Detection of α/β-hydrolase fold in the cell surface esterases of Acinetobacter species using an analysis of 3D profiles

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Abstract The primary sequence of esterases from *Acinetobacter lwoffii* RAG-1 and *A. calcoaceticus* BD413 were compared with linearized structural sequences of two hundred proteins selected from Brookhaven Protein DataBank using a modified version of the Bowie et al. algorithm [3]. Significant structural homology was found to α/β proteins and specifically to those with the α/β-hydrolase fold for which the crystal structure was reported. No such homology was detected using common primary sequence alignment programs such as FASTA or BLAST.

Key words: *Acinetobacter;* Esterase; α/β Hydrolase fold; Structural alignment

1. Introduction

Certain strains of Acinetobacter produce growth-dependent extracellular esterases and lipases [1,5–8,12,14,15]. In the oil-degrading bacterium *A. lwoffii* RAG-1, an exocellular esterase mediates the release of the amphipathic bioemulsifier emulsan from the cell surface by hydrolyzing the hydrophobic side chains which are connected by ester bonds to the sugar backbone of the emulsan, thereby weakening the association of the emulsan mini-capsule with the cell surface [14,15]. Previously, we described the cloning and expression of the *est* gene encoding the RAG-1 esterase [1,12]. The expression of the *est* gene conferred on *E coli* the ability to utilize triglycerides and other esters as sole carbon sources. An open reading frame of 909 bp was found whose expression in minicells led to the production of a unique peptide of M_r 32,500. Antibodies prepared against the over-expressed recombinant esterase in *E. coli* were used to locate the enzyme primarily in the membrane fractions of *A lwoffii* RAG-1 [1]. A cell-bound esterase from *A. calcoaceticus* BD413 has also been expressed in *E. coli* [8]. Analysis of the conserved motifs in the primary sequence of the two esterases and aligments with related proteins using BLAST and FASTA programs, suggested that the two Acinetobacter esterases may belong to the family of serine proteases [1,8]. However, no further insight into structural properties of these esterases has been forthcoming since the 3D structures of the most closely related proteins have not been determined [1].

In the present study, the 3D-1D algorithm of Bowie et al. [3] was used to generate a series of linearized structural protein sequences chosen from 200 3D protein structures of the Brookhaven Protein DataBank. A modification of this algorithm permitted a comparison of the RAG-1 and BD413 primary esterase sequences with each of the linearized representations. The results suggest that the Acinetobacter esterases share structural homology with a series of α/β proteins and are likely to have the α/β-hydrolase domain fold [11].

2. Materials and methods

2.1 *The algorithm for structural alignments*

The algorithm was based on the one-dimensional structural representation introduced by Bowie et al. [3] according to which amino acid residues in the protein sequence were characterized by their local environment (polarity, solvent-accessible surface area and secondary structure type). An 18-letter alphabet was thus generated.

Two hundred high resolution crystal structures from PDB (October, 1993) were chosen and each was converted into a 1D linear sequence. The alignments were performed using dynamic programming techniques [10] with a 3D-1D scoring matrix [3]. Since the score for an alignment depends strongly on both the length of the protein and its amino acid composition, a normalization procedure was introduced by which the scores were normalized after repeated alignments using randomly permuted sequences. *Z* scores were calculated as the number of standard deviations from the average score for the randomized sequences.

3. Results and discussion

3.1. *Primary sequence analysis of RAG-1 and BD413 esterases*

Screening of the amino acid sequences of the esterases from *A. lwoffii* RAG-1 (GenBank, EMBL and DDBJ accession no. M24890, [1]) and *A calcoaceticus* BD413 (accession no. X71598, [8]) against the GenEMBL, PIR and SwissProt libraries using either FASTA or BLAST programs, revealed characteristic homology to various esterases, lipases and acyl-transferases as previously described [1,8]. As illustrated in the Pretty Box alignment in Fig. 1, with the exception of a hypothetical protein from *E coli*, all the homologous proteins contain a consensus sequence Gly-X_1-Ser-X_2-Gly at the active site serine [4]. In most cases, X_1 was an aspartic acid residue and X_2 was primarily an alanine, although in the case of the RAG-1 enzyme, a cysteine appeared in place of the alanine. In addition, a significant sequence conservation was seen in the regions of the RAG-1 esterase between residues 79 and 156, and between residues 227 and 276.

