



Bioinformatics Study of the Sequence Analysis and Secondary Structure Prediction of *Avicennia marina* glyoxalase I protein

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Abstract:

*Glyoxalase 1 detoxifies α -oxoaldehydes, particularly methylglyoxal (MG) via different enzymatic and non-enzymatic reactions, a cytotoxic compound increased rapidly under stress conditions. The physiological significance of the glyoxalase system has not been clearly defined in plants. We used bioinformatics tools in this study; we performed sequence analysis; major part of *Avicennia marina* glyoxalase I proteins polypeptide consists of hydrophobic residues; comparison of salt tolerant glyoxalase I from *Avicennia marina* with that of salt sensitive plants multiple sequence alignment*

has shown that the catalytic residues Glu168, His122, Gln28 and Glu102 are highly conserved. Prediction of secondary structures have shown that glyoxalase I salt stress plant family consists of higher percentage of α -helix structure as compared to salt sensitive plant glyoxalase family. Prediction of *Avicennia marina* glyoxalase I secondary structures, consists of α -helix is 67.4%, β -sheet is 54.9%.

Key words: Secondary Structure Prediction, sequence Alignment, Glyoxalase, Salt Stress, Salt Sensitive, *Avicennia marina*.

Introduction

Salt tolerant plant either prevent the absorption of sodium or chloride ions by roots and leaves or tolerate the collection of sodium or chloride ions in its tissue (Bezona et al. 2001). Salt tolerance is a complex feature involving responses to cellular osmotic and ionic stresses and their consequent secondary stresses (e.g. oxidative stress) and whole plant coordination. *Avicennia marina* (Forssk.) Vierh belongs to grey mangrove and widely distributed along tropical and subtropical coastlines (Kathiresan & Bingham 2001). Salt tolerant proteins are synthesized in response to salinity and the sequence of these proteins are found to be highly conserved (Prashant et al. 2010). Many salt tolerant proteins such as glyoxalase, their role, activities and location were reported in salt tolerant plants like *Solanum lycopersicum*, *Brassica juncea* and *Arabidopsis thaliana*. Glyoxalase I has been shown to be up-regulated in plants in response to environmental stresses (Veena & Sopory 1999; Martins *et al.* 2001; Singla-Pareek *et al.* 2003). Glyoxalase I detoxifies α -oxoaldehydes, particularly methylglyoxal (MG) (Thornalley 1990, 1993, 2003; Mannervik 2008). The physiological significance of the glyoxalase system has not been clearly defined in plants; however, this system has been often regarded as a “marker for cell growth and division” (Paulus et al. 1993). The ubiquitous nature and high expression

of glyoxalase I in metabolically active cells/tissues, such as meristematic, newly dividing cells, or cells undergoing stress, indicate the fundamental importance of this system in plants. *Brassica juncea* glyoxalase I protein sequence revealed one serine and four threonine phosphorylation sites and two N-myristoylation sites. Over-expression of the *Brassica juncea* glyoxalase I in a tobacco transgenic line showed a significant increase in tolerance compared to wild-type plants when treated with methylglyoxal and sodium chloride (Veena & Sopory 1999). In addition, over-expression of either glyoxalase I or II in tobacco plants showed higher tolerance to salt and methylglyoxal than untransformed control plants (Singla-Pareek *et al.* 2003). Interestingly, when both glyoxalase I and II are over-expressed in the same plant, they act synergistically, increasing tolerance levels above that of the individual genes (Singla-Pareek *et al.* 2003). It is speculated that glyoxalase I is up-regulated in plants that are exposed to salt stress because glycolytic activity increases due to an increased demand for ATP (Veena & Sopory 1999). Bioinformatics is the multidisciplinary and revolutionary field that uses mathematical, statistical and computing methods to solve complex biological problems. A first step toward predicting the structure of a protein is to determine its secondary structure. The prediction of secondary structure from sequence is a fundamental and important component in the analytical study of protein structure and functions. In the present study, we analyzed the primary sequences of glyoxalase from unknown structure of various species. Sequence analysis was performed by aligning the amino acid sequences of salt-tolerant and salt-sensitive plant species and conserved and non conserved amino acid residues were analyzed. *Avicennia marina* glyoxalase I proteins as well as salt sensitive and salt tolerant plant glyoxalase I proteins amino acid sequence were also used for prediction of secondary structures.

