Prediction of Post Translational Modifications in *Avicennia marina* Cu-Zn Superoxide Dismutase: Implication of Glycation on the Enzyme Structure

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Summary: 3D homology model of Cu-Zn superoxide dismutase (SOD) from Avicennia marina (AMSOD) was constructed using the structural coordinates of Spinach SOD (SSOD). Prediction of post translational modification was done by PROSITE. The predicted sites were examined in the 3D model. AMSOD model was glycated using modeling software and changes in the structure was analyzed after glycation. The analysis revealed some potential sites and structural changes after glycation.

Introduction

In plants under abiotic stress or even under optimal conditions free radical reactions take place where active oxygen species (AOS) like superoxide, hydroxyl, alkoxyl radicals and hydrogen peroxide are produced as metabolic by products in different subcellular compartments [1-3]. These reactions are catalyzed by superoxide dismutases (SODs) that are divided into four groups according to their metal cofactor requirements. These are copper/zinc (Cu,Zn-SOD), manganese (MnSOD), iron (FeSOD) and NiSODs which are located in different compartments of the cell. NiSODs are present in prokaryotes [4]. Cu,Zn-SOD is the most abundant enzyme and has been localized in the cytosol, peroxisome, chloroplast, and apoplast [2, 3]. The enzymatic mechanism proposed for Cu,Zn-SOD is the reduction of oxidized Cu(II) form of the enzyme by superoxide releasing dioxygen, alternating with oxidation of the reduced Cu(I) form by another superoxide anion and two protons, generating hydrogen peroxide [5].

Cu,Zn-SOD is a 32kDa homodimeric metalloenzyme. Each monomer hosts a binuclear metal center (the Cu, Zn pair) responsible for catalytic dismutation of superoxide radical to hydrogen peroxide and oxygen [6]. Mangroves are salt tolerant plants and show increased SOD activities when transferred from fresh water to high salinity [7]**.** Residue His63 and the Cu ion are essential for the dismutase reaction, while Zn appears to be essential for the dissociation of the hydrogen peroxide formed and for the pH stability of the reaction.

In the present study we constructed the 3D homology model of *Avicennia marina* Superoxide Dismutase (AMSOD) bound with Cu and Zn ions present at the catalytic site. We also predicted the potential post translational modification sites in AMSOD and performed glycation studies by molecular modeling and analyzed the structural changes after glycation.

Results and Discussion

Multiple Sequence Alignment and Phylogenetic Analysis

A Phylogenetic tree was built by using the multiple sequence alignment of 69 sequences generated by Clustal X with the help of PHYLIP using default parameters. The species belonging to the same group of plants are placed in the same cluster i.e. Oleaceae, Paulowniaceae, Plantaginaceae, Plantaginaceae, Asteraceae and Asteraceae families (Fig. 1). According to the phylogenetic tree, SOD (gi|12744890|) from *Avicennia marina* belong to the phylum Magnoliophyta, dicotyledons, family *Acanthaceae* and subfamily *Avicennioideae* and is closely related to (gi|39840779|) from Olea europaea that belong to the same phylum but belong to family Oleaceae and subfamily Oleeae. Cu-Zn superoxide dismutase from Paulownia kawakamii Plantago major, Solidago canadensis var. scabra and Helianthus annuus belong to same phylum and in same clade but to a different cluster. The possible reason for such difference could be based on the assumption that superoxide dismutase genes have segregated as they belong to the different plant's family; *Avicennia marina* belong to Avicenaceace while *Spinacia oleracea* belongs to Amaranthaceae.

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Fig. 1: The evolutionary tree of Cu-Zn superoxide dismutase from *Avicennia marina* shows evolutionary relationship with superoxide dismutase was constructed by using PHYLIP- Phylogeny Inference Package.

Homology Model of AMSOD

The homology model of Cu, Zn-superoxide dismutase from *Avicennia marina* (AMSOD) was built using the 3D structural coordinates of spinach superoxide dismutase (Pdb id: 1srd) as the template as it showed highest sequence identity (Fig. 2). The stereochemical quality of the selected model was examined using PROCHECK [8]. The Rama-chandran plot obtained by PROCHECK showed that the quality of model was reasonably good. AMSOD closely resembles the structure of 1srd. The Ramachandran plot obtained with PROCHECK showed that 84.2% of the model's φ, ψ angles fall in the most favored region excluding Gly and Pro residues, 11.4% in the additionally allowed region and 4.4% in the generously allowed region The position of the functionally important residues in the structure is retained in the model when compared with the other Cu, Zn SODs and the overall packing of the flattened Greek-key nine-stranded beta-barrel is maintained (Fig. 3). The overall similarities between the AMSOD and spinach Cu, Zn-SOD structure were evaluated by superposition that showed root mean-square (r.m.s.d) deviations of 0.32Å which indicates that there is a strong structural conservation between the two proteins (Fig. 3b). Superposition of AMSOD with other Cu,ZnSODs illustrates that AMSOD is structurally related to the previously determined structures of bovine superoxide dismutase [9], human superoxide dismutase [10], mansoni superoxide dismutase [11] and yeast superoxide dismutase [5] with r.m.s.d. of 0.83Å, 0.88Å, 0.88Å and 0.84Å respectively. The ProSA energy plot shows the local model quality by plotting energies as a function of amino acid sequence position**.** In case of the homology models of AMSOD, ProSA analysis revealed Z-scores which lie in the low energy conformation states (Fig. 4). These results suggest that the models are of good quality.

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pdb|1SRD|A  Chain A, Three-Dimensional Structure Of Cu,Zn-Superoxide
Dismutase From Spinach
Score =  208 bits (530),  Expect = 6e-55, Method: Compositional matrix
adjust.Identities = 104/148 (70%), Positives = 113/148 (76%), Gaps = 0/148
(0%)

Query  3    KAVAVLSSNEGVRGTVYFTQEGDGPTTVTGNLSGFKSGPHGFHVHALGDTTNGCMSTGPH  62
Sbjct  4    KAVAVLKGTSNVEGVVTLTQEDDGPTTVNVRISGLAPGKHGFHLHEFGDTTNGCMSTGPH  63

Query  63   FNPAGKDHGAPEDEVRHAGDLGNITVGEDGTAAVNIVDKQIPLSGPHSIVGRAVVVHADP  122
Sbjct  64   FNPDKKTHGAPEDEVRHAGDLGNIVANTDGVAEATIVDNQIPLTGPNSVVGRALVVHELE  123

Query  123  DDLGKGGHELSKTTGNAGGRVACGIIGL  150
Sbjct  124  DDLGKGGHELSPTTGNAGGRLACGVVGL  151
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Fig 2: Pairwise sequence alignment of the target sequence, *Avicennia marina* superoxide dismutase against protein data bank entries using BLAST server. The template used was 1SRD.