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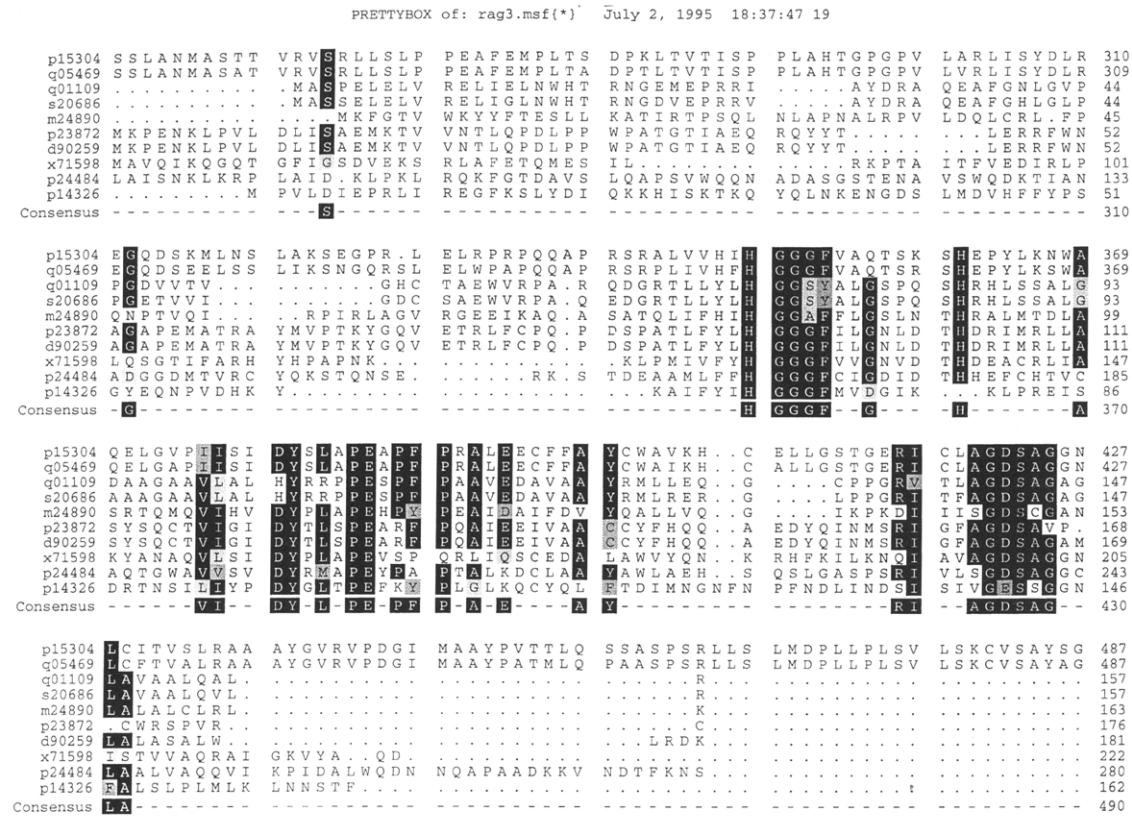
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q01109 .....MASPELELV RELIELNWHT RNGEMEPRRI .....AYDRA QEAFGNLGVP 44
s20686 .....MASSELELV RELIGLNWHT RNGDVEPRRV .....AYDRA QEAFGHLGLP 44
m24890 .....MKFGTV WKYYFTESLL KATIRTPSQL NLAPNALRPV LDQLCRL.FP 45
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x71598 MAVQIKQGQT GFIGSDVEKS RLAFETQMES IL..... .....RKPTA ITFVEDIRLP 101
p24484 LAISNKLKRP LAID.KLPKL RQKFGTDAVS LQAPSVWQQN ADASGSTENA VSWQDKTIAN 133
p14326 .....M PVLDIEPRLI REGFKSLYDI QKKHISKTKQ YQLNKENGDS LMDVHFFYPS 51
Consensus ---------- ---S------ ---------- ---------- ---------- ---------- 310
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x71598 LQSGTIFARH YHPAPNK... ..... .KLPMIVFYH GGGFVVGNVD THDEACRLIA 147
p24484 ADGGDMTVRC YQKSTQNSE. .....RK.S TDEAAMLFFH GGGFCIGDID THHEFCHTVC 185
p14326 GYEQNPVDHK Y..... ....KAIFYIH GGGFMVDGIK ...KLPREIS 86
Consensus -G-------- ---------- ---------- ---------H GGGF--G--- -H-------A 370
p15304 QELGVPIISI DYSLAPEAPF PRALEECFFA YCWAVKH..C ELLGSTGERI CLAGDSAGGN 427
q05469 QELGAPIISI DYSLAPEAPF PRALEECFFA YCWAIKH..C ALLGSTGERI CLAGDSAGGN 427
q01109 DAAGAAVLAL HYRRPPESPF PAAVEDAVAA YRMLLEQ..G ....CPPGRV TLAGDSAGAG 147
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m24890 SRTQMQVIHV DYPLAPEHPY PEAIDAIFDV YQALLVQ..G ....IKPKDI IISGDSCGAN 153
p23872 SYSQCTVIGI DYTLSPEARF PQAIEEIVAA CCYFHQQ..A EDYQINMSRI GFAGDSAVP. 168
