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CONTRIBUTION OF CATION-Π INTERACTION AND ITS EFFECT ON THE STRUCTURAL STABILITY OF LACCASE ENZYMES-A COMPUTATIONAL STUDY

K. RAMANATHAN, V. SHANTHI AND RAO SETHUMADHAVAN*

School of BioSciences and Technology, Vellore Institute of Technology University, Vellore 632014, Tamil Nadu, India.

* ***Corresponding author*** rsethumadhavan@vit.ac.in

ABSTRACT

The energy contribution resulting from cation-π interactions and free energy of folding has been computed for 18 laccase enzymes. The contribution of these cation-π interacting residues in secondary structure involvement, solvent accessibility, stabilization centers and structural stability has been evaluated. Secondary structure of the cation-π involving residues show that, Arg and Lys prefers to be in strand and coil structures respectively. Among the π residues, Phe and Tyr prefer to be in coil whereas Trp prefers to be in strand. Among the cation-π interacting residues Arg and Lys were in the exposed regions. Phe and Tyr were in the partially buried region and Trp in the fully buried region. Stabilization centers for these proteins showed that all the five residues found in cation-π interactions are important in locating one or more of such centers. We have also determined the stability of each enzymes by its ΔG value. On the whole, the results presented in this work suggest that *Bacillus Subtilis* Cota Laccase Adduct with ABTS (1UVW) exhibit the highest stability among the entire laccase enzyme studied in this investigation.

KEYWORDS

Cation–π interactions; Folding energy; Secondary structure; ASA; Stabilization centers; Interaction energy

INTRODUCTION

The three dimensional structure of a protein is determined by a delicate balance of weak interactions. In this, cation-π interactions are increasingly recognized as an important noncovalent binding interaction relevant to structural biology[1, 2]. The importance of this interaction has been stressed by several investigators for their role in enhancement of the stability of thermophilic proteins[3, 4], folding of polypeptides[5, 6] and the stability of membrane proteins[7, 8]. Influence of cation-π interactions in protein-DNA complexes is studied by Gromiha[9]. Also there are reports on theses kinds of interactions in a set of 62 non-reductant DNA binding proteins by the same author[10]. Recently, we have published the role of cation...π interactions on the structural stability of therapeutic proteins[11]. But till now, there are no reports on the stability of laccases due to cation-π interactions and free energy of folding (ΔG) which is used mainly for industrial and biotechnological applications.

Laccases have received much attention from researchers in last decades due to their

ability to oxidise both phenolic and nonphenolic lignin related compounds as well as highly recalcitrant environmental pollutants, which makes them very useful for their application to several biotechnological processes. Laccases are currently of interest in baking due to its ability to cross-link biopolymers[12]. Laccases have potential application in different aspects of the food industry such as bioremediation, beverage processing, ascorbic acid determination, sugar beet pectin gelation, baking and as a biosensor[13]. However, they suggested that more studies of laccase production and immobilisation techniques at lower costs are needed to improve the industrial application of this enzyme. The use of laccase in the textile industry is growing very fast, besides to decolourise textile effluents as commented above, laccase is used to bleach textiles and even to synthetise dyes[14]. Laccases are able to catalyse electron transfer reactions without additional cofactors. Their use has also been studied in biosensors to detect various phenolic compounds, oxygen or azides[15]. Also, Roy et al. found that cross-linked enzyme crystals (CLEC) of laccase from Trametes versicolor could be used in biosensor applications with great advantage over the soluble enzyme.

Polycyclic aromatic hydrocarbons (PAHs) together with other xenobiotics are a major source of contamination in soil. Therefore, their degradation is of great importance for the environment. The catalytic properties of laccases can be used to degrade such compounds also[16]. The cosmetic world has not been indifferent to the application of laccase: for example, laccase-based hair dyes are less irritant and easier to handle than current hair dyes, since laccases replace H_2O_2 as an oxidising agent in the dye formulation[17, 18].

The most important obstacles to commercial application of laccases are the lack of sufficient enzyme stocks and the cost of redox mediators. The general goal is to obtain stable catalysts with long life times and low cost. We think that the combination of these techniques will enhance: i) the adsorption of laccase on a suitable substrate, ii) the lifetime of the laccase activity and iii) reutilisation of the substrate/laccase product. Therefore there is a need to examine the stability of laccases, which is useful for the investigation on the specificity and selectivity of the enzyme and also for their structural studies. Hence, in the present work we have studied the stability of 18 laccase enzymes through cation-π interactions and folding energy. We are reporting that laccase enzyme (1UVW) exhibit the highest stability amongst all the laccase enzyme studied in this work. On the whole, the results presented in this work will be very useful for further investigations on the specificity and selectivity of laccase enzymes and also for their structural studies.

