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GUEST COMMENTARY

Update of the Standard Numbering Scheme for Class B β-Lactamases

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β-Lactamases represent the major cause of bacterial resistance against β-lactam antibiotics, and they have been divided into four classes (A to D) on the basis of their amino acid sequences (21). The class B enzymes have no sequence or structural similarity to the active-site serine enzymes of classes A, C, and D (6); require a bivalent metal ion (Zn^{2+}) for activity; and constitute group 3 in the Bush-Jacoby-Medeiros functional classification (2). The identification of Zn–β-lactamase-producing pathogenic strains of *Aeromonas*, *Bacteroides*, *Flavobacterium*, *Legionella*, *Serratia*, and *Stenotrophomonas* has greatly increased interest in this class of enzymes (2). The fact that they hydrolyze almost all β-lactam antibiotics, including carbapenems, underlines their clinical relevance. In consequence, the potential spreading of these enzymes among pathogenic bacteria is a frightening possibility, which emphasizes the importance of understanding their properties.

On the basis of the sequences, three subclasses of class B β-lactamases (B1 to B3) were identified, and a standard numbering scheme (BBL numbering) was proposed (13) by analogy to the ABL numbering scheme which has been widely used for class A β-lactamases. Due to the general low degree of identity between subclass sequences (<20%), classical alignment programs produce unreliable results. The proposed alignment (13) was facilitated by the availability of X-ray structures for B1 and B3 enzymes. Crystallographic structures have been described for several B1 enzymes: *Bacillus cereus* BcII (4, 11), *Bacteroides fragilis* CcrA (5, 8), *Pseudomonas aeruginosa* IMP-1 (7) and VIM-2 (unpublished data), and *Chryseobacterium meningosepticum* BlaB (14). Structural data are also available for two B3 enzymes: *Stenotroptromonas maltophilia* L1 (28) and *Legionella gormanii* FEZ-1 (15). Recently, we solved the first X-ray structure of a subclass B2 enzyme (CphA) produced by various species of *Aeromonas* (G. Garau, C. Bebrone, C. Anne, M. Galleni, J.-M. Frère, and O. Dideberg, unpublished data). Using all available three-dimensional structures, it is now possible to propose a bonafide structural alignment of the class B β-lactamases, and accordingly, to update the first proposed BBL scheme (Fig. 1).

For the three-dimensional structure comparison of the eight available structures, we used the program TOP (18) with the new option MAPS, allowing multiple alignments of protein structures. In addition, the program produces two ranking scores: the sequence identities of aligned residues and the structural diversity. The structural-diversity score was defined as $RMS/(N_{match}/N_0)^{3/2}$, where RMS is the root mean square deviation of the distances between matched Cα atoms, N_{match} is the number of matching residues, and (N_{match}/N_0) is the matching fraction of two compared structures. $N_0 = (N_1 + N_2/2)$, where N_1 and N_2 are the numbers of amino acids in the two compared proteins. This score estimates the evolutionary distance between proteins. These two scores are shown in Table 1 for all known X-ray structures.

Figure 1 displays the proposed alignment and numbering. Interestingly, the numbering of the important class B residues is conserved between old and new alignments. Improvements in the alignment concern mainly N and C termini and small shifts along the sequences. The main result of the new alignment is the identification of 14 sequence fragments of structurally conserved positions, which cover the entirety of all sequences (Fig. 1); they belong mainly to secondary-structure elements (α helices or β sheets). Notably, all Zn ligands are structurally aligned.

The following comments can be made. (i) Only sequences of proteins of known structures are shown. (ii) For residues in lightface, the fact that they have the same number does not imply that they are structurally equivalent. (iii) For newly discovered enzymes, any insertion departing from the present

TABLE 1. Sequence identities of aligned residues and structure diversity among proteins

Protein	Sequence identity/structural diversity[a]							
	CphA	BCII	CcrA	IMP-1	VIM-2	BlaB	L1	FEZ-1
CphA		0.32	0.30	0.24	0.28	0.29	0.20	0.15
BCII	1.19		0.37	0.39	0.39	0.37	0.22	0.15
CcrA	1.17	1.02		0.36	0.33	0.30	0.16	0.12
IMP-1	1.20	1.20	1.13		0.35	0.33	0.17	0.15
VIM-2	1.11	1.08	1.13	1.23		0.28	0.18	0.13
BlaB	1.26	1.19	1.17	1.38	1.19		0.18	0.18
L1	1.72	1.60	1.58	1.79	1.62	1.68		0.33
FEZ-1	1.78	1.61	1.57	1.67	1.72	1.70	1.31	

[a] In the upper right triangle (sequence diversity), the largest values characterize the most similar proteins; the opposite is true for the lower left triangle (structural diversity).

