


RESEARCH: BIOINFORMTICS

AMINO ACID FREQUENCY DISTRIBUTION AT ENZYMATIC ACTIVE SITE

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ABSTRACT

Enzyme's active site sequence is crucial to execute its function. The present study is based on the analysis of comparative frequency distribution of enzymes catalytic residues belongs to different organisms and localizations. The frequency distribution of enzymes catalytic residues was computed using the number of amino acids of each type and the total number of residues. The percentage composition of catalytic residue indicates the occurrence of 13 amino acids (out of 20 total amino acids) at enzymatic active site. These 13 catalytic residues are cumulatively constituted by five charged (E, D, H, K, R), six polar (C, Y, T, S, Q, N) and two hydrophobic residues (A/F/W, G). Viral, Prokaryotic and Eukaryotic enzymes (VEs, pEs and eEs) commonly show preference for four charged residues (H, D, E, R) and a single polar residue 'S' at their active site. The residues 'R>A' are predominantly distributed at VEs active site, while in pEs and eEs the order of preference of catalytic residues is H>D>K>E>R>Y>S. The analysis further indicate that both Prokaryotic and Eukaryotic membrane and non-membrane enzymes (MEs and nMEs) show high degree of similarity in the overall percentage distribution of charged and polar residues at their active site. In addition, VEs are significantly similar to nMEs in the frequency distribution of charged and polar residues. The catalytic residues 'H, D, S, Y, T, F' play crucial role in the catalysis of Prokaryotic membrane enzymes (pMEs), while the residues 'H, K, Y, S, C' are important for Eukaryotic membrane enzymes (eMEs) catalysis. However, in non-membrane enzymes (nMEs) five charged residues (E, D, H, K, R) are important for their catalytic function, while the polar residues have supportive function. The knowledge of amino acid frequency distribution at the active site can be exploited in designing novel enzyme active sites as well as the specific inhibitors and novel peptide based drugs.

Key words: enzyme catalytic residues; frequency distribution; viral enzymes; prokaryotic enzymes; eukaryotic enzymes

[1] INTRODUCTION

At each step, the metabolic processes involve the versatile catalytic molecules termed as enzymes [1]. Along with biochemical information, the three dimension structure of enzymes in complex with substrate has been used extensively to determine their catalytic mechanism. In general, the amino acids at the enzyme active site act as acid-base, nucleophiles or electrophiles and also make hydrogen bond either with the substrate or with other residues of an enzyme to stabilize the transition state [2].

Most of the enzymes are unique and specific in catalysing a particular biochemical reaction. The amino acid residues involved in making the active site of an enzyme are not sequentially arranged rather distributed all over the protein sequence. These residues come together during folding to make the enzymatic active site. Bartlett et al. (2002) used a dataset of 178 enzymes for determining the frequency distribution of enzyme's catalytic residues [3]. Since then the number of enzyme structures in Protein Data Bank (PDB) has been increased six fold. Furthermore, the comparative data for the frequency distribution of catalytic residues of enzymes of different origin and cellular localization is not available. This study is an attempt to find out either the substantial similarities

or significant differences among frequency distribution of catalytic residues of enzymes of Viral (VEs), Prokaryotic and Eukaryotic (pEs and eEs) origin. In addition, the Prokaryotic and Eukaryotic enzymes dataset was segregated for analysis into membrane and non-membrane enzymes (MEs and nMEs) depending upon their cellular localization. The improved understanding of the distribution of catalytic residues at the active site will be helpful in enzyme engineering and in designing novel inhibitors.

[II] MATERIALS AND METHODS

A dataset of 838 non-redundant enzymes was manually curated from the experimentally annotated entries of enzyme active site database called as 'Catalytic Site Atlas' (CSA) [4]. Out of 26,846 total entries, CSA (version - 2.3.11) possess 968 experimentally annotated entries of enzyme active site with a label 'literature reference'. For the purpose of this study, the whole dataset was divided in three categories namely Viral (VEs; 9), Prokaryotic (pEs; 481) and Eukaryotic (eEs; 348) enzymes based on the source organism. While preparing the database, repetitive CSA entries were excluded. Additionally, depending upon the cellular localization of the enzyme, the Prokaryotic and Eukaryotic dataset is further subdivided into membrane (pME and eME) and non-membrane (pnME and enME) enzymes by using the concerned journals and UNIPROT database [5]. Entire dataset was prepared in a fasta format by listing the amino acid residues present at the enzymatic active site in an ascending order. Residues constitute more than one active site of an enzyme are treated separately while preparing the dataset. The Viral protein dataset is small because till date only 72 viral protein structures, having 30% identity, are reported in PDB and only nine are viral enzymes. The viral protein dataset represents different type of proteins and categories of viruses such as chikunguniya, west Nile virus, dengue, encephalitis, pancreatic necrosis virus etc.

