Journal of Bioinformatics and Sequence Analysis Vol. 3(6), pp. 116-123, September 2011
Available online at http://www.academicjournals.org/JBSA
ISSN 2141-2464 ©2011 Academic Journals

Full length Research Paper

In silico analysis of amino acid sequences in relation to specificity and physiochemical properties of some aliphatic amidases and kynurenine formamidases

Naresh Kumar[1]* and T. C. Bhalla[2]

Department of Biotechnology, Himachal Pradesh University (HPU), Summer Hill, Shimla, Himachal Pradesh-171005, India.

Accepted 5 September, 2011

Computational analysis of amino acid sequences of aliphatic amidases and kynurenine formamidases for some of their physiochemical properties and their substrate specificity has been done. Multiple sequence alignment of 18 amino acid sequences shows a clear difference between the two classes of aminohydrolases. Statistical analysis indicated a clear distinction between aliphatic amidases and kynurenine formamidases. The kynurenine formamidases and aliphatic amidases mainly differ in the total number of amino acid and composition of amino acid. Catalytic triad was found to be conserved and difference in amino acids makes them substrate specific. The results of the present work will be quite useful in prediction and selection of kynurenine formamidases/aliphatic amidases either from reported amidases or from the large number of sequenced microbial genome.

Key words: Aliphatic amidases, kynurenine formamidases, amino acids, substrate specificity, multiple alignments.

INTRODUCTION

A variety of cyclic amide-metabolizing systems occur in nature and play important roles in various metabolisms such as pyrimidine and purine; amino acid and antibiotic (Soong et al., 2000).The enzyme physiologically functions in the second step of cyclic imide degradation, that is, the hydrolysis of monoamidated dicarboxylates (half-amides) to dicarboxylates and ammonia. Amidases (EC 3.5.1 and 3.5.2) a subclass of acylamide amidohydrolases are mainly involved in nitrogen exchange of pro and eukaryotes utilizing nitriles. Reaction catalyzed by these complex enzyme, are of primary interest for large scale production of acrylamide and acrylic acid in industry (Novo et al., 2002). The importance of amidases in biotechnology is growing rapidly, because of their potential applications that are span through chemical and pharmaceutical industries as well as in bioremediation (Jorge et al., 2007). Microbial Amidases are a class of enzymes that have potential value for the development of commercial bioprocesses (Wu et al., 1998). They are used in detoxification of industrial effluents containing toxic amides such as acrylamide and formamide.

Additionally, immobilized amidase can be used efficiently for production of acrylic acid from acrylamide, thus converting a toxic ambient contaminant into widely used industrial raw material (Nawaz et al., 1996; Nagasawa et al., 1989a; Madhavan et al., 2005). Most of the currently known amidases have been found and described in bacteria. Many genera are concerned: *Rhodococcus, Corynebacterium, Mycobacterium, Pseudomonas, Bacillus, Micrococcus, Brevibacterium, Nocardia, Streptomyces, Blastobacter, Arthrobacter, Alcaligenes, Helicobacter, Lactobacillus,* and *Methylophilus*. Amidases (EC 3.5.1.4) are ubiquitous enzymes in the living world and can be divided into two types. They include aliphatic amidases (hydrolyzing short and mid chain aliphatic amides) and broad range amidases (hydrolyzing various substrates, some arylamides, a-aminoamides, a-hydroxyamides and kynurenine formamidases). Amidases can be assigned to

*Corresponding author. E-mail: nareshkumariitr@gmail.com.

Table 1a. Name of microorganism with there SwissProt accession number for aliphatic amidases.