MATERIALS AND METHODS

(i) Data set

We have considered a set of 18 laccases from the Protein Data Bank[19] for our investigation the details of which are given in Table 1. The PDB ID's are as follows: 1HFU, 1GYC, 2H5U, 1A65, 1V10, 2FQD, 1UVW, 1KYA, 2IH8, 2HRG, 2HRH, 2FQE, 2FQF, 2FQG, 2QT6, 1GW0, 2HZH, 2IH9. Almost 90% of the structures were solved with <2.5 Å resolution.

Table 1
Data set of laccase enzymes

PDB ID	Title	Source	No. of residues
1HFU	Cu-Depleted Laccase at 1.68-A	*Coprinus Cinereus*	503
1GYC	Laccase at 1.90-A resolution containing a full complement of coppers	*Fungus Trametes versicolor*	499
2H5U	Laccase at 1.90-A	*Cerrena maxima*	499
1A65	Cu-Depleted Laccase at 2.23-A	*Coprinus Cinereus*	504
1V10	Laccase from Hemihedrally Twinned Crystals at 1.70-A	*Rigidoporus lignosus*	494
2FQD	Laccase CueO at different copper concentrations at 2.40-A	*E-Coli*	516
1UVW	Cota laccase adduct with ABTS at 2.75-A	*Bacillus Subtilis*	511
1KYA	Active laccase complexed with 2,5-Xylidine at 2.40-A	*Trametes versicolor*	499
2IH8	Laccase at 2.00-A	*Melanocarpus albomyces*	559
2HRG	Blue Laccase complexed with p-methylbenzoate at 1.58-A	*Trametes trogii*	496
2HRH	Blue Laccase at 2.60-A	*Trametes trogii*	496
2FQE	Laccase CueO at different copper concentrations at 1.92-A	*E-coli*	516
2FQF	Laccase CueO at different copper concentrations at 2.00-A	*E-coli*	516
2FQG	Laccase CueO at different copper concentrations at 2.30-A	*E-coli*	516
2QT6	Blue laccase at 1.50-A	*Lentinus Tigrinus*	498
1GW0	Laccase at 2.40-A	*Melanocarpus albomyces*	559
2HZH	Laccase at 2.60-A	*Coriolus zonatus*	499
2IH9	Laccase at 2.00-A	*Melanocarpus albomyces*	559

(ii) Computation of cation–π interactions energy

The cation–π interaction energy in each enzymes has been calculated using the program CaPTURE[20]. In the present study only energetically significant interactions ($E_{cat-\pi} \leq 2$ kcal/mol) were considered. The percentage composition of a specific amino acid residue contributing to cation–π interactions is obtained by the equation,

$$Comp_{cat\text{-}\pi}(i) = n_{cat\text{-}\pi}(i) \times [100/n(i)] \quad (1)$$

where i stands for the five residues, Lys, Arg, Phe, Trp and Tyr, $n_{cat-\pi}$ is the number of residues involved in cation–π interactions and n(i) is the number of residues of type i in the considered protein structures.

We have computed the energetic contribution of cation–π interactions for each enzymes in the data set and for all possible pairs of positively charged and aromatic amino acids. The total cation–π interaction energy ($E_{cat-\pi}$) has been divided into electrostatic (E_{es})

and van der Waals energy (E_{vw}) and were computed using the program CaPTURE, which has implemented a subset of OPLS force field[21] to calculate the energies. The electrostatic energy (E_{es}) is calculated using the equation

$$E_{el} = \sum q_i q_j e^2 / r_{ij}; \quad (2)$$

Where q_i and q_j are the charges for the atoms i and j, respectively, and r_{ij} is the distance between them.

The van der Waals energy is given by

$$E_{vw} = 4\varepsilon_{ij} [(\sigma_{ij}^{12}/r_{ij}^{12})-(\sigma_{ij}^{6}/r_{ij}^{6})] \quad (3)$$

Where $\sigma_{ij}= (\sigma_{ii}\sigma_{jj})^{1/2}$ and $\varepsilon_{ij}= (\varepsilon_{ii}\varepsilon_{jj})^{1/2}$; σ and ε are the van der Waals radius and well depth, respectively.

