one free and the other bound to the fibrin network. A temporary doubling of the number of apparent unfolding units (Table II) may be a reasonable observation under these circumstances.

Privalov & Medved' (1982) found that the transition envelope of isolated heavy D fragments can be deconvoluted into three closely spaced transitions. Our experiments of reversing the calcium effect in thrombin clots suggest that there must be at least two different kinds of unfolding units in the D nodule.

Finally, the reversal experiments also furnish additional proof that stabilization of the D nodules is not related to cross-linking of the clot; otherwise, no reversal would be observed on removal of calcium.

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Purification and Sequencing of the Active Site Tryptic Peptide from Penicillin-Binding Protein 1b of *Escherichia coli*†

Robert A. Nicholas,*,‡ Hideho Suzuki,§ Yukinori Hirota,‖ and Jack L. Strominger‡

Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138, Laboratory of Genetics, Department of Biology, Faculty of Science, University of Tokyo, Hongo, Tokyo 113, Japan, and National Institute of Genetics, Mishima, Shizuoka-ken 411, Japan

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ABSTRACT: This paper reports the sequence of the active site peptide of penicillin-binding protein 1b from *Escherichia coli*. Purified penicillin-binding protein 1b was labeled with [^{14}C]penicillin G, digested with trypsin, and partially purified by gel filtration. Upon further purification by high-pressure liquid chromatography, two radioactive peaks were observed, and the major peak, representing over 75% of the applied radioactivity, was submitted to amino acid analysis and sequencing. The sequence Ser-Ile-Gly-Ser-Leu-Ala-Lys was obtained. The active site nucleophile was identified by digesting the purified peptide with aminopeptidase M and separating the radioactive products on high-pressure liquid chromatography. Amino acid analysis confirmed that the serine residue in the middle of the sequence was covalently bonded to the [^{14}C]penicilloyl moiety. A comparison of this sequence to active site sequences of other penicillin-binding proteins and β-lactamases is presented.

The cytoplasmic membrane of *Escherichia coli* contains seven proteins that form a covalent bond with β-lactam antibiotics (Blumberg & Stromiger, 1974; Spratt & Pardee, 1975). These proteins, termed PBPs,[1] can be detected by incubation of *E. coli* membranes with [^{14}C]penicillin G, followed by $NaDodSO_4$–polyacrylamide gel electrophoresis and fluorography. PBPs are involved in the last stages of cell wall biosynthesis and the cross-linking of the peptidoglycan layer. Penicillin exerts its lethal effect by binding to these PBPs and

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‡ Department of Biochemistry and Molecular Biology, Harvard University.
§ Laboratory of Genetics, University of Tokyo.
‖ National Institute of Genetics.

[1] Abbreviations: PBP, penicillin-binding protein; $NaDodSO_4$, sodium dodecyl sulfate; CPase, D-alanine carboxypeptidase; TPCK trypsin, trypsin treated with L-1-(tosylamido)-2-phenylethyl chloromethyl ketone; PTH, phenylthiohydantoin.

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reacting with the proteins to form a stable covalent bond. The original hypothesis of Tipper & Strominger (1965) predicted that penicillin is a substrate analogue of the D-alanyl-D-alanine carboxy terminus of the growing peptidoglycan layer and that it reacts with an active site nucleophile on the transpeptidase involved in the final cross-linking of the cell wall. This hypothesis was later reinforced when both penicillin and the substrate analogue diacetyl-L-lysyl-D-alanyl-D-lactate were shown to react with the same serine residue in D-alanine carboxypeptidase (CPase), a low molecular weight PBP from *Bacillus subtilis* (Waxman & Strominger, 1980).

