

## GUEST COMMENTARY

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

MORENO GALLENI,<sup>1\*</sup> JOSETTE LAMOTTE-BRASSEUR,<sup>1</sup> GIAN MARIA ROSSOLINI,<sup>2</sup>  
JIM SPENCER,<sup>3</sup> OTTO DIDEBERG,<sup>4</sup> JEAN-MARIE FRÈRE,<sup>1</sup> AND  
THE METALLO- $\beta$ -LACTAMASE WORKING GROUP†

*Centre d'Ingénierie des Protéines, Université de Liège, B-4000 Liège, Belgium<sup>1</sup>; Dipartimento di Biologia Molecolare,  
Sezione di Microbiologia, Università di Siena, I-53100 Siena, Italy<sup>2</sup>; Division of Protein Structure,  
NIMR, London NW7 1AA, United Kingdom<sup>3</sup>; and Laboratoire de Cristallographie  
Macromoléculaire, Institut de Biologie Structurale Jean-Pierre Ebel  
(CNRS-CEA), F-38027 Grenoble, France<sup>4</sup>*

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

\* Corresponding author. Mailing address: Centre for Protein Engineering, B6 Sart Tilman, University of Liège, B4000 Liège, Belgium. Phone: 32-043663419. Fax: 32-043663364. E-mail: mgalleni@ulg.ac.be.

† Members are listed in the Appendix.

