the reaction (for the experimental conditions, see Leyh-Bouille *et al.*, 1981).

Measurement of β-lactamase activity

Samples were incubated with 1mM-[^{14}C]benzylpenicillin at 30°C in 10mM-potassium phosphate buffer, pH7.5, and the amount of [^{14}C]benzylpenicilloate formed was estimated after separation by paper electrophoresis (Marquet *et al.*, 1974).

Results

PBP pattern of the isolated membranes

The plasma membranes of *Streptomyces* K15 contained multiple proteins (Fig. 1, track 1). At least ten of them bound [^{14}C]benzylpenicillin and behaved as PBPs (Fig. 1, track 2). The most important PBPs had apparent molecular weights of 60000, 54000, 50000 (a doublet), 39000 and 26000 respectively. The two proteins that formed the 50000-M_r doublet showed identical properties with respect to their penicillin sensitivity and thermolability (see below). Saturating experiments (Fig. 2) showed that the 50000-, 54000- and 26000-M_r PBPs were half-saturated at 0.8, 4 and 25μM-[^{14}C]benzylpenicillin concentrations respectively, and that both 60000- and 39000-M_r PBPs were not saturated at 6mM-[^{14}C]benzylpenicillin. At 0.3mM-[^{14}C]benzylpenicillin, the relative abundance of the three penicillin-sensitive 54000-, 50000- and 26000-M_r PBPs was 12, 33 and 55% respectively (Table 1). At 6mM-[^{14}C]benzylpenicillin, the two highly penicillin-resistant 60000- and 39000-M_r PBPs largely superseded all the other PBPs (Table 1). A pre-incubation (before reaction with [^{14}C]benzylpenicillin) of freshly isolated membranes for 2h at 37°C and pH7.5 was sufficient to cause the loss of all the PBPs except the 39000- and 26000-M_r PBPs, which showed thermostability during prolonged incubations (up to 48h) under these conditions (Fig. 1, track 3). The presence of phenylmethanesulphonyl fluoride (0.33mM) had no effect.

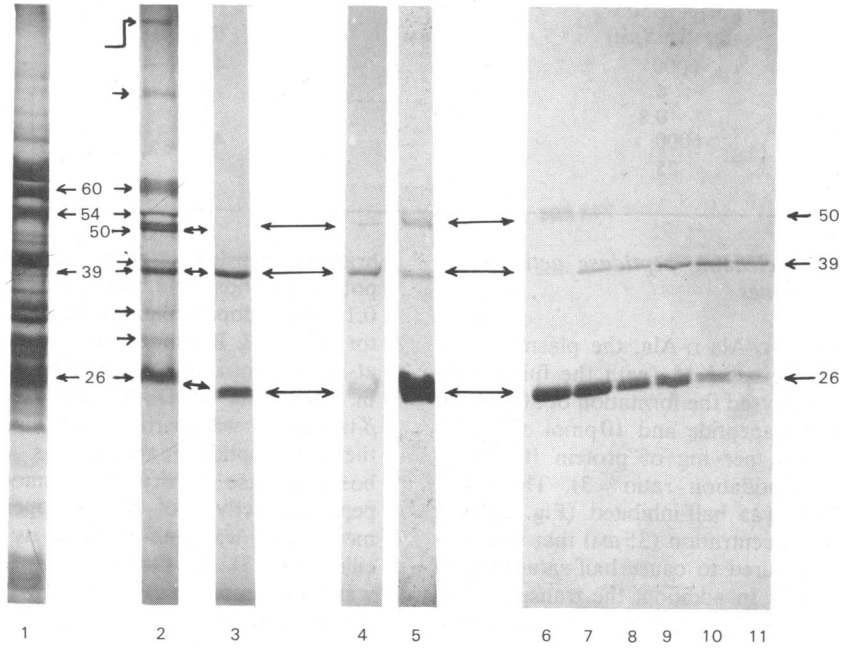

Fig. 1. *Sodium dodecyl sulphate/polyacrylamide-gel-electrophoresis analysis of (i) the isolated plasma membranes of Streptomyces K15 (tracks 1–3), (ii) fraction K_D 0.45 isolated from a Cetavlon extract of the mycelium (tracks 4–5) and (iii) the eluates obtained by successive hydroxylamine treatments of a column of ampicillin-linked Sepharose to which 90% of the transpeptidase activity of fraction K_D 0.45 had been previously bound (tracks 6–11)*

