Prediction of Post Translational Modifications of Salt Tolerant Plant Mangrove Specie Avicennia marina Glyoxalase: Implication of Casein kinase II Phosphorylation and N-myristoylation on the Enzyme Structure

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Abstract

In this current study, we performed sequence analysis and post translational modification of glyoxalase protein from Avicennia marina. It is important to analyze its sequence to improve our understanding of amino acids involved in glyoxalase functions. The conserved domains and functional sites of the Avicennia marina glyoxalase were predicted by ScanProsite. PROSITE entries consists of *two* Glyoxalase *signatures*, one N-myristoylation sites and six Casein kinase II phosphorylation site. Clustal alignment have shown Glyoxalase signatures-1 in N-terminal region consensus pattern: [HQ]-[IVT]-x-[LIVFY]-x-[IV]-x(4)-{E}-[STA]-x(2)-F-[YM]-x(2,3)-[LMF]-G-[LMF] in this sequence patch Met47 is present in Avicennia marina glyoxalase while Leu is present in place Met47 in most of glyoxalase family. However, the molecular mechanisms of plant stress tolerance are not fully understood and Glyoxalase signatures-2 is located in the central section of the protein G-[NTKQ]-x(0,5)-[GA]-[LVFY]-[GH]-H-[IVF][CGA]x-[STAGLE]-x(2)-[DNC] and contains a conserved histidine that could be implicated in the binding of the zinc atom.

Key words: Homology Modeling, Posttranslational modification, myristoylation, phosphorylation, glyoxalase, environmental stress, Avicennia marina.

1 INTRODUCTION

Glyoxalase I (EC 4.4.1.5) (lactoylglutathione lyase)) is part of the glyoxalase system present in the cytosol of all cells. Glyoxalase I in plant is the first enzyme of the glyoxalase system that can detoxify methylglyoxal. The glyoxalase system plays an important role in various physiological processes in plants, including salt stress tolerance [1]. Furthermore it has been reported in review [2] plant thiols play a significant role in abiotic stress tolerance. It has been reported that this enzyme catalyzes the first step of the glyoxal pathway, the transformation of methylglyoxal and glutathione into S-lactoylglutathione which is then converted by glyoxalase II to lactic acid [3] . Methylglyoxal is α-keto aldehyde metabolite formed mostly by the spontaneous dephosphorylation of triosephosphates and, in a small number of prokaryotes, by the enzyme-catalyzed dephosphorylation of dihydroxyacetone phosphate [4] . Glyoxalase I is a ubiquitous enzyme which binds one mole of zinc per subunit. The bacterial and yeast enzymes are monomeric while the mammalian one is

homodimeric. Methylglyoxal have present during normal growth conditions and it accumulates to higher levels under various environmental stresses, abiotic and heavy metal stresses also make reactive oxygen species and methylglyoxal in plants [1 and 5]. It is comprised of two enzymes, glyoxalase I and glyoxalase II (EC 3.1.2.6) and a catalytic amount of glutathione GSH. Glyoxalase I catalyses the isomerisation of the hemithioacetal, formed spontaneously from a-oxoaldehyde RCOCHO and GSH, to S-2-hydroxyacylglutathione derivatives RCH(OH)CO-SG, a cytotoxic compound increased rapidly under stress conditions. [5 and 6-8]. The scavenging systems of sugar- and lipid-derived reactive carbonyls (RCs) in the cyanobac-terium Synechocystis sp Slr0381 selected protein catalyzed the conversion of hemi-thioacetal to S-lactoylglutahione (SLG) in the glyoxalase (GLX) 1 reaction [9]. The molecular mechanisms of plant stress tolerance are not completely understood, and the information available is incomplete and sometimes conflicting. It has been reported that Casein Kinase2 is an evolutionarily conserved serine/threonine protein kinase was found in two catalytic α-subunits and two regulatory β-subunits in Arabidopsis plant [10]. In the present study, we have identified *two* Glyoxalase *signatures,* one N-myristoylation sites and six Casein kinase II phosphorylation site posttranslational modifications sites of Avicennia marina glyoxalase and analyzed the structural changes. The conserved domains and functional sites of the Avicennia marina glyoxalase were predicted by ScanProsite approaches and 3D homology model was constructed by the program MODELLER 9.10 and clustal alignment.

2 METHODS

2.1 Sequence Analysis

2.1.1 Pairwise Sequence Alignment

Primary amino acid sequence of *Avicennia marina* glyoxalase I protein (**A7LKM8**_AVIMR) consists of 184 amino acid was retrieved from UniProt database [11]. The sequence was then submitted to BLAST server [12] for search against PDB [13] in order to identify the template for molecular modeling. BLAST search gave highest homology (67%) with Mouse Glyoxalase I (PDB id: 2ZA0) [14]. The sequence was also submitted to PDBsum [15] (http://www.ebi.ac.uk/pdbsum) provides summary information about each experimentally determined structural model of glyoxalase protein in the Protein Data Bank (PDB).

The number of residues, 18-176 of the target sequence *Avicennia marina* glyoxalase I protein were aligned with residues, 24-181 of 2zao; chain A by the BLAST program. Some manual adjustments were done where needed.

2.1.2 Multiple sequence alignment

Primary amino acid sequences of *Avicennia marina* glyoxalase I protein were retrieved from UniProt database [11]. Multiple sequence alignment was performed by CLUSTAL X [16].

2.2 Model building

The three-dimensional homology model of *Avicennia marina* glyoxalase I was built using the structural coordinates of Mouse Glyoxalase I (Pdb id: 2zao) [14] by the protein structure modeling program MODELLER 9.10 [17]. The three dimensional (3D) coordinates of the templates were retrieved from PDB [13]. Protein structures were visualized and analyzed with SPDV viewer 3.7 [18] and DS viewer [19].

2.3 Model Assessment

Assessment of the predicted homology model was based on the analysis of geometry, stereochemistry and energy distributions. The consistency of the predicted homology models was assessed using the ENERGY command of the MODELLER. The stereochemical quality of best model was further conducted by the program PROCHECK [20] was used to generate Ramachandrans plot and ProSa [21-22]. 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.