S/N	Abbreviation	Accession No.	Source	No. of amino acids
1	AMIE_RHOER	Q01360	*Rhodococcus erythropolis*	345
2	AMIE_HELPY	O25067	*Helicobacter pylori*	339
3	AMIE_PSEAE	P11436	*Pseudomonas aeruginosa*	346
4	AMIE_BACSP	Q9L543	*Bacillus sp.*	348
5	AMIE_MARAV	A1U7G1	*Marinobacter aquaelei VT8*	348
6	AMIE_BURCH	A0B137	*Burkholderia cenocepacia I2424*	341
7	AMIE_ALCBS	Q0VN20	*Alcanivorax borkumensis SK2*	348
8	AMIE_NOCFA	Q5Z1VO	*Nocardia farcinia*	345
9	AMIE_DELAS	A9C011	*Delpha acidovorans SPH-1*	345

phylogenetically unrelated families on the basis of amino acid sequence. One family comprises the kynurenine formamidases group (3.5.1.9), and other may be termed as aliphatic amidase on the basis of substrate specificity (Fournand and Arnaud, 2001). Amidases turned out to be efficient tools for the synthesis of various compounds. Many microbial amidases have been purified and characterized.

To this end, these can be divided into two types based on their catalytic activity functions. Bacterial aliphatic amidases are the most extensively characterized, particularly as a consequence of their potential in the large-scale production of acrylic as well as other acidic products in industry (Hughes et al., 1998). Amidases exhibit a wide range of substrate specificities and some exhibit stereoselectivity (Mayaux et al., 1990; Mayaux et al., 1991; Hashimoto et al., 1991; Hirrlinger et al., 1996) a property that can be exploited to allow the production of enantiopure acids that would be difficult to produce by other methods. Microbial amidases with altered substrate specificity have attracted growing interest in the last decade because they can be used in detoxification of industrial effluents containing toxic amides such as acrylamide and formamide. Additionally, immobilized amidase can be used efficiently for production of acrylic acid from acrylamide, thus converting a toxic ambient contaminant into widely used industrial raw material (Jorge et al., 2007).

A number of physiochemical properties e.g. number of amino acid residues, molecular mass, theoretical pI, amino acid composition, negatively charged residues (Asp+Glu), positively charged residues (Arg+Lys), atomic composition, total number of atoms, extinction coefficients (M^{-1} cm^{-1}) at 280 nm, instability index/ aliphatic index, grand average hydropathicity (GRAVY), etc. of enzymes immensely influence their applications and need to be carefully studied. These properties can be either determined experimentally or deduced from the *in silico* analysis of amino acid sequences of enzymes available in the databases. Amidases are still not sufficiently investigated and their classification is not definitely formulated. The classification based on substrate specificity (Fournand and Arnaud, 2001) occasionally integrates enzymes with different structural organization, mechanism, and catalytic properties.

In spite of significant progress in expanding our knowledge of amidases, the spatial organization of these proteins remains unknown and systematic comparative analysis of formamidases and aliphatic amidases is far from complete. The novel approach taken in this paper is to study general physiochemical properties that are true for the sequences analysis and these properties of the sequence can then be used to predict there substrate specificity.

MATERIALS AND METHODS

Data collection and analysis

Information about the affinity (kynurenine formamidases /aliphaticity) of formamidases and aliphatic amidases of some microorganisms was obtained from the SwissProt data (Table 1a and b). Amino acid sequences for both from 18 microorganisms having experimentally proved substrate specificity as well as complete nucleotide sequences and these sequences are not fragmented, pseudo, putative or hypothetical. The amino acid sequences for formamidases and aliphatic amidases were downloaded from the ExPASy proteomic server. Physiochemical data were generated from the Swiss Prot and Expert Protein Analysis System (ExPASy) that is the proteomic server of Swiss Institute of Bioinformatics (SIB). FASTA format of sequences were used for analysis. Blastp (Protein BLAST) was performed to study the homology among the various amidase sequences and 18 sequences belonging to same E.C. number (E.C.3.5.1.4) of microbial amidases were selected (Table 1a and b). All the selected microorganism organisms have complete nucleotide sequences for its amidase gene as well as experimentally proved substrate specificity. Clustal W was used for multiple sequence alignment.