The seven PBPs of *E. coli* are numbered in order of decreasing apparent molecular weight: 1a, 1b, and 2–6. The role that each PBP has in maintaining cell viability has been studied by a number of different laboratories [reviewed in Waxman & Strominger (1983)]. PBPs 1a and 1b appear to be involved in cell elongation and are probably the major transpeptidases of the cell. A mutant missing either 1a or 1b, but not both, was shown to grow normally, and thus each of these PBPs is able to compensate for the absence of the other (Suzuki et al., 1978). PBP 2 is important in maintaining the rod shape of the bacterium and is unusual in that it is a highly specific target for the amidinopenicillin mecillinam (FL 1060) (Suzuki et al., 1978). PBP 3 has a role in septation at cell division. All four of these higher molecular weight PBPs are essential for cell propagation. First PBP 1b (Nakagawa et al., 1979; Tamura et al., 1980) and then PBP 1a (Ishino et al., 1980) was shown to catalyze both a penicillin-sensitive transpeptidase reaction and a penicillin-insensitive transglycosylase reaction using the lipid-linked precursor undecaprenol pyrophosphoryl disaccharide pentapeptide. Subsequently, PBP 3 was reported to possess similar catalytic activity (Ishino et al., 1981). Such bifunctionality has not been substantiated in PBP 2, although mecillinam-sensitive transpeptidation was detected in membranes from cells that overproduced PBP 2 (Ishino et al., 1982). PBPs 4, 5, and 6 appear to be the major carboxypeptidases in the cell membrane (Tamura et al., 1976; Matsuhashi et al., 1977, 1978), but although they account for over 85% of the total amount of [^{14}C]penicillin G bound to *E. coli* membranes (Spratt & Pardee, 1975), they do not appear to be essential for cell viability.

PBP 1b consists of three bands, termed α, β, and γ, on NaDodSO$_4$–polyacrylamide gels (Suzuki et al., 1978). It has been shown, however, that these three bands are the products of one gene (*ponB*), since a single mutation in the *ponB* gene results in the simultaneous loss of all three bands (Suzuki et al., 1978; Tamaki et al., 1977). There also exists a *ponB* mutant that produces PBP 1b with a higher electrophoretic mobility and yet with the same relative mobility among its three components (Suzuki et al., 1978). Each of the three components has been isolated from NaDodSO$_4$–polyacrylamide gels, reactivated, and shown to possess both transpeptidase and transglycosylase activities (Nakagawa & Matsuhashi, 1982). Recently, mapping and deletions of the *ponB* gene have suggested that the heterogeneity observed in α and γ is due to two different initiation sites within the *ponB* gene, and thus, these components probably differ at their amino termini (Kato et al., 1984). The origin of the β component was not as obvious, and may be due to proteolysis or posttranslational modification.

The sequence of the active site in the transpeptidase domain of PBP 1b has been undertaken. By utilizing both gel filtration and HPLC, the [^{14}C]penicilloyl peptide from PBP 1b was isolated and sequenced. The localization of the penicilloyl residue was determined, and this sequence is compared to the sequences of the active sites of other PBPs and β-lactamases.

Experimental Procedures

Materials. HPLC-grade water and CH_3CN were purchased from J. T. Baker; trifluoroacetic and constant-boiling HCl were from Pierce; TPCK trypsin was from Worthington Biochemicals; aminopeptidase M was from Sigma; [^{14}C]penicillin G (K^+ salt) was purchased from Amersham; En^{3}Hance was from New England Nuclear; all other reagents were of analytical-grade quality.

Growth of Bacteria and Preparation of Membranes. An *E. coli* strain carrying pHK231 (Kraut et al., 1981) was grown in L broth with a supply of 1/50 volume of buffered salt solution (Kato et al., 1984). The membranes were prepared as described previously (Tamura et al., 1980).

Purification of PBP 1b. The procedure for purification of PBP 1b was essentially as described previously (Tamura et al., 1980). Main modifications in the procedure were as follows: heat-labile PBPs were inactivated by incubating the membranes at 56 °C for 10 min; solubilization and separation of membrane proteins were carried out first in the absence and then in the presence of 1 M NaCl; PBP 1b was purified from the latter solubilized materials by using Affi-Gel 10 (Bio-Rad) as a ligand carrier for the affinity column.

NaDodSO$_4$–Polyacrylamide Gel Electrophoresis. The gel system of Laemmli (1970), as modified by Suzuki et al. (1978), was employed. The running gel was 8% acrylamide with 0.13% cross-linking, and the stacking gel was 4.5% acrylamide with 0.075% cross-linking. Gels were treated in En^{3}Hance, dried, and submitted to fluorography on Kodak XAR-5 film.