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x71598 KYANAQVLSI DYPLAPEVSP QRLIQSCEDA LAWVYQN..K RHFKILKNQI AVAGDSAGGN 205
p24484 AQTGWAVVSV DYRMAPEYPA PTALKDCLAA YAWLAEH..S QSLGASPSRI VLSGDSAGGC 243
p14326 DRTNSILIYP DYGLTPEFKY PLGLKQCYQL PTDIMNGNFN PFNDLINDSI SIVGESSGGN 146
Consensus ------VI-- DY-L-PE-PF P-A-E----A Y--------- --------RI --AGDSAG-- 430
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q01109 LAVAALQAL. .....R... 157
s20686 LAVAALQVL. .....R... 157
m24890 LALALCLRL. .....K... 163
p23872 .CWRSPVR.. .....C... 176
d90259 LALASALW.. ...LRDK... 181
x71598 ISTVVAQRAI GKVYA..QD. 222
p24484 LAALVAQQVI KPIDALWQDN NQAPAADKKV NDTFKNS... 280
p14326 FALSLPLMLK LNNSTF.... .... t..... 162
Consensus LA-------- ---------- ---------- ---------- ---------- ---------- 490
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q05469 AKTEDHSNSD QKALGMMGLV RRDTALLLRD FRLGASSWLN SFLELSGRKS QKMSE..... 542
q01109 157
s20686 157
m24890 163
p23872 176
d90259 181
x71598 222
p24484 280
p14326 162
Consensus ---------- ---------- ---------- ---------- ---------- ---------- 550
p15304 TAPHGFWALT ESMRRSVSEA ALAQPEGLLG TDSLKKLTIK DLSFKGNSE. PSDSPEMSQS 606
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q01109 .....DA GTPLPAAAVC ISPWADLACE GASHTTRKA. 188
s20686 .....DA GDPLPAAAVC ISPWADLACE GASHVTRKE. 188
m24890 .....QQ PELMPSGLIL MSPYLDLTLT SESLRFNQK. 194
p23872 .....GC VINRSIAVKL RAFCCGM... 195
d90259 .....QI DCGKVAGVLL WYGLYGLRDS VTRRLLGGV. 212
