

## GUEST COMMENTARY

### Standard Numbering Scheme for Class B $\beta$ -Lactamases

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Metallo- $\beta$ -lactamases constitute the molecular class B of Ambler (1) and group 3 according to the Bush-Jacoby-Medeiros functional classification (6). In recent years, many new enzymes of this class have been described and the sequences of the corresponding genes have been determined. Their clinical importance is highlighted by the fact that they hydrolyze carbapenems, compounds which most often escape the activity of active-site serine  $\beta$ -lactamases. Moreover, most metallo- $\beta$ -lactamases are broad-spectrum enzymes which also hydrolyze a variety of penicillins and cephalosporins (13, 21, 22, 26). On the basis of the known sequences, three different lineages, identified as subclasses B1, B2, and B3, can be characterized. Subclass B1 contains most known metallo- $\beta$ -lactamases, including the  $\beta$ -lactamase II (BcII) proteins from *Bacillus cereus* or other *Bacillus* spp. (15, 16, 19) and *Bacillus* sp. strain 170 (16), the CcrA (24) (also named CfiA [29]) proteins of *Bacteroides fragilis*, the BlaB proteins from *Chryseobacterium meningosepticum* (2, 26, 34), the IND-1 enzyme from *Chryseobacterium indologenes* (3), the IMP proteins found in some clinical isolates of *Pseudomonas aeruginosa* (17, 28), *Serratia marcescens* (21), *Klebsiella pneumoniae* (GenBank EMBL accession no. D29636), and *Acinetobacter baumannii* (25), and the VIM proteins found in some *P. aeruginosa* clinical isolates (18, 22). Subclass B2 includes the enzymes produced by various species of *Aeromonas* (CphA [20], ImiS [33], and CphA2 [23]) and the Sfh-I  $\beta$ -lactamase (GenBank accession no. AF197943) from *Serratia fonticola*. Finally, subclass B3 includes the L1 proteins from *Stenotrophomonas maltophilia* (27, 32), the GOB proteins from *C. meningosepticum* (2), the FEZ-1 enzyme from *Legionella gormanii* (5), and the THIN-B  $\beta$ -lactamase produced by *Janthinobacterium lividum* (25a).

The three-dimensional structures of several B1 (BcII [7, 9, 12], CcrA [8, 10], and IMP-1 [11]) enzymes and one B3 (L1 [31]) enzyme have been solved by X-ray crystallography. Despite a very low degree of sequence similarity between the two subclasses, the general structures and the relative positions of

the secondary structure elements are similar. Surprisingly, the L1 enzyme is a tetramer (4, 31), whereas the B1, B2, and other B3 (FEZ-1 [5; P. S. Mercuri, F. Bouillenne, L. Boschi, J. Lamotte-Brasseur, G. Amicosante, B. Devreese, J. van Beeumen, J. M. Frère, G. M. Rossolini, and M. Galleni, unpublished data] and GOB-1 [2])  $\beta$ -lactamases so far studied are monomers.

There are, however, no doubts that the proteins are homologous and the sequences of representatives of the three subclasses can be easily aligned. Indeed, in addition to the expected differences at the N and C termini, several insertions and deletions are necessary to allow the alignment of the few conserved residues acting, for instance, as ligands of the two zinc ions which can bind at the active site. Thus, homologous residues from the different class B sequences which are known to play a relevant role in the structure and function often differ in their numbering, even within each subclass.

In order to facilitate the comparative analysis of the structures and of the catalytic mechanisms, we would like to propose a standard numbering scheme for the class B  $\beta$ -lactamases, the BBL numbering, by analogy with the ABL numbering which has been widely accepted for class A  $\beta$ -lactamases. For the class B enzymes, the task was complicated by insertions and deletions and by the generally low degree of similarity but facilitated by the availability of some three-dimensional structures, which allowed the identification of homologous secondary structure elements, even when the sequence similarity was not obvious.

Figure 1 shows the proposed alignment and the derived numbering. The observed (B1 and B3) and expected (B2) secondary structure elements are indicated.