2.4 Prediction of Post-translational modification sites

Primary sequence of *Avicennia marina* glyoxalase I was searched at PROSITE database using ScanProsite [23] for the possible post-translational modification sites.

3 RESULTS AND DISCUSSION

3.1 3D Homology model of *Avicennia marina* glyoxalase I

3D Homology models of *Avicennia marina* glyoxalase I was constructed by the program MODELLER 9.10 using templates that gave highest sequence homology with the target sequence (Fig 1a). The stereochemical quality of the predicted models was evaluated using PROCHECK [20] . These plots showed no residue in the disallowed region (Fig 1b). The ProSA energy plot shows the quality of the models by plotting energies as a function of amino acid positions**.** In case of the homology models of *Avicennia marina* glyoxalase I, ProSA analysis revealed Z-scores value -4.69 which lie in the low energy conformation states (Fig. 1c). These results suggest that the models are of good quality.

The overall homology model glyoxalase I which consists of eight anti-parallel β-sheet packed against an five α-helix and loops (Fig.1a). 3D Homology models of *Avicennia marina* glyoxalase I closely resembled with the structure of Mouse Glyoxalase I (Pdb id:2zao) [14] as shown in figure 2.

3.2 Prediction of Post-translational modification sites

Post-translational modification plays a significant role in the regulation of biological activities and signal transduction. The conserved domains and functional sites of the *Avicennia marina* glyoxalase were predicted by ScanProsite [23] and 3D homology model was constructed by the program MODELLER 9.10 [17] (Fig 1). The results have shown 9 hits with four PROSITE entries, including *two* Glyoxalase *signatures,* one N-myristoylation sites and six Casein kinase II phosphorylation site (Table 1).

3.2.1 Glyoxalase Signatures

The PROSITE results have shown two signature patterns for glyoxalase I protein family. The PROSITE results have also shown *consensus pattern* of glyoxalase I sequence that is highly conserved in glyoxalase protein family. In bacteria and mammals, the enzyme is a protein of about 130 to 180 residues while in fungi it is about twice longer. In these organisms the enzyme is built out of the tandem repeat of a homologous domain.

3.2.2 Glyoxalase Signatures 1

Glyoxalase signatures one is located in the N-terminal region consensus pattern: [HQ]-[IVT]-x-[LIVFY]-x-[IV]-x(4)-{E}-[STA]-x(2)-F-[YM]-x(2,3)-[LMF]-G-[LMF]as shown in clustal alignment. The in clustalalignment that was found in all sequences of Glyoxalase and also matching with previously reported pdb structures. The PROSITE results were predicted *in* Avicennia marina glyoxalase I protein sequence patch: Gln28-Met49 as shown in table 2a in this sequence patch Met47 is present in Avicennia marina glyoxalase while in place of this amino acid Leu is present in most of glyoxalase family. It has been reported that Met could be involved in reactive oxygen species-mediated oxidation to methionine sulfoxide (MetO) and as a result could be to changes in protein conformation and activity [24].

3.2.3 Glyoxalase Signatures 2

The PROSITE results were predicted glyoxalase signature pattern 2 *in* Avicennia marina glyoxalase I protein sequence patch: Gly113-Asp129 as shown in table 2a and 2c. The second one is located in the central section of the protein and contains a conserved histidine that could be implicated in the binding of the zinc atom consensus pattern: G-[NTKQ]-x(0,5)-[GA]-[LVFY]-[GH]-H-[IVF][CGA]x-[STAGLE]-x(2)-[DNC]have shown in clustal alignment. This sequence pattern was found in all glyoxalase family except B.subtilis ywbc and also matching with previously reported pdb structures.

3.3 N-myristoylation site

The process of *N*-**myristoylation** is a co-translational modification that **in**volves the covalent reaction of myristate and the am**in**o-term**in**al glyc**in**e residue of a grow**in**g polypeptide. In our study, PROSITE search result has identified one N-myristoylation sites (Table 1). The enzyme myristoyl CoA:protein N-myristoyltransferase (NMT) recognizes certain characteristics within the N-termini of substrate proteins and finally attaches the lipid moiety to a required N-terminal glycine. Plant myristoylation is confirmed for proteins that are involved in growth regulation, disease resistance, salt tolerance and endocytosis [25]. Myristate appears to be critical for mediating protein-protein and/or protein-

membrane interactions [26-27]. PROSITE search result predicted one potetial myristoylation site in *Avicennia marina* glyoxalase in the amino acid sequence at Gly12 in the sequence patch: ^{12}**G**LHTSL^{17}. The analysis of N-terminal region of *Avicennia marina* Glyoxalase protein in homology model have shown (Fig 1) that Gly12 have surface accessibilities of the potetial myristoylation site is 17.3135Å^2, it is a part of the α -helix, are present on the surface and can be considered a potential myristoylation sites. It might be possible that roles of *Avicennia marina* Glyoxalase I is to interact with membrane lipids interaction is mediated through these glycines. This residue is highly conserved in plant including salt tolerant *Solanum lycopersicum* (tomato) (LGUL_SOLLC) [28], *Brassica juncea* (LGUL_BRAJU), *Arabidopsis thaliana* (thale cress) (LGUL_ARATH) [29-30] , and sensitive: salt sensitive *Cicer arietinum* (chickpea) (LGUL_CICAR), and *Glycine max* (soybean) (LGUL_SOYBN) [31] while in human pdb id: 1fro, 3vw9 , and mouse pdb id: 2zao, Ser is present in place of Gly12 in Glyoxalase protein family. Therefore this glycine can be considered potential myristoylation sites although experimental evidence is needed to confirm this.

