AMINO ACID SEQUENCE HOMOLOGIES BETWEEN *ESCHERICHIA COLI* PENICILLIN-BINDING PROTEIN 5 AND CLASS A β-LACTAMASES

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1. Introduction

Penicillin-sensitive D-alanine carboxypeptidases (CPases), membrane-bound enzymes of peptidoglycan biosynthesis, are inactivated by penicillins and other β-lactam antibiotics as a consequence of the formation of a covalent, stoichiometric penicilloyl–CPase complex (reviews [1,2]). That several CPases regain catalytic activity by an enzymatic hydrolysis of the bound penicilloyl moiety to penicilloic acid [3] supports the hypothesis [4] that penicillin-hydrolyzing β-lactamases and penicillin-sensitive CPases have a common evolutionary origin. More directly, sequence analysis of active site peptides derived from the CPases of two *Bacilli* indicates significant amino acid sequence homology between these CPases and the 4 class A β-lactamases [5] of known primary structure [6]. That this homology is most evident in the vicinity of the active site residue, serine 36, suggests that one might detect homology between β-lactamases and other CPases by NH_2-terminal sequence analysis. We present here NH_2-terminal sequences for 2 CPases purified from *E. coli*, penicillin-binding proteins (PBPs) 5 and 6 [7], and compare them to the corresponding regions of several β-lactamases and other CPases.

2. Experimental

PBPs 5 and 6 were each purified to protein homogeneity from membranes of *Escherichia coli* K12, strain PA 3092, as in [7]. Protein samples (~10 nmol each) were prepared and subjected to automated NH_2-terminal sequence analysis as in [6]. Phenylthiohydantoin amino acid derivatives were identified by thin-layer, gas, and high-pressure liquid chromatography, with some identifications also confirmed by back hydrolysis with HI, using the methods in [8].

NH_2-terminal sequences determined for *E. coli* PBPs 5 and 6 were aligned with those of the CPases from *Bacillus subtilis* [6] and *Bacillus stearothermophilus* [9] and with the class A β-lactamases [5] using computer programs from [10]. Computer analyses were kindly performed by Dr R. F. Doolittle, Dept. of Chemistry, University of California, San Diego. Optimal alignments of each of the 28 possible pairwise comparisons of the 4 CPase and 4 β-lactamase segments were generated using an algorithm (NEEWU) based on the method in [11]. The 6 β-lactamase–β-lactamase comparisons yielded essentially the same alignments described in [5] for the NH_2-terminal region. A single, uniform alignment of all 8 segments was obtained with the assistance of the computer-generated optimal alignments for the CPase–CPase, CPase–β-lactamase and β-lactamase–β-lactamase comparisons. As the degree of the homology exhibited by the first 2 groups is not as high as for the β-lactamase–β-lactamase comparisons, the alignments chosen (fig.1) do not correspond exactly to the computer-generated optimal alignment in every case.

The statistical significance of the homologies suggested by fig.1 was determined using a computer program (NWJUM, [10]) which generates 6 sets of randomized sequences of the same compositions and lengths as the 2 sequences being compared and then determines the mean score (with scores expressed here as % sequence identity for the optimal alignment)

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for all possible combinations of the randomized sequences. The significance of the authentic sequence comparisons (i.e., those shown in fig.1) are then expressed as the number of standard deviations above or below the mean score of the randomized comparisons (see table 2, values above the diagonal). Scores >3.0 SD above the scrambled mean scores are taken to indicate significant sequence homology [12]. Mean scores for the randomized comparisons varied 13.7–21.3% identity, with an average value of 16.2% identity for the 28 pairwise comparisons shown in table 2. Similarly, the observed standard deviations in the mean randomized scores varied from 2.3%–3.9% identity, with an observed average standard deviation of 3.3% identity for the comparisons made in table 2.