Tryptic Digestion of PBP 1b Labeled with [^{14}C]Penicillin G. PBP 1b (1.25 mL, 1.5 mg), in 100 mM Tris-HCl, pH 7.4, 250 mM NaCl, and 0.1% Triton X-100, was incubated with 60 μL of [^{14}C]penicillin G (54 Ci/mol, 1 mg/mL) for 40 min at 31 °C. Following this incubation, 30 μL of penicillin G (0.32 M) was added, and the mixture was allowed to sit at room temperature for 5 min. Five volumes of cold acetone (−20 °C) was then added, and the precipitate was collected by centrifugation at 3000g for 10 min. The pellet was resuspended in 1.4 mL of water, reprecipitated with acetone, and dried under a gentle stream of N_2.

The acetone precipitate (1.2×10^6 cpm) was dissolved in 100 μL of 88% formic acid, and 3 μL of 10% Triton X-100 was added. Water was added up to 1 mL, and the solution was lyophilized. The lyophilized protein was redissolved in 600 μL of 0.4% NH_4HCO_3, and 25 μL of TPCK-trypsin (10 mg/mL) in the above buffer was added. The solution was incubated at 37 °C for 30 min, another 25 μL of trypsin was added, and following a final 30-min incubation, the digest was lyophilized.

Purification of the Active Site Peptide of PBP 1b by Gel Filtration and HPLC. The tryptic digest was dissolved in 200 μL of 88% formic acid, and 500 μL of 95% ethanol and 3 μL of 2-mercaptoethanol were added. The solution was loaded onto a column of Sephadex LH-20 (1.5 cm × 85 cm) equilibrated with a mixture of formic acid/ethanol (1:4 v/v) and eluted with the same solvent (Khorana et al., 1979; Takagaki et al., 1980). The major peak of radioactivity, PI (6.6×10^5 cpm), was lyophilized and brought up in 600 μL of 0.1% trifluoroacetic acid.

Aliquots of PI were chromatographed on a Vydac 5-μm C_{18} column which was connected to a Waters Associates gradient system equipped with a Model 441 UV detector operating at 214 nm. Fractions of 0.5 mL were collected, and 15 μL of

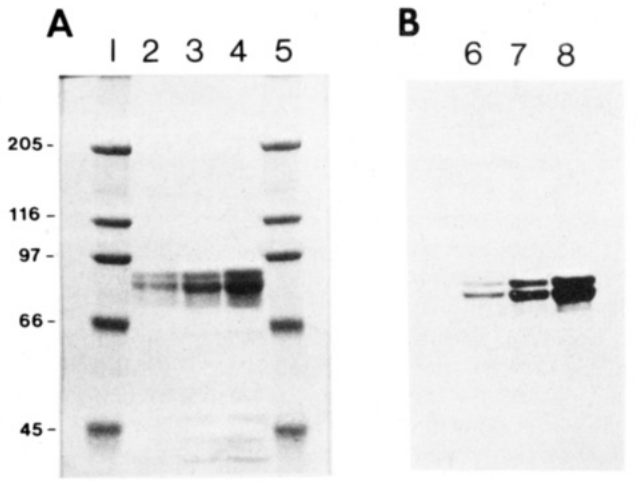

FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoresis of PBP 1b labeled with [^{14}C]penicillin G. PBP 1b labeled with [^{14}C]penicillin G (35 μg, 2 × 10^4 cpm) was submitted to electrophoresis on an 8% $NaDodSO_4$–polyacrylamide gel by the method of Laemmli (1970) as modified by Suzuki et al. (1978). (A) Coomassie Brilliant Blue staining; (B) fluorography; (Lanes 1 and 5) molecular weight standards (numbers are in kdaltons); (lanes 2 and 6) 2.5 μg of protein; (lanes 3 and 7) 5 μg of protein; (lanes 4 and 8) 10 μg of protein.