3.4 Casein kinase II (CK2) phosphorylation site

The multifunctional protein kinase CK2 is a well-conserved protein-kinase present in different organisms, including plants, mammals and serine/threonine kinase whose activity is independent of cyclic nucleotides and calcium. In addition, CK2 is involved in various biological phenomena which includes protein phosphorylation, modulation of DNA-binding ability, protein stability, intracellular localization etc. **[32-35]**. It has been reported that the role of CK2 in Arabidopsis plant, response to genotoxic agents of a CK2 dominant-negative mutant (CK2mut plants). CK2mutant plants were hypersensitive to a wide range of genotoxins that produce a variety of DNA lesions. **[36]**.

In our current study, PROSITE results predicted six potential casein kinase II phosphorylation sites in *Avicennia marina* Glyoxalase protein; two N-terminal sites are ^{15}Thr, and ^{16}Ser in the sequence patch; ^{15}Thr-^{16}Ser-Leu^{17}-Thr^{18}, and ^{16}Ser-Leu^{17}-Thr^{18},Glu^{19} respectively while four possible casien kinase II phosphorylation sites are found at the C-terminal sites; ^{101}Thr ^{103}Ser, ^{126}Thr ^{178}Ser, in the sequence patch ^{101}Thr-Glu-Ser-Asp^{104}, ^{103}Ser-Asp-Pro-Glu^{106}, ^{126}Thr -Val- Asp- Asp^{129}`, and ^{178}Ser -Thr-Ala-Asp^{181} respectively . Multiple alignment of glyoxalase protein family have shown that ^{15}Thr/Ser is almost

conserved while ^{16}Ser/ Thr is 36% conserved at N-terminal region, while at the C-terminal sites; ^{101}Thr ^{103}Ser, ^{126}Thr ^{178}Ser, are 81% , 63% , 63% and 27% conserved throughout glyoxalase protein family respectively (Table 2).

The analysis of N-terminal region of *Avicennia marina* Glyoxalase protein homology model have shown (Fig 1) that Thr15 and Ser16 have surface accessibilities of the possible phosphorylation sites are Thr15: 34.4286Å^2 and Ser16: 19.0836Å^2, are also part of loop which makes them a highly probable target for casein kinase II phosphorylation. These residues are highly conserved in the glyoxalase protein family. The other probable phosphorylation sites are Thr101, Ser103, Thr126 and Ser178 , homology model of *Avicennia marina* Glyoxalase protein have shown surface accessibilities of Thr101 is a part of loop, mainly buried (accessibility = 0.1559Å^2), Ser103 (26.2891Å^2) is also a part of loop, have a relatively higher surface accessibility than Thr126 (24.3011 Å^2), it is a part of the β-sheet, are present on the surface and can be considered a potential casien kinase II phosphorylation sites and Ser178 (3.8530Å^2) is a part of loop, cannot be considered as a phosphorylation site due to its location and low accessibility.

4 CONCLUSIONS

Glyoxalases change methylglyoxal to D-lactate by various catalytic strategies. It is involve in detoxifying glyoxals, Glx3 and its related homologs may have other significant roles in stress response [9]. The glyoxalase system plays an important role in various physiological processes in plants, including salt stress tolerance [1]. *Avicennia marina* that are salt-tolerant plants are better sheltered from oxidative damage under salt stress. They have developed an intricate series of enzymatic and non-enzymatic antioxidant defensive mechanisms. Plant thiols play a significant role in abiotic stress tolerance [2]. However, the molecular mechanisms of plant stress tolerance are not completely understood, and the data available is conflicting. PROSITE results, multiple alignment and homology model of *Avicennia marina* glyoxalase protein research has predicted potential residues that might be critical to alter the structural and functional properties of plant glyoxalase protein. Finally, we predicted various post-translational modification sites plays a significant role in the regulation of biological activities and signal transduction.

