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# Identification of the Major Penicillin-Binding Proteins of *Escherichia coli* as D-Alanine Carboxypeptidase IA

BRIAN G. SPRATT[1] AND JACK L. STROMINGER*

*Moffett Laboratories, Department of Biochemical Sciences, Princeton University, Princeton, New Jersey 08540, and The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138**

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Penicillin-binding proteins 5 and 6 of *Escherichia coli* have been identified as D-alanine carboxypeptidase IA.

Beta-lactam antibiotics exert their lethal effects on bacteria by inhibiting enzymes involved in the terminal stages of peptidoglycan metabolism (2). Recently a more precise understanding of the interaction of penicillins with bacterial cells has been achieved through the study of penicillin-sensitive enzymes (2, 4, 7) and penicillin-binding proteins (1, 2, 5-8; B. G. Sprott and A. B. Pardee, Fed. Proc. 34:668, 1975). Since penicillins are thought generally to acylate the enzymes that they inhibit (2), penicillin-binding proteins and penicillin-sensitive enzymes should in most cases be identical.

In *Escherichia coli*, three enzymatic activities are known to be penicillin sensitive (2): D-alanine carboxypeptidase I, peptidoglycan transpeptidase, and endopeptidase. D-alanine carboxypeptidase I activities have recently been purified from *E. coli*, and their properties have been described (5, 7, 8).

Pure carboxypeptidase IA was a doublet with apparent molecular weights of 32,000 and 34,000 on a sodium dodecyl sulfate (SDS)-polyacrylamide gradient gel (8). The enzyme is membrane bound, sensitive to beta-lactam antibiotics, and binds [$^{14}$C]benzylpenicillin reversibly. It also catalyzes a transpeptidase reaction and is a weak penicillinase (8). Carboxypeptidase IB is loosely membrane associated and can be extracted with LiCl; an apparently identical enzyme (carboxypeptidase IC) is found in the cytoplasm in larger amounts. This enzyme is more sensitive to beta-lactam antibiotics than carboxypeptidase IA; it has endopeptidase activity but is only a weak transpeptidase. It fails to bind [$^{14}$C]benzylpenicillin, although it is a weak penicillinase (8).

Since carboxypeptidase IA is membrane bound and binds [$^{14}$C]benzylpenicillin, it should be detected in studies of the penicillin-binding proteins of the *E. coli* cell envelope. Six penicillin-binding proteins have been detected in the inner membrane of *E. coli* (5, 6). Binding proteins 5 and 6 have several properties which suggest that these proteins are identical with carboxypeptidase IA. (i) The binding proteins (5 and 6) and carboxypeptidase IA are both membrane bound. (ii) Both bind [$^{14}$C]benzylpenicillin and release the bound penicillin with similar half-times (5 and 15 min at 30 C for binding proteins 5 and 6, respectively (B. Spratt, unpublished data) and 5 min at 37 C for carboxypeptidase IA (8). (iii) Both are doublets on SDS-polyacrylamide gels with molecular weights between either 32,000 and 34,000 (10) or 40,000 and 42,000 (B. C. Spratt and A. B. Pardee, Fed. Proc. 34:668, 1975).

Since the reported molecular weights of the carboxypeptidase IA doublet and binding proteins 5 and 6 were rather different, we have compared their mobility on the same SDS-polyacrylamide slab gel. [$^{14}$C]benzylpenicillin was bound to purified cell envelopes (prepared from *E. coli* KN126 - a K-12 strain - as previously described [5]) and to pure carboxypeptidase IA (prepared by Tamura et al. [8] from *E. coli* H2134 - a K-12 strain), and the proteins were fractionated as described in the legend to Fig. 1. The gel was stained with Coomassie brilliant blue, and an autoradiograph was made. Both components of the carboxypeptidase IA doublet bound [$^{14}$C]benzylpenicillin (Fig. 1a, slot a), and the mobilities of the doublet were identical to those of binding proteins 5 and 6 (Fig. 1a, slot c). The stained gel showed that the carboxypeptidase 1A doublet (Fig. 1b, slot a, b) corresponded to two minor bands in the inner membrane (Fig. 1b, slot c).