(iii) Secondary structure and solvent accessibility studies

Secondary structure and solvent accessibility are considered to be very important to understand the biochemical activity of proteins. Hence a systematic analysis of each cation–π interactions forming residue was performed based on their location in different secondary structures of enzymes and their solvent accessibility. Solvent accessibility was divided into three classes, buried, partially buried and exposed indicating, respectively, the least, moderate and high accessibility of the amino acid residues to the solvent. We used the program DSSP[22] to obtain the information about secondary structures and solvent accessibility.

(iv) Computation of stabilization center

Stabilization centers are clusters of residues that are involved in medium or long range interactions[23]. Residues can be considered part of stabilization centers if they are involved in medium or long range interactions and if two supporting residues can be selected from both of their flanking tetra peptides, which together with the central residues form at least seven out of the nine possible contacts. We used the server which is available at http://www.enzim.hu/scide for this purpose[24].

(v) Computation of enzyme stability by ∆G

Understanding the overall stability of laccase enzyme is major task for selecting laccase in industrial as well as biotechnological application. So to analyze the stability of laccase enzyme, we used the server Fold-x[25, 26] which calculate the free energy of folding for a given protein/enzyme structure. Protein stability is quantitatively described by the standard Gibbs energy change ∆G[27]. This server contains the FoldX force field (FOLDEF) which includes terms that have been found to be important for protein stability. The free energy from the unfolding (∆G) of a target protein is calculated using equation (4)

$$\begin{aligned}\Delta G = {} & W_{vdw} \times \Delta G_{vdw} + W_{solvH} \times \Delta G_{solvH} \\ & + W_{solvP} \times \Delta G_{solvP} + \Delta G_{wb} \\ & + \Delta G_{hbond} + \Delta G_{el} + \Delta G_{Kon} \\ & + W_{mc} \times T \times \Delta S_{mc} + W_{sc} \times T \\ & \times \Delta S_{sc} + W_{clash} \times \Delta G_{clash} \quad (4)\end{aligned}$$

Where ΔG_{vdw} is the sum of the van der Waals contributions of all atoms with respect to the same interactions with the solvent. ΔG_{solvH} and

ΔG_{solvP} are the differences in solvation energy for apolar and polar groups, respectively, when these groups change from the unfolded to the folded state. ΔG_{hbond} is the free-energy difference between the formation of an intramolecular hydrogen bond and the formation of an intermolecular hydrogen bond (with solvent). ΔG_{wb} is the extra stabilizing free energy provided by a water molecule that makes more than one hydrogen bond to the protein (water bridges) and that cannot be taken into account with nonexplicit solvent approximations[28]. ΔG_{el} is the electrostatic contribution of charged groups, including the helix dipole. ΔS_{mc} is the entropy cost of fixing the backbone in the folded state. This term is dependent on the intrinsic tendency of a particular amino acid to adopt certain dihedral angles[29]. Finally, ΔS_{sc} is the entropic cost of fixing a side chain in a particular conformation[30], and the ΔG_{clash} term provides a measure of the steric overlaps between atoms in the structure.

The energy value calculated above based on the fold-x does not include the energy due to cation- π interaction. Hence we report the total energy of the particular protein as the sum of the cation-π interaction energy and the free energy of folding.

RESULTS AND DISCUSSION

(i) Preference of cationic and aromatic residues for forming cation-π interaction in laccase enzymes

The preference of amino acid residues that are involved in cation-π interactions was analyzed and the results are presented in Table 2. We observed that in these enzymes, Phe has the highest occurrence among the aromatic residues involving in cation-π interactions. This trend is similar to those observed in transmembrane, globular proteins[20, 8,] DNA and RNA binding proteins[31]. Among the cationic residues Arg is preferred to Lys to be involved in the cation-π interaction in the set of the above enzymes studied.

