

BIOCHEMICAL JOURNAL LETTERS

A standard numbering scheme for the Class A β -lactamases

β -Lactamases catalyse the hydrolysis of the β -lactam ring of penicillins, cephalosporins and related compounds, and thereby protect the bacteria which elaborate the enzymes against the action of these antibiotics. A recent review of the molecular properties of the proteins is given by Coulson (1985).

The proteins have been classified on the basis of their sequences; the largest group is called 'Class A' (Ambler, 1980). Enzymes of this class are found in Gram-negative and Gram-positive organisms, cell-bound, periplasmic or secreted, and derived from plasmid or chromosomal genes. For a recent survey of this and the other sequence classes, and of the relation of β -lactamases to other proteins such as the cell-wall synthesis enzymes, see Joris *et al.* (1988). Mechanistic and structural studies (including X-ray crystallography) are in progress with at least half-a-dozen of the Class A enzymes. There is no doubt that the proteins are homologous, and it is to be expected that molecular studies of any of the Class A enzymes can be extended to other members of the class. However, the known sequences vary considerably in length. The leader peptides are not in general homologous, and have a wide range of lengths. Some sequences are known only from the protein (lacking the leader sequence), and some proteins show different processing for the cell-bound and secreted forms, etc. In addition, it is clear that there are differences in length internal to the processed forms of the proteins, and these are presumably associated with surface loops of different lengths connecting conserved internal structures.

For these reasons, homologous residues from different Class A sequences generally differ in their 'natural' or 'sequential' numbers. In order to avoid the confusion and inconvenience that arises in the comparison of molecular studies of different Class A enzymes, we propose here a standard numbering scheme for this group of proteins. The scheme (Fig. 1) has been generated by aligning 20 Class A sequences, and attaching numbers to the alignment in order to preserve as much as possible of the numbering used by Ambler (1980) for the first four members of the class.

It is not intended that the present schemes will replace the natural or sequential scheme for individual proteins. The scheme will be used in the context of comparison of homologous residues, and the standard numbers will be indicated by the label 'ABL' (for Class A β lactamase). Thus 'Val-77 (ABL80)' of the R-TEM enzyme will indicate a residue homologous to Leu-75 of PSE-4, with the same ABL number.

Alignment of protein sequences is most reliable when it is based on X-ray crystal structures of all the proteins concerned, and it cannot be ruled out that X-ray crystallography will suggest changes in the detail of the alignment in Fig. 1. However, there is no doubt the alignment is mostly correct. Fragments of sequence have been omitted in several places (particularly with

the more recently added sequences) where homology is uncertain. It is a virtue of the scheme we propose that future corrections and adjustments to individual residues will not alter the overall numbering and no changes will be made to accommodate new, longer sequences. Nor should the alignment of Fig. 1 be regarded as a definitive statement of the homology relations which exist amongst these proteins. For example, any worker who does not regard the DI (ABL 116–117) of the *Staphylococcus aureus* protein as equivalent to the GM sequence which is generally found here will simply not use the ABL numbers to refer to the *S. aureus* residues.

In order to give the active site serine residue the ABL number 70, it was necessary to start the numbering within some of the leader sequences. Expressed sequences start about ABL31, and though an alignment is shown for earlier residues, numbers 1–30 are unlikely to be used in practice since the leader sequences are not homologous.

A network of sequence relations has been recognized amongst many of the proteins which interact with β -lactams. It is possible, especially as X-ray crystal structures become available, that the current scheme can be extended to, for example, the Class C proteins.