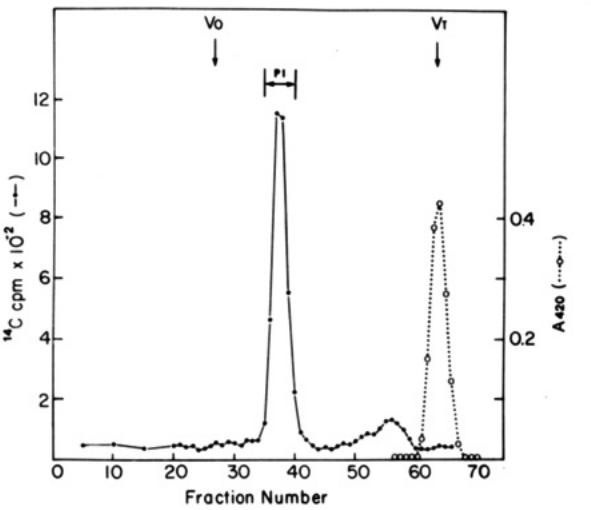

FIGURE 2: Gel filtration of a tryptic digest of [^{14}C]penicillin-labeled PBP 1b. PBP 1b labeled with [^{14}C]penicillin G (1.4 mg, 8.7 × 10^5 cpm) was digested with trypsin in the presence of 0.05% Triton X-100. Following lyophilization, the digest was dissolved in 200 μl of 88% formic acid, and then 500 μL of 95% ethanol and 3 μL of 2-mercaptoethanol were added. The solution was loaded onto a column of Sephadex LH-20 (1.5 × 85 cm) equilibrated in formic acid/ethanol (1:4 v/v), and the tryptic peptides were eluted with the same solvent at 8 mL/h. Fractions of 1.7 mL were collected, and 10 μL was analyzed for radioactivity. The indicated fractions, PI, were pooled for further analysis.

each fraction was analyzed for radioactivity.

Amino Acid Analysis. Selected fractions containing radioactivity were lyophilized and then hydrolyzed in 5.9 M HCl for 24 h at 110 °C. Analyses were performed on a Beckman 121 M amino acid analyzer equipped with an IBM integrator.

Sequencing. Purified [^{14}C]penicilloyl peptide (PI-B, 0.87 nmol) was sequenced on an Applied Biosystems gas-phase sequencer, using polybrene to prevent losses. PTH-amino acids were identified on a Hewlett-Packard HPLC system. All PTH-amino acids could be quantitated except for serine, threonine, and tryptophan, due to their fragmentation during conversion.

Identification of the Active Site Residues. The purified active site peptide, PI-B (2 × 10^5 cpm, 2.1 nmol), was digested with aminopeptidase M (14 μg, 220 milliunits; prepared by dialyzing the ammonium sulfate suspension into 60 mM sodium phosphate, pH 7.0) for 50 min at 37 °C. Two microliters of 88% formic acid was added to terminate the reaction, and the digest was lyophilized. The digest was dissolved in 150 μL of 0.1% trifluoroacetic acid and chromatographed exactly as described above. Fractions containing radioactivity were submitted to amino acid analysis. A blank containing protease but no peptide was treated identically, and the corresponding fractions from HPLC were also submitted to amino acid analysis.

Results

Analysis of Purified PBP 1b. The purity of PBP 1b was assessed by $NaDodSO_4$–polyacrylamide gel electrophoresis. As seen in Figure 1, the protein preparation consisted almost entirely of the several bands of PBP 1b observed previously (Suzuki et al., 1978). There are also several minor bands seen below the bands of PBP 1b, both in Coomassie Blue staining and in fluorography, but they constitute only about 1–5% of the total protein and thus did not interfere with isolation of the active site peptide of PBP 1b.

Isolation and Sequencing of the Active Site Peptide from PBP 1b. Purified PBP 1b, labeled with [^{14}C]penicillin G to a stoichiometry of 0.88 mol/mol protein, was digested with TPCK trypsin in the presence of 0.05% Triton X-100. Figure 2 shows the elution profile when the tryptic digest was chromatographed on Sephadex LH-20 in a formic acid/ethanol mixture (1:4 v/v). The resulting major peak of radioactivity (PI) was lyophilized and submitted to further purification by high-pressure liquid chromatography. When a portion of PI was chromatographed on a Vydac 5-μm C_{18} column, two peaks of radioactivity (PI-A and PI-B) were observed (Figure 3). The major peak (PI-B), representing 77% of the applied radioactivity, was submitted to both amino acid analysis and sequencing. Table I shows that PI-B contained two serine residues and one residue each of glycine, alanine, isoleucine, and lysine. The sequence of PI-B, shown in Table II, was Ser-Ile-Gly-Ser-Leu-Ala-Lys.