Table 2
Composition of cation-π forming residues in laccase

PDB Code	%Lys	%Arg	%Phe	%Tyr	%Trp
1HFU	1.4	3.8	4.4	3.0	1.6
1GYC	1.6	3.4	6.0	3.0	1.4
2H5U	1.4	2.8	6.0	2.4	1.4
1A65	1.4	3.8	4.4	3.0	1.6
1V10	0.6	4.1	4.3	3.1	1.4
2FQD	4.7	3.7	3.4	1.7	1.7
1UVW	4.8	5.6	3.2	5.2	1.8
1KYA	1.0	3.0	6.0	3.0	1.4
2IH8	1.6	4.5	3.8	3.4	2.9
2HRG	1.6	3.0	5.0	3.0	1.4
2HRH	1.6	3.0	5.0	3.0	1.4
2FQE	4.8	3.7	3.2	1.7	1.7
2FQF	4.7	3.7	3.4	1.7	1.7
2FQG	4.7	3.7	3.4	1.7	1.7
2QT6	1.2	2.6	5.8	3.0	1.6
1GW0	1.6	4.5	3.8	3.4	2.9
2HZH	1.4	2.4	5.2	3.2	1.4
2IH9	1.6	4.5	3.8	3.4	2.9
Mean	2.32	3.66	4.45	2.88	1.77

(ii) Cation-π residue pairs involved in laccase enzymes

There are six cation-π interacting pairs namely, Arg-Phe, Arg-Tyr, Arg-Trp, Lys-Phe, Lys-Tyr and Lys-Trp pairs. The specific pair wise residue involved in cation-π interaction and their position for all the laccase enzyme studied are given in Table 3. It could be seen from the table that the laccase enzyme with PDB code 1HFU and 1GYC does not have any interactions involving either Lys-Tyr or Lys-Trp residues. The enzyme *Bacillus Subtilis* Cota Laccase Adduct with ABTS (1UVW) showed a maximum of 10 energetically significant cation-π interactions. Out of the 10 interactions in this enzyme five interactions involve Arg-Tyr residues at different position. Except for the laccase enzyme 2FQD, 2FQF and 2FQG, all other enzymes studied showed an interaction between Arg-Tyr residues. Of the total 18 enzymes investigated, it was found that, 50% of the enzymes had more than 5 energetically significant cation-π interactions and remaining 50% of the enzyme showed a range from 1-5 energetically significant cation-π interactions.
The PyMol view of Arg-Phe, Arg-Tyr and Arg-Trp interacting pairs in 1UVW is shown in Fig. 1. Figure 2 shows the results of number of cation-π interaction in laccase enzymes. It was found that, among the cation- π interactions involving Arg residues, Arg-Tyr residues showed the highest percentage of interaction whereas Arg-Phe and Arg-Trp interactions were almost similiar. Among the cation-π interactions involving Lys residues, Lys-Tyr residues shows a slightly higher percentage of interaction than Lys-Trp. The interaction involving Lys-Phe is not found at all in any of the laccase enzyme studied. Hence from the results obtained, we could without ambiguity state that Arg-Tyr and Lys-Tyr interactions may be considered as important and significant interaction in further studies on the structural stability of laccase enzymes.

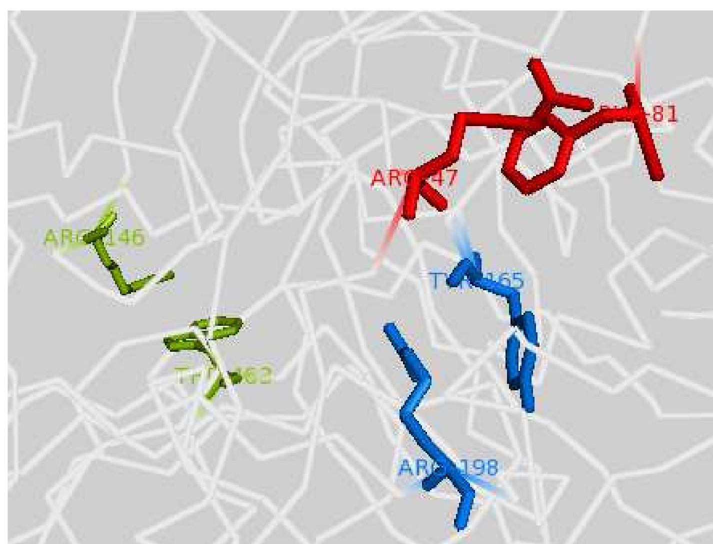

Fig. 1
PyMol view of Arg-Phe, Arg-Tyr, Arg-Trp interacting pairs in laccase enzyme ID 1UVW.