3. Results and discussion

NH_2-terminal amino acid sequence analysis of *E. coli* PBPs 5 and 6 established the sequences shown in table 1. These sequences were aligned (fig.1) with the corresponding portions of the CPases from *B. subtilis* [6] and *B. stearothermophilus* [9] and the 4 class A β-lactamases of known primary structure [5] as in section 2. Statistical analysis of the similarities between the NH_2-terminal portion of *E. coli* PBP 5 and the 4 β-lactamases indicated clear significance for ⩾3 of the comparisons (table 2B). These analyses also indicated a more significant homology of *E. coli* PBP 5 with the β-lactamases than with the CPases from *Bacillus* sp. (table 2A *vs* B). This is particularly interesting, as *E. coli* PBP 5 exhibits a low level β-lactamase activity resulting from hydrolysis of a bound penicilloyl moiety to yield penicilloic acid [2], a reaction not catalyzed by the 2 CPases from *Bacillus* sp.

In contrast to the significant sequence homologies detected for *E. coli* PBP 5, a statistically significant homology for the NH_2-terminal 25 residues of *E. coli* PBP 6 was only apparent when comparison was made with *E. coli* PBP 5 (table 2). These 2 polypeptides exhibit highly similar biochemical and catalytic properties [7], yet are distinct by peptide mapping [7] and by genetic analysis as well [13].

The comparisons presented in table 2 also suggest that the 4 CPases compared are much less homologous as a group than the class A β-lactamases (table 2A *vs* C). In fact, the limited data available do not provide strong support for a statistically significant homology between the CPases from *E. coli* and those from the *Bacilli*. It is also apparent from table 2

Table 1
NH_2-terminal sequence analysis of PBP 5 and PBP 6[a]

Cycle	PBP 5		PBP 6	
	Residue identified	Yield (nmol)	Residue identified	Yield (nmol)
1	Asp	2.4	Ala	5.5
2	Asp	2.6	Glu	4.6
3	Leu	4.3	Gln	2.2
4	Asn	1.3	Thr	1.2
5	Ilc	4.5	Val	4.2
6	Lys	2.4	Glu	3.4
7	Thr	1.0	Ala	3.0
8	Met	1.7	Pro	1.1
9	Ile	4.8	Ser	0.8
10	Pro	3.3	Val	3.1
11	Gly	2.5	Asp	0.8
12	Val	2.8	Ala	2.2
13	(Pro)	0.4	(Ser)	0.3
14	Gln	1.0	Ala	2.1
15	Ile	3.0	Trp	0.6
16	Asp	0.5	(Phe)	0.3
17	Ala	1.8	Leu	2.7
18	Glu	1.0	Met	1.1
19	(Ser)	0.2	Asp	0.3
20	Tyr	1.0	Tyr	0.5
21	Ile	3.0	Ala	1.3
22	Leu	3.1	(?)	–
23	Ile	2.6	Gly	1.6
24	Asp	1.3	Lys	0.4
25	Tyr	1.0	Val	0.8
26	Asn	0.4		
27	(Pro)	0.1		
28	Gly	0.8		

[a] Quantitative data was obtained by high-pressure liquid chromatography with unambiguous identifications made by ⩾2 additional methods (see section 2). Tentative identifications (residues shown in parentheses) were made when residues were obtained in unexpectedly low yields

that although *E. coli* PBP 6 exhibits significant homology to at least one other CPase (PBP 5), no homology to any of the β-lactamases is detectable from the pairwise comparisons. Similarly, *B. licheniformis* β-lactamase is not detectably homologous to any of the CPases by these methods. It is, however, reasonable to presume that the occurrence of significant structural and mechanistic homologies (see below) between some members from each group confers homology to all members of both groups. In other cases where pairwise comparisons have failed to indicate statistically significant homologies, consideration of ⩾3 sequences simultaneously using specially adapted computer methods has boosted the significance considerably