Table I: Amino Acid Analysis of Selected Fractions from Figures 3 and 4[a]

amino acid	fraction 116[b]	fraction 101–102[c]
Asp	93.1 (0.2)	33.9 (0.1)
Thr	17.6	ND
Ser	887.7 (1.9)	425.3 (1.0)
Glu	51.9 (0.1)	23.3 (0.1)
Pro	ND	ND
Gly	485.5 (1.1)	85.6 (0.2)
Ala	502.0 (1.1)	453.4 (1.1)
Val	12.3	ND
Met	8.6	ND
Ile	372.6 (0.8)	ND
Leu	456.9 (1.0)	405.7 (1.0)
Tyr	20.0	ND
Phe	25.3	ND
Lys	452.1 (1.0)	11.9
His	7.7	ND
Arg	40.5 (0.1)	53.5 (0.1)

[a] Values are presented as picomoles of amino acid. The values in parentheses represent the moles of amino acid per mole of leucine; no value signifies that the value was less than 0.1 mol of amino acid/mol of leucine. ND, not detected. [b] This HPLC fraction from Figure 3 was lyophilized and then hydrolyzed in 5.7 N HCl at 110 °C for 24 h. [c] These fractions from Figure 4 were lyophilized and hydrolyzed in 5.7 N HCl at 110 °C for 24 h. The values from the corresponding fractions of a blank run were then subtracted.

Localization of the Active Site Serine Residue. Of these amino acids, only serine would be capable of forming a co-

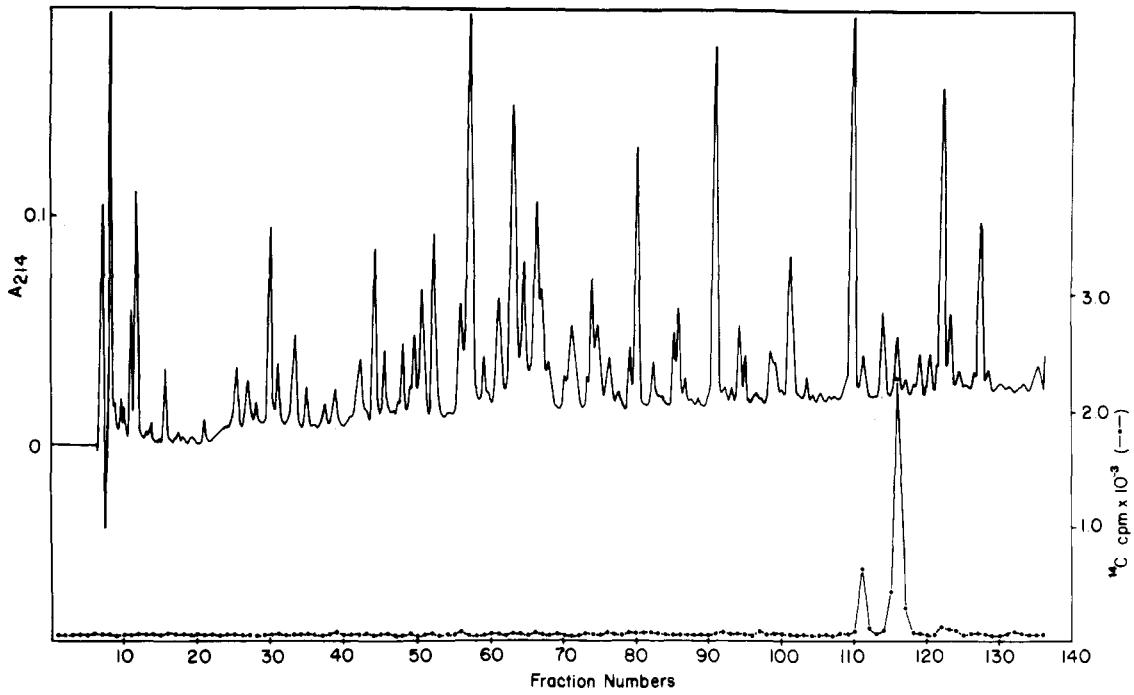

FIGURE 3: Purification of the active site [^{14}C]penicilloyl peptide by HPLC. PI from Figure 2 was dissolved in 500 μL of 0.1% trifluoroacetic acid and injected onto a Vydac 5-μm C_{18} column equilibrated with CH_3CN/H_2O (1:9 v/v) containing 0.1% trifluoroacetic acid. The peptides were eluted with a linear gradient over 75 min from CH_3CN/H_2O (1:9 v/v) containing 0.1% trifluoroacetic acid to CH_3CN/H_2O (3:7 v/v) containing 0.1% trifluoroacetic acid. Fractions of 0.5 mL were collected, and 15 μL was analyzed for radioactivity.