Various tools in the Proteomic server {ProtParam, Protein calculator, Compute pI/Mw, ProtScale (14)} were applied to calculate/deduce different physiochemical properties of amidases from the protein sequences. The molecular weights (Kda) of kynurenine formamidases and aliphatic amidases were calculated by the addition of average isotopic masses of amino acid in the protein and deducting the average isotopic mass of one water molecule. The pI of amidases was calculated using pK values of amino acid (Bjellqvist et al., 1993). The atomic composition of amidases was derived using the ProtParam tool, available at

Table 1b. Name of microorganism with there SwissProt accession number for Kynurenine formamidases.

S/N	Abbreviation	Accession No.	Source	No. of amino acids
1	KYNB_BURA4	B1YVH0	*Burkholderia ambifaria Str. C40-6*	213
2	KYNB_CUPTR	B3R5Q1	*Cupriavidus taiwanensis (Str. R1 / LMG 19424)*	216
3	KYNB_ERYLH	Q2N5X0	*Erythrobacter litoralis (Str. HTCC2594)*	216
4	KYNB_PSEFL	Q84HF4	*Pseudomonas fluorescens*	218
5	KYNB_RALME	P0C8P4	*Ralstonia metallidurans ATCC 43123*	218
6	KYNB_DEIGD	Q1IY56	*Deinococcus geothermalis (Str. DSM 11300)*	213
7	KYNB_ACIAC	A1TLB1	*Acidovorax avenae Subsp. Citruli (Str. AAC00-1)*	216
8	KYNB_BORPE	Q7VYS5	*Bordetella pertussis*	209
9	KYNB_MARMM	Q0APM5	*Maricaulis maris (strain MCS10)*	209

ExPASy. The extinction coefficient of various amidases was calculated using the following equation (Stanley et al., 1989):

E(Prot) = Numb(Tyr)*Ext(Tyr) + Numb(Trp)*Ext(Trp) + Numb(Cystine)*Ext(Cystine)

The values of aliphatic index of various amidase sequences were obtained using Prot Param (ExPASy) tool (Kyte and Doolittle, 1982). The instability index and grand average of hydropathicity (GRAVY) were estimated following the method of Guruprasad et al. (1990), and Kyte and Doolittle (1982) respectively.

Statistical analysis

An analysis of variance (ANOVA) was conducted on various physiochemical parameter variables for each study with the statistical packages 'Asistat version-7.4 beta 2008'. F-tests were used to determine the statistical significance. When significant effects were detected, a Tukey test was applied for all pairwise comparisons of mean responses.

RESULTS

In the present study, attempts to find differences between the physiochemical properties of eighteen (18) amino acid sequences of aliphatic amidases (9) and kynurenine formamidases (9) has been done (Table 2a and b). The total number of amino acid residues in these amidases differed substantially as kynurenine formamidases have lesser number of amino acid residues ranging between 209 to 218 amino acid as whereas aliphatic amidases ranging between 339 to 348. The molecular weight of kynurenine formamidases ranges between 22546.4 to 24015.0 and that of aliphatic amidases ranges between 37712.9 to 38596.9. Theoretical pI varied between 4.61 to 6.07 in case of kynurenine formamidases and it was found to be 4.94 to 6.20 for aliphatic amidases. It was further found that the average pI value of aliphatic amidases were insignificantly higher than that of kynurenine formamidases (Table 2a). Negative charge residues (Asp and Glu) were found to be substantially higher in aliphatic amidases that is 45.33 in aliphatic amidases and 26.77 in kynurenine formamidases. Significant difference was found between kynurenine formamidases (16.15) and aliphatic amidases (34.00) for positively charged amino acid residues also. Instability indices of these two groups of amidases indicated that aliphatic amidases (33.98) were more stable as compared to kynurenine formamidases (45.53) (Table 2a).