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	1		50		100
Klebsiella pneumoniae	MRYVRL	CVISLLATLP	LVVYAGPQPL	EQIKQSESQ	SGRVGVEMD
PIT-2			SPQPL	EQIKLSESQL	SGRVGMIEHD
R-TEM	MSIQHFRV	ALIPFFAAFC	LPVFAPHTLD	VYKVADEQTL	GARYGVYIELD
Pseudomonas aeruginosa	CHFLSVPAI	LGCVGLICTS	AYAMDTGILD	LAVTQEETTL	GARYGVAVID
PSE-4	GVTYMKFLLA	FSLLIPSVVF	ASSSKFQVVE	QDVKAIEVSL	SARIGVSVLD
Rhodopseudomonas capsulata	TVLSRVATGL	ALGLSHATAS	LAGTPVEALLS	ETVARIEEQL	GARYGLSLME
Actinomadura R39		AEP	SAEVTAEDLS	GEFERLSEF	DARLGVYAVD
Bacillus cereus 569H	TSLEAFTGES	LQVEAKEKTG	QVKHKNQATH	KEFSQLEKFF	DARLGVYAVD
Bacillus cereus 5/B	TSLVFTTGG	LQVEAKEKTG	QVKHKNQATH	KEFSQLEKFF	DARLGVYAVD
Bacillus cereus III	LIGCSNSNTQ	SESNKQTNQT	NQVKQENKRN	HAFAKLEKEY	NAKLGIVYALD
Bacillus licheniformis	LFSCVALAGC	ANNGTNASQP	AEKNEKTEMK	DDFAKLEEQF	DAKLGIFALD
Streptomyces badius	..SDSTAPPS	SAKPATSASA	SLP.RPKPYT	GDFKLEREF	DARLGVYAVD
Streptomyces cacaoi blaU	ESSADAPEA	GSAPSSSAAA	HKPGVEPEYA	AELKALEDEF	DVRLGVYAVD
Streptomyces cacaoi ULg	ACGQASGSES	GQQPGLGGAD	EAHVSADAEH	KEFRALKEFF	DAHPPVYAVD
Klebsiella oxytoca		MAA	AAVPLLASG	SLWASADAIQ	QKLDLEKRS
Staphylococcus aureus		MKKL	IFLIVLIALVL	SACNSNSSHA	KLNDLEKCY
Streptomyces aureofaciens	TMAALLPAGG	AAYASTSTAK	APAAEADLSG.	.RLRALEKQY	DAHPPVYAVD
Streptomyces albus	ALAAATLVPG	TAAHSSGGRG	HGSGSVSDAE	RRLAGLERAS	GARLGVYAVD
Streptomyces lavendulae	AVAGPLPGGS	TFAA.....	.APRGNPDVE	RQLRALEQY	SARLGVYAVD
Streptomyces fradiae	ALAATAAAG	PAHA.....	.APGRGARVE	RQLRALERTH	DARLGVYAVD
Consensus	.saa.aa.g.	aavpslaaag	.apgsnpa..	ke.kalEKqf	darlGvya.d

	101		150		200
Klebsiella pneumoniae	DLVDYSPVSE	KHLVDGMTIG	ELCAAAITLS	DNSAGNLLLA	TVGGPAGLTA
PIT-2	DLVDYSPVSE	KHLADGMTVG	ELCAAAITMS	DNSAANLLLT	AVGGPAGLTA
R-TEM	DLVEYSPVTE	KHLTDGMTVR	ELCSAAITMS	DNTAANLLLT	TIGGPKelta
Pseudomonas aeruginosa	ALVTVSPVTE	LTLR	ELCRAAVSIS	DNTAANLLD	AIGGARTFTA
PSE-4	DLVTVSPVIE	KQVQAATLD	DACFATHTS	DNTAANILIS	AVGGPKGKTD
Rhodopseudomonas capsulata	DLVVPYAPVTE	MTLD	ELCLAADMS	DNVAANILIG	HLGGPEAVTQ
Actinomadura R39	DLVDYSPITE	QHVDTGMTLL	EVADAAYRHS	DNTAANLLFE	ELGGPEGFE
Bacillus cereus 569H	DLVDYSPVTE	KHVDGTMLKLG	EIAEAAYRVS	DNTAGNIFL	KIGGPKGYEK
Bacillus cereus 5/B	DLVDYSPVTE	KHVDGTMTLG	EIAEAAYRVS	DNTAGNIFL	KIGGPKGYEK
Bacillus cereus III	DLVSNYPITE	KHVDGTMLK	ELADASLRYS	DNAAQNLLK	QIGGPESLKK
Bacillus licheniformis	DLVSNYPITE	KHVDGTMLK	ELADASLRYS	DNAAQNLLK	QIGGPESLKK
Streptomyces badius	DLVAHSPVTE	KHVDGTMLK	ELCDASVRY	DNTAANLLFD	GPKGLDA
Streptomyces cacaoi blaU	DLVDNSPVTE	KHVEDGMTL	ALCDAAYRVS	DNTAANLLFE	TVGGPKGLDK
Streptomyces cacaoi ULg	AILPNSPVTE	KHVDGMSLR	ELCDAAYRVS	DNTAANLLFD	QLGGRRGSTR
Klebsiella oxytoca	DLVWVSPITE	KHLQSGHTLA	ESLAAALQYS	DNTAMNKHS	YLGGPEKVT
Staphylococcus aureus	DIVAYSPILE	KYVGKIDITL	ALIEASHTYS	DNTAMNKIK	EIGGKIKVKQ
Streptomyces aureofaciens		PVT	GMTGA	ELCAAAVSES	DNGAGNLLLR
Streptomyces albus	DV	APETG K	GHTVE	ELCEVSTIS	DNCRAEHLR
Streptomyces lavendulae		FGPVT	GHTVE	RLCAAAICQS	DNAANLLLR
Streptomyces fradiae		YSPV	GHTVA	ELCEATLRS	DNTAANLLLR
Consensus	dlvdyspvte	khvdtgmtl	elcdaayr	DntAaNLlr	elggpkgvta