Tracks 1, 4 and 6–11 are protein patterns. Tracks 2, 3 and 5 are PBP patterns as obtained at a 0.3mM-[^{14}C]benylpenicillin concentration. The time of exposure of the gels to X-ray films (at −70°C) was 5 weeks (tracks 2 and 3) and 7 days (track 5). In track 3, the membranes were pre-incubated at 37°C and pH7.5 for 24h before sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The amounts of protein used were: 214μg (tracks 1–3); 15μg (tracks 4 and 5); 4μg (track 6); 3.25μg (track 7); 1.5μg (track 8); 2μg (track 9); 1.2μg (track 10); 0.7μg (track 11). The numbers by the arrows in the photograph represent $10^{-3} \times M_r$.

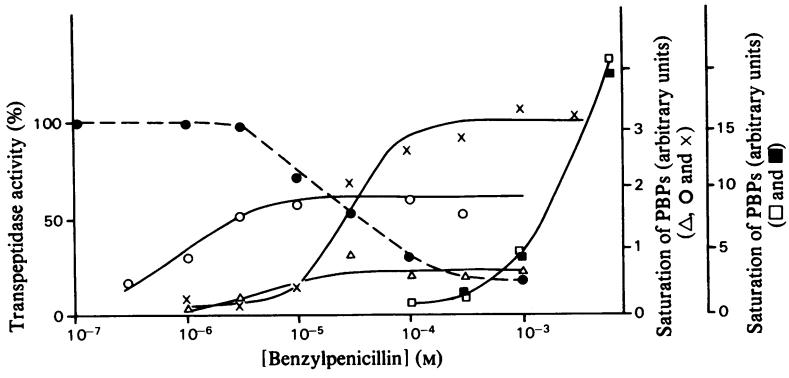

Fig. 2. *Saturation of the membrane-bound PBPs (continuous lines) and inhibition of the membrane-bound transpeptidase activity (broken line) by increasing concentrations of [^{14}C]benzylpenicillin and non-radioactive benzylpenicillin respectively*

□, 60000-M_r PBP; △, 54000-M_r PBP; ○, 50000-M_r PBP; ■, 39000-M_r PBP; ×, 26000-M_r PBP. The amount of proteins used was 214 μg (for PBPs) or 947 μg (for enzyme activity).

Table 1. *PBP pattern of the isolated membranes of Streptomyces K15*

M_r of PBP	[^{14}C]Benzylpenicillin concentrations required to achieve 50% saturation (μM)	Relative abundance (%) at [^{14}C]benzylpenicillin concentrations of: 0.3 mM	6 mM	Effect of pre-incubation of the membranes for 2 h at 37°C and pH 7.5
60000	>6000		44	Thermolabile
54000	4	12	2	Thermolabile
50000 (doublet)	0.8	33	3	Thermolabile
39000	>6000		44	Thermostable
26000	25	55	7	Thermostable

D-Alanyl-D-alanine-cleaving peptidase activities of the isolated membranes

From Ac_2-L-Lys-D-Ala-D-Ala, the plasma membranes of *Streptomyces* K15 (with the full assortment of PBPs) catalysed the formation of 30 pmol of transpeptidated tetrapeptide and 10 pmol of hydrolysed dipeptide/min per mg of protein (transpeptidation/carboxypeptidation ratio = 3). The transpeptidase activity was half-inhibited (Fig. 2) at a benzylpenicillin concentration (35 μM) that was very similar to that required to cause half-saturation of the 26000-M_r PBP. In addition, the transpeptidase activity showed high thermostability at 37°C and pH 7.5. On this basis, in the saturating experiments shown in Fig. 2, the 100% level of transpeptidase activity, as measured in the absence of benzylpenicillin, was adjusted to the 100% level of labelling of the 26000-M_r PBP, as measured at saturating concentrations of [^{14}C]benzylpenicillin.