The following comments can be made. (i) Not all the known sequences are shown. When variants of an enzyme are known and the amino acid alignment exhibits more than 80% sequence identity, only the first described sequence is included in the alignment.

(ii) Alignments at the N and C termini are rather uncertain, due to a high variability even within each subclass. As is done for the class A enzymes, residue no. 1 is the first residue of the leader peptide sequence of the *S. maltophilia* L1 protein (32). Since they are highly divergent and irrelevant to the functional structure, the other leader sequences have not been included

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<sup>†</sup> Members are listed in the Appendix.

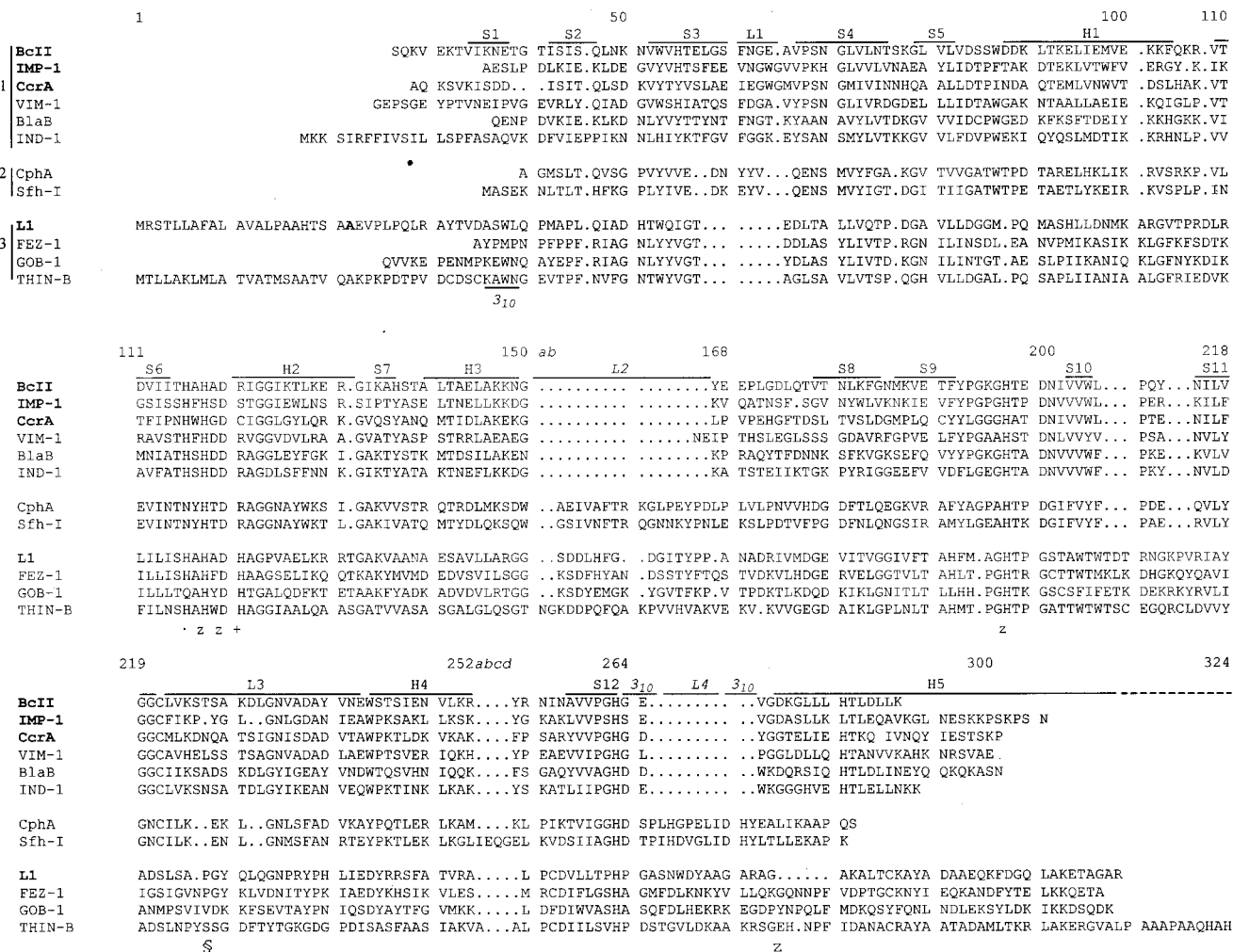


FIG. 1. Alignment of 12 class B  $\beta$ -lactamases numbered according to the BBL scheme. The sequences are referred to by their familiar names. BcII, *Bacillus cereus* 569H (15); IMP-1, *Pseudomonas aeruginosa* 101/477 (17); CcrA, *Bacteroides fragilis* TAL3636 (24); VIM-1, *Pseudomonas aeruginosa* VR-143/97 (18); BlaB, *Chryseobacterium meningosepticum* NCTC10585 (26); IND-1, *Chryseobacterium indologenes* 001 (3); CphA, *Aeromonas hydrophila* AE036 (20); Sfh-I, *Serratia fonticola* UTAD54 (GenBank accession no. AF197943); L1, *Stenotrophomonas maltophilia* IID1275 (32); FEZ-1, *Legionella gormanii* ATCC33297<sup>T</sup> (5); GOB-1, *Chryseobacterium meningosepticum* PINT (2); and THIN-B, *Janthinobacterium lividum* JAC1 (25a). The names written in bold refer to the enzymes for which the three-dimensional structure is known. The amino acid in bold (Ala 22 of L1) represents the first amino acid of the mature  $\beta$ -lactamase. Conserved secondary structure elements of subclasses B1 and B3 are indicated above the sequences:  $\beta_{10}$ ,  $\beta_{30}$  helix; S,  $\beta$  strand; H, helix. Secondary structure elements specific to subclasses B1 and B3 are highlighted by