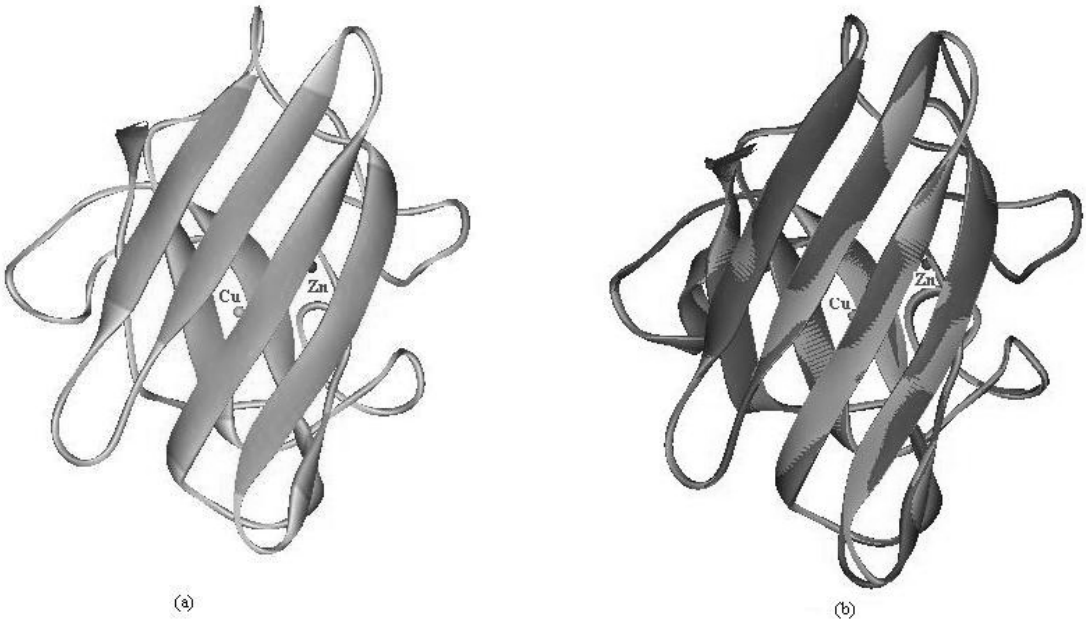

Fig 3: (a) The 3D Homology Model of *Avicennia marina* Superoxide Dismutase showing the secondary structural features. Also shown are the Cu and Zn ions (b) Superposition of Superoxide Dismutase from *Avicennia marina* (grey) and Spinach (blue).

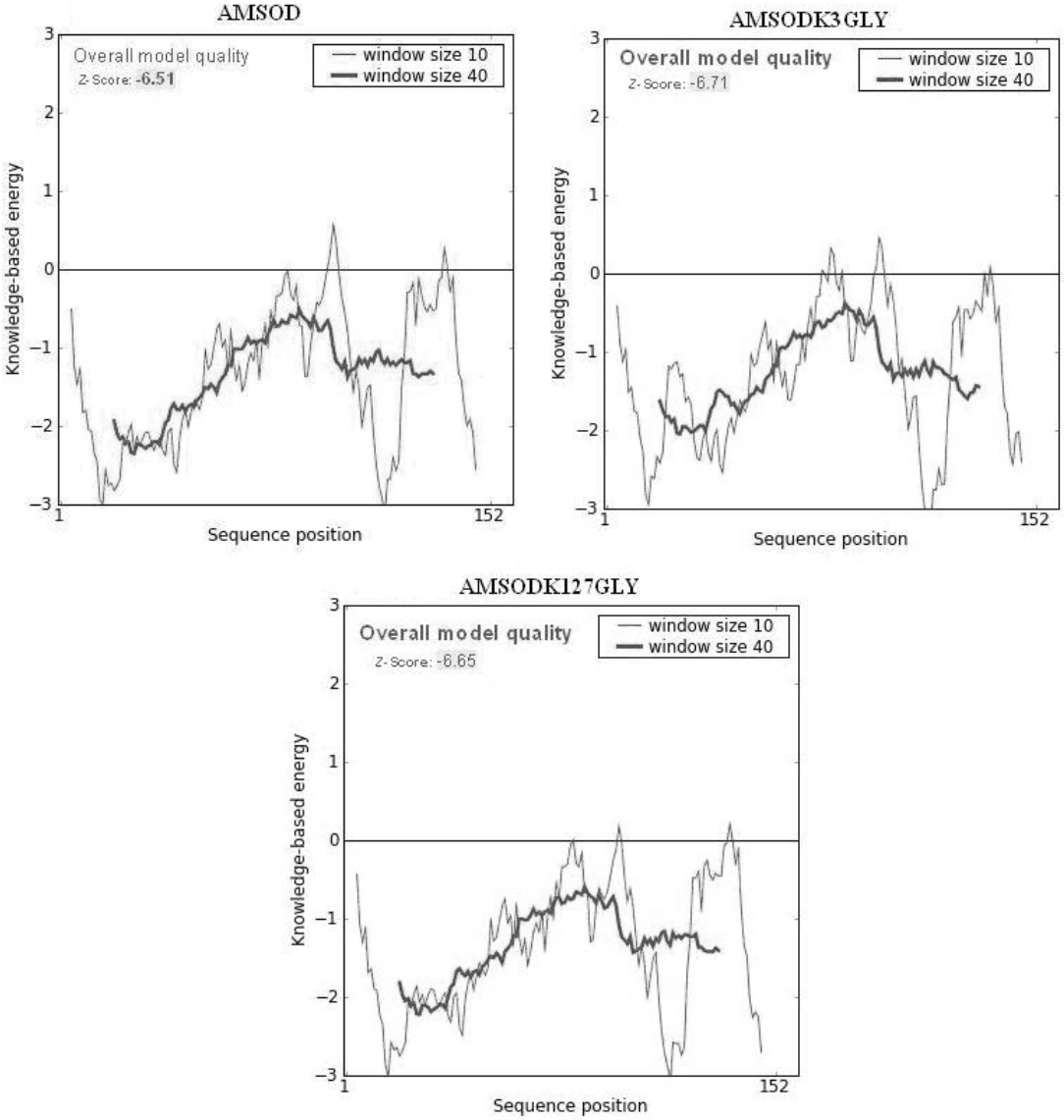

Fig 4: Protein Structure Analysis (ProSA) plots of homology models of *Avicennia marina* superoxide dismutase and glycated models (glycation at Lys3 and Lys127) with z scores of -6.51-6.71 and -6.65 respectively. ProSA plot shows local model quality by plotting energies as a function of amino acid sequence position. The thick line shows the average energy over each 40-residue fragment while the thin line shows a smaller window size of 10 residues.