The plasma membranes of *Streptomyces* K15 showed β-lactamase activity, hydrolysing benzylpenicillin (at an initial concentration of 1 mM) at a rate of 0.87 nmol/min per mg of protein. A membrane suspension (22.8 mg of protein in 3.2 ml of potassium phosphate buffer) was supplemented with 0.1 mM-β-iodopenicillanate (a β-lactamase inactivator; Moore & Brammer, 1981), incubated for 10 min at 37°C, centrifuged, washed with, and resuspended in, phosphate buffer. At least 80% of the original β-lactamase was permanently inactivated, whereas the transpeptidase activity (as well as the carboxypeptidase activity) was unmodified. The transpeptidase activity of the β-iodopenicillanate-treated membranes was half-inhibited by 5 μM-benzylpenicillin (Fig. 3). As shown below, this concentration was that required to inhibit by 50% the purified transpeptidase.

The inhibition by benzylpenicillin of the carboxypeptidase activity of the β-iodopenicillanate-treated membranes appeared to be biphasic (Fig. 3), suggesting the presence of two enzymes of different penicillin sensitivities. The highly penicillin-sensitive carboxypeptidase activity was half-inhibited at about 0.2 μM-benzylpenicillin and was thermolabile (after 16 h of incubation at 37°C and pH 7.5; results not shown). It was tentatively attributed to the 50000-M_r PBP. In turn, the moderately penicillin-sensitive

carboxypeptidase activity was half-inhibited at about 10 μM-benzylpenicillin and was thermostable (under the same conditions as above). Most likely, at least part of it was attributable to the 26000-M_r PBP.

Isolation of the 26000-M_r PBP/D-alanyl-D-alanine-cleaving transpeptidase (Table 2)

Fraction K_D 0.45 was obtained by direct treatment of the mycelium with Cetavlon and filtration of the extract on Sephadex G-100 in the presence of the detergent (see the Materials and methods secton). It had no detectable β-lactamase activity and, in agreement with previous studies (Leyh-Bouille *et al.*, 1977), it was substantially enriched in transpeptidase activity. From Ac_2-L-Lys-D-Ala-D-Ala, it catalysed the formation of 14nmol of transpeptidated tetrapeptide and 1.7nmol of hydrolysed dipeptide/min per mg of protein (transpeptidation/carboxypeptidation = 8).

Sodium dodecyl sulphate/polyacrylamide-gel-electrophoresis analysis and microdensitometry measurements of the Coomassie-stained gels (Fig. 1, track 4) revealed that fraction K_D 0.45 contained essentially only the 39000- and 26000-M_r PBPs occurring in the ratio 0.7:1. This is in contrast with a ratio of at least 7:1 found in the isolated membranes after reaction with 6mM-[^{14}C]benzylpenicillin and densitometry measurements of the fluorograms. The 39000-M_r PBP was not saturated by 6mM-benzylpenicillin, whereas the 26000-M_r PBP was half-saturated and both transpeptidase and carboxypeptidase activities were half-inhibited at very similar (40–15 μM) benzylpenicillin concentrations (Fig. 4). Fraction K_D 0.45 also contained trace amounts of a diffused 50000-M_r doublet of PBPs (Fig. 1, tracks 4 and 5). This latter doublet was distinguished from the 50000-M_r PBP doublet present in the membranes by a low affinity for benzylpenicillin and by a high thermostability. The

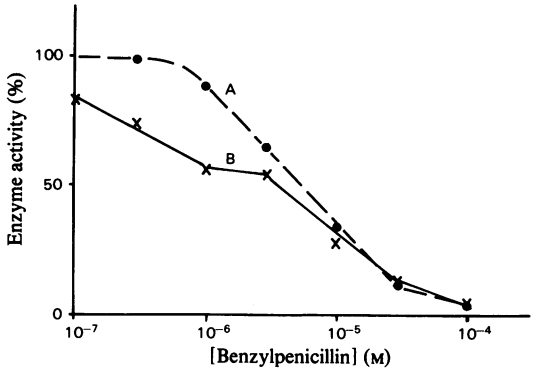

Fig. 3. *Inhibition of the transpeptidase (A) and carboxypeptidase (B) activities of the β-iodopenicillanate-treated membranes of Streptomyces K15 by increasing concentrations of benzylpenicillin*
For more details, see the text.