x71598 ....AQFL IYPVVDFKSR HPSFYAYKD. 245
p24484 .....LA DLPRPLAQLP LYPVTDYEAE YPSWELYGE. 311
p14326 ..... .FKKISKVLV YYPITDCNLK HHPIIDLRES 191
Consensus ---------- ---------- ----P----- --P--DL--- --S------- ---------- 610
p15304 METLGPSTPS DVNFFLRSGN SQEEAETRDD ISPMDGIPRV RAAFPDGFHP RRSSQGVLHM 666
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q01109 ....REILLD TADLRRMAER 204
s20686 ....REILLD TEDLLRMAGR 204
m24890 ....HDALLS IEALQAGIKH 210
p23872 ....GFTD YGIP..... 203
d90259 ....WDGLTQ QDLQMYEEAY 228
x71598 ....GLVLTG NDVDYVTDYY 261
p24484 ....GLLLDH NDAEVFNSAY 327
p14326 ....IN KGSMKWCWNH 203
Consensus ---------- ---------- ---------- ---------- -------L-- ---------- 670
p15304 PLYSSPIVKN PFMSPLLAPD VMLKTLPPVH LVACALDPML DDSVMFARRL KDLGQPVTLK 726
q05469 PLYSSPIVKN PFMSPLLAPD SMLKSLPPVH IVACALDPML DDSVMLARRL RNLGQPVTLR 715
q01109 YLA.GTDPRH PLASPAHGD. ..LTGLPPLL IQVGSEEVLH DDARALEQAA LKAGTPVTFE 260
s20686 YLA.GTDPRN PLASPAHGD. ..LTGLPPLL IQVGSEEVLY DDARALEQAA LKAGVPVTFD 260
m24890 YLTDDIQPGD PRVSPLFDD. ..HDGLPPTL VQVGSKEILL DDSKRFREKA EQADVKVHFK 267
p23872 203
d90259 LSNDADRESP YYCLFNNDL. ..TREVPPCF IAGAEFDPLL DDSRLLYQTV SCASAA.... 281
x71598 ATKHAVHLDD PIISPTYGN. ..EKKLAPAY IVTAGHDVLH DEGEIYSHKL RQAGVKIHFE 318
p24484 TQHSGLPQSH PLISVMHGD. ..NTQLCPSY IVVAELDILR DEGLAYAELL QKEGVQVQTY 384
p14326 YTNNDSERDE ITCCPLKATI DQLKDFPETL VITAETDVLS SEGEQFGLKL SNANVKVSVL 263
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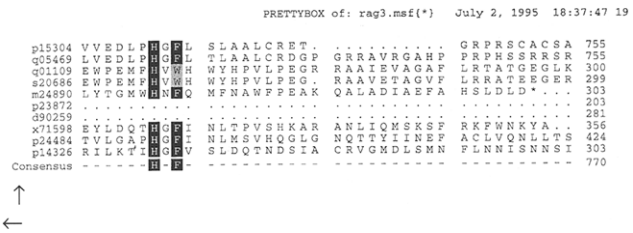