The frequency distribution of 20 amino acid residues at enzymatic active site was computed using the number of amino acids of each type and the total number of residues. It is defined as Residue composition

$$(\%) (r) = \sum nr/N \times 100 \dots \dots \dots (1)$$

where 'r' stands for one of the 20 amino acid residues. $\sum nr$ is the sum total of residue of each type and N is the total number of residues in the dataset. The prepared dataset contains 2683 catalytic residues in total. The catalytic residues in sub-datasets are Viral (27), Prokaryotic membrane (76) and non-membrane (1493) and Eukaryotic membrane (72) and non-membrane (1015) respectively.

The probability of each amino acid occurrence as a catalytic residue at the enzymatic active site was calculated as follows:

$$\text{Amino acid probability} = \frac{\text{Sum total of an amino acid at the active site}}{\text{Sum total of an amino acid in the whole enzyme}} \dots \dots \dots (2)$$

[III] RESULTS

3.1. General

The composition of catalytic residues is calculated by using equation 1. The percentage composition of catalytic residue indicates the occurrence of only 13 amino acid residues (out of 20 total amino acids) at enzymatic active site [Table-1]. Five charged residues (E, D, H, K, R), six polar residues (C, Y, T, S, Q, N) and two hydrophobic residues (A/F/W, G) constitute the

total 13 catalytic residues. Depending upon the organism, the charged, polar and hydrophobic catalytic residues show percentage frequency distribution range of 55-65%, 22-40%, and 8-11% respectively [Table-2].

In contrast to nMEs, MEs show higher percentage frequency of polar catalytic residues ~10-15%; [Table-2]. In MEs, two polar catalytic residues 'S' and 'Y' possess higher percentage frequency and responsible for creating the difference between MEs and nMEs. In general, 'S' forms hydrogen bonds with water and other neighbouring molecules and helps in the stabilization of transmembrane domains, while some of the membrane proteins such as ion-channels are regulated via phosphorylation and dephosphorylation of 'Y' residue [6].

The comparison of frequency distribution of catalytic residues among VEs, pEs and eEs revealed that these enzymes possess preference for four charged residues (H, D, E, R) and a single polar residue 'S' at their active site [Table-3]. This implies that at least five catalytic residues i.e. four charged (H, D, E, R) and one polar 'S', are extremely favoured at the active site of enzymes of different origin irrespective of their cellular localization. Furthermore, pEs and eEs also show preferential occurrence of another charged 'K' and a polar residue 'Y' at their active site. Therefore, the active site of pEs and eEs prefer five charged and two polar residues with their frequency distribution order of H>D>K>E>R and Y>S respectively. It can be concluded that the seven catalytic residues (H>D>K>E>R>Y>S) constitute the functional elements of Prokaryotic and Eukaryotic enzymes and mutation of any of these residues might affect the enzyme kinetics most significantly [7-9]. The probability occurrence calculation revealed that 'H' residue has highest chance of occurrence as catalytic residue among all the 13 frequently distributed amino acids. 'D' residue has almost equal probability of occurrence except eMEs (eq. 2). 'E' has exactly half the chances of occurrence in pMEs in comparison to other enzymes. Comparatively, the amino acid 'K' has least possibility of occurrence in VEs. 'R' strongly favours the VEs active site, while 'S' best occupy the MEs active site. 'Y' has the highest probability of occurrence at the active site of eMEs [Table-1].

3.2. Frequency distribution

3.2.1. Viral enzymes (VEs)

The frequency distribution comparison of active site residues revealed unique features among enzymes of different origin. In VEs, order of charged residue distribution is R>H>D>E>K (66.65% of total), polar residue order is S>Y>T>C>Q (22.21% of total). In addition, VEs also possess high frequency of a hydrophobic residue 'A' (7.41% of total). VEs are significantly similar to nMEs in the overall frequency distribution of charged and polar residues probably due to the usage of host cell machinery for their production. VEs show high dependency on four charged catalytic residues R>H>D>E for catalysis. Interestingly, except 'S', frequency distribution indicates that the rest of the polar residues are equally contributing to the

catalytic activity of VEs. VEs uniquely possess 'A' residue at their active site, while pMEs and eMEs totally lack it. pnMEs and enMEs have in-significant distribution of 'A' at their active

site. Among charged residues 'R' have highest percentage frequency distribution (22.22%), which is almost double over pEs and eEs (8%-10%).