Table 3

Cation – π interaction energy in laccase enzymes

PDB Code	R-Y (-kcal/mol)	R-W (-kcal/mol)	R-F (-kcal/mol)	K-Y (-kcal/mol)	K-W (-kcal/mol)	N-Cat π
1HFU	R196 – Y137 (2.85) R409 – Y416 (3.62)	R23 – W151 (4.32)	R259 – F273 (5.77)			4
1GYC	R197 – Y137 (2.78) R408 - Y415 (3.59)	R22 – W151 (4.07)	R161 – F162 (3.66) R260 – F270 (5.36)			5
2H5U	R408 – Y415 (3.97)	R22 – W151 (4.4)	R260 – F270 (6.16)			3
1A65	R196 – Y137 (2.3) R409 – Y416 (3.7)	R23 – W151 (4.89)	R259 – F273 (5.56)			4
1V10	R409 – Y416 (3.62)	R22 – W151 (2.94)	R265 – F275 (4.87)			3
2FQD		R150 – W67 (2.76) R234 – W176 (2.57) R453 – W469 (7.19)	R242 – F310 (7.51) R252 – F293 (8.47) R349 – F422 (6.06)	K485 – Y429 (3.46)		7
1UVW	R198 – Y165 (5.67) R248 – Y189 (8.41) R261 – Y263 (3.04) R338 – Y242 (8.11) R434 – Y449 (4.72)	R146 – W463 (5.78) R429 – W463 (3.4)	R47 – F81 (4.54)		K5 – W240 (3.32) K101 – W122 (4.84)	10
1KYA	R197 – Y137 (3.34) R408 – Y415 (3.27)	R22 – W151 (4.32) R121 – W151 (2.11)	R260 – F270 (5.92)			5
2IH8	R10 – Y163 (6.79) R123 – Y125 (7.11) R226 – Y163 (2.81)		R178 – F186 (3.95)	K386 – Y391 (5.19)	K554 – W384 (5.1)	6
2HRG	R196 – Y137 (2.48) R198 – Y127 (2.8) R407 – Y414 (3.96)	R22 – W151 (4.26)	R259 – F269 (5.45)			5
2HRH	R407 – Y414 (3.97)		R259 – F269 (5.05)			2
2FQE	R31 – Y221 (4.52)	R150 – W67 (2.61) R234 – W176 (2.29) R453 – W469 (6.95)	R242 – F310 (8.34) R252 – F293 (8.94) R349 – F422 (6.19)	K485 – Y429 (3.18)		8
2FQF		R150 – W67 (2.53) R234 – W176 (2.44) R453 – W469 (6.88)	R242 – F310 (7.8) R252 – F293 (8.44) R349 – F422 (6.22)	K485 – Y429 (3.31)		7
2FQG		R150 – W67 (2.63) R234 – W176 (2.39) R453 – W469 (7.09)	R242 – F310 (8.06) R252 – F293 (8.48) R349 – F422 (6.23)	K370 – Y371 (2.35) K485 – Y429 (3.64)		8
2QT6	R197 – Y137 (2.51) R407 – Y414 (3.44)	R22 – W151 (3.93) R121 – W151 (2.2)	R161 – F162 (3.63) R260 – F270 (5.65)			6
1GW0	R10 – Y163 (4.49) R123 – Y125 (7.45) R226 – Y163 (3.5) R476 – Y273 (2.93)		R178 – F186 (4.15)	K386 – Y391 (4.81)	K554 – W384 (5.27)	7
2HZH	R197 – Y137 (2.92) R408 – Y415 (4.14)	R22 – W151 (3.9)	R260 – F270 (5.63)			4
2IH9	R10 – Y163 (6.3) R123 – Y125 (7.56) R226 – Y163 (2.6)		R178 – F186 (4.26)	K386 – Y391 (3.54)	K554 – W384 (4.66)	6