Figure 2 shows that the apparent subunit molecular weights of the doublet and binding proteins 5 and 6 were 40,000 and 42,000 when compared with eight molecular-weight standards. It is not clear why the molecular weight

[1] Present address: Department of Genetics, University of Leicester, Leicester LE1 7RH, England.

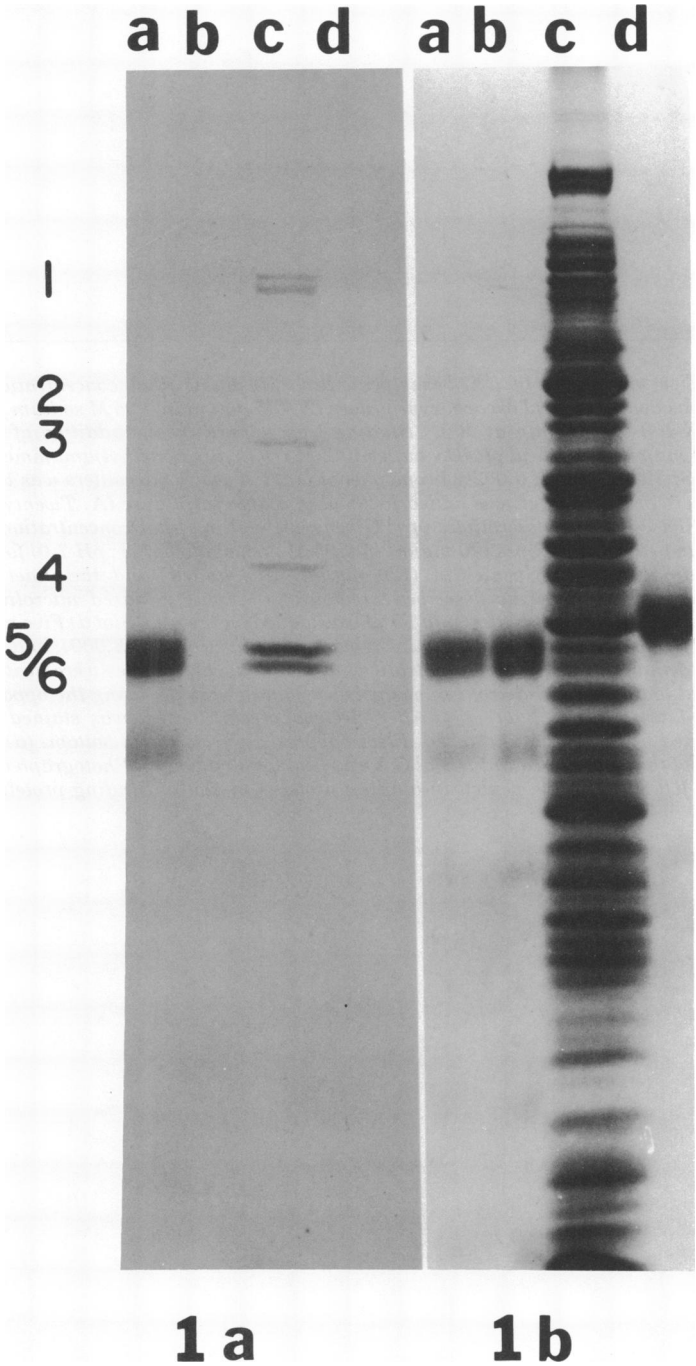
a b c d a b c d
1
2
3
4
5/6
1a
1b

FIG. 1. *(Slot a) Five microliters of [$^{14}$C]benzylpenicillin (31 μg/ml final concentration; 54 mCi/mM, Amersham/Searle) was bound to 50 μl of carboxypeptidase IA (0.5 mg/ml in 0.05 M sodium phosphate buffer, pH 7.0; 1% Triton X-100) for 10 min at 30 C. Binding was stopped by the addition of 5 μl of unlabeled benzylpenicillin (120 mg/ml) and 30 μl of SDS buffer (0.2 M tris(hydroxymethyl)aminomethane-hydrochloride, pH 6.8; 3% SDS; 30% glycerol; 0.002% bromophenol blue). Twenty microliters was loaded on the gel. (Slot b) Ten microliters of SDS buffer was added to 20 μl of carboxypeptidase IA. Twenty microliters were loaded on the gel. (Slot c) Twenty microliters of [$^{14}$C]benzylpenicillin (final concentration, 31 μg/ml) was bound to 200 μl of purified cell envelopes (20 mg/ml in 0.05 M phosphate buffer, pH 7.0) for 10 min at 30 C. Five microliters of unlabeled benzylpenicillin (120 mg/ml) was added, and the inner membrane was selectively solubilized with Sarkosyl as described previously (5). One hundred microliters of the inner membrane was added to 50 μl of SDS buffer, and 55 μl was loaded on the gel. (Slot d) Five microliters of SDS buffer was added to 100 μl of ovalbumin (1 mg/ml; Sigma; molecular weight, 43,000), and 10 μl was loaded on the gel. β-mercaptoethanol (10% final concentration) was added, the samples were heated to 100 C for 2 min, and then applied to a 12% SDS-discontinuous polyacrylamide slab gel using the apparatus and buffer system described by Laemmli and Favre (3). After electrophoresis, the gel was stained with Coomassie brilliant blue, destained by diffusion, and dried. Electrophoresis is from top to bottom. (a) Autoradiograph prepared by exposure of the gel to Ilford industrial G X-ray film for 30 days. (b) Photograph of the stained gel. The numbers on the left indicate the penicillin-binding proteins in slot c. Binding protein 1 appears as a doublet on this gel.*