Post-translational Modification

Post translational modifications are involved in regulating a wide variety of mechanisms that include changes in protein activities, interactions, and subcellular localizations Recently, superoxide and its related reactive oxygen (ROS) and nitrogen species (RNS) have been found to affect not only toxic species in cells but also serve as possible modifiers of cell signal processes [12-13], therefore regulation of SOD activity by the post-translational modifications could open a new area of interest for regulating various signal transduction processes [14]. The conserved domains and functional sites of the *Avicennia marina* superoxide dismutase was predicted by ScanProsite [15], including, two superoxide dismutase signatures, signature 1: [GA] - [IMFAT] - H - [LIVF] - H - {S} - x - [GP] - [SDG] - x - [STAGDE] and signature 2: G - [GNHD] - [SGA] - [GR] - x - R - x - [SGAWRV] - C - x(2) - [IV], 2 Casein kinase II phosphorylation sites, 5-myristoylation sites and 2 N-glycosylation sites (Table-1).

Table-1: Predicted post translational modification sites Avicennia marina superoxide dismutase.

Glycosylation site	Casein kinase II phosphorylation site	N-myristoylation site
33 – 36 NLSG	9 – 12 SSNE	32 -37 GNlSGF
85 – 88 NITV	87 - 90 TVGE	55-60 GCMSTG

(i) Glycosylation Sites

Naturally occurring glycosylated superoxide dismutase is found in yeast strain *Kluyveromyces marxianus* NBIMCC 1984 [16]. ScanProsite revealed three potential N-linkage sites (Asn11, Asn33, and Asn85) in the AMSOD sequence suggesting that the enzyme might be glycosylated. These sites were examined in the the AMSOD homology model and the proposed sequence patches were compared with the proposed criteria for such a site in glycosylated proteins [17-19]. Two sites can be termed as the potential N-glycosylation sites i.e. Asn33 in the sequence patch ^{33}Asn-Leu-Ser-Gly^{36} and Asn85 in the sequence patch ^{85}Asn-Ileu-Thr-Val^{88}. Asn33 and Asn85 were found on the surface (accessibility = 21.4265 Å^2 and 14.7258 Å^2 respectively) and are part of the loop structure. Asn33 is ~37% conserved in the SOD family whereas Asn85 is highly conserved (Table-2). As Asn33 and Asn85 are present in the loop region (Fig. 5) and also fulfil other criteria of being glycosylated, there is a strong probability that glycosylation can take place at these residues.

Table-2: CLUSTAL X multiple sequence alignment the sequence of members of the superoxide dismutase family.

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gi|12744890|      --------------MPKAVAVLSSNEGVRGTVYFTQEGDG-PTTVTGNLSGFKSGPHGFH 45
gi|58616005|      --------------MVKAVVVLNSKEGVSGTIYFTEEEDG-STKVTGSVSGLKPGLHGFH 45
gi|58616003|      --------------MVKAVVVLNSKEGASGTIYFTEEEDG-STKVTGSVSGLKPGLHGFH 45
gi|240148052|     --------------MVKAVVVLNSKEGVSGTIYFTEEEDG-STKVTGSVSGLKPGLHGFH 45
gi|240148050|     --------------MVKAVAVLNSKEGVSGTIYFTEEEDG-STKVTGSVSGLKPGLHGFH 45
gi|58615999|      --------------MVKAVVVLGSNAGVNGTIYFTEEEDG-STKVTGSVSGLKPGLHGFH 45
gi|225451120|     --------------MVKAVAVLNSNEGACGTIYFAEEGDG-STTVTGSLSGLKPGLHGFH 45
gi|13431904|      --------------MVKAVAVLGSSEGVKGTIYFTQEGDG-PTTVTGSISGLKPGLHGFH 45
gi|39840779|      --------------MVKAVTVLNSSEGVTGTVYFTQEGDG-PTTVTGNLSGLKPGLHGFH 45
gi|12230565|      --------------MVKGVAVLSSSEGVSGTIYFTQEGDG-PTTVTGNVSGLKPGPHGFH 45
gi|53748479|      --------------MVKGVAVLSSSEGVSGTVLFSQEGEG-PTTVTGNLSGLKPGLHGFH 45
gi|3914998|       --------------MVKAVAVLSSSEGVSGTIFFSQEAEGAPTTVTGDLSGLKPGPHGFH 46
gi|50978416|      --------------MVKAVTVLNSSEGVSGTIFFTQEAEGAPTTVTGDISGLKPGPHGFH 46
gi|171854653|     --------------MVKAVAVLSSSECVSGTILFSQDGDA-PTTVTGNVSGLKPGLHGFH 45
gi|3915000|       --------------MVKAVAVLSSSECVSGTILFSQDGDA-PTTVTGNVSGLKPGLHGFH 45
gi|134616|        --------------MVKAVAVLSSSEGVSGTIFFTQDGDA-PTTVTGNVSGLKPGLHGFH 45
gi|3334337|       --------------MVKAVAVLNSSEGVSGTILFTQDGAA-PTTVNGNISGLKPGLHGFH 45
gi|134612|        --------------MVKAVAVLNSSEGVSGTYLFTQVGVA-PTTVNGNISGLKPGLHGFH 45
gi|13445918|      ------------------VAVLSSSEGVCGTILFTQDGDA-PTTVNGNISGLKPGLHGFH 41
gi|586004|        --------------MVKAVAVLSSSEGVSGTIFFSQEGDG-PTTVTGNVSGLKPGLHGFH 45
gi|170784983|     --------------MAKGVAVLSSSEGVAGTILFTQEGDG-PTTVTGNISGLKPGLHGFH 45
gi|224042462|     --------------MAKGVAVLSSSEGVAGTILFTQEGDG-PTTVTGNISGLKPGLHGFH 45
gi|38228697|      SVPTARGVLRSHRTMAKGVAVLSSNEGVCGTIYFAQEGDG-PTTVTGNISGLKPGLHGFH 59
gi|3334334|       --------------MVKAVVVLSSSEGVSGTVQFTQEGSG-PTTVTGNVSGLRPGLHGFH 45
gi|1773326|       --------------MVKAVVVLSSSEGVSGTVQFTQEGSG-PTTVTGNVSGLRPGLHGFH 45
gi|134628|        --------------MGKAVVVLSSSEGVSGTVYFAQEGDG-PTTVTGNVSGLKPGLHGFH 45
gi|17385628|      --------------MVKAVVVLGSSAGVTGTVFFNQEGDG-PTTVTGNVSGLKSGLHGFH 45
gi|297843670|     --------------MAKGVAVLNSSEGVKGTIFFTQEGDG-VTTVTGTVSGLKPGLHGFH 45
gi|3288850|       --------------MAKGVAVLNSSEGVKGTIFFTQEGDG-ATTVTGTVSGLKPGPHGFH 45
gi|49615353|      --------------MVKGVAVLGSSEGVKGTISFVQEGDG-PTTVTGSVSGLKPGLHGFH 45
gi|90186542|      --------------MVKGVAVLGSSEGVKGTINFVQEGDG-PTTVTGCISGLKPGLHGFH 45
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gi|71040665|      --------------MVKAVAVLSSSEGVSGTIQFSQEGNG-PTTVTGNLAGLKPGLHGFH 45
gi|146215972|     --------------MVKAVAVLGSNEGVTGTISFSQEGNG-PTTVTGNLAGLKPGLHGFH 45
gi|217071256|     --------------MVKAVAVLGNSNDVSGTISFTQEGNG-PTTVTGNLSGLKPGLHGFH 45
gi|3860329|       --------------MVKAVAVLGSSDTVSGTINFSQEGDG-PTTVTGNLAGLKPGLHGFH 45
gi|32441511|      --------------MVKAVAVLGSSDNVKGTITFSQEGDG-PTNVSGTISGLKPGLHGFH 45
gi|50540929|      --------------MVKAVAVLNSSEGVAGTIFFTQEGDG-PTTVTGSVSGLKPGLHGFH 45
gi|5726592|       --------------MVKAEAVLTSSEGVSGTIFFTQEGDG-PTTVTGNISGLKPGLHGFH 45
gi|12230571|      --------------MVKAVAVLSSSEGVSGTIFFTQAADG-PTTVTGEISGLKPGHHGFH 45
gi|118482799|     --------------MVKAVAVLNSSEGVSGTIFFTQEGDG-PTTVTGNLSGLKPGLHGFH 45
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gi|13274150|      --------------MVKAVAVLNSSEGVKGTINFTQEGDG-PTTVTGSLCGLKPGLHGFH 45
gi|259016718|     --------------MVKAVVVLNSSAGVSGTVHFSQEGDG-PTTVIGNLSGLKPGLHGFH 45
gi|56549631|      --------------MVKAVVVLNSSAGVSGTVQFTQEGDG-PTKVTGSLSGLQPGPHGFH 45
gi|295979335|     --------------MVKAVVVLGNSEGVSGTVYFTQEGDG-PTTVTGSLSGLKPGLHGFH 45
gi|255542450|     --------------MVKAVTVLGSSEGVKGTIFFTQEADG-PTTVTGSLSGLKPGLHGFH 45
gi|164654158|     --------------MVKAVAVLNGSEGVKGTVNFTQEGDG-PTTVTGSLSGLKPGLHGFH 45
gi|162135925|     --------------MVKAVAVLNGSEGVKGTVNFTQEGDG-PTTVTGSLCGLKPGLHGFH 45
gi|19172405|      --------------MVKAVAVLNGSEGVKGTVFFTQEGDG-PTTVTASLSGLKPGLHGFH 45
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Table-2: Continued…