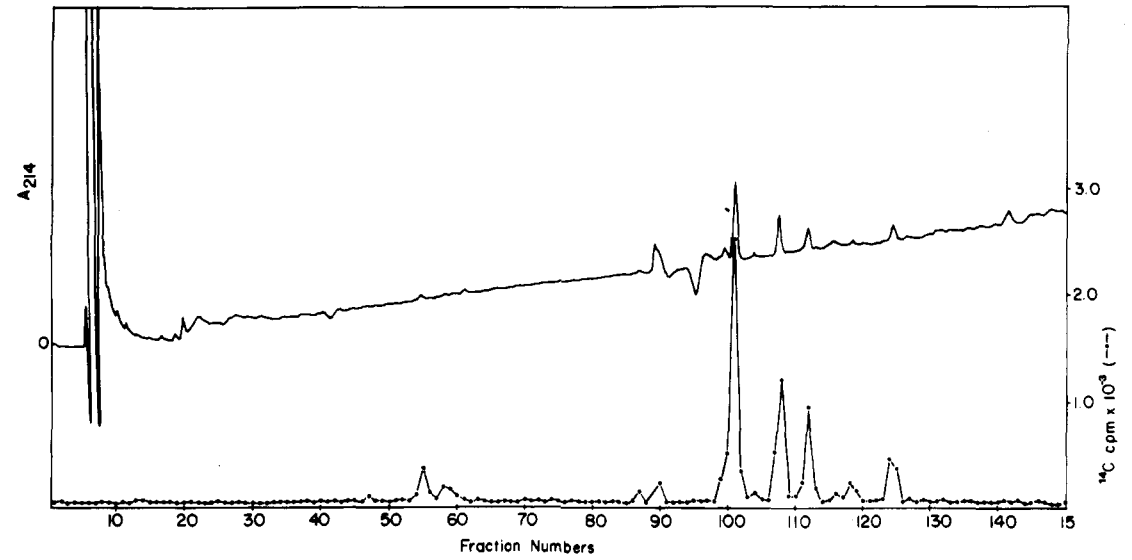

FIGURE 4: Separation by HPLC of the radioactive products from the digestion of purified [^{14}C]penicilloyl peptide using aminopeptidase M. Purified PI-B (2×10^5 cpm) was digested with 220 milliunits of aminopeptidase M for 50 min at 37 °C. The digest was chromatographed on a Vydac C_{18} column exactly as described in Figure 3. Fractions from the major radioactive peak were submitted to amino acid analysis, and the values are shown in Table I.

valent bond with penicillin. The presence of two serine residues, however, makes assigning the active site amino acid equivocal within this sequence. It was therefore necessary to isolate a penicilloyl peptide with only one serine residue in its sequence. This was accomplished by treating PI-B with aminopeptidase M. The specificity of this enzyme predicts that it should sequentially release amino-terminal amino acids until it reaches an amino acid with a bulky substituent (Delange & Smith, 1971). When PI-B was treated with aminopeptidase M and then submitted to HPLC, the profile in Figure 4 was obtained. When the fractions containing the major radioactive peak (fractions 101 and 102) were pooled and analyzed, only serine, leucine, and alanine were obtained (Table I). These amino acids could only be derived from a penicilloyl peptide with the sequence Ser-Leu-Ala, which comprise residues 4, 5, and 6 of PI-B. This result confirms that the serine in the middle of the sequence of PI-B is the active site nucleophile of PBP 1b. It is interesting to note that no lysine was observed; evidently, the commercial preparation of aminopeptidase M also contained carboxypeptidase B activity.