The aliphatic index values were found to be higher (1.31 fold) in kynurenine formamidases and values for grand average hydropathicity (GRAVY) was substantially higher (5.61 fold) for aliphatic amidases than that of kynurenine formamidases. Results of amino acid analysis of two types of amidases are shown in Table 2a and b. These enzymes contained all the common amino acids. The comparison of the amino acid composition of the kynurenine formamidases and aliphatic amidases has shown that alanine (Ala), one of the simplest amino acid is found to be the predominant residue in kynurenine formamidases and glycine (Gly) in aliphatic amidases. The amino acid cysteine (Cys) is considered to be an important parameter in the calculation of extinction co-efficient of proteins and its content was 1.61 fold higher in aliphatic amidases as compared to kynurenine formamidases. The amino acid Ser, Thr, Trp and Val are (1.10, 1.11, 1.28 and 1.04) non-significantly higher in kynurenine formamidases while the amino acid, Ala, Arg, Asp, His, Leu and Pro are (1.39, 1.38, 1.43, 1.78, 2.20 and 2.12) significantly higher in kynurenine formamidases. The amino acid Asn, Glu, Gly, Ile, Lys, Met, Phe and Tyr are (4.5, 1.73, 1.43, 1.19, 3.56, 2.58, 2.03 and 2.70) significantly higher in aliphatic amidases while only Gln (1.19x) is found to be non-significantly higher in aliphatic amidases.

The multiple sequence alignment of aliphatic amidases and kynurenine formamidases showed some unique differences for the position specific presence of some amino acids. The position specific (conserved) amino acid in these amidases comprised of active site domain and the N-terminal regions. However, no differences were observed in the conserved amino acid in the C-terminal regions of the enzymes compared in the present study. The present work has contributed to conclude that

Table 2a. Comparative analysis of physiochemical properties of aliphatic amidases and kynurenine formamidases.

Parameter	SS[1]	1	2	3	4	5	6	7	8	9	Avg.	(P < 0.01)
Number of amino acids	AA[2]	345	339	346	348	348	348	345	346	345	345.55	7871.12**
	KF[3]	213	216	216	218	218	213	216	209	209	214.22	
Molecular weight	AA	38200.1	37712.9	384947	38596.9	38460.5	38359.4	38183	38441.7	38564.7	38334.88	6704.56**
	KF	22699	23164.4	24015	23629.1	23382.7	22784.8	23229.6	22776	22546.4	23136.33	
Theoretical pI	AA	4.94	6.20	5.31	5.38	5.06	4.96	4.98	5.65	5.69	5.35	0.01 ns
	KF	5.24	5.57	4.61	6.07	5.73	5.11	5.37	5.47	4.80	5.33	
Negatively charged residues (Asp+Glu)	AA	48	41	45	45	47	47	48	43	44	45.33	100.14**
	KF	26	22	38	22	24	29	25	25	30	26.77	
Positively charged residues (Arg+Lys)	AA	32	38	35	36	33	31	31	35	35	34.00	312.01**
	KF	17	14	20	16	15	18	17	16	16	16.55	
Extinction coefficients ($M^{-1}cm^{-1}$) at 280 nm	AA	60320	58955	58830	57340	57465	57465	60320	64790	54945	58936.67	90.59 **
	KF	28210	28210	48595	24200	28210	21200	29575	39085	33710	32221.67	
Instability index	AA	30.15	40.47	35.61	30.20	28.78	32.92	31.57	38.05	38.09	33.98	21.16**
	KF	46.28	47.36	50.54	40.54	51.15	42.18	50.03	49.57	32.14	45.53	
Aliphatic index	AA	74.96	77.35	74.74	74.57	68.13	68.16	76.90	73.58	69.54	73.10	70.87 **
	KF	99.39	100.83	80.06	102.06	96.79	94.88	101.67	102.78	89.28	96.41	
Grand average of hydropathicity (GRAVY)	AA	-0.319	-0.347	-0.330	-0.324	-0.360	-0.333	-0.279	-0.348	-0.397	-0.337	45.16 **
	KF	0.081	-0.001	-0.302	0.002	-0.052	-0.168	0.040	-0.013	-0.128	-0.060	

[1]-Substarte specificity; [2]-Aliphatic amidase ; [3]- Kynurenine formamidases. (1) *Rhodococcus erythropolis* Q01360 (2). *Helicobacter pylori* O25067 (3). *Pseudomonas aeruginosa* P11436 (4). *Bacillus sp.* Q9L543 (5). *Marinobacter aquaelei* VT8 A1V7G1 (6) *Burkholderia cenocepacia* HI2424 A0B137 (7). *Alcanivorax borkumensis* SK2 Q0VN20 (8). *Nocardia farcinia* Q5Z1VO (9). *Delpha acidovorans* SPH-1 A9C011. ** Significant at a level of 1% of probability (P < 0.01); * Significant at a level of 5% of probability (0.01 =< P < 0.05); ns: Non-significant (P >= 0.05).