Methods

Sequence Analysis

Primary amino acid sequence of *Avicennia marina* glyoxalase I protein (A7LKM8_AVIMR) consists of 184 amino acid was retrieved from UniProt database (Bairoch et al. 2005). Sequence identity searches were performed using BLAST server (Altschul et al. 2001) against non-redundant (nr) database using default parameters.

Multiple sequence alignment and phylogenetic analysis

Primary amino acid sequences of *Avicennia marina* glyoxalase I protein as well as that of salt stress and salt sensitive plants were retrieved from UniProt database (Bairoch et al., 2005). Multiple sequence alignment was performed by CLUSTAL X (Thompson et al. 1997). Sequences of salt sensitive and salt tolerant plant glyoxalase I proteins were used for constructing phylogenetic tree (Felsenstein 1989; Howe et al. 2002). Phylogenetic analysis based on Neighbor-Joining (NJ) method was performed for reconstructing phylogenetic tree from evolutionary distance data by using Drawgram program and Drawtree of PHYLIP tree package version 3.67.

Protein secondary structure prediction

Avicennia marina glyoxalase I proteins as well as salt sensitive and salt tolerant plant glyoxalase I proteins amino acid sequence were also used for prediction of secondary structures by using Chou-Fasman algorithm (Hang Chen et al. 2006), (<http://www.biogem.org>). The Chou–Fasman process based on analyses of the relative frequencies of each amino acid in alpha helices, beta sheets, and turns based on known protein structures solved by X-ray crystallography.

Results and Discussion

Sequence Analysis and Comparison of salt tolerant and salt sensitive glyoxalase

Sequence analysis of *Avicennia marina* glyoxalase I (Q9AXH1_AVIMR) has shown that the major part of this polypeptide consists of hydrophobic residues. A **BLASTP alignment** search against non-redundant (nr) database (Altschul et al. 1997) has shown significant sequence similarities with other proteins. The amino acid composition of *Avicennia marina* glyoxalase I retrieved from ProtParam (Gasteiger et al. 2005) predicted that protein sequence contains 32.7% non polar residues (a major contribution in the non-polar residue pool), 18.5% of polar uncharged residues (0.5% cysteine), 15.7% negatively charged acidic residues, and 14.6% positively charged basic residues; 11.4% aromatic residues are found in the sequence. Furthermore, the multiple sequence alignment of *Avicennia marina* glyoxalase I has shown the major conserved region of the plant glyoxalase I family. Multiple sequence alignment has shown that the catalytic residues Glu168, His122, Gln28 and Glu102 are highly conserved in the family of salt sensitive *Cicer arietinum* (chickpea) (LGUL_CICAR) and Glycine max (soybean) (LGUL_SOYBN) (Skipsey et al. 2000) and salt stress *Solanum lycopersicum* (*Lycopersicon esculentum*) (LGUL_SOLLC) (Espartero et al., 1995), *Brassica juncea* (LGUL_BRAJU) and *Arabidopsis thaliana* (thale cress) (LGUL_ARATH) (Yamada et al. 2003; Theologis et al. 2000) glyoxalase. At position 181 Asp is present in *Avicennia marina* glyoxalase 1 while in most of glyoxalase family proteins Gly/Thr, is present at this positions in salt sensitive and Asn/Ser is present at this positions in salt stress; this residue is involved in hydrogen bonding interactions with methyl-gerfelin inhibitor.

Phylogenetic analysis

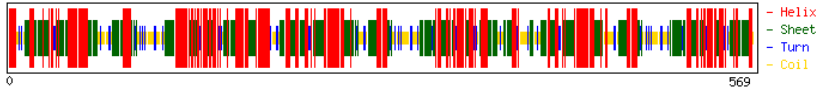
Avicennia marina (Q9AXH1_AVIMR) glyoxalase I sequence was compared with glyoxalase of two salt sensitive plant species *Cicer arietinum* (chickpea) (LGUL_CICAR) and *Glycine max* (soybean) (LGUL_SOYBN) (Skipsey et al. 2000) and glyoxalase of salt stress plant species *Solanum lycopersicum* (*Lycopersicon esculentum*) (LGUL_SOLLC) (Espartero et al., 1995), *Brassica juncea* (LGUL_BRAJU) and *Arabidopsis thaliana* (thale cress) (LGUL_ARATH) (Yamada et al., 2003; Theologis et al. 2000) by the Phylip tree that read the aligned sequences in Clustal-format. On cladogram of glyoxalase of salt tolerant species, two distinct clusters are formed and the homologies between related species was observed. The sequence of *Avicennia marina* (Q9AXH1_AVIMR) glyoxalase I is closely related to glyoxalase of salt tolerant *Solanum lycopersicum* (*Lycopersicon esculentum*) (LGUL_SOLLC) (Espartero et al., 1995). On cladogram with glyoxalase of salt sensitive species, *Avicennia marina* (Q9AXH1_AVIMR) glyoxalase I was found in separate clade.