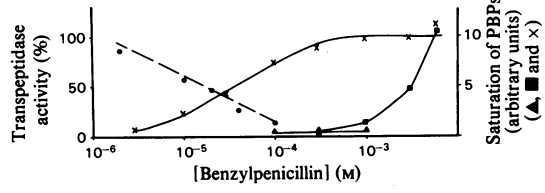

Fig. 4. *Saturation of the 26000-, 39000- and 50000-M_r PBPs of fraction K_D 0.45 (continuous lines) and inhibition of the transpeptidase activity of the same fraction K_D 0.45 (broken line) by increasing concentrations of [^{14}C]benzylpenicillin and non-radioactive benzylpenicillin respectively*
▲, 50000-M_r PBP; ■, 39000-M_r PBP; ×, 26000M_r PBP. The amount of proteins used was 15 μg (for PBPs) or 7.5 μg (for enzyme activities). (Note that the above 50000-M_r PBP is probably different from the membrane-bound 50000-M_r PBP of Fig. 2.)

Table 2. *Purification of the D-alanyl-D-alanine-cleaving transpeptidase of Streptomyces K15*
Abbreviations: Tpase, transpeptidase activity; Cbpase, carboxypeptidase activity; T/C, transpeptidation/carboxypeptidation. All the experiments were carried out at the substrate concentrations indicated in the Materials and methods section.

Enzyme preparation	Total protein (mg of protein/litre of culture)	Total activity (nmol/min per litre of culture)		Specific activity (nmol/min per mg of protein)		T/C	Enrichment in transpeptidase activity (fold)
		Tpase	Cbpase	Tpase	Cbpase		
Isolated membranes	70	2.1	0.7	0.03	0.01	3	1
Mycelium extract (Cetavlon)							
Original extract	28	14	14	0.5	0.5	1	17
Fraction K_D 0.45*	0.8	11.7	1.4	14	1.7	8	470
Purified transpeptidase (90% purity)	0.01	6.5	0.38	250	14.5	17	8300

* The protein concentration was determined by measuring A_{260} and A_{280}.

doublet could not be detected after reaction with 10 μM-[^{14}C]benzylpenicillin, whereas at this antibiotic concentration the membrane-bound 50000-M_r PBP doublet was saturated and its ability to fix penicillin was not lost after pretreating fraction K_D 0.45 for 10 min at 50°C.

Further purification of the 26000-M_r PBP was carried out by affinity chromatography on ampicillin-linked CH Sepharose 4B under the conditions described in the Materials and methods section. The procedure made use of the fact that this PBP had a higher propensity to form a stable adduct with ampicillin than the 39000-M_r PBP and that the adduct formed with the 26000-M_r PBP was more susceptible to hydroxylaminolysis than that formed with the 39000-M_r PBP. Most (90%) of the 26000-M_r PBP/transpeptidase of fraction K_D 0.45 was fixed on the ampicillin-linked column and about 56% of it was recovered in eluates 1, 2 and 3 (Fig. 1, tracks 6–8). These three eluents had virtually the same high specific transpeptidase activity, catalysing the conversion of 250, 220 and 230 nmol of tripeptide/min per mg of protein respectively. In turn, eluates 4–6 (Fig. 1, tracks 9–11) contained most of the 39000-M_r PBP that had been absorbed on the affinity column. They exhibited low and decreasing specific transpeptidase activities of 150, 75, 44 and 10 nmol/min per mg of protein respectively. Altogether, they contained 9% of the transpeptidase activity present in fraction K_D 0.45.