	1	50	100	110						
	S1	S2	S3	L1	S4	S5	H1			
BcII	SQKV EKTVKINETG TISIS QLNK NVWVHTELGS	FNGE AVPSN GLVLNTSKGL	VLDVSSWDDK	LTKELEIEMVE	.KKFKQKR.VT					
<b>IMP-1</b>	AESLP DLKIE KLDE	GVYVHTSSEE VNGWGVVKH	GLVLVNAEA	YLIDTPFTAK	DTEKLVTWFV	, ERGY. K. IK				
B1 CcrA	AQ KSVKISD.. .ISIT.QLSD KVTYVSLAE	IEGWGMVPSN GMIVINNHQA	ALLDTPINDA	QTEMVLNWWT	.DSLHAK. VT					
VIM-1	GEPSGE YPTVNEIPVG EVRLY QIAD	GWVSHIATQS FDGA. VYPSN	GLIVRQDDEL	LLIDTAWGAK	NTAALLAEIE	.KQIGLP. VT				
BlaB	QENP DVKIE KLKD	NLYVYTNTYNT	FNGT.KYAAN	AVYLVTDKGV	VVIDCPWGKD	KFKSFTDEIY	.KKHGKK. VI			
IND-1	MKK SIRFIFIVSIL LSPFASAAVK	DFVIEPPIKN NHIIYKTFGV	FGGR.EYSAN	SMYLVTKG	VLFDPVWSKI	QYQSLMDTIK	.KRHNLP. VV			
B2 CphA			A GMSLT.QVSC PVYVVE..DN YYV...QENS	MVYFGA.KGV	TVVGATWTPD	TARELHKLIK	.RVSRLPK.VL			
Sfh-I	MASEK NLTLT.HFKG	PLVYVE..DK EYV...QENS	MVYIGT.DGI	TIIGATWTPE	TAETLYKIEIR	.KVSPLP. IN				
<b>L1</b>	MRSTLLAFAL AVALPAHTS AAEVPLPQLR AYTVDASWLQ	PMAPL.QIAD HTWQIGT...	EDLTA LLVQTP.DGA	VLLDGGM.PQ	MASHLLDNMR	ARGVTPRDLR				
FEZ-1	AYPMPN PFPF.RIAG NLYYVG...	DDIAS YLIVTP.RGN	ILINSDL.EA	NVPMIKASIK	KLGKFESDTK					
GOB-1	QVVK E PENNMKEWNQ AYEPF.RIAG NLYYVG...	YDLAS YLIVTD.KGN	ILINIGT.AE	SLPIKANIQ	KLGFNKYKD1K					
THIN-B	MTLLAKLMLA TVATMSAATV QAKPKPDTPV	DCDSCKAWNG	EVTPF.NVFG	NTWYVGT...	AGLSA VLVTSP.QGH	VLLDGAL.PQ	SAPLIANIA ALGFRIEDVK			
			3 <sub>10</sub>							
	111	150 ab	168	200	218					
	S6	H2	S7	H3	L2	S8	S9	S10	S11	
BcII	DVIITHAHAD RIGGIKTLKE R.GIKAHSTA	LTAELAKKNG	.....	YE EPLGDLQTVT	NLKFGNMKVE	TFYPGKGHT	DNIIVVWL...	PQY...	NIUV	
IMP-1	GSISSHFHSD STGGIEWLNS R.SIPTYSE	LTNNEKKDG	.....	....KV	QATNSF.SGV	NYLWVKNQ	VFYPGPGHTP	DNVVVWL...	PER...	KILF
CcrA	TFIPNWHGHD CIGGLGYLQR K.GVQSYANQ	MTIDLAKEGK	.....	....LP	VPEHGTDSL	TVSLDGMPLQ	CYLYGGHAT	DNIIVVWL...	PTE...	NIUF
VIM-1	RAVSTHFHDD RVGGVDSLRA A.GVATYASP	STRRLAEEAG	.....	....NEIP	THSLEGLLSS	GDAVRFGPVE	LFYPPGAHST	DNLVYYV...	PSA...	NVLY
BlaB	MNIATHSHD RAGGLEYFGK I.GAKYIST	MTDSLAKEN	.....	....KP	RAQYTFDNNK	SFKVGKSEFQ	VVYFGKGHTA	DNVVVWF...	PKE...	KVIL
IND-1	AVFATHSHD RAGDLSFFNN K.GIKTYATA	KTNEFLKKDG	.....	....KA	TSTEIIKTKG	PYRIGEEFQ	VDFLGEHTA	DNVVVWF...	PRY...	NVLD
CphA	EVINTNHYTD RAGGNAYWKS I.GAKVUSTR	QTRDLMKSDW	..AEIVAFTR	KGLPEYPDPL	LVLNVVHDG	DFTLQKGKVR	AFYAGPAHTP	DGIFVYF...	PDE...	QVY
Sfh-I	EVINTNHYTD RAGGNAYWK L.GAKIVATQ	MTYDQKQSOW	..GSIVNFTR	QGNKNYPNL	KSLPDTVEP	DFNLQNGSIR	AMYLGEAHTK	DGIFVYF...	PAE...	RVLY
<b>L1</b>	LILLISHAHAD HAGPVALLKR RTGAKVAANA	ESAVLLARGG	..SDDLHFG.	DGITYPP.A	NADRIVMDGE	VITVGGIVFT	ARHFM.AGHTP	GSTAFTWTDT	RNGKPVRIAY	
FEZ-1	ILLISHAHFD HAAGSELIKQ QTAKAYMVMD	EDVSVILSGG	..KSDFHYAN	.DSSTYFTQS	TVDKVLHGE	RVELGGTVLT	AHLT.PGHTR	GCTTWTMFLK	DHGKQYQAVI	
GOB-1	ILLLTQAHYD HTGALQDFKT	ETAAKFYADK	ADVDTVRLTGG	..KSDYEMGK	.YGVTFKP.V	TPDKTLQD	KJLGNITLT	LLHH.PGHTK	GSCSFIEFTK	DEKRKYRVL
THIN-B	FILNSHAWD HAGGIALQA ASCATVVASA	SGALGLQSGT	NGKDPQFQA	KPVVHVAKVE	KV.KVVGEGD	AIKLGPLNL	AHMPT.PGHTP	GATTWTWTSC	EGQRCLDVYV	
	z z +							z		
	219	252abcd	264	300					324	
	L3	H4	S12 3 <sub>10</sub>	L4 3 <sub>10</sub>	H5					
BcII	GGCLVKSTSA KDLGNVADAY VNEWSTSIEN	VLKR....R	NINAVPGHG	E.....	VGDKGLLL	HTLDDLLK				
IMP-1	GGCFIKP.YG L..GNLGDAN IEAWPKSAKL	LKSK....YG	KAKLVPVSHS	E.....	VGDASSLK	LTLEQAVKNG	NESKPKSKPS	N		
CcrA	GGCMLKDQNA TSIGNISDAD VTAWPKTLDK	VKAK....FP	SARVVPVGHQ	D.....	YGGTELIE	HTKQ IVNQY	IESTSKP			
VIM-1	GGCAVHELLS TSAGNVADAD LAEWPTSVER	IQKH....YP	EAEVVPIVGHG	L.....	PGGLDQQ	HTANVVKAHK	NRSVAE.			
BlaB	GGCIKKSADS KDLGYIGEAY VNDWTPQSVN	IQKQ....FS	GAQIVVAGHD	D.....	WKDQRSIQ	HTLDLINEYQ	QKQKASN			
IND-1	GGCLVKSNSA TDLYKIEKAN VEQWPKTINK	LKAK....YS	KATLIIPGHD	E.....	WKGGGHVE	HTLELLNNK				
CphA	GNCLIK..EK L..GNLFSAD	VKAYPQTLER	LKAM....KL	PIKTVIGGHD	SPLHGPELID	HYEALIKAP	QS			
Sfh-I	GNCLIK..EN L..GNMSFAN	RTEYPKTLEK	LKGLEIQQEL	KVDSIAGHD	TP1HDVGLID	HYTLLEKEAP	K			
<b>L1</b>	ADSLSA.PGY QLQNPYRPH	LIEDYRRSFA	TVRA....L	PCDVLLTPH	GASNWYDIA	ARAG....	AKALTCKAYA	DAAEQKFDQ	LAKETAGR	
FEZ-1	IGSIGVNPYK	KLVNDITYPK	IAEDYKHSIK	VLES....M	RCDIFLGS	GMFDLNRKVY	LLQKGQNNPF	VDPFGCKNYI	EQKANDFYTE	
GOB-1	ANMPSVLKD KFSEVTTAYPN	IQSDFYAYTFG	VMKK....L	DFDIWASHA	SQFDLIEKRR	EGDPYNPLF	MDQKSYFQNL	NDLEKSYLDK	IKKDSQDK	
THIN-B	ADSLNPYSSG DFTYTGKGDG	PDISASFAAS	IAKVA...AL	PCDIILSVHP	DSTGVLDKAA	KRSGEH.NPF	IDANACRAYA	ATADAMLT	KR LAKERGVALP	AAAPAAQHAH
	§									