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gi|33340236|      --------------MVKAVAVLGGTEGVKGTVSFTQEGDG-PTTVSGSLSGLKPGPHGFH 45
gi|261746204|     --------------MVKAVAVLASSEGVKGTIYFVQEGDG-PTTVTGSVSGLKPGLHGFH 45
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gi|301073308|     --------------MVKAVAVLGSNEGVKGTIFFTQEGDG-PTTVTGSVSGLKPGLHGFH 45
gi|301153720|     --------------MVKAVASLGSSEGVKGTIFFTQEGDG-PTTVTGSVSGLNPGLHGFH 45
gi|194695572|     --------------MVKAVAVLGSSDGVKGTIFFTQEGDG-PTTVTGSVSGLKPGLHGFH 45
gi|134598|        --------------MVKAVAVLGSSDGVKGTIFFTQEGDG-PTAVTGSVSGLKPGLHGFH 45
gi|134597|        --------------MVKAVAVLGSSEGVKGTIFFTQEGDG-PTTVTGSVSGLKPGLHGFH 45
gi|145388988|     --------------MVKAVVVLASSEGVKGTIHFTQEGDG-PTTVTGSVSGLKPGLHGFH 45
gi|122170280|     --------------MVKAVVVLGSSEIVKGTIHFVQEGDG-PTTVTGSVSGLKPGLHGFH 45
gi|538430|gb|     --------------MVKAVVVLGSSEIVKGTIHFVQEGDG-PTTVTGSVSGLKPGLHGFH 45
                                 . * .   . **  * :   .  * * . : *:. * ****

gi|12744890|      VHALGDTTNGCMSTGPHFNPAGKDHGAPEDEVRHAGDLGNITVGEDGTAAVNIVDKQIPL 105
gi|58616005|      VHALGDTTNGCMSTGPHFNPQSKEHGAPEDENRHAGDLGNVTVGEDGTVNITIVDKQIPL 105
gi|58616003|      VHALGDTTNGCMSTGPHFNPQSKEHGAPEDENRHAGDLGNVTVGEGGTVNITIVDKQIPL 105
gi|240148052|     VHALGDTTNGCMSTGPHFNPQSKEHGAPEDENRHAGDLGNVTVGEGGTVNITIVDKQIPL 105
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gi|39840779|      VHALGDTTNGCMSTGPHFNPVGKEHGAPGDENRHAGDLGNITVGEDGTAAINIVDKQIPL 105
gi|12230565|      VHALGDTTNGCLSTGPHFNPAGKEHGAPDDEVRHAGDLGNVTVGEDGTAAFTIVDKQIPL 105
gi|53748479|      VHALGDTTNGCMSTGPHFNPAAKEHGAPDDEVRHAGDLGNVTVGDDGTASFTIVDKLIPL 105
gi|3914998|       VHALGDTTNGCMSTGPHYNPHGKDHGAPDDEHRHAGDLGNVTVGEDGTAKFTIVDKQIPL 106
gi|50978416|      VHALGDTTNGCMSTGPHXNPHGKEHGAPDDEIRHAGDLGNVTVGEDGTAKFTIVDKQIPL 106
gi|171854653|     VHALGDTTNGCMSTGPHYNPAGKEHGAPEDENRHAGDLGNITVGEDGTASFTITDEQIPL 105
gi|3915000|       VHALGDTTNGCMSTGPHYNPAGKEHGAPEDENRHAGDLGNITVGEDGTASFTITDEQIPL 105
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gi|211906512|     VHALGDITNGCMSTGPHFNPAGKEHGAPEDENRHAGDLGNVTVGDDGCASFSITDKQIPL 105
gi|209419744|     VHALGDTTNGCMSTGPHFNPAGKEHGAPEDVNRHAGDLGNVTVGDDGCASFSITDKQIPL 105
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gi|71040665|      VHALGDTTNGCLSTGPHFNPNNKEHGAPEDENRHAGDLGNVNVGDDGTVSFSISDSQIPL 105
gi|146215972|     VHALGDTTNGCLSTGPHFNPQGKEHGAPEDVNRHAGDLGNVNVGDDGTAKFTITDSQIPL 105
gi|217071256|     IHALGDTTNGCLSTGPHFNPNGKEHGAPEDETRHAGDLGNVTVGDDGTASFTITDNQIPL 105
gi|3860329|       IHALGDTTNGCISTGPHFNPNGKEHGSPEDPIRHAGDLGNINVGDDGTVSFSITDNQIPL 105
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gi|261746204|     VHALGDTTNGCMSTGPHFNPAGKEHGAPEDENRHAGDLGNVTAGADGVANVNVVDSQIPL 105
gi|134596|        VHALGDTTNGCMSTGPHFNPTGKEHGAPQDENRHAGDLGNITAGADGVANVNVSDSQIPL 105
gi|134613|        VHALGDTTNGCMSTGPHFNPVGKEHGAPEDEDRHAGDLGNVTAGEDGVVNVNITDSQIPL 104
gi|301073308|     VHALGDTTNGCMSTGPHYNPAGKEHGAPEDEIRHAGDLGNVTAGADGVANINVTDCQIPL 105
gi|301153720|     VHALGDTTNGCMSTGPHYNPEGKEHGAPEDEIRHAGDLGNVTAGQDGVANVNVTDSQIPL 105
gi|194695572|     VHALGDTTNGCMSTGPHYNPASKEHGAPEDENRHAGDLGNVTAGADGVANINVTDSQIPL 105
gi|134598|        VHALGDTTNGCMSTGPHYNPASKEHGAPEDENRHAGDLGNVTAGADGVANINVTDSQIPL 105
gi|134597|        VHALGDTTNGCMSTGPHYNPASKEHGAPEDENRHAGDLGNVTAGADGVANINVTDSQIPL 105
gi|145388988|     VHALGDTTNGCMSTGPHYNPAGKEHGAPEDENRHAGDLGNVTAGADGVANINVTDSQIPL 105
gi|122170280|     IHALGDTTNGCMSTGPHYNPAGKEHGAPEDETRHAGDLGNVTAGEDGVANIHVVDSQIPL 105
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Table-2: Continued…