there is a clear difference between kynurenine formamidases and aliphatic amidases in terms of position specific presence of certain amino acid. Amino acid Proline (Pro) at 50 and Glycine (Gly) at positions 247, 252 and 291 is conserved aliphatic and kynurenine formamidases, respectively. Amino acid residues at position 59, 60, 87, 91, 97, 100, 117, 119, 184, 244, 254, 259, 263, 281, 304, 308 and 320 in aliphatic amidases are invariable Glu, Tyr, Phe, Cys, Trp, Phe, Asn, Leu, Glu, Gly, Gln, Ser, Ile, His, Phe, Trp and Glu respectively, while these positions are occupied by Gly, Asp, Ser, Gly, Pro, Tyr, Gly, Cys, Arg, Ala, Asp, Asp, Ser, Glu, Leu, Pro and Arg respectively in kynurenine formamidases.

Table 2b. Comparison between amino acids presents in aliphatic amidases and kynurenine formamidases.

Amino acid composition	SS[1]	Micro-organism***									Avg.	(P<0.01)
		1	2	3	4	5	6	7	8	9		
Ala (A)	AA[2]	9.6	7.4	9.2	8.3	8.9	9.5	9.3	8.7	7.2	8.86	24.62**
	KF[3]	12.7	14.4	7.9	11	14.2	11.3	13	12.9	13.9	12.36	
Arg (R)	AA	4.6	3.5	5.8	3.7	4.0	4.0	5.2	4.3	5.2	4.47	27.02**
	KF	6.6	6.0	6.5	5.0	6.0	7.0	6.9	6.2	5.7	6.21	
Asn (N)	AA	4.1	4.4	4.0	4.6	4.9	4.3	2.3	4.6	4.9	4.23	109.34**
	KF	0.5	1.4	0.5	0.9	0.5	1.9	0.9	1.4	0.5	0.94	
Asp (D)	AA	7.0	5.6	4.9	4.6	6.6	6.3	7.2	5.2	5.2	5.84	15.50**
	KF	8.5	8.8	10.2	6.9	7.3	6.1	8.3	7.7	11.5	8.36	
Cys (C)	AA	2.3	3.2	2.6	2.3	2.9	2.9	2.6	2.3	2.9	2.66	39.95**
	KF	1.9	1.9	0.9	1.8	1.8	1.9	1.4	1.4	1.9	1.65	
Gln (Q)	AA	3.5	4.7	4.0	3.2	3.2	3.4	4.1	2.6	2.6	3.47	1.38^{ns}
	KF	0.9	5.1	2.3	4.1	3.7	1.4	2.8	2.9	2.9	2.90	
Glu (E)	AA	7.0	6.5	8.1	7.8	6.9	7.2	6.7	7.2	7.5	7.21	19.10**
	KF	3.8	1.4	7.4	3.2	3.7	7.5	3.2	4.3	2.9	4.15	
Gly (G)	AA	9.6	9.4	9.8	9.8	9.8	9.8	9.6	9.5	9.9	9.68	59.72**
	KF	6.6	5.6	8.3	6.0	6.4	8.9	6.5	5.7	6.7	6.74	
His (H)	A	2.3	2.1	2.0	2.3	2.3	2.3	2.9	2.6	3.2	2.44	28.08**
	KF	3.8	4.6	3.2	6.0	6.0	3.8	3.7	3.8	4.3	4.35	
Ile (I)	AA	7.0	6.2	6.1	7.8	5.7	5.7	5.2	6.4	6.4	6.27	6.99**
	KF	4.7	5.1	5.1	6.4	6.0	3.8	5.6	6.2	4.3	5.24	
Leu (L)	AA	4.6	5.6	5.8	4.3	4.3	4.6	6.1	4.9	4.6	4.99	211.78**
	KF	9.9	11.6	10.6	11.5	10.1	13.1	10.6	11.5	10.0	10.98	
Lys (K)	AA	4.6	7.7	4.3	6.6	5.5	4.9	3.8	5.8	4.9	5.34	66.41**
	KF	1.4	0.5	2.8	2.3	0.9	1.4	0.9	1.4	1.9	1.50	
Met (M)	AA	3.8	2.1	4.3	3.7	4.9	5.5	3.2	4.3	4.6	4.04	34.20**
	KF	1.9	0.9	3.2	1.4	1.4	0.5	1.9	1.0	1.9	1.56	
Phe (F)	A	2.6	3.2	2.6	3.2	2.9	2.6	2.6	2.0	2.9	2.73	59.80**
	KF	0.9	1.4	1.9	1.4	0.9	1.4	0.9	1.4	1.9	1.34	
Pro (P)	AA	4.0	5.0	4.6	4.3	4.3	4.6	4.6	5.2	5.2	4.64	58.84**
	KF	11.3	12.5	6.0	9.2	11.9	9.9	10.2	9.6	8.1	9.85	
Ser (S)	AA	4.1	4.4	4.6	4.0	4.3	4.9	4.6	5.2	4.6	4.52	1.16^{ns}
	KF	5.2	3.7	7.4	6.0	3.7	3.8	4.6	5.7	4.8	4.98	
Thr (T)	AA	5.2	3.8	3.8	6.0	5.5	4.9	4.9	4.0	4.6	4.74	1.40^{ns}
	KF	5.6	4.2	4.2	6.0	5.0	7.5	5.6	3.8	5.7	5.28	