italic characters above and under the sequences, respectively. Amino acid insertions in newly sequenced enzymes are represented by small letters. The residues acting as zinc ligands in at least one subclass are characterized as follows: z, conserved residues in the three subclasses; \*, conserved residues in subclass B1 and some enzymes of subclass B3; +, conserved residue in subclass B3; \$, conserved residues in subclasses B1 and B2.

unless the site of action of the signal peptidase has not been verified (Sfh-I [GenBank accession no. AF197943], IND-1 [3], and THIN-B [25a]).

(iii) This is only a numbering scheme. The fact that residues in different proteins have been assigned the same number does not imply that they occupy exactly the same relative spatial position. Indeed, if the Zn ions and their ligands are superimposed, the G<sub>232</sub>N<sub>233</sub> dyad of BcII is more than 3 Å away from the corresponding residues in the *S. maltophilia* enzyme.

(iv) The loop which can close the active site of B1 enzymes extends between residues BBL 61 and 65 (11, 14, 30). It is absent in subclass B3 (31) and probably in B2.

(v) Any insert in a newly discovered enzyme can be charac-

terized by small letters following the number of the last residue of the consensus sequence. Accordingly, residues N<sub>140</sub>G<sub>141</sub> of THIN-B are defined as BBL 150a and -b and residues I<sub>198</sub>E<sub>201</sub> of Sfh-I are defined as BBL 252a, -b, -c and -d, respectively.

(vi) Table 1 shows a cross-reference of the BBL numbering of the residues identified as or suspected to be the Zn1 and Zn2 ligands and that used for the individual enzymes up to the present time. Note that in subgroup B3, one of the Zn2 ligands (H121) originates with a very different part of the polypeptide chain compared to subgroup B1. Similarly, in subclass B2 and for the B3 GOB-1 enzyme, the sequence alignments unambiguously point to residues H118, H196, and N116 (B2) or Q116