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gi|538430|gb|          IHALGDTTNGCISTGPHYNPAGKEHGAPEDETRHAGDLGNVTAGEDGVANIHVVDSQIPL 105
                       :**: * ****:***** **  : **:* *  ********: .. .* . .   *  :**

gi|12744890|           SGPHSIVGRAVVVHADPDDLGKGGHELSKTTGNAGGRVACGIIGLQG- 152
gi|58616005|           TGPYSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRVACGIVGLQG- 152
gi|58616003|           TGPYSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRVACGVVGLQG- 152
gi|240148052|          TGPYSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRVACGIIGWQG- 152
gi|240148050|          TGPYLIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRVACGIIGLQG- 152
gi|58615999|           SGPNSIVGRAVVVHADPDDLGKGGHELSKSTGNAGGRVACGIVGLQG- 152
gi|225451120|          TGSNSIVGRAVVVHADPDDLGKGGHELSKSTGNAGGRVACGVIGLQG- 152
gi|13431904|           SGSNSIIGRAVVVHADPDDLGKGGHELSKTTGNAGGRVACGIIGLQG- 152
gi|39840779|           TGPHSIIGRAVVVHSDPDDLGRGGHELSKSTGNAGGRVACGIIGLQG- 152
gi|12230565|           TGPHSIIGRAVVVHADPDDLGKGGHELSKTTGNTGGRVACGINGLQG- 152
gi|53748479|           TGPHSIIGRAVVVHADPDDLGRGGHELSKTTGNAGGRVACGIIGLQG- 152
gi|3914998|            IGAQSIIGRAVVVHADPDDLGKGGHELSKTTGNAGGRVACGIIGLQG- 153
gi|50978416|           IGGQSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRVACGIIGLQG- 153
gi|171854653|          TGPQSIIGRAVVVHADPDDLGKGGHELSKTTGNAGGRVACGIIGLQG- 152
gi|3915000|            TGPQSIIGRGVVVHADPDDLGKGGHELTKTTGNAGGRVACGIIGLQG- 152
gi|134616|             AGPQSIIGRAVVVHADPDDLGKGGHELSKTTGNAGGRVACGIIGLQG- 152
gi|3334337|            TGPQSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRIACGIIGLQG- 152
gi|134612|             TGPQSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRIACGIIGLQG- 152
gi|13445918|           TGSQSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRIACGIIGLQG- 148
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gi|170784983|          TGPHSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRIACGIIGLQG- 152
gi|224042462|          TGLTSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRIACGIIGLQG- 152
gi|38228697|           CGPNSIIGRAVVVHGDPDDLGKGGHELSKSTGNAGGRIACGIIGLQG- 166
gi|3334334|            TGPNSIVGRAVVVHADPDDLGRGGHELSKATGNAGGRVACGVIGLQG- 152
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gi|134628|             SGPNSIVGRAVVVHAEPDDLGRGGHELSKTTGNAGGRVACGIIGLQG- 152
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gi|297843670|          TGPNSIVGRAVVVHADPDDLGKGGHELSLATGNAGGRVACGIIGLQG- 152
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gi|217071256|          TGPNSIIGRAVVVHADPDDLGKGGHELSKTTGNAGGRVACGIIGLQG- 152
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gi|50540929|           SGPHSIVGRAVVVHADPDDLGKGGHELSKTTGNAGGRVACGVIGLQG- 152
gi|5726592|            SGQNSIIGRAVVVHADPDDLGRGGHELSKTTGNAGGRVACGIIGLRG- 152
gi|12230571|           TGPNSIVGRAVVVHADPDDLGKGGHELSKTTGNAGGRVACGVIGLQG- 152
gi|118482799|          TGPHSIIGRAVVVHGDPDDLGKGGHELSKTTGNAGGRVACGIIGLQG- 152
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gi|4102859|            TGPHSIIGWAVVVHGDPDDLGKGGHELSKTTGNAGGRVACGIIGLQG- 152
gi|222857965|          TGPNSIVGRAVVVHADPDDLGKGGHELSKTTGNAGGRVACGVIGLQG- 152
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gi|259016718|          DGPNSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRIACGIIGRQG- 152
gi|56549631|           SGPHSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRIACGIIGLQG- 152
gi|295979335|          FGPNSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRIACGIIGLQG- 152
gi|255542450|          SGPNSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRIACGIIGLQG- 152
gi|164654158|          TGPNSVVGRAVVVHSDPDDLGKGGHELSKSTGNAGGRLACGVIGLQA- 152
gi|162135925|          TGPNSVVGRAVVVHSDPDDLGKGGHELSKSTGNAGGRLACGVIGLQA- 152
gi|19172405|           TGPHSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRVACGIIGLQA- 152
gi|33340236|           SGPNSIIGRAVVVHADPDDLGKGGHELSKTTGNAGGRVACGIIGLQG- 152
gi|261746204|          TGPQSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRVACGIIGLQG- 152
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gi|134613|             AGPHSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRVACGIIGLQG- 151
gi|301073308|          TGPSSIIGRAVVVHADPDDLGKGGHELSKTTGNAGGRVACGIIGPQG- 152
gi|301153720|          TGPHSIIGRAVVVHADPDDLGKGGHELSKTTGNAGGRFACEIIELQG- 152
gi|194695572|          TGPNSIIGRAVVVHADPDDLGKGGHELRKSTGNAGGRVACGIIGLQG- 152
gi|134598|             TGPNSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRVACGIIGLQG- 152
gi|134597|             TGPNSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRVACGIIGLQG- 152
gi|145388988|          TGPNSIIGRAVVVHADPDDLGRGGHELSKSTGNAGGRIACGIIGLQG- 152
gi|122170280|          TGPNSIIGRAVVVHADPDDLGKGGHELSKTTGNAGGRVACGIIGLQG- 152
gi|538430|gb|          TGPNSIIGRAVVVHADPDDLGKGGHELSKTTGNAGGRVACGIIGLQG- 152
                        *   ::* .****.: ****:*****  :***:*. .** :
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Amino acid sequence of superoxide dismutase (A.marina: gi|12744890|)
*** = fully conserved residues; : = strongly conserved; . = weakly conserved**
ScanProsite revealed Asn33 and Asn85 (in red), Ser9 and Thr87 (in pink), Gly32 and Gly55 (in aqua) and Lys3 and Lys127 (in blue) as potential sites for glycosylation, casein kinase II phosphorylation, myristoylation and glycation respectively.