Discussion

The sequence of the active site peptide of PBP 1b has been determined by a combination of gel filtration and HPLC, and the active site nucleophile within this sequence has been identified. Upon elution from HPLC, however, a second radioactive penicilloyl peptide was observed. The possibility that this second peptide was actually a different active site peptide

Table II: Yields and Identification of PTH-Amino Acids from PI-B by Automated Degradation[a]

cycle	PTH-amino acid	amount (pmol)	cycle	PTH-amino acid	amount (pmol)
1	Ser	NQ[b]	5	Leu	780
2	Ile	680	6	Ala	730
3	Gly	900	7	Lys	330
4	Ser	NQ			

[a] The active site peptide (870 pmol of PI-B, Figure 3), purified on HPLC from PI (Figure 2), was submitted to automated degradation on an Applied Biosystems gas-phase sequencer. [b] NQ, not quantitated; the fragmentation of the serine derivative into its characteristic byproducts during conversion prevented the quantitation of this amino acid.

Table III: Active Site Sequences of PBPs and β-Lactamases

enzyme	microorganism	active site sequence
CPase[a]	*B. subtilis*	-Leu-Pro-Ile-Ala-Ser-Met-Thr-Lys-
CPase[b]	*B. stearothermophilus*	-Leu-Gly-Ile-Ala-Ser-Met-Thr-Lys-
PBP 5′[c]	*E. coli*	-Arg-Asp-Pro-Ala-Ser-Leu-Thr-Lys-
PBP 1b[d]	*E. coli*	-Ser-Ile-Gly-Ser-Leu-Ala-Lys-
β-lactamase (class A)[e]	*S. aureus*	-Phe-Ala-Tyr-Ala-Ser-Thr-Ser-Lys-
	B. cereus	-Phe-Ala-Phe-Ala-Ser-Thr-Tyr-Lys-
	B. licheniformis	-Phe-Ala-Phe-Ala-Ser-Thr-Ile-Lys-
	E. coli	-Phe-Pro-Met-Met-Ser-Thr-Phe-Lys-

[a] From Waxman & Strominger (1980). [b] From Yocum et al. (1980) [c] From Nicholas et al. (1985). [d] This study. [e] From Ambler (1980).

from the lighter staining component on $NaDodSO_4$–polyacrylamide gels (Figure 1) is highly unlikely for the following reasons. Genetic studies indicate that the α and γ components of the *ponB* gene share primary DNA sequence and are both transcribed in the same direction. It would be very unusual if these two components were read in a different reading frame, and thus, it is most probable that these two components have an identical active site sequence. Moreover, the penicilloyl moiety is known to undergo chemical fragmentation, and even minor changes could be distinguished by HPLC. The heterogeneity of a radioactive penicilloyl peptide has been observed previously in this laboratory (Nicholas et al., 1985). Sequence analysis of PI-A was complicated by the presence of two peptides; however, the secondary sequence Ser-Ile-Gly-X-Leu-Ala-Lys could be identified along with the primary sequence of the contaminating peptide (data not shown). Serine was present in the major contaminating peptide at residue 3, and carry-over to the fourth cycle prevented unambiguous assignment of the serine residue anticipated at this position in the penicilloyl peptide. Thus it seems almost certain that the minor radioactive peak is an artifact due to the chemical properties of the penicilloyl moiety.

Now that the sequences of the active sites of several penicillin-binding proteins are known, they can be compared with one other and to β-lactamases. When the active site serine residue is lined up for all of these proteins, several similarities become evident (Table III). The lysine residue at the end of the peptide is conserved throughout the sequences. The glycine residue at the amino-terminal side of the active site serine in the PBP 1b sequence is a relatively conservative substitution for the alanine present in all but one of the other sequences, although it is possible that this substitution may have an effect upon the secondary structure in this region of the protein. The isoleucine residue at the second position of the PBP 1b peptide is also seen in the same position for the sequences of the CPases from two different bacterial strains. The homology between the peptide from PBP 5′ and PBP 1b shows that three out of seven residues are identical, as well as glycine for alanine as a conservative replacement.