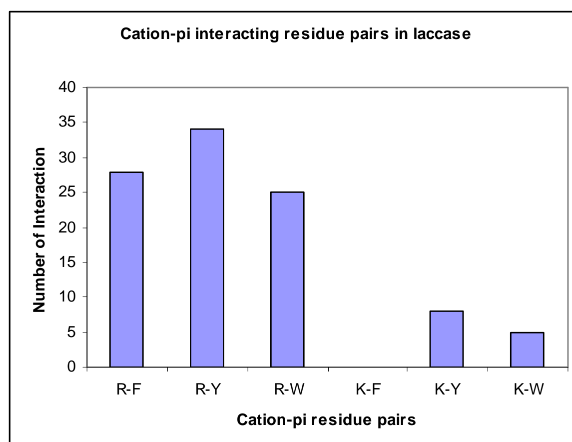

Fig. 2
Cation-π interacting residues pairs in laccase enzymes

(iii) Cation-π interaction energies in laccase enzymes

The pair wise cation- π interaction energy between the cationic and aromatic residues shows that Arg-Phe energy is the strongest and Lys-Tyr is the lowest among the six possible pairs as shown in Fig. 3. The strength of cation-π interaction energy differs significantly in the laccase enzyme. For instance, for 1UVW it was -51.83 (kcal/mol) and in 2HRH it was -9.02 (kcal/mol). Of the total 18 laccase enzymes investigated, it was found that 6, 44, 11, 22, 11, 6% of enzymes had total cation- π interaction energy between -1 to -10 (kcal/mol), -10 to -20 (kcal/mol), -20 to -30 (kcal/mol), -30 to -40 (kcal/mol), -40 to -50 (kcal/mol), and greater than -50 (kcal/mol) respectively. We observed an average energetic contribution of -25.95 (kcal/mol) in the group of laccase enzyme investigated in the present work. The composition of cation- π interaction energy into electrostatic and van der Waals energy terms showed that, among the 18 laccase enzymes, 15 enzymes had stronger electrostatic energy than van der Waals energy.

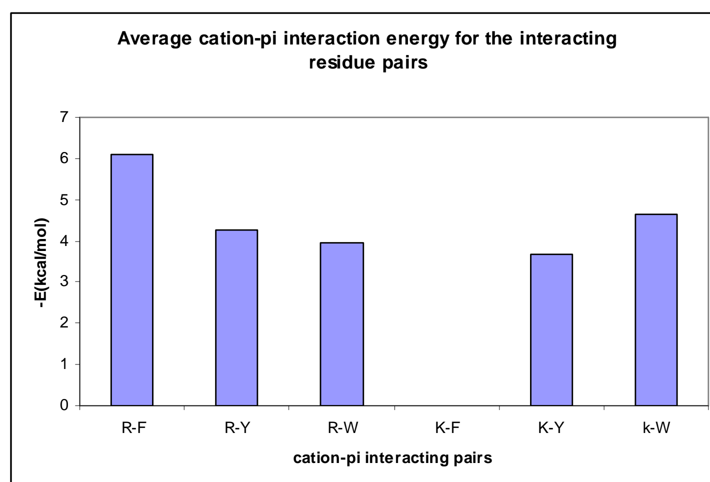

Fig. 3
Average Cation-π interaction energy for the interacting residue pairs

(iv) Secondary structure prediction of amino acid residues in the laccase enzymes

The propensities of the amino acid residues to favor a particular conformation are well known. Such conformational preference is not only dependent on the amino acid alone but is also dependent on the local amino acid sequence. We have computed the preference of cation- π interaction forming residues in different secondary structures and the results are shown in Table 4. It was found that, in the cationic group, Arg preferred to be in strand and Lys preferred to be in coil. In the aromatic group it was found that, Phe and Tyr prefers to be in coil whereas Trp preferred to be in strand. We used the program DSSP[22] to obtain the information about secondary structures.

Table 4

Frequency of occurrence of cation-π interaction forming residue in different secondary structures

Residue	Coil	Strand	Helix
Arg	34.48 (28.33)	60.92 (41.45)	4.60 (30.22)
Lys	92.30 (41.78)	NI (24.55)	7.65 (33.66)
Phe	60.71 (51.33)	28.57 (21.62)	10.71 (27.05)
Tyr	69.04 (58.4)	16.66 (22.95)	14.28 (18.65)
Trp	36.66 (15.33)	63.33 (50.85)	NI (33.82)

Parenthesis value shows % of cation – π interaction forming residues in different secondary structures of whole data set, NI: no interaction.

(v) Solvent accessibility of the cation-π interacting residues in laccase enzymes

We used DSSP[22] to estimate the solvent accessibility of the residues involved in cation- π interactions. The average solvent accessibility of the residues Lys, Arg, Tyr, Phe and Trp which are involved in cation- π interactions are 84.62, 62.32, 38.1, 46.43, and 10, respectively, as shown in Fig. 4. The solvent accessibility of Arg and Lys residues are significantly higher than other cation- π forming residues. The normalized ASA has been divided into three categories, buried, partially buried and exposed for different ranges of ASA; <20, 20–50 and >50, respectively[32-34]. From this classification, we observed that Arg and Lys preferred to be in exposed region. Among the aromatic residues, it was observed that Phe and Tyr preferred to be in partially buried region, while Trp preferred to be in the fully buried regions. This observation is quite reasonable in the sense that, the aromatic residues are in principle, non polar residues, and tend to be buried. Since Arg and Lys are polar in nature they tend be exposed to the solvent surface.