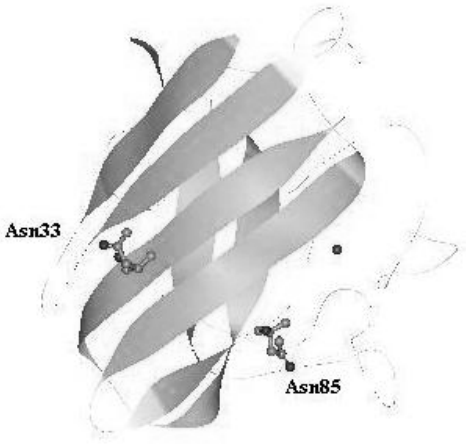

Fig 5: Potential N-linked glycosylation sites (Asn33 and Asn85) in the 3D Homology Model of *Avicennia marina* superoxide dismutase.

(ii) Casein Kinase II Phosphorylation Sites

CK2 is a well-conserved protein-kinase present in different organisms, including plants, mammals and yeast [20-22]. PROSITE results predicted two potential casein kinase II phosphorylation sites in *Avicennia marina* superoxide dismutase, Ser9 and Thr87 in the sequence patch ^{9}Ser-Ser-Asn-Glu^{12} and ^{87}Thr-Val-Gly-Glu^{90}. Ser9 is 30% conserved while Thr87 is almost conserved in superoxide dismutase family (Table-2). The analysis of AMSOD homology model (Fig. 6) showed that the surface accessibilities of the possible phosphorylation sites are Ser9: 20.9001Å^2 and Thr87: 25.4294 Å^2. Ser9 and Thr87 are also a part of loop which makes it a highly probable target for casein kinase II phosphorylation.

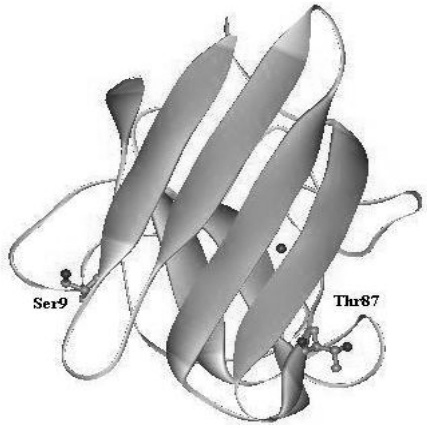

Fig 6: Two potential casein kinase II phosphorylation sites in the 3D Homology Model of *Avicennia marina* superoxide dismutase.

(iii) N-myristoylation Sites

The PROSITE pattern search results (Table-1) has identified two N-myristoylation sites in the amino acid sequence of AMSOD i.e. at Gly32 and Gly55 in the sequence patch ^{32}Gly-Asn-Ileu-Gly-Phe^{36} and ^{55}Gly-Cys-Met-Ser-Thr-Gly^{60} respectively. These regions were further analyzed at http://mendel.imp.univie.ac.at/myristate/prediction. These residues are highly conserved in superoxide dismutase family (Table-2). Therefore these glycines (Fig. 7) can be considered potential myristoylation sites although experimental evidence is needed to confirm this.

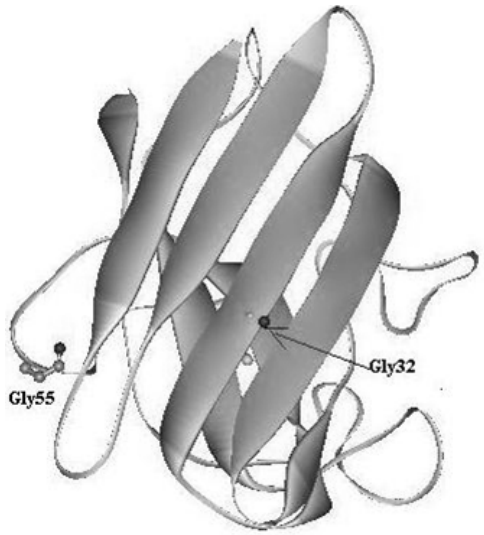

Fig 7: Two potential myristoylation sites in the 3D Homology Model of *Avicennia marina* superoxide dismutase.

(iv) Glycation Sites

Nonenzymatic glycosylation or glycation of proteins takes place through the formation of a schiff base between glucose and ε amino group of lysine residues. After the unstable Amadori reorganization it finally results in the formation of a comparatively stable ketoamine. Glycation in HSOD has been observed as the chronic complication of diabetes mellitus and is assumed to participate in the aging and pathogenesis of neurodegenerative diseases [23]. It has been demonstrated experimentally that glycation proceeds faster in mutated G37R, G93A, and I113T HSOD mutants. Increased glycation was observed in familial amyotrophic lateral sclerosis [7]. Studies have shown that glycation of proteins may modify some of their functions and properties [24]. It has been documented that the potential sites for glycation in HSOD are Lys3, Lys9, Lys30, Lys36, Lys122, and Lys128 [7]. Likewise, AMSOD contains 6 potential Lys residues, out of which Lys3 and

Lys127 were selected for this study. We attached alpha-D-glucose to these Lysine residues and predicted the effect of binding on the AMSOD models. Stereochemical evaluation of the predicted homology models of glucose bound AMSOD i.e. Lys3-AMSOD (Fig. 8a-b) and Lys127-AMSOD shows that 97.3% and 96.5% residues fall in the most favored and additionally allowed regions while 2.6% and 3.5% residues fall in the generously allowed regions respectively. In Lys3-AMSOD model, the coordination of Cu with amino acid residues was decreased at the catalytic site. It has been documented that as a result of glycation, ROS is produced by glucose autooxidation which causes the fragmentation of Cu,Zn-SOD [7]. On the other hand, Lys27-AMSOD model did not show significant change in the Cu and Zn coordination with the catalytic residues. Changes predicted in terms of spatial positions of amino acids were also measured (Table-3). The overall change was observed on some of the charged residues thus causing charge disruption in the molecule. No change was observed in the interior of the molecule as the hydrophobic residues showed no change in their positions.