TABLE 1. Numbering of the important class B residues<sup>a</sup>

$\beta$ -Lactamase	Zn1 ligands			Zn2 ligands		
Subclass B1						
<b>Consensus BBL</b>	<b>His116</b>	<b>His118</b>	<b>His196</b>	<b>Asp120</b>	<b>Cys221</b>	<b>His263</b>
BcII	His86	His88	His149	Asp90	Cys168	His210
IMP-1	His77	His79	His139	Asp81	Cys158	His197
CcrA	His99	His101	His162	Asp 103	Cys181	His223
<u>VIM-1</u>	<u>His88</u>	<u>His90</u>	<u>His153</u>	<u>Asp92</u>	<u>Cys172</u>	<u>His214</u>
<u>BlaB</u>	<u>His76</u>	<u>His78</u>	<u>His139</u>	<u>Asp80</u>	<u>Cys158</u>	<u>His200</u>
<u>IND-1</u>	<u>His96</u>	<u>His98</u>	<u>His159</u>	<u>Asp100</u>	<u>Cys178</u>	<u>His220</u>
Subclass B2						
<b>Consensus BBL</b>	<b>Asn116</b>	<b>His118</b>	<b>His196</b>	<b>Asp120</b>	<b>Cys221</b>	<b>His263</b>
CphA	Asn69	His71	His148	Asp73	Cys167	His205
Sfh-I	Asn72	His74	His151	Asp76	Cys170	His212
Subclass B3						
<b>Consensus BBL</b>	<b>His/Gln116</b>	<b>His118</b>	<b>His196</b>	<b>Asp120</b>	<b>His121</b>	<b>His263</b>
L1	His84	His86	His160	Asp88	His89	His225
<u>FEZ-1</u>	<u>His71</u>	<u>His73</u>	<u>His149</u>	<u>Asp75</u>	<u>His76</u>	<u>His215</u>
<u>GOB-1</u>	<u>Gln80</u>	<u>His82</u>	<u>His157</u>	<u>Asp84</u>	<u>His85</u>	<u>His213</u>
<u>THIN-B</u>	<u>His105</u>	<u>His107</u>	<u>His185</u>	<u>Asp109</u>	<u>His110</u>	<u>His253</u>

<sup>a</sup> For CcrA, the numbering is reported in references 10 and 24. When not confirmed by a three-dimensional structure, the ligands are underlined. The consensus (bold) and putative consensus (bold and underlined) ligand numbers are given for each subgroup.

(B3), but such a function is rather unusual for asparagine and glutamine side chains.

#### APPENDIX

The metallo- $\beta$ -lactamase group also includes the following: G. Amicosante and N. Franceschini, Dipartimento di Scienze e Tecnologie Biomediche, Università di L'Aquila, I-67100 Coppito, L'Aquila, Italy; K. Bush, The R. W. Johnson Pharmaceutical Research Institute, Raritan, NJ 08869; N. O. Concha, Department of Structural Biology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406; O. Herzberg, Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Rockville, MD 20850; D. M. Livermore, Antibiotic Resistance Monitoring and Reference Laboratory, Central Public Health Laboratory, London NW9 5HT, United Kingdom; P. Nordmann, Service de Bactériologie-Virologie, Hôpital de Bicêtre, Faculté de Médecine Paris-Sud, 94275 Le Kremlin-Bicêtre, France; B. A. Rasmussen, Wyeth-Ayerst Research, Pearl River, NY 10965; J. Rodrigues and M. J. Saavedra, Department of Animal Health, University of Trás-os-Montes e Alto Douro, 5000-911 Vila Real, Portugal; B. Sutton and S. M. Fabiane, The Randall Centre, King's College London, London SE1 1UL, United Kingdom; and J. H. Toney, Department of Biochemistry, Merck Research Laboratories, Rahway, NJ 07065-0900.

#### ACKNOWLEDGMENTS

This work was supported in part by a grant from the European Union (grant ERB3512-IC15-CT98-0914) as part of the training and mobility of researchers program and by the Belgian Program Pôles d'Attraction Interuniversitaire initiated by the Belgian state, prime minister's office, Services Fédéraux des Affaires Economiques, Techniques et Culturelles (PAI P4/03).

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