Table: 1. Percentage frequency distribution of catalytic residues of enzymes belongs to different organisms. The probability occurrence of active site amino acids is shown in bracket.

Amino acid residues	Viral enzymes (VEs)	Prokaryotic Membrane enzymes (pMEs)	Eukaryotic Membrane enzymes (eMEs)	Prokaryotic non-Membrane enzymes (pnMEs)	Eukaryotic non-Membrane enzymes (enMEs)
L	0%	0%	0%	0.59%	0.65%
I	0%	0%	1.16% (0.01)	0.39%	0.47%
F	0%	4.23% (0.04)	2.33% (0.03)	1.84% (0.01)	1.49% (0.02)
W	0%	0%	0%	1.90% (0.02)	1.40% (0.02)
V	0%	0%	0%	0.26%	0.19%
M	0%	1.40% (0.01)	0%	0.85% (0.01)	0.28%
A	7.41% (0.08)	0%	0%	1.97% (0.02)	1.02% (0.01)
G	3.70% (0.04)	2.82% (0.03)	4.65% (0.04)	2.95% (0.03)	3.63% (0.05)
P	0%	0%	0%	0.41% (0.004)	0.19% (0.002)
C	3.73% (0.04)	2.82% (0.03)	8.14% (0.09)	5.38% (0.06)	3.72% (0.04)
Y	3.70% (0.04)	9.86% (0.08)	13.95% (0.12)	5.77% (0.05)	6.42% (0.06)
T	3.70% (0.04)	7.04% (0.07)	3.49% (0.04)	2.69% (0.03)	3.72% (0.04)
E	11.11% (0.11)	5.63% (0.06)	9.30% (0.11)	11.42% (0.13)	13.58% (0.14)
S	7.41% (0.07)	11.27% (0.11)	11.63% (0.13)	5.31% (0.05)	6.42% (0.06)
Q	3.70% (0.04)	0%	2.33% (0.03)	1.64% (0.02)	2.42% (0.02)
D	14.81% (0.15)	14.08% (0.14)	5.81% (0.07)	15.94% (0.16)	15.81% (0.02)
H	14.81% (0.15)	18.31% (0.18)	15.12% (0.17)	15.16% (0.14)	19.06% (0.19)
N	0%	4.23% (0.04)	1.16% (0.02)	4.13% (0.04)	3.81% (0.04)
K	3.70% (0.04)	9.86% (0.10)	11.63% (0.13)	10.70% (0.11)	7.53% (0.07)
R	22.22% (0.22)	8.45% (0.08)	9.30% (0.11)	10.70% (0.11)	8.19% (0.08)

Table: 2. Percentage frequency distribution of three broad classes of catalytic residues of enzymes of different origin and cellular localization. Polar 'H' is categorised under charged group of residues due to its pK_a alteration in a protein.

Enzyme origin	Catalytic residue type (%)		
	Charged (E, D, H, K, R)	Polar (C, Y, T, S, Q, N)	Hydrophobic
VE	66.65	22.24	11.11
pME	56.33	35.22	8.45
eME	51.16	40.70	8.14
pnME	63.92	24.92	11.16
enME	64.17	26.51	09.32

Table: 3. Comparison of most frequently occurring catalytic residues (highlighted in bold) of enzymes of different origin and cellular localization.

Enzyme origin	Enzyme's catalytic residues having highest frequency of occurrence										
VE	A		D	E		H		R	S		
pME			D	E	F	H	K	R	S	T	Y
eME		C	D	E		H	K	R	S		Y
pnME			D	E		H	K	R	S		Y
enME			D	E		H	K	R	S		Y

