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** **b**eta **l**actamase). 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 EQIKQSESQL SGRVGMVEMD LANGRTLAAW RADERFPMVS TFKVLLCGAV LARVDAGLEQ LDRRIHYRQQ
PIT-2                                                  SPQPL EQIKLSESQL SGRVGMIEMD LASGRTLTAW RADERFPMMS TFKVVLCGAV LARVDAGDEQ LERKIHYRQQ
R-TEM                         MSIQHFRV ALIPFFAAFC LPVFAHPETL VKVKDAEDQL GARVGYIELD LNSGKILESF RPEERFPMMS TFKVLLCGAV LSRVDAGQEQ LGRRIHYSQN
Pseudomonas aeruginosa      CHFLSVPVAI LGCVGLICTS AYAMDTGILD LAVTQEETTL QARVGVAVID TDSGLTW.QH RGDERFPLNS THKAFSCAAV LAQADRHKLN LEQAIPIERT
PSE-4                       GVTYMKFLLA FSLLIPSVVF ASSSKFQQVE QDVKAIEVSL SARIGVSVLD TQNGEYW.DY NGNQRFPLTS TFKTIACAKL LYDAEQGKVN PNSTVEIKKA
Rhodopseudomonas capsulata  TVLSRVATGL ALGLSMATAS LAGTPVEALS ETVARIEEQL GARVGLSLME TGTGWSW.SH REDELFLMNS TVKVPVCGAI LARWDAGRLS LSDALPVRKA
Actinomadura R39                             AEPA SAEVTAEDLS GEFERLESEF DARLGVYAVD TGTGEEV.FH RADERFGYAS THKAFTAALV LGQ..NTPEE LEEVVTYTEE
Bacillus cereus 569H        TSLEAFTGES LQVEAKEKTG QVKHKNQATH KEFSQLEKKF DARLGVYAID TGTNQTI.SY RPNERFAFAS TYKALAAGVL LQQ..NSIDS LNEVITYTKE
Bacillus cereus 5/B         TSLVTFTGGA LQVEAKEKTG QVKHKNQATH KEFSQLEKKF DARLGVYAID TGTNQTI.AY RPNERFAFAS TYKALAAGVL LQQ..NSTKK LDEVITYTKE
Bacillus cereus III         LIGCSNSNTQ SESNKQTNQT NQVKQENKRN HAFAKLEKEY NAKLGIYALD TSTNQTV.AY HADDRFAFAS TSKSLAVGAL LRQ..NSIEA LDERITYTRK
Bacillus licheniformis      LFSCVALAGC ANNQTNASQP AEKNEKTEMK DDFAKLEEQF DAKLGIFALD TGTNRTV.AY RPDERFAFAS TIKALTVGVL LQQ..KSIED LNQRITYTRD
Streptomyces badius         ..SDSTAPPS SAKPATSASA SLP.RPKPYT GDFKKLEREF DARLGVYAID TGTGREV.TH NDRARFAYHS TFKALQAAVV LS    SLDG LDKRVTYTRE
Streptomyces cacaoi blaU    ESSADAAEPA GSAPSSSAAA HKPGEVEPYA AELKALEDEF DVRLGVYAVD TGSGREV.AY RDGERFPYNS TFKALECGAV L           DRVVKYSED
Streptomyces cacaoi ULg     ACGQASGSES GQQPGLGGAD EAHVSADAHE KEFRALEKKF DAHPGVYAID TRDGQEI.TH RADERFAYGS TFKALQAGAI LAQV        DKVVHYGQD
Klebsiella oxytoca                 MAA AAVPLLLASG SLWASADAIQ QKLADLEKRS GGRLGVALIN TA  QTL  Y RGDERFAMCS TGKVMAAAAV LKQ        VNKRLEIKKS
Staphylococcus aureus             MKKL IFLIVIALVL SACNSNSSHA KELNDLEKKY NAHIGVYALD TKSGKEV.KF NSDKRFAYAS TSKAINSAIL LEQV..PYNK LNKKVHINKD
Streptomyces aureofaciens   TMAALLPAGG AAYASTSTAK APAAEGISG. .RLRALEKQY AARLGVFALD TGTGAGR.SY RAGERFPMCS VFKALAAAAV LRDVDA     LTKRIHY
Streptomyces albus          ALAAATLVPG TAHASSGGRG HGSGSVSDAE RRLAGLERAS GARLGVYAYD TGSGRTV.AY RADELFPMCS VFKTLSSAAV LRD        LDR      D
Streptomyces lavendulae     AVAGIPLGGS TAFA...... .APRGNPDVL RQLRALEQEH SARLGVYARD TATGRTV.LH RAEERFPMCS VFKTLAVAAV LR         LDR
Streptomyces fradiae        ALAATAAAAG PAHA...... .APGRGARVE GRLRALERTH DARLGAFAYD TGTGRTV.AY RADERFPIAS MFKTIAVAAV LR         LDR
Consensus                   .saa.aa.g. aavpslaaag .apgsnpa.. ke.kalEkqf darlGvya.d tgtgrtv.ay raderFpmaS tfKala..av L.q.....e. l..ritytk.