Fig. 1. Sequence alignments of RAG-1 and BD413 esterases to homologous proteins. The RAG-1 (M24890) and BD413 (X71598) esterases amino-acid sequences were screened using the GenEMBL, Pir and SwissProt libraries (by FASTA and BLAST). The screening revealed extensive homology to the translation products of the following genes: Q01109, *S hygroscopicus* acetyl-hydrolase; S20686, *S viridochromogenes* deacetylase; P23872, *E. coli* hypothetical protein; D90259, *E. coli* lipase; P15304, rat lipase; Q05469, human lipase; P24484, *Moraxella sp* lipase; P14326, slime mold protein. Identical amino acid residues are boxed

3.2. Structural alignments of Acinetobacter esterases

In order to gain some additional insight into the structural characteristics of the RAG-1 and BD413 esterases, we used the method of Bowie et al. [3], in an attempt to align the linearized structural sequence of 200 proteins selected from the PDB with the modification as described in section 2. As a control, the sequence of acetylcholinesterase from *T. californica* [13] was aligned with the linearized structures of the same 200 proteins. The distribution of normalized Z-score for each of the three proteins is shown in Fig. 2 and Table 1. Not surprisingly, the vast majority of the proteins exhibited little structural homology when aligned with any of the three sequences (clustering around a normalized Z-score of zero). In the case of the positive control (Fig. 2A, Table 1A), the primary sequence of acetylcholinesterase was aligned with the structural representation of that enzyme with an extremely high score of almost 13 standard deviation (Z-score = 12.97). Moreover, the four best aligned structures of the six proteins which yielded Z-score > 2.0 were members of the α/β-hydrolase family. Of these, two lipases (1crl and 1thg) exhibited Z-score of 7.98 and 6.08, respectively. When the primary sequences of both *A lwoffii* RAG-1 and *A calcoaceticus* BD413 esterases were screened against the same 200 structural representations, a similar distribution was observed although maximum Z-scores were lower. The RAG-1 sequence (Fig 2B, Table 1B) exhibited a maximum Z-score of 4.92 when aligned with 5rub (ribulose-1,5-biphosphate carboxylase/oxygenase) and a Z-score of 4.63 when aligned with the lipase, 1crl. The lipase protein also exhibited the second highest score when aligned with the acetylcholinesterase control (Fig. 2A, Table 1A). Eighteen proteins were found to align with the RAG-1 esterase sequence with Z-score > 2.0 standard deviations. Of these, 5 proteins belonging to the α/β-hydrolase family including 1crl, 3sc2, 1ede, 1ace (acetylcholinesterase). The primary sequence of the BD413 esterase showed an even better alignment with acetylcholinesterase, i.e. exhibiting a maximum Z-score of 6.73 (Fig. 2C, Table 1C). However, in this case only one of the α/β-hydrolase fold enzymes, 1thg, had a Z-score > 2.0.

It should be noted that as shown in Table 1 for both RAG-1 and BD413 esterases, more than 2/3 of the highest Z-score enzymes are part of the α/β-class proteins according to the SCOP [9] classification, while 5/6 of the top scores for acetylcholinesterase are members of this class. Therefore, it is very likely that both RAG-1 and BD413 esterases are members of the α/β class. The results of the threading procedure described here points to the possibility that the two Acinetobacter esterases may, in fact, be members of the α/β-hydrolase family. This observation could not have been predicted according to the amino acid alignments shown in Fig. 1. It is interesting to note that the results obtained here are in line with those obtained when a sequence search was performed against just the sequences found in the PDB. When the esterase sequences were run through such a procedure, via the SCOP WEB home page [9], with BLAST [2], only very weak alignments were obtained. However, the highest scores observed were with members of the α/β-hydrolase family (data not shown).

All the five enzymes displaying the α/β-hydrolase fold which were compared by Ollis et al. [11]. show a similar core consisting of eight β-sheets connected by α-helices. Furthermore, they have similar overall topology, a conserved sequence order for the catalytic triad residues, and conserved loops for the catalytic triad. However, they show variation in the identity of the amino acid constitution of the catalytic triad. The only conserved residue is the histidine. The negatively charged amino