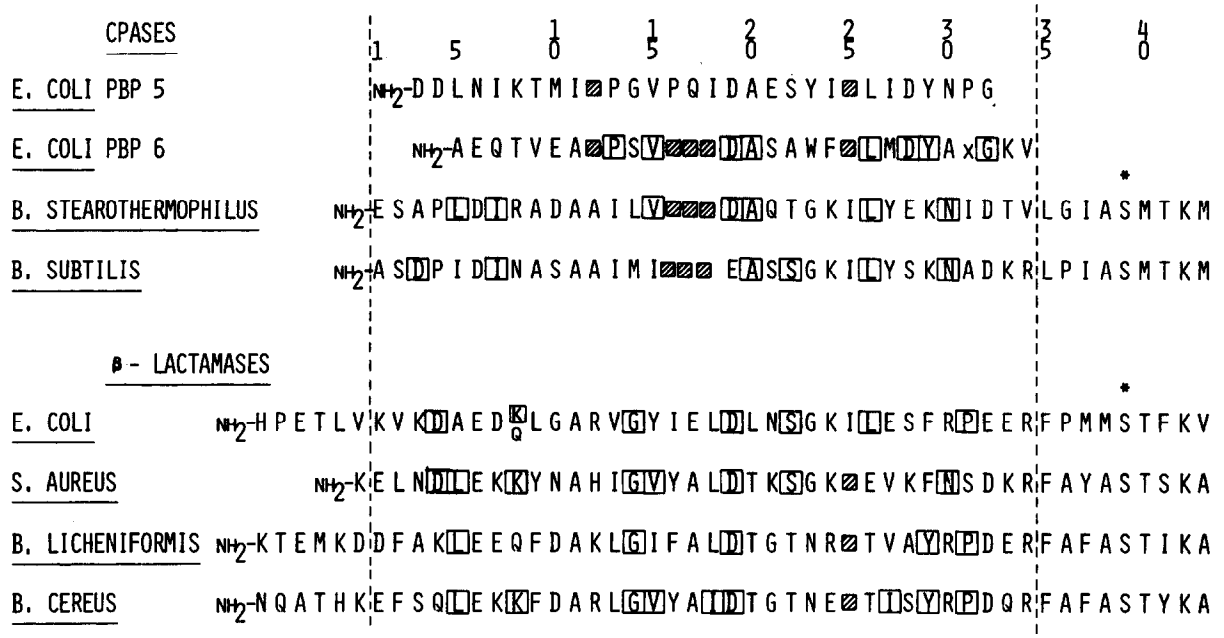

Fig.1. Comparison of the NH_2-terminal sequences of *E. coli* PBPs 5 and 6 to those of other CPases and β-lactamases. The uniform alignment shown for the 8 sequences was determined as in section 2. This alignment differs somewhat from that shown for the CPases from *Bacillus* sp. [6] in the placement of the triple gap (shaded spaces) after position 15. Positions are numbered from the NH_2-terminus of the *B. stearothermophilus* CPase. The active site serine, both of the *Bacillus* CPases [6,9] and of the class A β-lactamases [15,16] is indicated * at position 39. X = unidentified residue. Residues identical to those of *E. coli* PBP 5 are boxed. Sequences used for the statistical analyses (table 2) are shown within the vertical dashed lines.

[10,14]. Such an increase in statistical significance is most likely to occur in cases where multiple homologies occur at a given site. The data in table 3 indicate that the CPase–β-lactamase comparisons clearly fall into that category.

Both structural and mechanistic data support the hypothesis [4] that these and possibly other penicillin-sensitive CPases might be related evolutionarily to penicillin-inactivating β-lactamases:

(1) CPases from ⩾3 bacterial species exhibit statistically significant NH_2-terminal sequence homologies to β-lactamases. Alignment of active-site serines (fig.1) utilized by the 2 groups of enzymes [6,15,16] adds significance to the observed homologies. These homologies extend to the level of secondary structure as demonstrated by use of predictive methods [17]. Additional sequence data is clearly necessary to clarify the extent of structural relatedness between the CPases and β-lactamases. To this end, the availability of NH_2-terminal sequences for *E. coli* PBPs 5 and 6 should facilitate further structural analysis by DNA sequencing using the cloned PBP genes [18].

(2) Both CPases and β-lactamases are acylated at the same unique residue, serine 36 (shown at position 39 in fig.1) during catalysis [1,19]. In the case of CPases, the acyl group can be either β-lactam- or *R*-D-alanyl–D-alanine-derived [6,9].

(3) Several CPases, including *E. coli* PBP 5, can catalyze deacylation of a bound penicilloyl moiety with formation of penicilloic acid, i.e., they exhibit a weak β-lactamase activity. One might predict that these CPases are more closely related to the β-lactamases than are other CPases (e.g., those which fragment the bound penicilloyl moiety to yield phenylacetylglycine).