                           101                                               150                                                  200
Klebsiella pneumoniae       DLVDYSPVSE KHLVDGMTIG ELCAAAITLS DNSAGNLLLA TVGGPAGLTA FLRQIGDNVT RLDRWETALN EALPGDARDT TTPASMAATL RKLLTAQHLS
PIT-2                       DLVDYSPVSE KHLADGMTVG ELCAAAITMS DNSAANLLLT AVGGPAGLTA FLRQIGDNVT RLDRWETELN EALPGDARDT TTPASMAATL RKLLTSQRLS
R-TEM                       DLVEYSPVTE KHLTDGMTVR ELCSAAITMS DNTAANLLLT TIGGPKELTA FLHNMGDHVT RLDRWEPELN EAIPNDERDT TMPAAMATTL RKLLTGELLT
Pseudomonas aeruginosa      ALVTYSPVTE       LTLR ELCRAAVSIS DNTAANLALD AIGGARTFTA FMRSIGDDKT RLDRREPELN EATPGDARDT TTPIAAARSL QTLLLDGVLS
PSE-4                       DLVTYSPVIE KQVGGQAITLD DACFATMTTS DNTAANIILS AVGGPKGVTD FLRQIGDKET RLDRIEPDLN EGKLGDLRDT TTPKAIASTL NKFLFGSALS
Rhodopseudomonas capsulata  DLVPYAPVTE       MTLD ELCLAAIDMS DNVAANILIG HLGGPEAVTQ FFRSVGDPTS RLDRIEPKLN DFASGDERDT TSPAAMSETL RALLLGDVLS
Actinomadura R39            DLVDYSPITE QHVDTGMTLL EVADAAVRHS DNTAANLLFE ELGGPEGFEE DMRELGDDVI SADRIETELN EVPPGETRDT STPRAMAGSL EAFVLGDVLE
Bacillus cereus 569H        DLVDYSPVTE KHVDTGMKLG EIAEAAVRSS DNTAGNILFN KIGGPKGYEK ALRHMGDRIT MSNRFETELN EAIPGDIRDT STAKAIATNL KAFTVGNALP
Bacillus cereus 5/B         DLVDYSPVTE KHVDTGMTLG EIAEAAVRYS DNTAGNILFH KIGGPKGYEK ALRKMGDRVT MSDRFETELN EAIPGDIRDT STAKAIARNL KDFTVGNALP
Bacillus cereus III         DLSNYNPITE KHVDTGMTLK ELADASVRYS DSTAHNLILK KLGGPSAFEK ILREMGDTVT NSERFEPELN EVNPGETHDT STPKAIAKTL QSFTLGTVLP
Bacillus licheniformis      DLVNYNPITE KHVDTGMTLK ELADASLRYS DNAAQNLILK QIGGPESLKK ELRKIGDEVT NPERFEPELN EVNPGETQDT STARALVTSL RAFALEDKLP
Streptomyces badius         DLVAHSPVTE KHVDTGMTLK ELCDASVRYS DNTAANLLFD    GPKGLDA SLEKLGDDIT RMDREEPELS RWVPGEKRDT STPRALAEDL RAFVLGKALR
Streptomyces cacaoi blaU    DLVDNSPVTE KHVEDGMTLT ALCDAAVRYS DNTAANLLFE TVGGPKGLDK TLEGLGDHVT RMERVEPFLS RWEPGSKRDT STPRAFAKDL RAYVLGDVLA
Streptomyces cacaoi ULg     AILPNSPVTE KHVADGMSLR ELCDAIVAYS DNTAANLLFD QLGGRRGSTR VLKQLGDHTT SMDRYEQELG SAVPGDPRDT STPRAFA