3.2.2. Membrane enzymes (MEs)

The comparison of catalytic residue frequency distribution of MEs in Prokaryotes and Eukaryotes show the definite preferences. The order of charged residues distribution is H>D>K>R>E and H>K>R>E>D in pMEs (56.33% of total) and eMEs (50.96% of total), while polar residues are distributed in order S>Y>T>N>C and Y>S>C>T>Q>N in pMEs (35.22% of total) and eMEs (40.7% of total) respectively. The comparison revealed that the type and overall proportion of charged catalytic residues is similar in both pMEs and eMEs. The noteworthy feature is the distinct preference of 'D' and 'E' charged catalytic residues by pMEs and eMEs. The respective composition of 'D' in pMEs and eMEs is 14.08% and 5.81%, while the composition of 'E' in pMEs and eMEs is 5.63% and 9.30% respectively. The 'D' residue is a strong nucleophilic as well as electrophilic agent normally involved in the activation of molecules such as water and also forms metal co-ordination complexes [10]. The charged catalytic residues distribution indicates that 'H' (18.31%) and 'D' (14.08%) plays crucial role in the catalysis of pMEs, while 'H' (15.12%) and 'K' (11.63%) are important for eMEs catalysis. Also, the polar residues distribution indicates that 'S' (11.27%), 'Y' (9.86%) and 'T' (7.04%) plays crucial role in the catalysis of pMEs, while 'Y' (13.95%), 'S' (11.63%) and 'C' (8.14%) are important for eMEs catalysis [Table-1]. Among polar residues, pMEs prefer 'T' instead of 'C' probably because hydroxyl moiety of 'T' side chain acts as a potent nucleophile for binding and catalyzing the substrate [11]. The reactive hydroxyl and thiol group in side chain of 'Y' and 'C' have the ability to de-protonate and re-protonate for stabilizing the enzyme-substrate transition state complex [12]. The main chain atoms of 'Y' also involve in the formation of hydrogen bonds for stabilization of active site during catalysis [13]. As the probability occurrence of 'Y' (0.12) is higher than 'D' (0.07) in eMEs active site, it is possible that 'Y' can partly contribute in activation of substrate molecule as it is a weaker nucleophile than 'D'. The amino acid 'S' is equally distributed in both pMEs (11.27%) and eMEs (11.63%). Comparatively, MEs have almost double distribution of 'S' with respect to VE and nMEs. The short side chain of 'S' may help in maintaining the stability of the active site while its reactive

hydroxyl group may function like 'Y' in acid-base catalysis, formation of transition state complex [14]. In contrast to other class of enzymes, pMEs exclusively possess high percentage of one hydrophobic 'F' (4.23%) amino acid at their active site. The presence of 'F' at the active site helps in stabilizing the ligand and enzyme interaction [15]. Depending upon the frequency distribution of catalytic residues, we can conclude that the 'H, D, S, Y, T, F' and 'H, K, Y, S, C' residues are most important in pMEs and eMEs catalysis.

3.2.3. Non-membrane enzymes (nMEs)

Comparison of composition of catalytic residues of nMEs in Prokaryotes and Eukaryotes revealed that the charged residues are distributed in order of D>H>E>R>K and H>D>E>R>K in pMEs (63.92% of total) and eMEs (64.18% of total), while polar residues are distributed in order Y>C>S>N>T>Q and Y>S>N>C>T>Q in pMEs (24.92% of total) and eMEs (26.51% of total) respectively [Table-1]. In contrast to MEs, nMEs have dominance of charged catalytic residues at their active site as evident by their percentage composition. This implies that all the five charged residues are critical for the function of nMEs, while the polar residues having supportive functions like stabilization of the substrate/intermediate molecule. In pnMEs, three polar catalytic residues (Y, C and S) are equally important for catalytic function, while in enMEs 'Y and S' are the two key residues having broader spectrum of distribution and possibly more suitable for supportive catalytic functions in comparison to other polar residues.

3.2.4. Comparison of MEs and nMEs

The comparison of catalytic residue composition and distribution between pMEs and pnMEs and eMEs and enMEs was carried out separately [Figure-2]. In pnMEs the role of 'H' is partly shared by 'D' and 'E' residues, while in pMEs the polar catalytic residues (Y, S and T) have greater importance. Interestingly, both pMEs and pnMEs have more or less equal distribution of 'K, R and N'. The conserve distribution of these three residues indicates that the terminal hydrogen atom of these

residues necessary for the stabilization of the intermediate via hydrogen bonding and keeping the intermediate at the active site during catalysis. The high proportion of 'C' in pnMEs shows that the terminal sulphur atom 'C' plays a key role in attacking the substrate and initiating the catalytic reaction [16]. The absence of 'Q' amino acid in pMEs and its less significant proportion in pnMEs indicate that this polar residue is not vital for executing the catalytic function of enzymes. Overall, pMEs depends more or less equally on charged (56.33%) and polar (35.22%) residues for catalysis, while pnMEs primarily dependent on charged residues (63.92%) for catalysis and polar residue (24.92%) support the catalytic function.