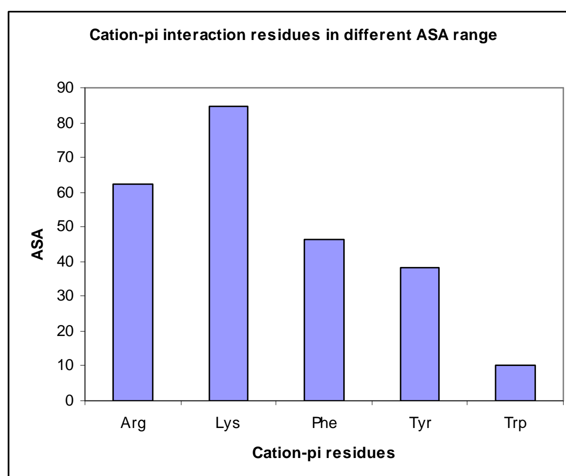

Fig. 4
Cation-π interaction residues in different ASA range

(vi) Stabilization centers of cation-π interacting residues in laccase enzymes

We have computed the stabilization center for all cation- π interaction forming residues in laccase enzyme using the program SCide and the results are depicted in Fig. 5. It was found that 54% of cationic residues and 50% of π residues were found to have one or more stabilization centers. Cationic residues were found to have more stabilization centers than π residues. This trend was different with the earlier report on RNA binding proteins[31]. It was interesting to note that all the five residues found in cation- π interactions are important in locating one or more stabilization centers. These observations strongly reveal that these residues may contribute significantly to the structural stability of these proteins in addition to participating in cation-π interactions.

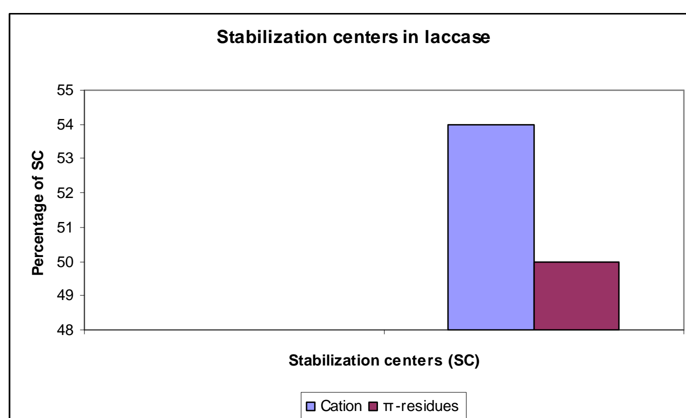

Fig. 5
Stabilization centers in Laccase enzymes

(vii) Folding free energy determination of laccase enzymes

We used the server FoldX[25, 26] which calculate the free energy of folding for a given protein or polypeptide structure. This server contains the

FoldX force field (FOLDEF) which includes terms that have been found to be important for protein stability. In our analysis, we have determined the total energy of the all the 18 lacccase enzymes determined and it is presented in Table 5. The results shows that out of the 18 enzymes studied, *Bacillus Subtilis* Cota Laccase Adduct with ABTS (1UVW) exhibit an energy of -214.11 (kcal/mol). It indicates that, the stability of this enzyme is the highest as compared to the other enzyme studied in our analysis. The enzyme with the lowest energy is 1V10. This enzyme has the less number of amino acid residue and also less number of energetically significant cation-π interactions in their structure as compared to 1UVW. This result is shown in Figure 6. The result reported by our work in this study is well supported by an experimental study carried out earlier on CotA laccase from the spore coat of *Bacillussubtilis*[35].