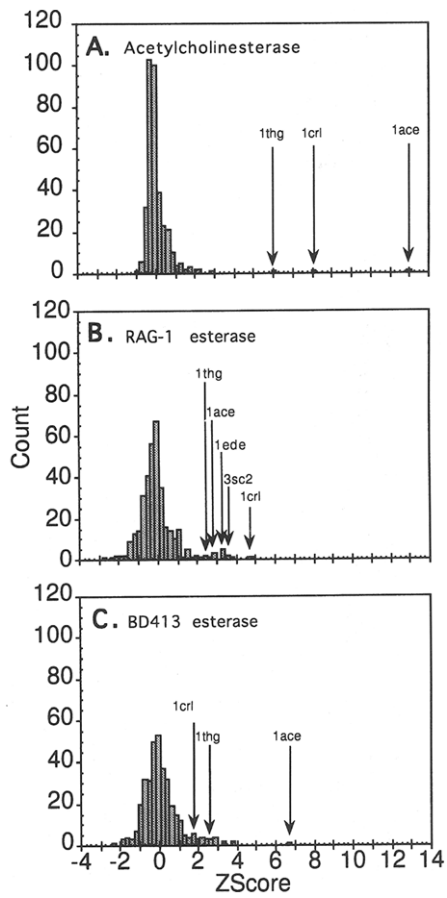

Fig. 2. Normalized Z-scores of *T. californica* acetylcholinesterase (A), *A. lwoffii* RAG-1 esterase (B) and *A calcoaceticus* BD413 esterase (C) The normalized Z-score (X-axis) are plotted against frequency of their occurrence (Y-axis). The arrows point to proteins that are members of the α/β-hydrolase family and for which the Z-score was greater then two standard deviations. Protein abbreviations: 1ace, *T californica* acetylcholinesterase; 1crl, *Candida rugosa* lipase; 1thg, *Geotrichum candidum* lipase triacylglycerol hydrolase; 3sc2, *Triticum vulgaris* serine carboxypeptidase II; 1ede, *Xanthobacter autotrophicus* haloalkane dehalogenase

Table 1
Proteins that gave Z-scores > 2.0 for *T. californica* acetylcholinesterase (A), Acinetobacter RAG-1 esterase (B) and BD413 esterase (C)

A. *T. californica* acetylcholinesterase

ID Code	Z-score	Protein	Class	Fold
1ace	12.97	Acetylcholinesterase	α/β	α/β hydrolase
1crl	7.98	Lipase	α/β	α/β hydrolase
1thg	6.08	Triacylglycerol hydrolase	α/β	α/β hydrolase
4cpa	2.89	Carboxypeptidase A	α/β	Zn-dependent exopeptidase
1nca	2.25	N9 neuraminidase	all β	β propellers
1atn	2.22	Actin	α/β	Ribonuclease H-like

B. *A. lwoffii* RAG-1 esterase

ID Code	Z-score	Protein	Class	Fold
5rub	4.92	Carboxylase/oxygenase	α + β	Ferredoxin-like
1crl	4.63	Lipase	α/β	α/β hydrolase
1btc	3.68	β-amilase	α/β	β/α-(TIM)-barrel
1gpb	3.61	Phosphorylase B	α/β	β-glucozyl transferase
2pia	3.44	Reductase	α/β	Ferredoxin reductase
2liv	3.42	leu/ile/val binding protein	α/β	Periplasmic binding
1ldb	3.41	Lactate dehydrogenase	α/β	NAD(P)-Binding
1pha	3.37	Cytochrome P450-CAM	all α	Cytochrom P450
3sc2	3.25	Serine carboxypeptidase	α/β	phosphofructokinase
1ede	3.20	Haloalkane dehalogenase	α/β	α/β hydrolase
1thl	2.96	Thermolysin	α + β	Metzincins
1pfk	2.85	Phosphofructokinase	α/β	Phosphofructokinase
1ace	2.80	Acetylcholinesterase	α/β	α/β hydrolase
7tim	2.75	Triosephosphate isomerase	α/β	β/α-(TIM)-barrel
1thg	2.37	Triacylglycerol hydrolase	α/β	α/β hydrolase
1tro	2.33	trp repressor	all α	Trp repressor
4mdh	2.20	Malate dehydrogenase	α/β	NAD(P)-Binding
1rla	2.03	Rhinovirus coat	all β	Viral coat & capsid