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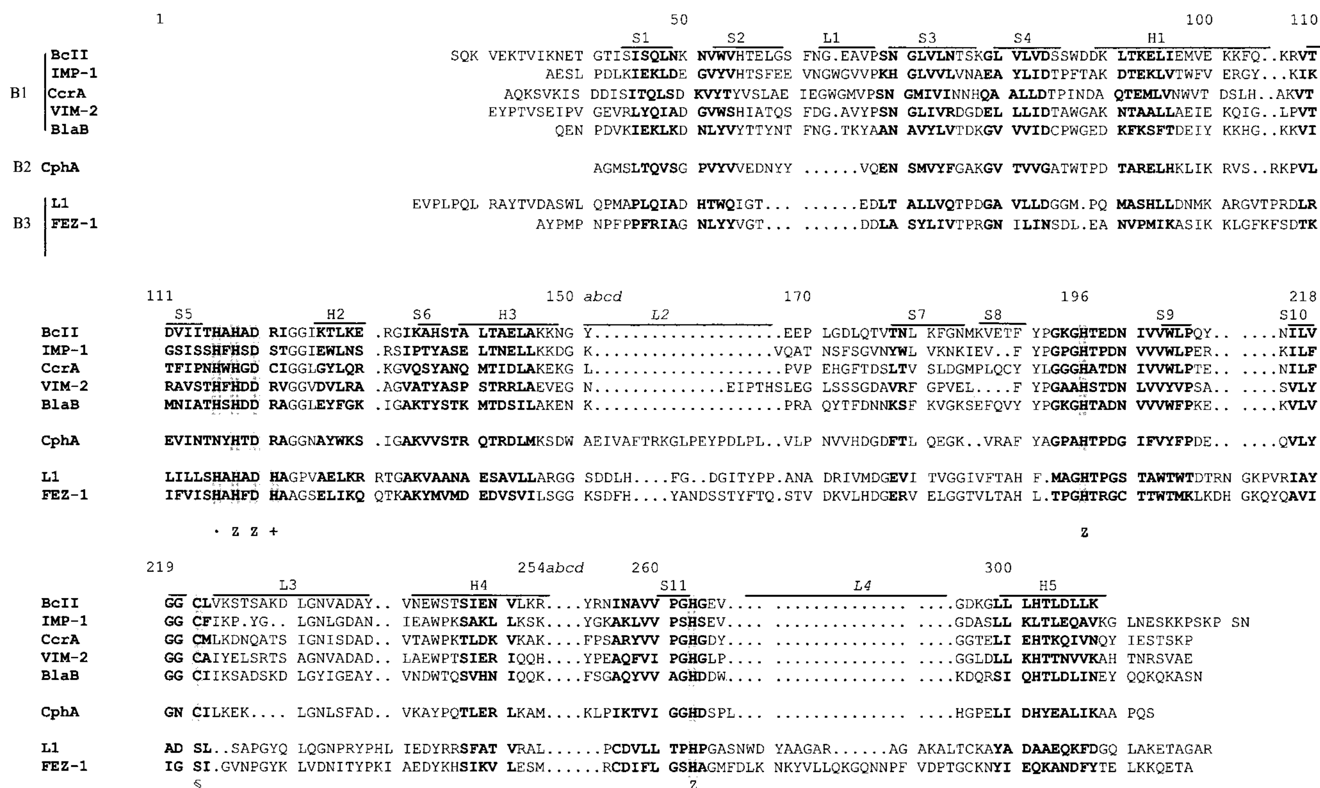

FIG. 1. Structural alignment of eight class B β-lactamases with known X-ray structures. The sequences are referred to by their familiar names. BCII, *B. cereus* 569H (16); IMP-1, *P. aeruginosa* 101/477 (17); CcrA, *B. fragilis* TAL3636 (25); VIM-2, *P. aeruginosa* species (24); BlaB, *C. meningosepticum* NCTC10585 (26); CphA, *Aeromonas hydrophila* AE036 (20); L1, *S. maltophilia* IID1275 (29); FEZ-1, *L. gormanii* ATCC33297[T] (1). The BBL numbering is defined. Conserved secondary-structure elements for the three subclasses are indicated above the sequences: S, β-strand; H, helix; L, loop. Amino acid insertions in newly sequenced enzymes are represented by lowercase letters. The zinc ligands in at least one subclass are shaded and labeled as follows: Z, residues conserved in the three subclasses; ●, residues conserved in subclass B1 and some enzymes of subclass B3; +, residue conserved in subclass B3; §, residues conserved in subclasses B1 and B2. *L2* and *L4* represent largely variable regions. The 14 sequence fragments of structurally conserved positions, which cover the entirety of all sequences, are shown in boldface.

numbering can be characterized by lowercase letters following the number of the last residue of the consensus sequence.