Table 2b. Contd.

Trp (W)	AA	1.7	1.8	1.7	1.7	1.7	1.7	1.7	1.7	1.4	1.67	3.44^{ns}
	KF	1.9	1.9	3.7	1.4	1.8	1.4	1.9	2.9	2.4	2.14	
Tyr (Y)	AA	5.2	5.0	4.9	4.6	4.6	4.6	5.2	6.1	5.2	5.04	277.27**
	KF	1.9	1.9	1.4	2.3	1.8	1.4	2.3	1.9	1.9	1.86	
Val (V)	AA	7.0	8.3	6.6	6.6	6.9	6.3	8.1	7.2	6.7	7.07	0.62^{ns}
	KF	10.3	7.4	6.5	7.3	6.9	6.1	8.8	7.2	6.7	7.40	

[1-]Substarte specificity; [2-]Aliphatic amidase; [3-] Kynurenine formamidases. (1) *Burkholderia ambifaria* Str. MC40-6 B1YVH0 (2). *Cupriavidus taiwanensis* (Str. R1 / LMG 19424) B3R5Q1 (3). *Erythrobacter litoralis* (Str. HTCC2594) Q2N5X0 (4). *Pseudomonas fluorescens* Q84HF4 (5). *Ralstonia metallidurans* ATCC 43123 P0C8P4 (6). *Deinococcus geothermalis* (Str. DSM 11300) Q1IY56 (7). *Acidovorax avenae Subsp. Citruli* (Str. AAC00-1) A1TLB1 (8). *Bordetella pertussis* Q7VYS5 (9). *Maricaulis maris* (strain MCS10) Q0APM5. ** Significant at a level of 1% of probability ($P < 0.01$); * Significant at a level of 5% of probability ($0.01 =< P < 0.05$); ns: Non-significant ($P >= 0.05$).