Table 5
Total energy calculations in the laccase enzymes

PDB Code	Cat- π Energy (kcal/mol)	Folding Energy (ΔG) (kcal/mol)	Total Energy of the Enzyme (kcal/mol)
1HFU	-16.56	-127.77	-144.33
1GYC	-19.46	-147.58	-167.04
2H5U	-14.53	-93.89	-108.42
1A65	-16.45	-102.22	-118.67
1V10	-11.43	-83.05	-94.48
2FQD	-38.02	-84.31	-122.33
1UVW	-51.83	-162.28	-214.11
1KYA	-18.96	-101.39	-120.35
2IH8	-30.95	-158.01	-188.96
2HRG	-18.95	-131.97	-150.92
2HRH	-9.02	-103.36	-112.38
2FQE	-43.02	-87.06	-130.08
2FQF	-37.62	-92.26	-129.88
2FQG	-40.87	-98.32	-139.19
2QT6	-21.36	-164.57	-185.93
1GW0	-32.6	-116.41	-149.01
2HZH	-16.59	-86.28	-102.87
2IH9	-28.92	-94.99	-123.91

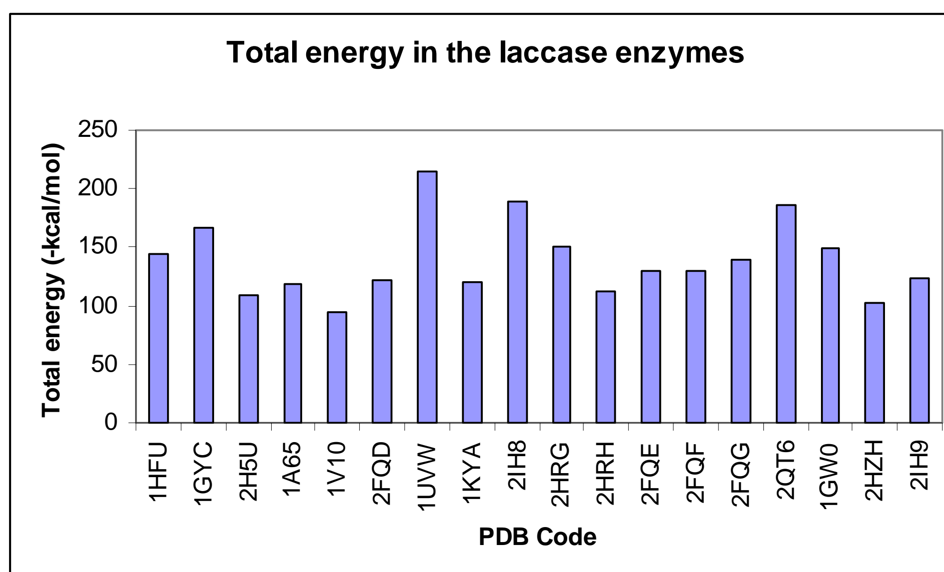

Fig. 6
Total energy in Laccase enzymes

CONCLUSIONS

We investigated the cation- π interactions and free energy of folding for 18 laccase enzymes and the following conclusions were made. Phe has the highest occurrence in this interaction. Among the cationic residue, Arg is found to involve in this interaction more than the Lys residues. All the laccase enzymes studied showed significant cation-π interactions. Among the cation- π residue pairs that were involved in these interaction, Arg-Trp residue pair showed the strongest cation-π interaction. Among the cation-π residue pair the highest percentage of interaction was with Arg-Tyr, Lys-Tyr and the lowest was with Lys -Trp residues. There are no cation-π interactions between Lys-Phe residues at all in all the laccase enzymes studied. In the secondary structure arrangement of cationic group, Arg preferred to be in strand and Lys preferred to be in coil. In the aromatic group it was found that, the residues namely Phe and Tyr preferred to be in coil whereas Trp prefers to be in strand. In the cationic residues Lys and Arg preferred to be in exposed region. Among the aromatic residues, Phe and Tyr preferred to be in partially buried region, while Trp preferred to be in the fully buried regions. We found that, all the five residues found in cation- π interactions are important in locating one or more stabilization centers. The total energy of the entire enzyme calculated based on the cation-π energy and folding energy. It shows that out of the 18 enzymes studied, *Bacillus Subtilis* Cota Laccase Adduct with ABTS (PDB Code 1UVW) posses an energy of -214.11 (kcal/mol). It indicates that, the stability of this enzyme is the highest as compared to other enzymes studied in our analysis. On the whole, the results presented in this work will be very useful for further investigations on the specificity and selectivity of laccase enzymes especially *Bacillus Subtilis* Cota Laccase Adduct with ABTS (1UVW) for its chemical and industrial applications.

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