About 90% of the protein content of eluates 1, 2 and 3 consisted of the 26000-M_r PBP. However, minor amounts of both 50000-M_r PBP doublet and 39000-M_r PBP were still present and occurred in various proportions in different eluates. Eluate 1, for example, contained 3% of the 39000-M_r PBP and 7% of the 50000-M_r doublet PBPs (Fig. 5). Varying the temperature (100, 60 and 37°C) during pretreatment of the eluate with sodium dodecyl sulphate and mercaptoethanol before sodium dodecyl sulphate/polyacrylamide-gel electrophoresis had no effect on the protein or PBP pattern. Omitting mercaptoethanol during pretreatment at 100°C made it impossible to detect the minor 50000-M_r doublet PBPs by Coomassie staining or fluorography of the gel, but had no effect on the 26000-M_r PBP (nor the residual 39000-M_r PBP).

Properties of the 26000-M_r PBP/transpeptidase

The final 26000-M_r PBP preparation (90% purity) behaved essentially as a transpeptidase. Its ability to react with water and perform carboxypeptidase activity was low. It exhibited a transpeptidation/carboxypeptidation ratio of 17 when the two activities were estimated in separate experiments (standard procedure) and of at least 50 when they were estimated concomitantly with the co-substrate system [^{14}C]Ac_2-L-Lys-D-Ala-D-Ala + *meso*-A_2pm (see the Materials and methods section). Both transpeptidase and carboxypeptidase activities were inhibited by 50%, and the 26000-M_r protein was half-saturated by very similar benzylpenicillin concentrations (5 and 15 μM respectively).

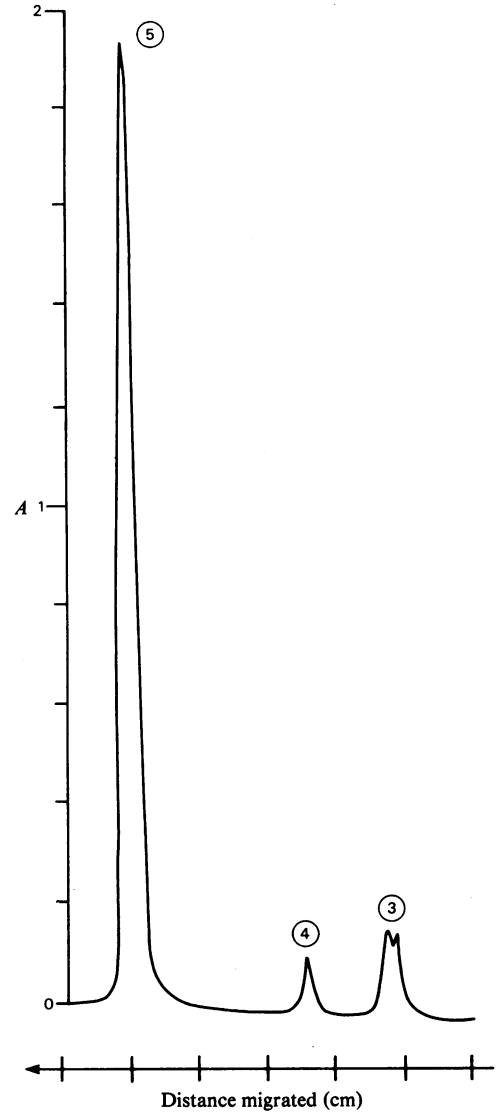

Fig. 5. *Relative abundance of the 26000-M_r PBP (peak 5), 39000-M_r PBP (peak 4) and 50000-M_r doublet PBPs (peak 3) in eluate 1 (see track 6 in Fig. 1) obtained by hydroxylamine treatment of ampicillin-linked Sepharose to which 90% of the transpeptidase activity of fraction K_D 0.45 had been bound previously*

Microdensitometry (at 550 nm) of the gel was performed after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and Coomassie staining. The amount of protein used was 8 μg.