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: 3<sub>10</sub>, 3<sub>10</sub> 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>EQG<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		
<b>Subclass B1</b>				
<b>Consensus BBL</b>	<b>His116</b>	<b>His118</b>	<b>His196</b>	<b>Asp120</b>
BcII	His86	His88	His149	Asp90
IMP-1	His77	His79	His139	Asp81
CcrA	His99	His101	His162	Asp 103
<u>VIM-1</u>	<u>His88</u>	<u>His90</u>	<u>His153</u>	<u>Asp92</u>
BlaB	<u>His76</u>	<u>His78</u>	<u>His139</u>	Asp80
IND-1	<u>His96</u>	<u>His98</u>	<u>His159</u>	Asp100
<b>Subclass B2</b>				
<b>Consensus BBL</b>	<b>Asn116</b>	<b>His118</b>	<b>His196</b>	<b>Asp120</b>
CphA	<u>Asn69</u>	<u>His71</u>	<u>His148</u>	<u>Asp73</u>
Sfh-I	<u>Asn72</u>	<u>His74</u>	<u>His151</u>	<u>Asp76</u>
<b>Subclass B3</b>				
<b>Consensus BBL</b>	<b>His/Gln116</b>	<b>His118</b>	<b>His196</b>	<b>Asp120</b>
L1	His84	His86	His160	Asp88
FEZ-1	<u>His71</u>	<u>His73</u>	<u>His149</u>	<u>Asp75</u>
GOB-1	Gln80	His82	His157	Asp84
THIN-B	His105	His107	His185	Asp109

<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.

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