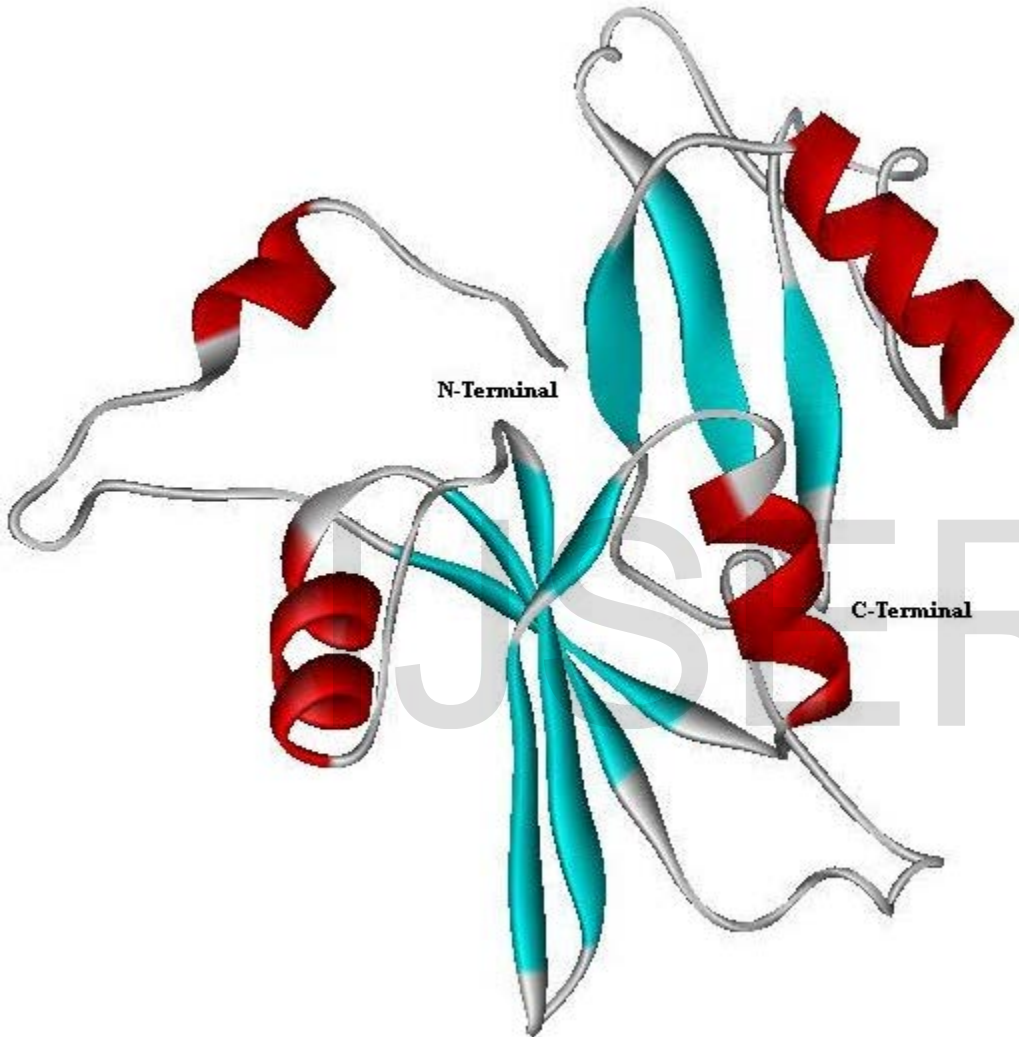
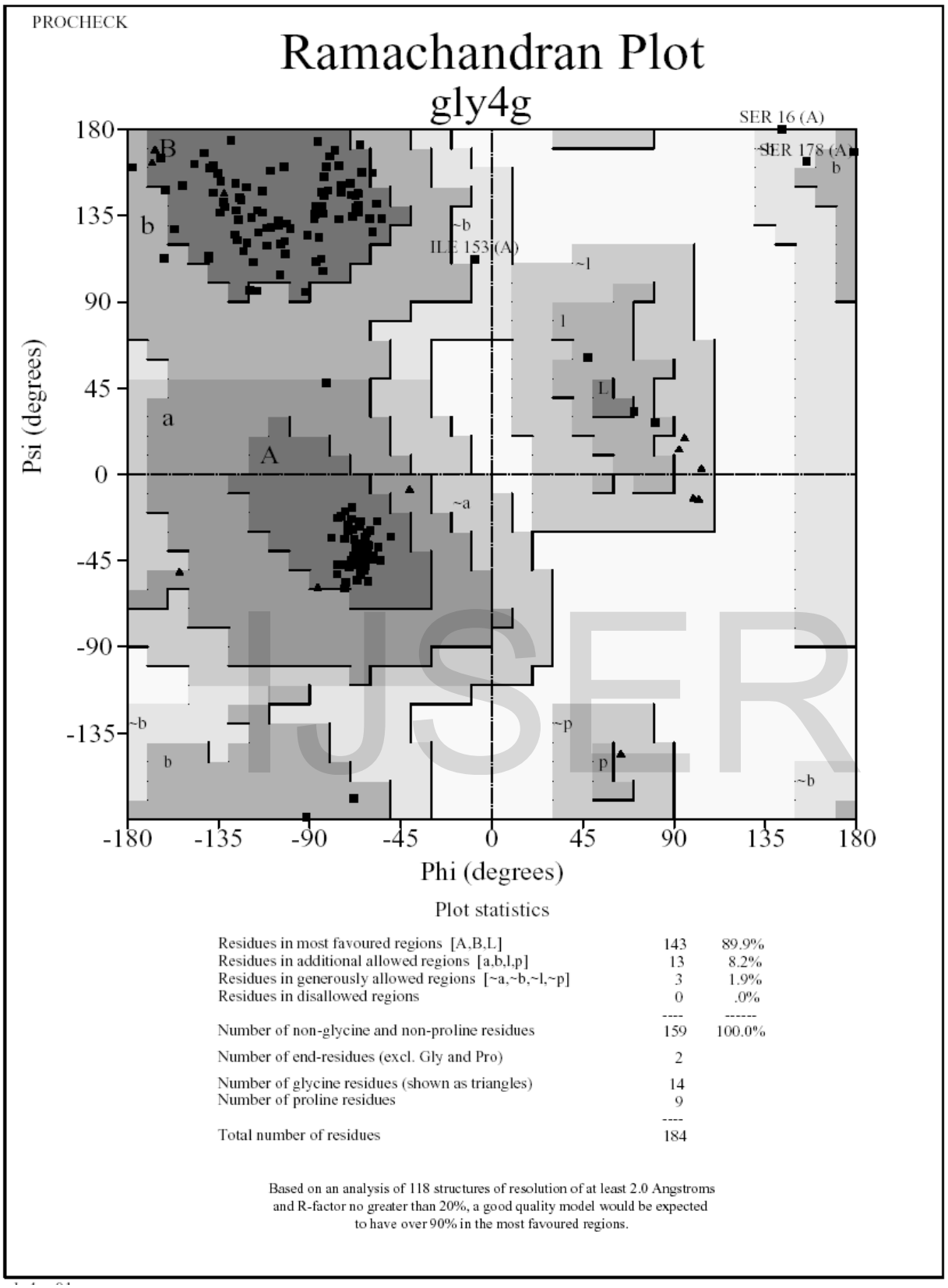


Fig.1a. 3D Homology models of *Avicennia marina* glyoxalase



Plot statistics

Residues in most favoured regions [A,B,L]	143	89.9%
Residues in additional allowed regions [a,b,l,p]	13	8.2%
Residues in generously allowed regions [~a,~b,~l,~p]	3	1.9%
Residues in disallowed regions	0	.0%
Number of non-glycine and non-proline residues	159	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	14	
Number of proline residues	9	
Total number of residues	184	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Fig.1b. PROCHECK summary of the homology model of *Avicennia marina* glyoxalase protein showing 0% residue in the disallowed region.