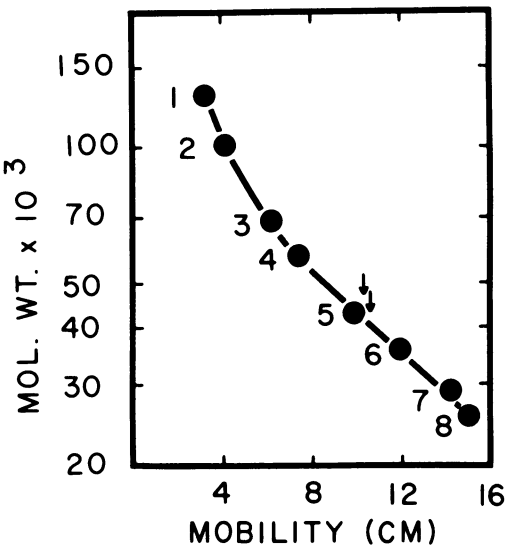


FIG. 2. *Ten microliters of* D-*alanine carboxypeptidase IA (300 μg/ml in SDS buffer) was subjected to electrophoresis on a 12% SDS-polyacrylamide slab gel together with 20 μl of a solution containing the following standard proteins (each protein, 80 μg/ml): (1) β-galactosidase (molecular weight, 130,000), (2) phosphorylase a (molecular weight, 100,000), (3) serum albumin (molecular weight, 68,000), (4) pyruvate kinase (molecular weight, 57,000), (5) ovalbumin (molecular weight, 43,000), (6) glyceraldehyde-3-phosphate dehydrogenase (molecular weight, 36,000), (7) carbonic anhydrase (molecular weight, 29,000), and (8) chymotrypsinogen (molecular weight, 25,700). The mobilities of the proteins are plotted against the log (molecular weight). Arrows show the position of the two components of* D-*alanine carboxypeptidase IA.*

reported here differs from one of the previous estimates (8), although different standard proteins and a different gel system were used.

D-Alanine carboxypeptidase IA is identical with penicillin-binding proteins 5 and 6 in *E. coli*. It is not clear why the protein forms a doublet on SDS-polyacrylamide gels. The situation in *E. coli* is similar to that in *Bacillus subtilis:* the major penicillin-binding protein corresponds to a D-alanine carboxypeptidase (1). In both organisms the major carboxypeptidase (IB in the case of *E. coli*) appears to be unimportant for cell growth (1, 5; M. Iwaya, T. Tamura, and J. L. Strominger, Fed. Proc. 33: 1240, 1974). Minor penicillin-sensitive enzymes are probably involved in the lethal effect of beta-lactam antibiotics in these organisms (1, 2, 5, 6).

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