The comparison of eMEs and enMEs revealed that enMEs show marked dependency on 'H, D' and 'E' charged residues for their catalytic activity, while eMEs preferentially dependent on 'H and K' residues for their catalytic activity and least dependent

on 'D' residue. It is difficult to understand the significant compositional difference for 'D' residue between eMEs and enMEs, at least this difference does not reflect the biased dataset. The three polar catalytic residues 'Y, S and C' contribute actively in eMEs catalysis; their proportion is more or less half in enMEs. It is possibly that these three residues are involved in executing certain specific catalytic function in eMEs which are not carried out by enMEs. With respect to eMEs, enMEs have significantly high frequency of 'N' residue, possible it is related to the size of the substrate. eMEs depends equally on charged (50.96%) and polar (40.7%) residues for catalysis, while enMEs show high dependence on charged residues (64.18%) and polar residue (26.51%) support the catalytic function. In total, both Prokaryotic and Eukaryotic MEs and nMEs show high degree of similarity in the percentage distribution of charged and polar residues at their active site.

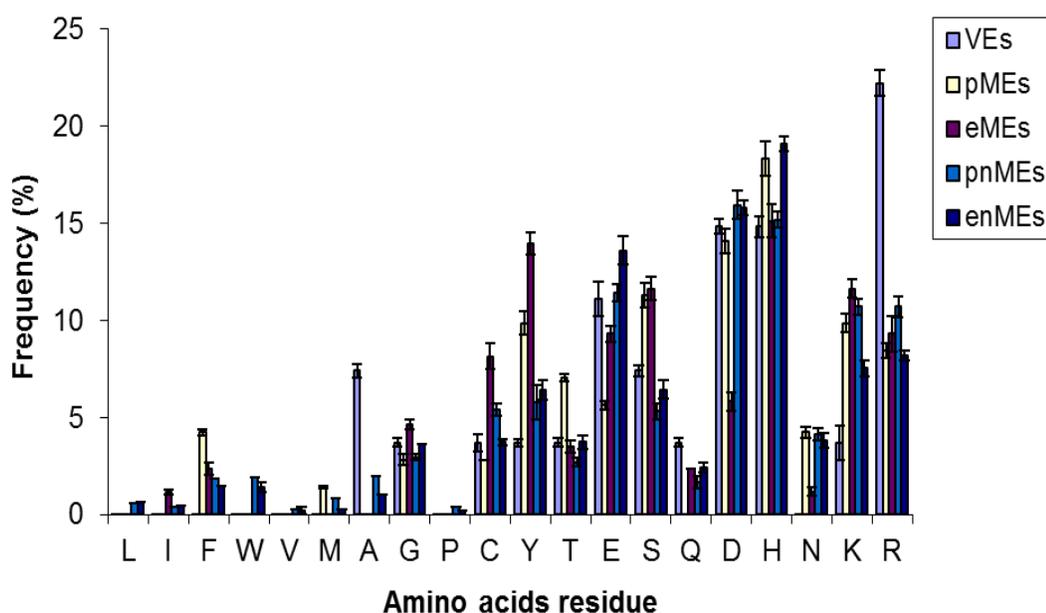


Fig: 1. Amino acid frequency distribution at the enzymatic active site of different organisms. The amino acids are arranged in decreasing order of hydrophobicity. The standard deviation for frequency distribution of a particular catalytic active site residue of an enzyme belongs to different organisms is represented separately as a bar. VE: Blue bar; pME: brown bar; eME: yellow bar; pnME: purple bar; enME: black bar.

[IV] DISCUSSION

The study is based on the comparative frequency distribution of catalytic residues occurring in enzymes belongs to different organism and cellular localization [Table-1 and Figure-1], which is different from the earlier study where the frequency distribution of enzyme catalytic residues was reported irrespective of their origin and cellular localization (Charged - 65%; Polar - 27%; Hydrophobic - 8%) [3]. In general, the significant occurrence of charged and polar residues at the

enzymatic active site is expected as they provide the electrostatic force necessary for the movement of electrons and protons during catalysis. The results indicate that the catalytic residues of membrane enzymes (MEs) show significant variation in the distribution in contrast to the composition reported by Bartlett et al. (2002). In addition, the slight deviation of catalytic residues frequency distribution in non-membrane enzymes (nMEs) is probably due to the large dataset used in this study in comparison to 178 proteins (from undefined origin) included in the dataset of Bartlett et al.