Table 2 shows the numbering of the putative zinc ligands. Not all proteins of known sequence are shown. Only enzymes with <50% sequence identity compared to the first reported sequence are included in the table.

In 1997, Neuwald et al. (23) detected a few proteins that have sequence similarities to (and may have given rise to) Zn–β-lactamases. They include enzymes with large variations in function (sulfatase; DNA cross-link repair enzyme) and which are encoded by yeast, plant, or bacterial open reading frames. Human glyoxalase II was also shown to belong to the superfamily. More recently, 17 groups with known functions were identified (9). In order to evaluate the structural diversity of the Zn–β-lactamase superfamily, human glyoxalase II (3) and rubredoxin oxygen-oxidoreductase from *Desulfovibrio gigas* (12) were also aligned using TOP, along with one member of each subclass. Table 3 shows the sequence identities and structural diversity of the two proteins and BCII, CphA, and FEZ-1. As expected, low sequence identity corresponds to a high structural-diversity score. The structural-diversity scores for proteins belonging to a superfamily range from 1.4 to 2, in contrast to 3.5 to 4 for proteins with different folds (18). In-

TABLE 2. Numbering of important class B residues

Protein	Zn1 ligand			Zn2 ligand		
Consensus BBL B1	His116	His118	His196	Asp120	Cys221	His263
BcII	His86	His88	His149	Asp90	Cys168	His210
IMP-1	His77	His79	His139	Asp81	Cys158	His197
CcrA	His99	His101	His162	Asp103	Cys181	His223
VIM-2	His88	His90	His153	Asp92	Cys172	His214
BlaB	His76	His78	His139	Asp80	Cys158	His200
IND-1	His96	His98	His159	Asp100	Cys178	His220
SPM-1[a]	His76	His78	His165	Asp80	Cys184	His221
JOHN-1[a]	His76	His78	His159	Asp80	Cys178	His220
TUS-1[a]	His94	His96	His157	Asp98	Cys176	His217
Consensus BBL B2	Asn116	His118	His196	Asp120	Cys221	His263
CphA	Asn69	His71	His148	Asp73	Cys167	His205
Sfh-I	Asn72	His74	His151	Asp76	Cys170	His212
Consensus BBL B3	His/Gln116	His118	His196	Asp120	His121	His263
L1	His84	His86	His160	Asp88	His89	His225
FEZ-1	His71	His73	His149	Asp75	His76	His215
GOB-1[a]	Gln80	His82	His157	Asp84	His85	His213
THIN-B[a]	His105	His107	His185	Asp109	His110	His253
CAU-1[a]	His96	His98	His172	Asp100	His101	His237

[a] For SPM-1, JOHN-1, TUS-1, THIN-B, and CAU-1, sequences are reported in references 27, 22, 19, 26, and 10, respectively.

TABLE 3. Sequence identities of aligned residues and structural diversity among proteins

Protein	Sequence identy/structural diversity[a]				
	CphA	BCII	FEZ-1	ROO	GOX
CphA		0.32	0.15	0.20	0.23
BCII	1.19		0.15	0.17	0.24
FEZ-1	1.78	1.61		0.16	0.21
ROO	1.78	1.64	1.47		0.21
GOX	1.61	1.62	1.40	1.47	

[a] Sequency identities, upper right triangle; structural diversity, lower left triangle. ROO, rubredoxin oxygen-oxidoruductase; GOX, human glyoxylase II.

terestingly and surprisingly, FEZ-1 is closer to glyoxalase II and rubredoxin oxygen-oxidoreductase than to BcII or CphA.

In the structural alignment, a large number of amino acid changes and insertions-deletions are observed. One hypothesis is that an ancient protein gave rise to the different subclasses of Zn–β-lactamases. A few candidates for the ancient protein are those related to essential biological functions within the cell, such as DNA or RNA processing or DNA repair (9). Nature used a limited number of scaffolds to generate a large variety of biological functions. Zn–β-lactamases are good examples of such a selection.

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