C. *A. calcoaceticus* BD413 esterase

ID Code	Z-score	Protein	Class	Fold
1ace	6.73	Acetylcholinesterase	α/β	α/β hydrolase
1gox	3.82	Glycolate oxidase	α/β	β/α-(TIM)-barrel
1ath	3.72	Actin	α/β	Ribonuclease H
1mee	3.41	Eglin C	α + β	cI-2 protease inhibitors
1nca	3.31	N9 Neuraminidase	all β	β propellers
1mpp	2.91	Pepsin	all β	Acid proteases
2pia	2.88	Reductase	α/β	Ferredoxin reductase
1st3	2.87	Subtilicin BL	α/β	Subtilases
1chr	2.81	Cycloisomerase	α/β	β/α-(TIM)-barrel
1nar	2.73	Narbonin	α/β	β/α-(TIM)-barrel
1gla	2.70	Glycerol kinase	α/β	Ribonuclease H
1thg	2.57	Triacylglycerol hydrolase	α/β	α/β hydrolase
1ldb	2.46	Lactate dehydrogenase	α/β	NAD(P)-binding
1sub	2.43	Subtilicin BPN'	α/β	Subtilases
1sib	2.36	Eglin C	α + β	cI-2 protease inhibitors
1sbn	2.30	Subtilicin novo BPN'	α/β	Subtilases
1lct	2.16	Lactoferrin	α/β	Periplasmic binding
1eca	2.09	Hemoglobin III	all α	Globin-like
3pgm	2.08	Phosphoglycerate mutase	α/β	Phosphoglycerate mutase

acid can be either glutamate or aspartate and the nucleophile, which can either be serine, cysteine or aspartate, is embedded in a tight turn between the 5th β-strand and the next α-helix. Based on the amino-acid alignments and 3-D structural analysis of RAG-1 esterase, ser^{149}, either asp^{196} or glu^{244}, and either his^{265}, his^{274}, or his^{298}, could be members of the catalytic triad. Site-directed mutagenesis is currently being employed to ressolve this issue, and attempts are underway to crystallize both wild-type and mutant proteins.

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References

[1] Alon, R.N and Gutnick, D.L. (1993) FEMS Microbiol. Lett. 112, 275–280.
[2] Altschul, S.F., Gish, W., Miller, W., Meyers, E.W. and Lipman, D.J. (1990) J. Mol. Biol. 215, 403–410.
[3] Bowie, J.U., Lüthy, R. and Eisenberg, D. (1991) Science 253, 164–170.
[4] Brenner, S. (1988) Nature 334, 528–530.

[5] Breuil, C. and Kushner, D.J. (1975) Can. J. Microbiol. 21, 423–428.
[6] Claus, R., Fischer, B.E. and Kleber, H.-P. (1985) J. Basic Microbiol. 25, 299–304.
[7] Fischer, B.E., Koslowski, R. and Kleber, H.-P. (1987) J. Biotech. 6, 271–280.
[8] Kok, R.B., Christoffels, V.M., Vosman, B. and Hellingwerf, K.L. (1993) J. Gen. Microbiol. 139, 2329–2342.
[9] Murzin, A.G., Brenner, S.E., Hubbard, T. and Chothia, C. (1995) J. Mol. Biol. 247, 536–540.
[10] Needelman, S.B. and Wunsch, C.D. (1970) J. Mol. Biol. 48, 443–453.
[11] Ollis, D.L., Cheah, E., Cygler, M., Dijkstra, B., Frolow, F., Franken, S.M., Harel, M., Remington, S.J., Silman, I., Schrag, J., Sussman, J.L., Verschueren, K.H.G. and Goldman, A. (1992) Protein Engineering 5, 197–211.
[12] Reddy, P.G., Allon, R., Mevarech, M., Mendelovitz, S., Sato, Y. and Gutnick, D.L. (1989) Gene 76, 145–152.
[13] Schumacher, M., Camp, S., Maulet, Y., Newton, M., MacPhee-Quigley, K., Taylor, S.S., Friedmann, T. and Taylor, P. (1986) Nature 319, 407–409
[14] Shabtai, Y. and Gutnick, D.L. (1985) J. Bacteriol. 161, 1176–1181.
[15] Shabtai, Y., Pines, O. and Gutnick, D.L. (1985) Dev Indust. Microbiol. 26, 291–299.