Klebsiella oxytoca          DLVVWSPITE KHLQSGMTLA ESLAAALQYS DNTAMNKMIS YLGGPEKVTA F    GDVTF RLDRTEPALN SAIPGDKRDT TTPLAMAESL RKLTLGNALG
Staphylococcus aureus       DIVAYSPILE KYVGKDITLK ALIEASMTYS DNTANNKIIK EIGGIKKVKQ RLKELGDKVT NPVRYEIELN YYSPKSKKDT STPAAFGKTL NKLIANGKLS
Streptomyces aureofaciens         PVT       GMTGA ELCAAAVSES DNGAGNLLLR ELDGPTGITR FCRSLGDTTT RLDRWEPALN SAEPDRVTDT TSPGAIGRTF GRLIVGSALR
Streptomyces albus          DV   APETG K    GMTVE ELCEVSITAS DNCAANLMLR ELGGPAAVTR FVRSLGDRVT RLDRWEPELN SAEPGRVTDT TSPRAITRTY GRLVLGDALN
Streptomyces lavendulae         FGPVT       GMTVE RLCAAAICQS DNAAANLLLR ELGGPEAVTR FCRSVGDRTT RLDRWEPELN SAEPGRLTDT TTPRAIGATY GELVLGDALA
Streptomyces fradiae            YSPV        GMTVA ELCEATLTRS DNTAANLLLR DLGGPTAVTR FCRSVGDHVT RLDRWEPELN SAEPGRVTDT TSPRAIGRTY GRLILGDLLA
Consensus                   dlvdyspvte khvdtgmtl. elcdaav.yS DntAaNlllr elgGpkgvta flrslGD.vt rldRwEpeLn eaepgdkrDT ttpraiartl r.lllgdaLs

                            201                                              250                                        295
Klebsiella pneumoniae       ARSQQQLLQW MVDDRVAGPL IRAVLPPGWF IADKTGAG.E RGARGIVALL GP.DGKPERI VVIYLRDTPA SMAERNQHIA GIGQR
PIT-2                       ARSQRQLLQW MVDDRVAGPL IRSVLPAGWF IADKTGAG.E RGARGIVALL GP.NNKAERI VVIYLRDTPA SMAERNQQIA GIGAALIEHW QR
R-TEM                       LASRQQLIDW MEADKVAGPL LRSALPAGWF IADKSGAG.E RGSRGIIAAL GP.DGKPSRI VVIYTTGSQA TMDERNRQIA EIGASLIKHW
Pseudomonas aeruginosa      APARNELTQW MLGDQVADAL LRAGLPRDWQ IADKSGAG.G HGSRSIIAVV WP.PKRSAVI VAIYITQTAA SMSASNQAVS RIGSALAKAL Q
PSE-4                       EMNQKKLESW MVNNQVTGNL LRSVLPAGWN IADRSGAG.G FGARSITAVV WS.EHQAPII VSIYLAQTQA SMEERNDAIV KIGHSIFDVY TSQSR
Rhodopseudomonas capsulata  PEARGKLAEW MRHGGVTGAL LRAEAEDAWL ILDKSGSG.S H.TRNLVAVI QP.EGGAPWI ATMFISDTDA EFEVRNEALK DLGRAVVAVV RE
Actinomadura R39            EGPRDVLTEM LLNNTTGDEL IRAGVPEDWR VGDKTGGG.S HGSRNDIAVV WP.PEDDPIV IAVMSTREQE DAEFDNALVS GATEVVVEAL AP
Bacillus cereus 569H        AEKRKILTEW MKGNATGDKL IRAGIPTDWV VGDKSGAG.S YGTRNDIAVV WP.PNRAPII IAILSSKDEK EAIYDNQLIA EATKVIVKAL R