Methods

(a) Sequence Analysis

(i) Pairwise Sequence Alignment

Primary amino acid sequence of AMSOD was retrieved from the SWISSPORT data bank [25]. The sequence was submitted to the BLAST server that searched for the appropriate template using Gapped BLAST and PSI-BLAST algorithms [26] against the sequences in the Protein data bank, PDB [27]. The highest sequence homology was found to be 70% with spinach Cu,Zn-SOD (Pdb id: 1srd) [28].

(ii) Multiple Sequence Alignment and Phylogenetic Analysis

Primary amino acid sequences of members of the cu-zn superoxide dismutase family were retrieved from UniProt database [25]. Sequence identity searches were performed using BLAST server [26] against non-redundant (nr) database using default parameters. All cu-zn superoxide dismutase sequences were subjected to multiple sequence alignment using the program, Clustal X [29]. About 69 sequences of superoxide dismutase were used for constructing phylogenetic tree with the help of online neighbor joining method and for reconstructing phylogenetic tree from evolutionary distance data by using Drawgram program of PHYLIP tree package version 3.67 [30, 31].

(b) Model Building

The three-dimensional homology model of AMSOD with Cu and Zn ions was built using the structural coordinates of spinach Cu,Zn-SOD (Pdb id: 1srd) [28] by the protein structure modeling program MODELLER 9v3 [32]. The three dimensional (3D) coordinates of the template was retrieved from PDB [27]. Protein structures were visualized and analyzed with SPDV viewer 3.7 [33] and DS viewer [34].

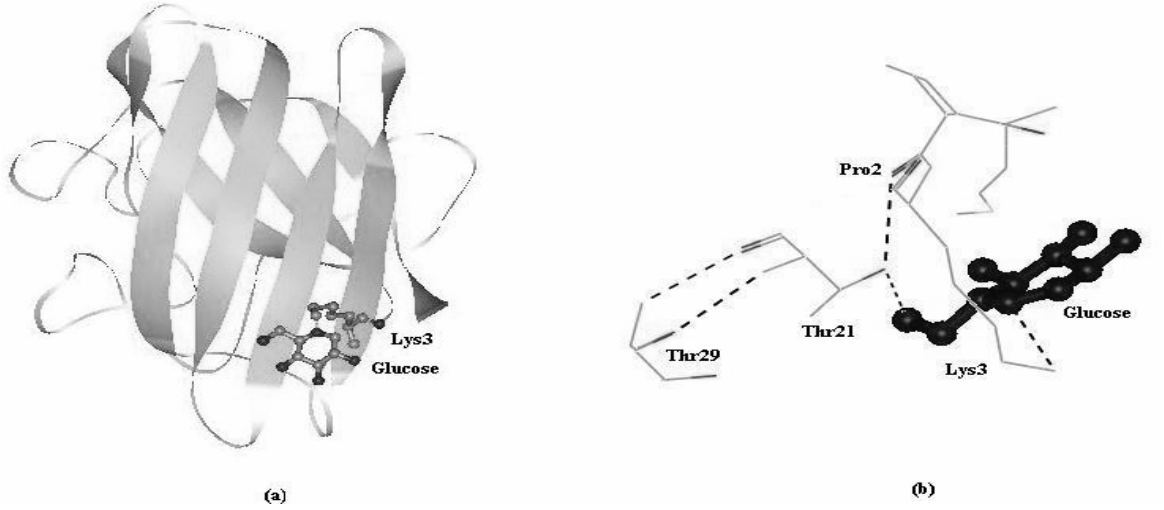

Fig 8: (a) Predicted homology models of glucose bound *Avicennia marina* superoxide dismutase (Lys3-AMSOD) (b) Hydrogen bond interaction between glucose and Lys 3 and other nearest residues.

Table-3: Predicted surface accessibilities (Å^2) of amino acid residues altered in the glycated models

Amino Acids	Original Model	Glycated Model (LYS-3)	Glycated Model (LYS-127)
Met1	20.5549	1.8704	23.8901
Lys3	25.7972	20.7814	23.7990
Thr21	7.9691	2.7959	6.4655
Asn11	36.7666	24.7827	35.0942
Arg15	45.1289	42.6345	45.3463
Thr17	13.5911	17.1140	13.5911
Tyr19	29.8120	24.3540	27.5727
Thr21	7.9691	2.7959	6.4655
Thr29	10.3940	12.9439	10.3940
Thr31	8.0382	11.3302	8.0382
Asn33	20.9713	24.4710	19.0486
Lys38	45.2551	49.6566	50.0347
His42	0.8548	2.2528	2.9517
His47	0.000	0.6990	0.000
Ala48	7.1644	8.9119	5.9413
Gly36	10.8831	7.5156	12.0874
Gly50	3.7424	2.7560	2.0015
Asp51	9.5008	14.8186	10.7327
Thr52	19.4741	11.9278	16.8853
Thr53	27.3116	25.6613	23.2019
Gly55	13.1154	8.4966	11.9488
Cys56	4.7159	1.2944	3.8120
Met57	39.0647	35.7489	40.1843
Ser58	3.3331	4.0450	2.1746
His62	1.3791	2.5645	2.0780
Phe63	2.6211	3.8443	2.9706
Ala94	0.1747	0.1747	0.8737
Gln102	15.0263	13.8845	9.9587
His119	0.3306	0.7982	0.4865
Lys127	36.1210	37.9062	21.064
His130	25.2347	28.8099	25.7023
Ser133	0.0000	0.8457	0.0000
Lys134	33.3397	41.6707	28.6583
Thr136	6.2282B	8.1590	7.5614
Asn138	15.3137	8.1277	15.6756
Gln151	28.8250	15.7576	23.7682

(c) Model Assessment

Assessment of the predicted homology models was based on the analyses of geometry, stereochemistry and energy distributions in the model. The consistency of the predicted homology models were assessed using the ENERGY command of the MODELLER. Assessment was further conducted by programs PROCHECK [8] and ProSa [35-36]. The variability among the models was compared by superposition of the Cα traces of the model and the template and the RMSD between the equivalent atoms was determined.