It is interesting to note that the threonine residue present in all of the CPases is replaced with an alanine residue in PBP 1b. This might be due to the different functions that these two enzymes display in vivo, i.e., carboxypeptidase vs. transpeptidase activity. Another interesting feature of the comparison is the presence of the conserved threonine residue on the carboxy terminal side of the active site serine in the β-lactamases. This residue is adjacent to the active site nucleophile, while in the CPases and PBP 5′ the conserved threonine residue is one amino acid removed from this serine. This could explain the difference in the specific activity of these enzymes, which are in themselves weak β-lactamases, compared to actual β-lactamases. It has been reported that mutation of this threonine to an isoleucine leads to a β-lactamase with a much reduced specific activity (Ambler, 1980), although it is not known if the mutation affects both acylation and deacylation or only one of the two reactions. This speculation is complicated by the fact that a mutation in PBP 5 over 60 residues away in the primary sequence from the active site serine abolishes the deacylation of the acyl-enzyme intermediate from both substrate and penicillin (Matsuhashi et al., 1979; Broome-Smith & Spratt, 1984). It is clear that more work needs to be done to establish which residues of these proteins are involved in the enzymatic hydrolysis of β-lactams and the acyl-enzyme intermediate.

The sequence of the *ponB* gene encoding PBP 1b has recently been reported (Broome-Smith et al., 1985), and the active site sequence reported in this paper comprises residues 507–513 of the gene sequence.

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Registry No. CPase, 9077-67-2; β-lactamase, 9073-60-3; serine, 56-45-1; penicillin G, 61-33-6.

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Pressure-Induced Conformational Changes in a Human Bence–Jones Protein (Mcg)†

James N. Herron, Kathryn R. Ely, and Allen B. Edmundson*
Department of Biology, University of Utah, Salt Lake City, Utah 84112
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ABSTRACT: The effect of high static pressures on the internal structure of the immunoglobulin light chain (Bence–Jones) dimer from the patient Mcg was assessed with measurements of intrinsic protein fluorescence polarization and intensity. Depolarization of intrinsic fluorescence was observed at relatively low pressures (<2 kbar), with a standard volume change of −93 mL/mol. The significant conformational changes indicated by these observations were not attributable to major protein unfolding, since pressures exceeding 2 kbar were required to alter intrinsic fluorescence emission maxima and yields. Fluorescence intensity and polarization measurements were used to investigate pressure effects on the binding of bis(8-anilinonaphthalene-1-sulfonate) (bis-ANS), rhodamine 123, and bis(*N*-methylacridinium nitrate) (lucigenin). Below 1.5 kbar the Mcg dimer exhibited a small decrease in affinity for bis-ANS (standard volume change ~5.9 mL/mol). At 3 kbar the binding activity increased by >250-fold (volume change −144 mL/mol) and remained 10-fold higher than its starting value after decompression. With rhodamine 123 the binding activity showed an initial linear increase but plateaued at pressures >1.5 kbar (standard volume change −23 mL/mol). These pressure effects were completely reversible. Binding activity with lucigenin increased slightly at low pressures (standard volume change −5.5 mL/mol), but the protein was partially denatured at pressures >2 kbar. Taken in concert with the results of parallel binding studies in crystals of the Mcg dimer, these observations support the concept of a large malleable binding region with broad specificity for aromatic compounds. The complementarity between this binding region and a suitable ligand can be improved by the imposition of external pressures far below those causing general disruption of the protein structure.

The light chain found in the Bence–Jones dimer and monoclonal IgG1 protein from the patient Mcg exhibits a high degree of conformational flexibility. Three-dimensional structural studies revealed that the Mcg dimer contained two light chains identical in amino acid sequence but folded in different conformations (Schiffer et al., 1973; Edmundson et al., 1975). Noncovalent interactions between the two variable domains produced a hydrophobic binding cavity. The Bence–Jones dimer formed trigonal crystals in ammonium sulfate and orthorhombic crystals in water (Abola et al., 1980; Ely et al., 1983). Conformational differences were found in the crystal structures of analogous light chains in the trigonal and orthorhombic forms, an indication that at least four different conformational isomers existed in the light chain dimers. Another conformational isomer was observed in the X-ray analysis of the Mcg IgG1 protein (Rajan et al., 1983).

In this report we consider the question of analogous conformational flexibility of the Mcg light chain in aqueous solution. Environmental perturbation of proteins in solution can be investigated by varying either temperature or pressure. Temperature studies produce changes in both protein thermal energy and protein volume, and it is difficult to separate the

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