Overall model quality

Z-Score: -4.69

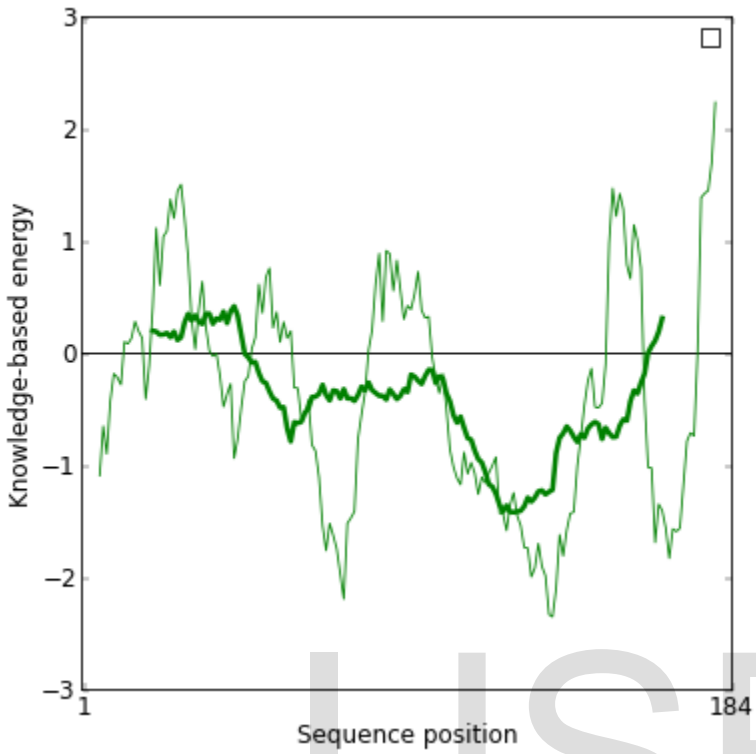


Fig.1c. Protein Structure Analysis (ProSA) plots of homology model of *Avicennia marina* glyoxalase protein with z score of - 4.69. ProSA plot shows local model quality by plotting energies as a function of amino acid sequence position. The program calculates an overall quality score for a specific input structure. The thick line shows the average energy over each 40-residue fragment while the thin line shows a smaller window size of 10 residues .

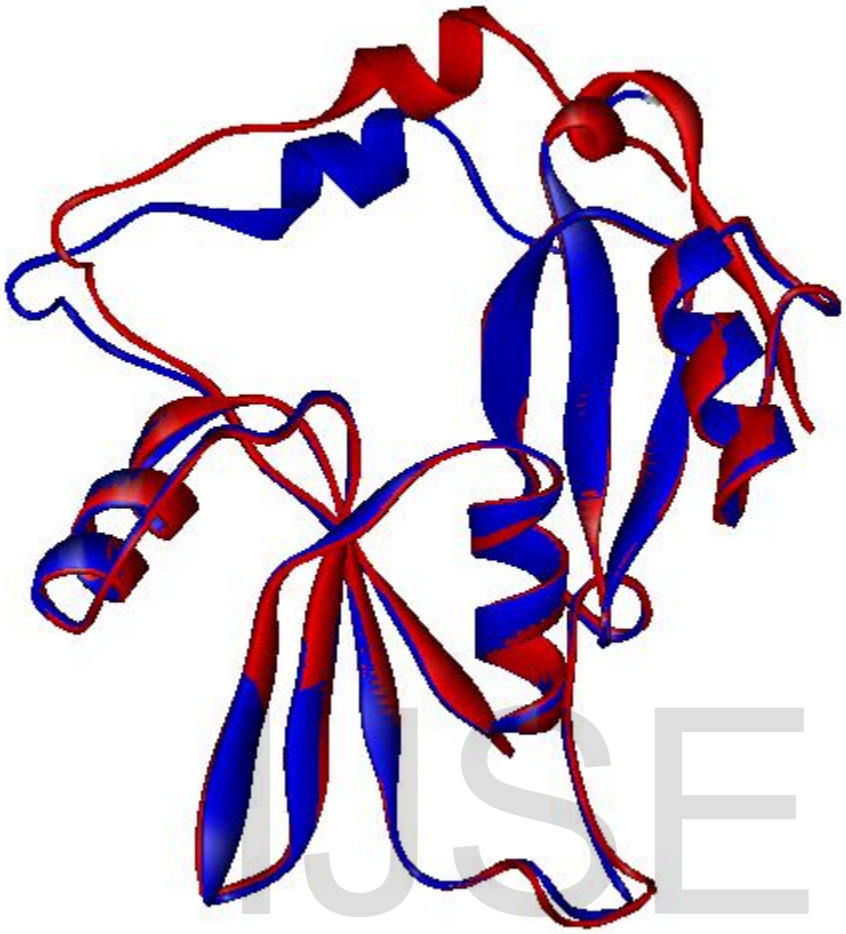


Fig.2. Superposition of glyoxalase from *Avicennia* marina (red) and mouse (blue) with r.m.s.d. 0.42Å