Bacillus cereus 5/B         HQKRNILTEW MKGNATGDKL IRAGVPTDWV DADKSGAG.S YGTRNDIAIV WP.PNRSPII IAILSSKDEK EATYDNQLIK EAAEVVIDAI K
Bacillus cereus III         SEKRELLVDW MKRNTTGDKL IRAGVPKGWE VADKTGAG.S YGTRNDIAII WP.PNKKPIV LSILSNHDKE DAEYDDTLIA DATKIVLETL KVTNK
Bacillus licheniformis      SEKRELLIDW MKRNTTGDAL IRAGVPDGWE VADKTGAA.S YGTRNDIAII WP.PKGDPVV LAVLSSRDKK DAKYDDKLIA EATKVVMKAL NMNGK
Streptomyces badius         APERAQLTTW LRTNTTGDAV IRAGVPENWV VGDKTGTG.S YGARNDIAVV WP.PDSAPIV IAILSHRGTK DAEPDDELIA EAASVVVDSL SS
Streptomyces cacaoi blaU    EGDRKQLTTW LRNNTTGDGL IRAGVRQGWV VGDKTGTG.S YGARNDMAVV WR.PDGRPLV LNVMVHGHTK DAELDSELIA RATEVVADRL G
Streptomyces cacaoi ULg        RLQLNDW MSGKPTGDAL IRAGVPKDWK VEDKSGQV.K YGTRNDIAVV RP.PGRAPIV VSVMSHGDTQ DAEPHDELVA EAGLVVADGL K
Klebsiella oxytoca          EQQRAQLVTW LKGNTTGGQS IRAGLPASWA VGDTKGAG.D YGTTNDIAVI WP.ENHAPLV LVTYFTQPQQ DAKSRKEVLA AAKIVTEGL
Staphylococcus aureus       KENKKFLLDL MLNNKSGDTL IKDGVPKDYK VADKSGQAIT YASRNDVAFV YPKGQSEPIV LVIFTNKDNK SDKPNDKLIS ETAKSVMKEF
Streptomyces aureofaciens   AGDRKRLTGW LVANTTNRPT FRAGLPDDWV LADKTGGGEQ YGVANDVGVV QP.PGRAPLV LSVLSTKFDP KGPTDNPLVA KAAALVAGEL T
Streptomyces albus          PRDRRILTSW LLANTTSGDR FRAGLPDDWT LGDKTGAG.R YGTNNDAGVT WP.PGRAPIV LTVLTAKTEQ DAARDDGLVA DAARVLAETL G
Streptomyces lavendulae     PRDRERLTGW LLANTTSTER FRKGLPADWT LGDKTGGG.A YGTNNDAGVT WP.PHRPPVV MVVLTTHDRP DAVADNPLVA KTAALLASAL G
Streptomyces fradiae        AHDRERLTRW MLDNRTSDER FRKGLPADWL LADKTGGG.D YGTNNDAGVA WP.PGRPPVV LAVQTTRFTP DAEADNVLVA EAARLLAEAM TD
Consensus                   ae.rkqLtdw mlgnttgdal iraglpadwv vaDktGag.s ygtrndiavv wp.pgrapiv lailstkd.. dae.dn.lia eaakvvaeal .s..k
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Fig. 1. Alignment of 20 Class A β-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., Scahill, 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: Houba, 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 Beeumen, 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 Beeumen, J., Erpicum, T., Frère, J.-M. & Ghuysen, J.-M. (1987) Eur. J. Biochem. **166**, 345.