(2002).

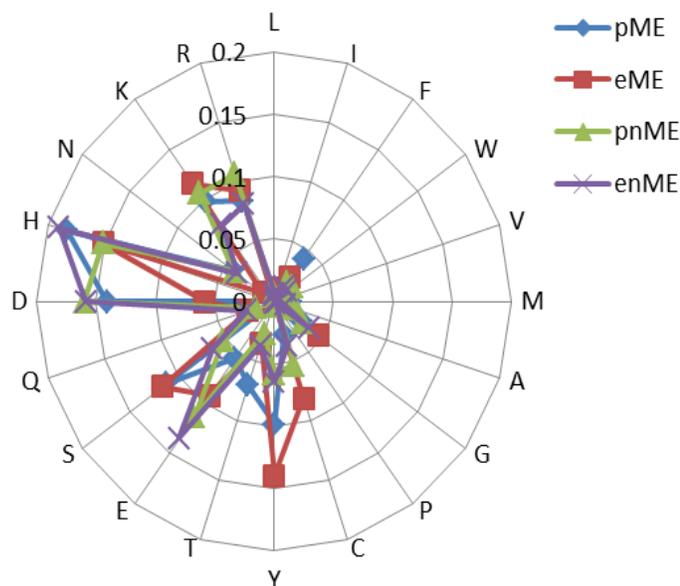


Fig: 2. Radar projection highlighting the difference between amino acid frequency distribution of membrane and non-membrane enzyme active site.

The highest probability occurrence of 'H' as catalytic residue among all the 13 frequently distributed amino acids indicates its multiple roles at enzymatic active site such as nucleophilic and electrophilic agent, acid and base, and stabilization of enzyme-substrate transition state complex. It has been recently reported that the shape of 'H' is also important in enzyme catalysis [17]. 'D' residue has almost equal probability of occurrence at enzymes active site except in eMEs [Table-1]. It is possible that not all the eMEs possess 'D' residues at their active site except those belong to particular family [18]. 'E' has exactly half the chances of occurrence in pMEs and also shows low distribution in eMEs in comparison to other enzymes. This pattern of frequency distribution of 'E' may be due to the occurrence of less number of membrane enzymes in general and its role in selected category of membrane proteins [19]. Comparatively, the amino acid 'K' has least possibility of occurrence in VEs. 'R' strongly favors the VEs active site, while 'S' best occupy the MEs active site. 'Y' has the highest probability of occurrence at the active site of eMEs [Table-1].

The Viral dataset used in this study is relatively smaller in comparison to Prokaryotes and Eukaryotes because only nine viral enzyme (VEs) structures, having 30% sequence identity, are currently available in PDB. Furthermore, the viral enzyme homologues active site is identified with the help of multiple sequence alignment by using viral enzymes, having solved structure in PDB, as reference [4]. Though the viral dataset used here is small, it is a representative of protein mosaic (e.g. Protease, Helicase, RNA polymerase etc.) belongs to different types of viruses. The VEs show distinct high occurrence of 'R' and 'A' catalytic residues in comparison to prokaryotic and eukaryotic enzymes. The replication and production of viral

particles inside the host cell require viral enzymes to catalyse exponentially the replication and other reactions. Probably three nitrogen atoms in the side chain of 'R' help viral enzymes to make stronger and stable electrostatic interactions with the substrate to facilitate the enzymatic reactions at an exponential rate. Also 'R' side-chain has a good geometry to stabilize a pair of oxygen atoms on a phosphate group, which facilitate the interaction of Viral enzymes with host DNA/RNA molecules [20]. The significantly high occurrence of an aliphatic and short side chain 'A' residue probably helps in assisting the proper positioning of the substrate at the enzyme active site and also keeps the intermediate ions/substrate stable.

The high occurrence of polar catalytic residues in MEs play an essential role in functioning of transmembrane helices via mediating and stabilizing helical interactions [21]. In addition, enzyme frequently uses polar and non-polar groups to make weak interactions with the substrate in their specificity sub-site of the active site [22].

[V] CONCLUSION

Enzymes of different origin have distinct preference of charged and polar amino acids at their active site. Certainly, high probability occurrence of these amino acids does not indicate their conservation at the active site. The knowledge of amino acid frequency distribution at the active site can be exploited in designing novel enzymes active site as well as their specific inhibitors.

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