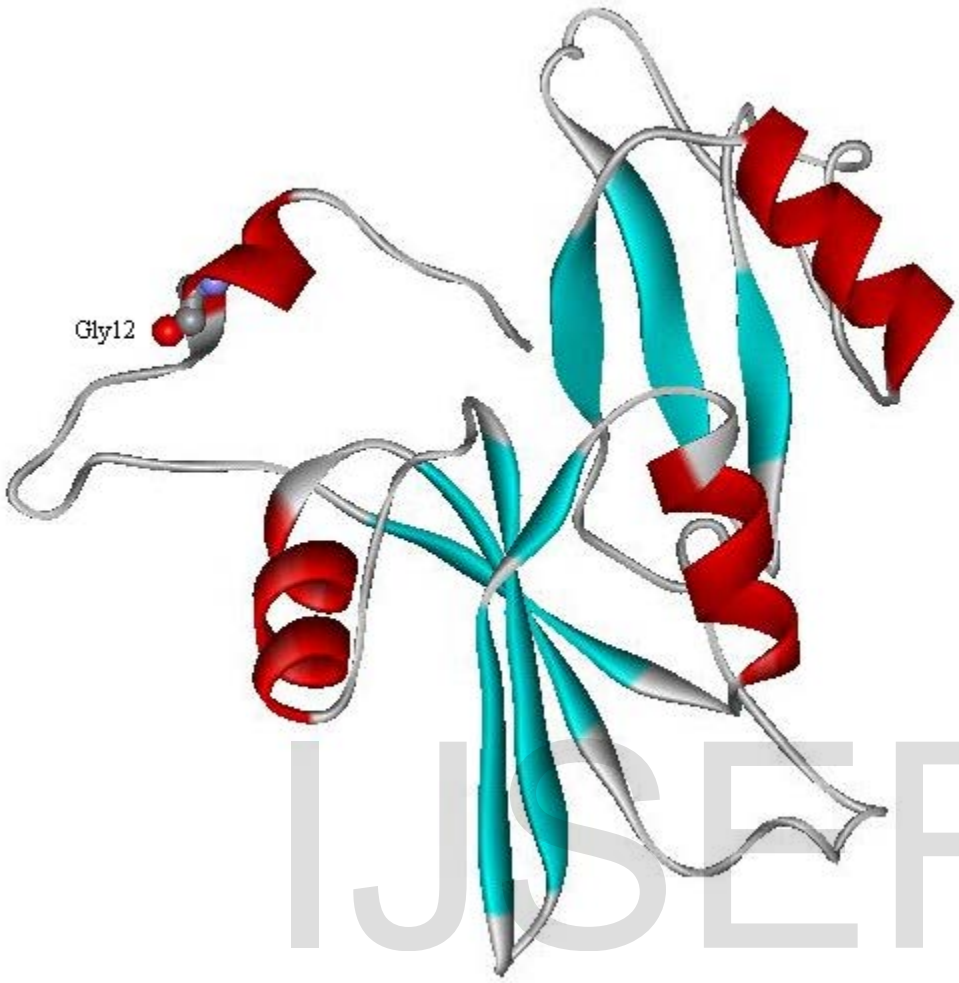


Fig.3. One potential myristoylation site in 3D-homology model of *Avicennia marina* glyoxalase protein**.**

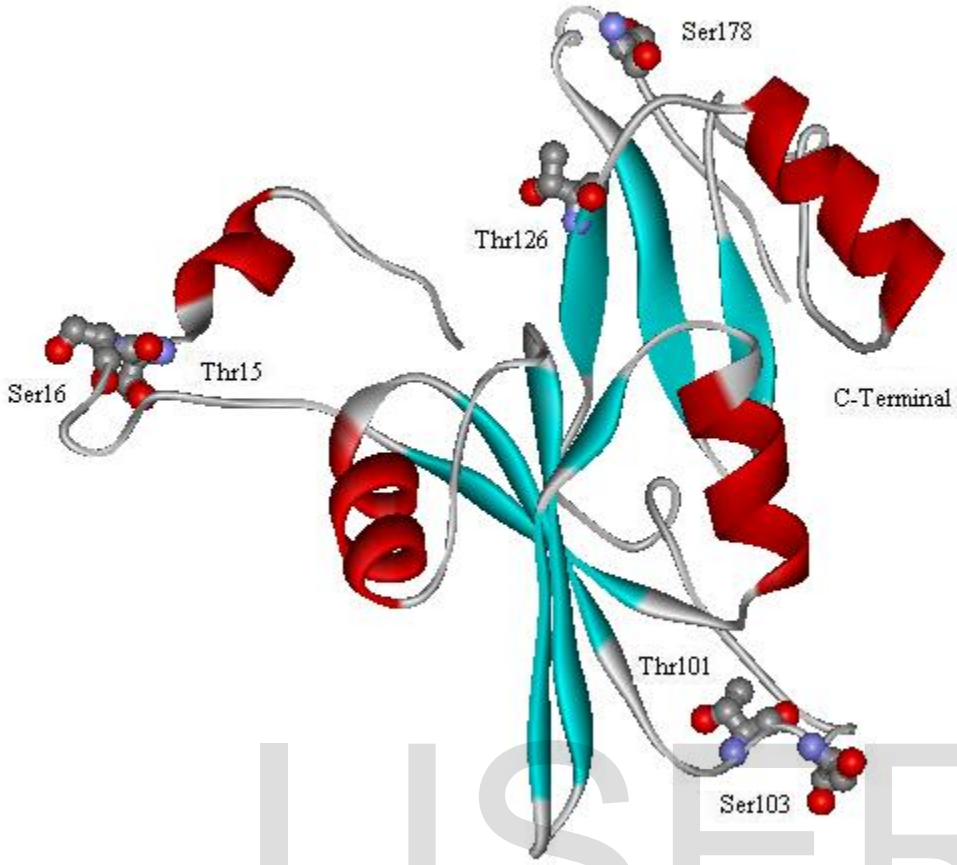


Fig. 4.Homology model of *Avicennia marina* glyoxalase protein have shown six possible casein kinase II phosphorylation sites.

Table 1: Sequence patches that contain the predicted post translational modification sites in *Avicennia marina* glyoxalase protein as predicted by PROSITE. The predicted residue in the patch is denoted by asterisk (*). In case of phosphorylation and myristoylation, Ser (S) / Thr (T) and Gly (G) are modified respectively.

Casein kinase II phosphorylation site	N-myristoylation site
*15 - 18 TSLD	*12 - 17 GLHTSL
*16 - 19 SLDE	
*101 - 104 TESD	
*103 - 106 SDPE	
*126 - 129 TVDD	
*178 - 181 STAD	