Heating the preparation for 10min at 50°C did not affect the enzyme activity nor the penicillin-binding capacity of the protein but removal of Cetavlon by successive filtrations on Sephadex G-100 in buffer not supplemented with the detergent caused irreversible inactivation.

Previous assays carried out with a non-purified transpeptidase preparation (Dusart *et al.*, 1977) had shown that the K_m values were 4mM for the carbonyl donor Ac_2-L-Lys-D-Ala-D-Ala and 0.2mM for the amino acceptor Gly-Gly. A K_m value of 3.6mM for Ac_2-L-Lys-D-Ala-D-Ala was found with the purified 26000-M_r PBP transpeptidase preparation (results not shown). At saturating concentrations of both carbonyl donor and amino acceptor, the tripeptide was utilized at a maximal velocity of 0.66μmol/min per mg of protein, indicating a catalytic-centre activity of about $0.3\,s^{-1}$.

Discussion

On the basis of the present and previous studies (Dusart *et al.*, 1977; Leyh-Bouille *et al.*, 1977, 1981), *Streptomyces* K15 possesses a set of at least nine major PBPs. Two PBPs (M_r 54000 and 40000) are excreted into the medium during growth; two PBPs (M_r 40000 and 38000) are released from the mycelium during protoplast formation under lysozyme action; and at least five PBPs (M_r 60000, 54000, 50000, 39000 and 26000) are bound to the plasma membrane. All these PBPs show wide variations in their penicillin sensitivity. The four water-soluble (i.e. exocellular and lysozyme-releasable) PBPs, the membrane-bound 26000-M_r PBP and, probably, the membrane-bound 50000-M_r PBP are D-alanyl-D-alanine-cleaving peptidases catalysing the transfer of the Ac_2-L-Lys-D-alanyl group from Ac_2-L-Lys-D-Ala-D-Ala to both water (carboxypeptidase activity) and a proper amino nucleophile such as Gly-Gly or *meso*-A_2pm (transpeptidase activity). No enzyme activity can be attributed to the membrane-bound 60000-, 54000- and 39000-M_r PBPs, at least with the substrates used. The four water-soluble and probably the membrane-bound 50000-M_r PBPs/peptidases behave mainly as carboxypeptidases. In marked contrast, the membrane-bound 26000-M_r PBP/peptidase functions almost exclusively as a transpeptidase and continues to do so once it has been solubilized by the cationic detergent Cetavlon. This 26000-M_r PBP/transpeptidase thus appears to be able to scavenge amino groups present in millimolar concentration in a 55.5M-H_2O medium and to effectively use them as acceptors of the transfer reaction. Another remarkable property of the enzyme is its high thermostability (10min at 50°C).

The 26000-M_r PBP/transpeptidase of *Streptomyces* species is analogous to the 90000-M_r PBP 1B of *Escherichia coli* (Nakagawa *et al.*, 1979*a*,*b*) in several respects. Indeed, this *E. coli* PBP is a transpeptidase apparently devoid of carboxypeptidase activity (note that as it has been isolated, it also performs a penicillin-insensitive transglycosylase activity); it shows high thermostability and is active in the presence of the anionic detergent sodium dodecyl *N*-sarcosinate (Sarkosyl). Cetavlon and Sarkosyl are known to be powerful denaturing agents for most enzymes and proteins. Finally, both the *Streptomyces* 26000-M_r PBP/transpeptidase (Marquet *et al.*, 1974) and the *E. coli* 90000-M_r PBP/transpeptidase (Spratt, 1980) seem to play important roles in the life cycle of the corresponding organisms and appear to be important targets for penicillin action.

Direct treatment of the mycelium of *Streptomyces* K15 is a much more effective means of isolating the membrane-bound 26000-M_r PBP/transpeptidase than a procedure involving prior isolation of the plasma membranes. The extracted PBP/transpeptidase can then be isolated to 90% purity by a two-step procedure (Sephadex filtration and affinity chromatography) with a yield of about 50% and, at least, an 8000-fold specific enrichment (when compared with the transpeptidase activity of the isolated membranes).

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