(d) Post-translational Modification

Primary sequence of AMSOD was searched at PROSITE database using ScanProsite for the possible post translational modification sites. For nonenzymatic glycosylation or glycation studies, alpha D-glucose was added separately to Lys3 and Lys127 in the AMSOD to get two modified glycated models. 3D structural coordinates of alpha D-glucose was obtained from the theoretical model of human gamma B crystallin, 1mz1 [37] and attached manually to the predicted model by using DS Viewer. The modified models were then used as templates for constructing the new homology model by MODELLER 9v3 [32]. This was done in order to obtain the homology model with minimized energy. After many runs of the MODELLER, the best model was chosen after evaluating each model by the program PROCHECK [8]. Comparative structural analyses were carried out between the original and the glycated model. Hydrogen bonding, molecular surface accessibility, protein-cofactor contacts and ion pairs were calculated by the program WHATIF [38].

References

1. N. T. Barkasdjieva, K. N.Chrostov and N. K. Christina, *Journal of Plant Biology,* **43**,73 (2000)
2. F. Navari-Izzo, M. F. Quartacci, C. Pinzino, F. D. Vecchia, and C. L. M. Sgherri, *Physiologia Plantarum*,**104**, 630 (1998).
3. K. Ogawa, S. Kanematsu, and K. Asada, *Plant and Cell Physiology,* **38**, 1118 (1997).
4. J. Wuerges, J. W. Lee, Y.I. Yim, H. S. Yim, S. O. Kang, and C. K. Djinovic, *Proceding of the Naturall Academy of Science U S A.,* **101**, 8569 (2004).
5. P. J. Hart, M. M. Balbirnie, N. L. Ogihara, A. M. Nersissian, M. S. Weiss, J. S. Valentine, and D. Eisenberg, *Biochemistry* **,38**, 2167 (1999).
6. F. Arnesano, L. Banci, I. Bertini, M. Martinelli, Y. Furukawa, T.V. O'Halloran, *Journal of Biological Chemistry.,* 279, 47998 (2004).
7. T. Takemura, N. Hanagata, K. Sugihara, I. B. S. Karube, Z. Dubinsky, *Aquatic Botany,* **68**, 15 (2000).
8. R. A. Laskowski, M. W. McAurthur, D. S. Moss, and J. M. Thornton, *Journal of Applied Crystallography*, **26**, 283 (1993).
9. Ferraroni, M, Rypniewski, W. R., Bruni, B. Orioli, P. and S. Mangani, *Journal of Biological. Inorganic.Chemistry* **3,** 411 (1998).
10. R. M. Cardoso, M. M. Thayer, M. Lo, T. P. DiDonato, C. K. Bruns, E. D. Getzoff, and J. A. Tainer, *Journal of Molecular Biology.* **324**, 247 (2002).
11. R. M. Cardoso, C. H. Silva, A. P. Ulian de Araujo, T. Tanaka, M. Tanaka, and Garratt, R. C. *Acta Crystallography.* D *Biological. Crystallography,* **60**, 1569 (2004).
12. C. C. Winterbourn and M. B. Hampton, *Free Radical Biology and Medicine,* **45**, 549 (2008).
13. L. Liaudet, V. Giuseppe and P. Pacher, *Frontiers in Bioscience,* **14**, 4809 (2009).
14. F. Yamakura and H. Kawasaki, *Biochimica et Biophysica Acta,* **1804**, 318 (2010).

15. J. C. Obenauer L. C. Cantley and M. B. Yaffe, *Nucleic Acids Research,* **31**, 3635 (2003).
16. P. D. Angelova, V. Moshtanska, A. Kujumdzieva, B. Atanasov, V. Petrova W. Voelter and J. V. Beeumen, *Journal of Molecular Structure,* **980**, 18 (2010)
17. J. P. Aubert, N. Helbecque, M. H and Loucheux-Lefebvre, *Archieves of Biochemistry and Biophysics,* **208**, 20 (1981).
18. M. Yontem, M. Bilge, S. Kaleli, S. Kurban and G. Celik, *Journal of the Chemical Society of Pakistan*, **33,** 389 (2011).
19. K. Jilani, M. Asgher, H. N. Bhatti and Z. Mushtaq, *Journal of the Chemical Society of Pakistan*, **33,** 209 (2011).
20. F. Meggio, L. A. Pinna and *FASEB. J.,* **17**, 349 (2003).
21. M. E. Olsten and D. W. Litchfield, *International Journal of Biochemistry and Cell Biology,* **82**, 681 (2004).
22. E. Ogiso*,* Y. Takahashi, T. Sasaki, M. Yanom and T. Izawa, *Plant Physiology*, **152**, 808 (2010).
23. K. Arai, S. Maguchi, S. Fujii H. Ishibashi, K. Oikawa and N. Taniguchi, *Journal of Biological. Chemistry,* **262**, 16969 (1987).
24. M. U. Ahmed, S. R. Thorpe, and J. W. Baynes, *Journal of Biological Chemistry,* **261**, 4889 (1986).
25. A. Bairoch, and R. Apweiler, *Nucleic Acids Research,* **28**, 45 (2000).
26. S. F. Altschul, T. L Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman, *Nucleic Acids Research,* **25,** 3389 (1997).
27. H. M. Berman, T. Battistuz, T. N. Bhat, W. F. Bluhm, P. E. Bourne, K. Z. Burkhardt. Feng, G. L. Gilliland, L. Iype, S. Jain, P. Fagan, J. Marvin, D. Padilla, V. Ravichandran, B. Schneider, N. Thanki, H. Weissig, J. D. Westbrook, C. Zardecki. *Acta Crystallography.* D *Biological Crystallography,* **58**, 899 (2002)
28. Y. Kitagawa, N. Tanaka, Y. Hata, M. Kusunoki, G. P. Lee, Y. Katsube, K. Asada, S. Aibara, and Y. Morita, *Journal of Biochemistry,* **109**, 477 (1991).
29. Thompson, J. D., Gibson, T. J, Plewniak, F. Jeanmougin, F. and D. G. Higgins, *Nucleic Acids Research*. **25**, 4876 (1997).
30. J. Felsenstein and Cladistics, **5**, 164 (1989).
31. K. Howe, A. Bateman, R. Durbin, *Bioinformatics,* **18**, 1546 (2002).
32. A. Sali. and T. L. Blundell, *Journal of Molecular Biology,* **234,** 779 (1993).
33. N. Guex and M. C. Peitsch, *Electrophoresis,***18**, 2714 (1997).
34. http://accelrys.com/products/discovery-studio/
35. M. J. Sippl, *Proteins,* **17**, 355 (1993).
36. M. Wiederstein and M. J. Sippl, *Nucleic Acids Research*, **35,** W407 (2007).
37. A. Salim, A. Bano, and Z. H. Zaidi, *Proteins; Structure Function and Bioinformatics* **53**, 162-(2003).
38. R. Rodriguez, G. Chinea, N. Lopez, T. Pons, and G. Vriend, *CABIOS.,* **14**, 523 (1998).