CLUSTAL W (1.83) multiple sequence alignment

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                                                          Glyoxalase signature 1
                                  12 1516          28                   47 49
Q9AXH1_AVIMR      ---------MASKESADNNPGLHTSLDEATKGYFLQQTMLRVKDPKVSLDFYSRIMGMSL
LGUL_SOLLC        -------MASESKDSPSNNPGLHATPDEATKGYFLQQTMFRIKDPKVSLEFYSKVLGMSL
LGUL_BRAJU        -------MASEAKESPANNPGLSTVRDEATKGYIMQQTMFRVKDPKASLDFYSRVLGMSL
LGUL_ARATH        -------MASEARESPANNPGLSTNRDEATKGYIMQQTMFRIKDPKASLDFYSRVLGMSL
LGUL_CICAR        ------MAASESKESPANNPGLHTTIDEATKGYFMQQTMFRIKDPKVSLDFYSRVLGMSL
LGUL_SOYBN        -------MAAEPKESPSNNPGLHTTPDEATKGYIMQQTMFRIKDPKVSLDFYSRVLGMSL
1FRO              ----AEPQPPSGGLTDEAALSCCSDADPSTKDFLLQQTMLRVKDPKKSLDFYTRVLGMTL
3VW9              GSHMAEPQPPSGGLTDEAALSCCSDADPSTKDFLLQQTMLRVKDPKKSLDFYTRVLGMTL
1F9Z              --------------------------------MRLLHTMLRVGDLQRSIDFYTKVLGMKL
2ZA0              ---MAEPQPASSGLTDETAFSCCSDPDPSTKDFLLQQTMLRIKDPKKSLDFYTRVLGLTL
2C21              -------------------------GSHMPSRRMLHTMIRVGDLDRSIKFYTERLGMKV
                                           : :**:*: * . *:.**:. :*:.:
                                                                101 103
Q9AXH1_AVIMR      LKRLDFPEMKFSLYFLGYEDTSSAPSDPVERTSWTFGQKAVLELTHNWGTESDPEFKGYH
LGUL_SOLLC        LKRLDFPEMKFSLYFMGYEDTASAPSDPVERTAWTFSQKSTLELTHNWGTESDPNFTGYH
LGUL_BRAJU        LKRLDFSEMKFSLYFLGYEDTSTAPTDPTERTVWTFGRPATIELTHNWGTESDPEFKGYH
LGUL_ARATH        LKRLDFSEMKFSLYFLGYEDTTTAPTDPTERTVWTFGQPATIELTHNWGTESDPEFKGYH
LGUL_CICAR        LKRLDFPEMKFSLYFMGYEDTTEAPSNPVDRTVWTFAQKATIELTHNWGTESDPEFKGYH
LGUL_SOYBN        LKRLDFPEMKFSLYFMGYENTAEAPSNPIDKVVWTFSQKATIELTHNWGTESDPEFKGYH
1FRO              IQKCDFPIMKFSLYFLAYEDKNDIPKEKDEKIAWALSRKATLELTHNWGTEDD-ETQSYH
3VW9              IQKCDFPIMKFSLYFLAYEDKNDIPKEKDEKIAWALSRKATLELTHNWGTEDD-ETQSYH
1F9Z              LRTSENPEYKYSLAFVGYG---------------PETEEAVIELTYNWGVDK--------
2ZA0              LQKLDFPAMKFSLYFLAYEDKNDIPKDKSEKTAWTFSRKATLELTHNWGTEDD-ETQSYH
2C21              LRKWDVPEDKYTLVFLGYG---------------PEMSSTVLELTYNYGVTS--------
                  ::  : .  *::* *:.*                .    :.:***:*:*. .
              Glyoxalase signature 2
                   113            129
Q9AXH1_AVIMR      NGNSDPRGFGHIGVTVDDVHKACERFESLGVEFVKKPR--DGKIMDVAFIKDPDGYWIEI
LGUL_SOLLC        NGNSEPRGFGHIGVTVDDVYKACERFESLGVEFVKKPL--DGKMKGIAFIKDPDGYWIEI
LGUL_BRAJU        NGNSEPRGFGHIGVTVDDVHKACERFEQLGVEFVKKPH--DGKMKNIAFIKDPDGYWIEI
LGUL_ARATH        NGNSEPRGFGHIGVTVDDVHKACERFEELGVEFAKKPN--DGKMKNIAFIKDPDGYWIEI
LGUL_CICAR        NGNSDPRGFGHIGITVDDTYKACERFQNLGVEFVKKPD--DGKMKGIAFIKDPDGYWIEL
LGUL_SOYBN        NGNSEPRGFGHIGVTVDDTYKACERFQNLGVEFVKKPE--DGKMKGIAFIKDPDGYWIEI
1FRO              NGNSDPRGFGHIGIAVPDVYSACKRFEELGVKFVKKPD--DGKMKGLAFIQDPDGYWIEI
3VW9              NGNSDPRGFGHIGIAVPDVYSACKRFEELGVKFVKKPD--DGKMKGLAFIQDPDGYWIEI
1F9Z              --YELGTAYGHIALSVDNAAEACEKIRQNGGNVTREAGPVKGGTTVIAFVEDPDGYKIEL
2ZA0              NGNSDPRGFGHIGIAVPDVYSACKRFEELGVKFVKKPD--DGKMKGLAFIQDPDGYWIEI
2C21              --YKHDEAYGHIAIGVEDVKELVADMRKHDVPIDYED-----ESGFMAFVVDPDGYYIEL
                     .   .:***.: * :. .    :.. .  .  :          :**: ***** **:
                          178
Q9AXH1_AVIMR      FDTRTIAKSTADAAV-----
LGUL_SOLLC        FDTKIIKDAAGSAS------
LGUL_BRAJU        FDLKTIGTTAGNAA------
LGUL_ARATH        FDLKTIGTTTVNAA------
LGUL_CICAR        FDRKTIGNVTEGNA------
LGUL_SOYBN        FDRKTIGNVTQTAA------
1FRO              LNPNKMATLM----------
3VW9              LNPNKMATLM----------
1F9Z              IEEKDAGRGLGN--------
2ZA0              LNPNKIATII----------
2C21              LNEKTMMEKAEADMKEQGTA
                  :: .
```

Table 2a: multiple sequence alignment showing the sequence of members of glyoxalase family .
* = fully conserved residues; : = strongly conserved; . = weakly conserved
Amino acid sequence patch of *Avicennia marina* (Q9AXH1_AVIMR) glyoxalase signature 1: Q28-M49 and signature 2: G113-D129 . casein kinase II phosphorylation residues Thr15, and Ser16 and Thr101, Ser103, Thr126 Ser178 and myristoylation residue is Gly12

Table 2b

CLUSTAL format alignment of Glyoxalase family protein signature 1 pattern