(4) Although there has been no conclusive demonstration that β-lactamases can recognize the cell wall-related CPase substrates (e.g., [20]), CPases clearly bind and are acylated by many β-lactam antibiotics. In addition, treatment of CPases with cefoxitin, a 7-α-methoxycephalosporin, yields acyl-enzymes which are much more stable to hydrolysis than are the corresponding penicilloyl-CPases [2], a situa-

Table 2
Statistical analysis of the CPase and β-lactamase sequence homologies[a]

	(A) CPase–CPase				(B) CPase–β-lactamase			
	E. coli PBP 5	*E. coli* PBP 6	*B. stearothermophilus* CPase	*B. subtilis* CPase	*E. coli* β-lactam	*S. aureus* β-lactam	*B. licheniformis* β-lactam	*B. cereus* β-lactam
E. coli PBP 5		*4.9* 1 g	2.5 3 g	1.9 3 g	*3.5* 2 g	*5.1* 1 g	1.8 1 g	*5.4* 1 g
E. coli PBP 6	36%		1.4 2 g	−0.1 2 g	1.1 3 g	2.7 2 g	0.0 2 g	1.4 2 g
B. stearother. CPase	28%	25%		*11.3* 0 g	1.7 1 g	*4.5* 2 g	2.0 2 g	*4.1* 2 g
B. subtilis CPase	24%	21%	55%		*4.0* 1 g	*4.4* 2 g	−0.4 2 g	−0.4 2 g
	(B) CPase–β-lactamase				(C) β-Lactamase–β-lactamase			
E. coli β-lactamase	25%	21%	19%	26%		*7.7* 1 g	*5.8* 1 g	*6.2* 1 g
S. aureus β-lactamase	29%	21%	33%	30%	36%		*5.6* 0 g	*13.2* 0 g
B. licheniformis β-lactamase	18%	17%	23%	13%	27%	33%		*18.2* 0 g
B. cereus β-lactamase	32%	21%	27%	13%	30%	42%	61%	

[a] Results were obtained utilizing the residues shown between positions 1 and 34 as aligned in fig.1. Listed below the diagonal is the percent sequence identity for each of the 28 pairwise sequence comparisons. Listed above the diagonal is the statistical significance of each of these comparisons as expressed by the number of standard deviations above (positive values) or below (negative values) the mean score obtained from randomized comparisons as in section 2. The relative number of gaps for each comparison is as indicated (*g*). Significant values are in italics

Table 3
Sites of homology between CPases and β-lactamases[a]

Position	Residue(s)	No. matches	Position	Residue(s)	No. matches
1	Asp, Glu	4/6	24	Lys, Arg	5/8
5	Leu	5/8	25	Ile	3/3
6	Asp, Glu	7/8[b]	26	Leu	5/8
8	Lys, Arg	5/8	27	Ile, Val	4/8
10	Asp, Glu	4/8[b]	29	Tyr, Phe	6/8
11	Ala	7/8	30	Asn	4/8
13	Ile, Val	4/8	31	Pro	4/7
14	Gly	5/8	32	Asp, Glu	6/8
15	Ile, Val	7/8	34	Arg	5/7
19	Asp, Glu	8/8	38	Ala	5/6
22	Ser, Thr	7/8	39	*Ser	6/6
23	Gly	4/8	42	Lys	6/6

[a] Listed are sites (numbered as in fig.1) which are ⩾50% conserved between the CPases and β-lactamases. Thus, sites with 4 conserved CPase residues with no β-lactamase matches (position 20) or 4 conserved β-lactamase residues with no CPase matches (positions 35 and 40) are not included. Included as homologous are pairs of closely related amino acids (e.g., Asp, Glu; Lys, Arg; Ile, Val), as defined by the mutation data matrix [12] used in [6]. Note that Ile is more closely related to Val then to Leu by this method; * = active site serine

[b] An additional 1 of the 8 sequences has Asn at this position

tion which is quite analogous to the formation of a long-lived acyl-enzyme upon treatment of *E. coli* RTEM β-lactamase with the same antibiotic [19].

Further studies of the mechanisms and structural relatedness of these 2 groups of enzyme may help to clarify their functions in vivo.

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