The other conserved amino acid residues in both aliphatic amidases included Ala -16, 39, 76, 90, 179, 208, 216, 218, 220, 256 and 298; Gln- 130, 190, 199, 200, 271 and 273; Pro-23, 50, 80, 115, 140, 146, 156, 172, 195, 301 and 338; Glu-71, 83, 105, 108, 127, 142, 173, 245 and 249 ; Tyr- 20, 67, 116, 151, 171, 194, 214, 227, 229, 255 and 305; Arg-2, 24, 133, 176, 188, 264 and 282 ; Lys- 113, 157, 166, 197, 205 and 278, ; Gly- 4, 14, 44, 48, 51, 64, 81, 98, 104, 126, 147, 155, 158, 169, 182, 191, 222, 225, 231, 237, 240, 286, 293, 296 and 328 ; Cys-166, 178, 189 and 332; Trp-144 and 175; Val-13, 17, 18, 31, 129, 152, 187, 215 and 226; Thr-75, 84, 103, 118 and 242; Asp- 5, 11, 53, 167, 168, 177, 224, 239, 265 and 311; His- 26, 107 and 232 (Figure 1). The conserved amino acid residues present in kynurenine formamidases comprised His- 90, 94, 99 and 269; Tyr- 302; Asp-40, 95 and 315; Asp- 40, 95 and 315 (Figure 1).

DISCUSSION

The present article aims to differentiate two groups of amidases that is kynurenine formamidases and aliphatic amidase on the basis of their amino acid sequences and physiochemical properties. The sequence redundancy was removed by 40, 60, 80 and 100% by the use of CD-HIT software. The selected sequences in each group that is aliphatic amidases and kynurenine formamidases were 100% homologous and more than 40% identical with respect to model sequence of each group that is aliphatic amidase amino acid sequence of *Marinobacter aquaelei* VT8 (A1U7G1,UniProtKB/Swiss-Prot) were selected. Variation in total number of amino acid residues between aliphatic amidases and kynurenine formamidases result shows that total number of amino acid and molecular weight might be playing some role in providing the substrate specificity to these two groups of amidases. No significant difference for pI values was found between the two groups of amidases. This indicates that pI value is not responsible with the substrate specificity of the amidase enzymes considered in the present study.

As 20 amino acids differ in their chemical composition and physiochemical properties, also proteins differ widely in physiochemical properties as well as in substrate specificity (O'Reilly and Turner, 2003; Yeom et al., 2008). The results of this work have confirmed that amino acid number and their percentage composition in amidases significantly affect the substrate specificity. Ala is the predominant amino acid in the kynurenine formamidases and Gly in the aliphatic amidases has confirmed there presence in amino acid sequences of compared amidases in the present investigation. An important amino acid that is found to play a significant role in amidases is Cys (Kobayashi et al., 1993) that generally provides stability to proteins due to the formation of disulphide bonds. The results in the present work also indicate that aliphatic amidases are more stable as is shown by the instability index values (Table 2a) of various amidases considered in present work. This finding is further supported by the fact that aliphatic amidases have higher percentage of Cys content as compared to kynurenine formamidases.

Conclusion

A number of physiochemical properties of aliphatic amidases and kynurenine formamidases have been derived from amino acid sequences of some amidases. The kynurenine formamidases and aliphatic amidase mainly differs in the total number of amino acid, molecular weights and composition of amino acid. This study has clearly found differences between these two types of amidases in conserved amino acid residues at several positions. The results of the present work will be quite useful in prediction and selection of kynurenine formamidases / aliphatic amidases from hitherto reported amidases or from the large number of sequenced

Figure 1. Alignment of the amino acid sequences of aliphatic amidases and kynurenine formamidases. #Reference sequence A1U7G1 (*Marinobacter aquaelei* VT8).

microbial genomes.

REFERENCES

Bjellqvist B, Hughes GJ, Pasquali C, Paquet N, Ravier F, Sanchez JC, Frutiger S, Hochstrasser D (1993). The focusing positions of polypeptides in immobilized pH gradients can be predicted from their amino acid sequences. Electrophoresis, 14: 1023-1031.

Fournand D, Arnaud A (2001). Aliphatic and enantioselective amidases: from hydrolysis to acyl transfer activity. J. Appl. Microbiol., 91: 381-393.

Guruprasad K, Reddy BVB, Pandit MW (1990). Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. Protein Eng., 4: 155-161.

Hashimoto Y, Nishiyama M, Ikehata O, Horinouchi S, Beppu T (1991). Cloning and characterization of an amidase gene from *Rhodococcus* species N-774 and its expression in *Escherichia coli*. Biochim. Biophys. Acta, 1088: 225-233.