	201		250		295
Klebsiella pneumoniae	ARSQQQLLQW	MVDDRAGPL	IRAVLPPGF	IADKTGAG.E	RGARGIVALL
PIT-2	ARSQRQLLQW	MVDDRAGPL	IRSVLPAGVF	IADKTGAG.E	RGARGIVALL
R-TEM	LASRQQLDWD	MEADKAVAGPL	LRSLAPAGVF	IADKSGAG.G	RGSRGIIAAL
Pseudomonas aeruginosa	APARNELTGW	HLGDDQVADAL	LRAGLPRDQW	IADKSGAG.G	HGSRISIAVV
PSE-4	EMNQKLGES	MVNNQVTGNL	LRSLVLPAGW	IADRSAGG.G	FGARSITAVV
Rhodopseudomonas capsulata	PEARQKLAEW	MRHGGVTGAL	LRAEAEADWL	ILDKSGGG.S	H.TRLNLVAVI
Actinomadura R39	EGPRDVLTE	LLNNTTGDDEL	IRAGVPEDWR	VGDKTGTTG.S	HGSRNDIAVV
Bacillus cereus 569H	AEKRIKILTEW	MKGNATGDGL	IRAGIPTDWW	VGDKSGAG.S	YGRNDIAVV
Bacillus cereus 5/B	HQKRNILTEW	MKGNATGDGL	IRAGVPTDWW	DADKSGAG.S	YGRNDIAVV
Bacillus cereus III	SEKRELLVDW	MKRNITGDGL	IRAGVPPKGE	VADKTGAG.S	YGRNDIAVV
Bacillus licheniformis	SEKRELLVDW	MKRNITGDGL	IRAGVPPKGE	VADKTGAA.S	YGRNDIAVV
Streptomyces badius	APERAGLTTW	LRTNTTGDVAV	IRAGVPEDWR	VGDKTGTTG.S	YGARNDIAVV
Streptomyces cacaoi blaU	EGDRKQLTTW	LRNNTTGDGL	IRAGVROGUV	VGDKTGTTG.S	YGARNDAVV
Streptomyces cacaoi ULg	RLQLNDW	MSGKPTGDAL	IRAGVPPKDW	VEDKSGQV.K	YGRNDIAVV
Klebsiella oxytoca	EQQRQLLVTW	LKGNNTGGQS	IRAGLPASWA	VGDTKGAG.D	YGTNDIAVV
Staphylococcus aureus	KENKFLLLDL	MLNKNKSGDTL	IKDGVPKDYK	VADKSGGAT	YGVANDVAVV
Streptomyces aureofaciens	AGDRKRLTW	LVANTTNRPT	FRAGLPDDTW	LADKTGQGEY	YGVANDVAVV
Streptomyces albus	PRDRRLTSW	LLANTTSGDR	FRAGLPDDTW	LGDKTGAG.R	YGTNDAGVT
Streptomyces lavendulae	PRDRRLTSW	LLANTTSTER	FRKGLPADWT	LGDKTGAG.A	YGTNDAGVT
Streptomyces fradiae	AHDRERLRGW	MLDNRTSDER	FRKGLPADWT	LADKTGGS.D	YGTNDAGVA
Consensus	ae.rkQLtdw	mlgnttgdal	iraglpadvw	vadktgag.s	ygrndiavv