```
Q9AXH1_AVIMR/28-49      QTMLRVKDPKVSLDFYSRIMGM
HPPD_DANRE/21-42        HIKFWVGNAKQAAVFYCDKFGF
LGUC_ARATH/91-112       HVVYRVGDMDRTIKFYTECLGM
LGUC_ARATH/221-242      QVMLRVGDLDRAIKFYEKAFGM
LGUL_ARATH/30-51        QTMFRIKDPKASLDFYSRVLGM
LGUL_BRAJU/30-51        QTMFRVKDPKASLDFYSRVLGM
LGUL_BRAOG/20-41        HVVYRVGDLDRTIQFYTECFGM
LGUL_BRAOG/150-171      QVMLRVGDLDRAVKFMEKALGM
LGUL_CICAR/31-52        QTMFRIKDPKVSLDFYSRVLGM
LGUL_ECO57/5-26         HTMLRVGDLQRSIDFYTKVLGM
LGUL_ECOLI/5-26         HTMLRVGDLQRSIDFYTKVLGM
LGUL_HAEIN/5-26         HTMLRVGDLDRSIKFYQDVLGM
LGUL_HUMAN/34-55        QTMLRVKDPKKSLDFYTRVLGM
LGUL_MACFA/34-55        QTMLRVKDPKKSLDFYTRVLGM
LGUL_MOUSE/34-55        QTMLRIKDPKKSLDFYTRVLGL
LGUL_NEIMA/5-26         HTMLRVGNLEKSLDFYQNVLGM
LGUL_NEIMB/5-26         HTMLRVGNLEKSLDFYQNVLGM
LGUL_ORYSJ/158-179      QVMLRVGDLDRSIKFYEKALGM
LGUL_PSEAE/26-47        HTMLRVKDPKRSLDFYSRVLGM
LGUL_PSEPU/27-48        HTMLRVKDIEKSLDFYTRVLGF
LGUL_RAT/34-55          QTMLRIKDPKKSLDFYTRVLGL
LGUL_SALTI/5-26         HTMLRVGDLQRSIAFYTNVLGM
LGUL_SALTY/5-26         HTMLRVGDLQRSIAFYTNVLGM
LGUL_SCHPO/14-35        HTMIRVKDLDKSLKFYTEVFGM
LGUL_SCHPO/169-189      HTMVRVKDPEPSIAFYEK.LGM
LGUL_SHIFL/5-26         HTMLRVGDLQRSIDFYTKVLGM
LGUL_SOLLC/30-51        QTMFRIKDPKVSLEFYSKVLGM
LGUL_SOYBN/30-51        QTMFRIKDPKVSLDFYSRVLGM
LGUL_SYNY3/5-26         HTMIRVGDLDKSLQFYCDILGM
LGUL_VIBCH/8-29         HTMLRVGDLDKSIEFYTQVMGM
LGUL_VIBPA/8-29         HTMLRVGDLDKSIKFYTEVMGM
LGUL_YEAST/25-46        HTCLRVKDPARTVKFYTEHFGM
LGUL_YEAST/185-206      HTMIRIKNPTRSLEFYQNVLGM
YQJC_BACSU/6-27         HIGIAVFSIKDARSFYENVLGL
YRAH_BACSU/5-26         QIRLLVNDFKKSVEFYKDSLGL
YWBC_BACSU/7-28         HTGIMVRDINASITFYEEVLGM
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Table 2c

CLUSTAL format alignment Glyoxalase family protein signature 2 pattern

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Q9AXH1_AVIMR/113-129    GNSDPR-GFGHIGVTVDD
LGUL_ARATH/115-131      GNSEPR.GFGHIGVTVDD
LGUL_BRAJU/115-131      GNSEPR.GFGHIGVTVDD
LGUL_BRAOG/84-96        GT.....GFGHFAISTQD
LGUL_CICAR/116-132      GNSDPR.GFGHIGITVDD
LGUL_ECO57/69-81        GT.....AYGHIALSVDN
LGUL_ECOLI/69-81        GT.....AYGHIALSVDN
LGUL_HAEIN/69-81        GT.....AYGHIAIGVDD
LGUL_HUMAN/118-134      GNSDPR.GFGHIGIAVPD
LGUL_MACFA/118-134      GNSDPR.GFGHIGIAVPD
LGUL_MOUSE/118-134      GNSDPR.GFGHIGIAVPD
LGUL_NEIMA/69-81        GN.....AYGHIAVEVDD
LGUL_NEIMB/69-81        GN.....AYGHIAVEVDD
LGUL_PSEAE/109-125      GNQDPR.GFGHICFSVPD
LGUL_PSEPU/111-127      GNTDPR.GFGHICVSVPD
LGUL_RAT/118-134        GNSDPR.GFGHIGIAVPD
LGUL_SALTI/69-81        GN.....AYGHIALSVDN
LGUL_SALTY/69-81        GN.....AYGHIALSVDN
LGUL_SCHPO/93-110       GNTEPKRGFGHICFTVDN
LGUL_SCHPO/241-258      GNDGDEKGYGHVCISVDN
LGUL_SHIFL/69-81        GT.....AYGHIALSVDN
LGUL_SOLLC/115-131      GNSEPR.GFGHIGVTVDD
LGUL_SOYBN/115-131      GNSEPR.GFGHIGVTVDD
LGUL_SYNY3/69-81        GN.....GFGHIALGVED
LGUL_VIBCH/72-84        GN.....AYGHIAIGVDD
LGUL_VIBPA/72-84        GT.....AFGHIAIGVDD
LGUL_YEAST/107-124      GNEEPHRGFGHICFSVSD
LGUL_YEAST/260-276      GNSEPQ.GYGHICISCDD
NKD1_HUMAN/353-367      GKSV...GVGHVARGARN
YQJC_BACSU/72-84        GQ.....GLHHIAFLCNC
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