Hirrlinger B, Stolz A, Knackmuss HJ (1996). Purification and properties of an amidase from *Rhodococcus erythropolis* MP50 which enantioselectively hydrolyzes 2- arylpropionamides. J. Bacteriol., 178: 3501-3507.

Hughes J, Armitage YC, Symes KC (1998). Application of whole cell rhodococcal biocatalysts in acrylic polymer manufacture. Antonie Van Leeuwenhoek, 74: 107-118.

Jorge A, Amin K, Maria A, Carrondo, Carlos F (2007). Structure of Amidase from *Pseudomonas aeruginosa* showing a Trapped Acyl Transfer Reaction Intermediate State. J. Biol. Chem., 282(27): 19598-19605.

Kobayashi M, Komeda H, Nagasawa T, Nishiyama M, Horinouchi S, Beepu T, Yameda H, Shimizu S (1993). Amidases coupled with low molecular mass nitrile hydratase from *Rhodococcus rhodochrous* J1 Eur. J. Biochem., 217: 327- 336.

Kyte J, Doolittle RF (1982). A simple method for displaying the hydropathic character of a protein. J. Mol. Biol., 157: 105-132.

Madhavan NK, Roopesh K, Chacko S, Pandey A (2005). Competitive study of amidase production by free and immobilized *Escherichia coli* cells. Appl. Biochem. Biotechnol., 120:97-108.

Mayaux JF, Cerbelaud E, Soubrier F, Yeh P, Blanche F, Petre D (1991). Purification, cloning, and primary structure of a new enantiomer-selective amidase from a *Rhodococcus* strain: structural evidence for a conserved genetic coupling with nitrile hydratase. J. Bacteriol., 173: 6694-6704.

Mayaux JF, Cerebelaud E, Soubrier F, Faucher D, Petre D (1990). Purification, cloning, and primary structure of an enantiomer-selective amidase from *Brevibacterium* sp. strain R312: structural evidence for genetic coupling with nitrile hydratase. J. Bacteriol., 172: 6764-6773.

Nagasawa T, Ryuno K, Yamada H (1989a). Superiority of Pseudomonas chlororaphls B23 nitrile hydratase as a catalyst for the enzymatic production of acrylamide. Experientia, 45: 1066-1070.

Nawaz MS, Khan AA, Bhattacharya D, Silton PH, Cerniglia CE (1996). Physical, biochemical and immunological characterization of a thermostable amidase from *Klebsiella pnumoniae* NCTR1. J. Biotechnol., 178: 2397-2401.

Novo C, Fournand S, Tata R, Clemente A, Brown PR (2002). Support for a three-dimensional structure predicting a Cys-Glu-Lys catalytic triad for *Pseudomonas aeruginosa* amidase comes from site-directed mutagenesis and mutations altering substrate specificity. Biochem. J., 365: 731-738.

O'Reilly C, Turner PD (2003). The nitrilase family of CN hydrolyzing enzymes – A comparative study. J. Appl. Microbiol., 95: 1161-1174.

Soong CL, Ogawa J, Shimizu S (2000). A Novel Amidase (Half-Amidase) For Half-Amide Hydrolysis involved in the Bacterial Metabolism of Cyclic Amides. Appl. Environ. Microbiol., 66: 1947-1952.

Stanley C, Gill, Peter H, Von-Hippel (1989). Calculation of protein extinction coefficients from amino acid sequence data. Analytical Biochemistry, 1 November, 182(2): 319-326.

Wu S, Fallon RD, Payne MS (1998). Cloning and nucleotide sequence of amidase gene from *Pseudomonas putida*. DNA Cell Biol., 17(10): 915-920.

Yeom SJ, Kim HJ, Lee JK, Kim DE, Oh DK (2008). An amino acid at position 142 in nitrilase from *Rhodococcus rhodochrous* ATCC 33278 determines the substrate specificity for aliphatic and aromatic nitrile. Biochem. J., 415: 401- 407.