Fig. 1. Alignment of 20 Class A beta-lactamases numbered according to the ABL scheme

The sequences are referred to by their most familiar names. '.' indicates a postulated deletion; blank spaces indicate one or more residues omitted from the alignment. Leader sequences before position 1 are omitted. Note that single tyrosine residues have been omitted from the *Streptomyces badius* and *Streptomyces cacaoi* sequences at position 241. Publication references are as follows: *Klebsiella pneumoniae*: Arakawa, Y., Ohta, M., Kido, N., Fujii, Y., Komatsu, T. & Kato, N. (1986) FEBS Lett. **207**, 69-74; PIT-2: Barthelemy, M., Peduzzi, J. & Labia, R. (1988) Biochem. J. **251**, 73-79; R-TEM: Sutcliffe, J. G. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 3737-3741; *Pseudomonas aeruginosa* and *Rhodopseudomonas capsulata*: Campbell, J. I. A., Scatell, S. A., Gibson, T. & Ambler, R. P. (1989) Biochem. J. **260**, 803-812; PSE-4: Boissinot, M. & Levesque, R. C. (1990) J. Biol. Chem. **265**, 1225-1230; *Actinomadura R39*: Houbba, S., Molitor, C., Willem, S., Ghuysen, J.-M., Frère, J.-M., Duez, C. & Dusart, J. (1989) FEMS Microbiol. Lett. **65**, 241-246; *Bacillus cereus* 569H and 5/B: Madgwick, P. J. & Waley, S. G. (1987) Biochem. J. **248**, 657-662 and Madonna, M. J., Zhu, Y. F. & Lampen, J. O. (1987) Nucleic Acids Res. **15**, 1877; *Bacillus cereus* III: Husain, M., Pastor, F. I. J. & Lampen, J. O. (1987) J. Bacteriol. **169**, 579-586; *Bacillus licheniformis*: Neugebauer, K., Sprengel, R. & Schaller, H. (1981) Nucleic Acids Res. **9**, 2577-2588; *Streptomyces badius*, *cacaoi* blaU, *lavendulae* and *fradiae*: Forsman, M., Haggstrom, B., Lindgren, L. & Jaurin, B. (1990) J. Gen. Microbiol. **136**, 589-598; *Streptomyces cacaoi* ULg: Lenzini, M. V., Ishihara, H., Dusart, J., Ogawara, H., Joris, B., Van Beumen, J., Frère, J.-M. & Ghuysen, J.-M. (1988) FEMS Microbiol. Lett. **49**, 371-376; *Klebsiella oxytoca*: Arakawa, Y., Ohta, M., Kido, N., Mori, M., Ito, H., Komatsu, T., Fujii, Y. & Kato, N. (1989) Antimicrob. Agents Chemother. **33**, 63-70; *Staphylococcus aureus*: Ambler, R. P. (1975) Biochem. J. **151**, 197-218 and McLaughlin, J. R., Murray, C. J. & Rabinowitz, J. C. (1981) J. Biol. Chem. **256**, 11273-11282; *Streptomyces aureofaciens*: G. Tiraby, unpublished work; *Streptomyces albus* G: Dehottay, P., Dusart, J., De Meester, F., Joris, B., Van Beumen, J., Ercipum, T., Frère, J.-M. & Ghuysen, J.-M. (1987) Eur. J. Biochem. **166**, 345.