Secondary Structure Prediction

The secondary structure prediction of *Avicennia marina* glyoxalase I protein is done by Chou–Fasman method. Glyoxalase I consists of 184 amino acids. *Avicennia marina* (Q9AXH1_AVIMR) glyoxalase I have content of α -helix is 67.4%, β -sheet is 54.9% and turns 14.7% (as shown in fig). The secondary structure prediction of salt sensitive and salt tolerant species is also done by using Chou–Fasman process as shown in table and figure. Salt sensitive plant consists of higher percentage of β -sheet structure as compared to salt stress plant glyoxalase family. Furthermore salt stress glyoxalase family; *Solanum lycopersicum* (*Lycopersicon esculentum*) (LGUL_SOLLC) (Espartero et al. 1995), *Brassica juncea* (LGUL_BRAJU) and *Arabidopsis thaliana* (thale cress)

Target Sequence:

MASKESADNN PGLHTSLDEA TKG YFLQQTM LRVKDPK VSL DFYSRIMGMS LLKRLDFPEM KFSLYFLGYE
 DTSSAPSDPV ERTSWTFGQK AVLELTHNWG TESDPEFKGY HNGNSDPRGF GHIGVTVDV HKACERFESL
 GVEFVKKPRD GKIMDVAFIK DPDGYWIEIF DTRTIAKSTA DAAV



Secondary Structure:

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                *   *   *   *   *

Query 1  MASKESADNNPGLHTSLDEATKG YFLQQTMLRVKDPK VSLDFYSRIMGMSLLKRLDFPEM 60
Helix 1                                <---->  <----->  <-----> 60
Sheet 1                                EEEE  EEEEEEEEEEEEE  EEEEEEEEEEEEEEEEEEEEE 60
Turns 1                                TT TT  T T  T  TT  60

                *   *   *   *   *

Query 61  KFSLYFLGYEDTSSAPSDPVERTSWTFGQKAVLELTHNWGTESDPEFKGYHNGNSDPRGF 120
Helix 61                                ---->  <----->  120
Sheet 61                                EEEEEEEE  EEEEEEEEEEEEEEEEEEEEE  E 120
Turns 61                                T  TT  T  T  T  TTT  TT  T 120

                *   *   *   *   *

Query 121 GHIGVTVDVHVKACERFESLGVEFVKKPRDGKIMDVAFIKDPDGYWIEIFDTRTIAKSTA 180
Helix 121                                <----->  <----->  180
Sheet 121                                EEEEEEEEE  EEEEEEEE  EEEEE  EEEEEEEEE  180
Turns 121                                T  T  TT  T  T  T  180

Query 181 DAAV 184
Helix 181  --> 184
Sheet 181  184
Turns 181  184
    
```

Total Residues: H: 124 E: 101 T: 27
 Percent: H: 67.4 E: 54.9 T: 14.7

FIGURE 5. Secondary structure prediction of *Avicennia marina* (Q9AXH1_AVIMR) glyoxalase protein.

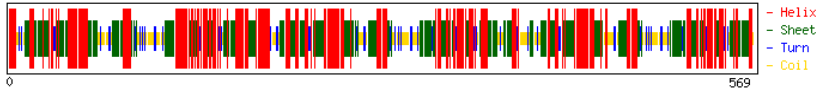
Name of the sequence is *LGUL_CICAR*.

Sequence consists of 186 amino acids.

Target Sequence:

MAASESKESP ANNPGLHTTI DEATKGYFMQ QTMFRIKDPK VSLDFYSRVL GMSLLKRLDF PEMKFSLYFM

GYEDTTEAPS NPVDRTVWTF AQKATIELTH NWGTESDPEF KGYHNGNSDP RGFHIGITV DDTYKACERF
 QNLGVEFVKK PDDGKMKGIA FIKDPDGYWI ELFDRKTIGN VTEGNA



Secondary Structure:

```

                *   *   *   *   *
Query 1  MAASESKESPANNPGLHTTIDEATKGYFMQQTMFRIKDPKVS LDFYSRVLGMSLLKRLDF 60
Helix 1          <-----> <-----> <-----> 60
Sheet 1          EEEE EEEEEEEEEEEEE EEEEEEEEEEEEEEEEEEEEE 60
Turns 1          T T TT T T T TT 60
    
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                *   *   *   *   *
Query 61 PEMKFSLYFMGYEDTTEAPSNPVDRTVWTFQAQKATIELTHNWGTESDPEFKGYHNGNSDP 120
Helix 61          -----> <-----> 120
Sheet 61          EEEEEEEEEEEEEEEEE EEEEEEEEEEEEEEEEEEEEE 120
Turns 61          T TT T T T TTT TT 120
    
```

```

                *   *   *   *   *
Query 121 RGFHIGITVDDTYKACERFQNLGVEFVKKPDDGKMKGIAFIKDPDGYWIELFDRKTIGN 180
Helix 121          <-----> <-----> 180
Sheet 121          EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE 180
Turns 121          T T T T TT TT 180
    
```

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Query 181 VTEGNA 186
Helix 181      186
Sheet 181      E 186
Turns 181      T 186
    
```

Total Residues: H: 110 E: 115 T: 29
 Percent: H: 59.1 E: 61.8 T: 15.6

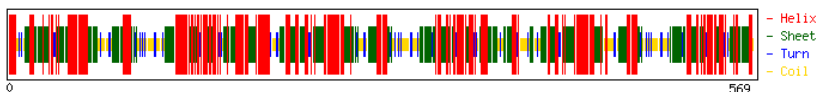
FIGURE 6. Secondary structure prediction of salt sensitive plant *Cicer arietinum* (LGUL_CICAR) glyoxalase protein.

Name of the sequence is *LGUL_SOYBN*.

Sequence consists of 185 amino acids.

Target Sequence:

MAAEPKESPS NNPGLHTTPD EATKGYIMQQ TMFRIKDPKV SLDYFSRVLG MSLKRLDFP EMKFSLYFMG
 YENTAEAPSN PIDKVWTFQ QKATIELTHN WGTESDPEFK GYHNGNSEPR GFGHIGITVD DTYKACERFQ
 NLGVEFVKKP EDGKMKGIAF IKDPDGYWIE IFDRKTIGNV TQTAA



Secondary Structure:

```

      *   *   *   *   *
Query 1  MAAEPKESPSNPNGLHTTPDEATKGYIMQQTMFRIKDPKVSFLDFYSRVLGMSELLKRLDFP 60
Helix 1                                <----> <-----> <-----> 60
Sheet 1                                EEE EEEEEEEEEEE EEEEEEEEEEEEEEEEEEE 60
Turns 1                                T T T T T TT 60

      *   *   *   *   *
Query 61 EMKFSLYFMGYENTAEAPSNPIDKVVWTFQKATIELTHNWGTESDPEFKGYHNGNSEPR 120
Helix 61                                -----> <> <-----> 120
Sheet 61                                EEEEEEEEEEE EEEEEEEEEEEEEEEEEEE 120
Turns 61                                T TT T T TTT TT T 120

      *   *   *   *   *
Query 121 GFGHIGVTVDITYKACERFQNLGVEFVKKPEDGKMKGIAFIKDPDGYWIEIFDRKTIGNV 180
Helix 121                                <-----> <-----> 180
Sheet 121                                EEEEEEEEEEEEEEEEEEEEEEE EEEEEEE EEEEEEEEEEEEEEE 180
Turns 121                                T T T T TT 180

Query 181 TQTAA 185
Helix 181 ---> 185
Sheet 181 EE 185
Turns 181 185
    
```

Total Residues: H: 120 E: 112 T: 25
 Percent: H: 64.9 E: 60.5 T: 13.5

FIGURE 7. Secondary structure prediction of salt sensitive plant *Glycine max* (LGUL_SOYBN) glyoxalase protein.

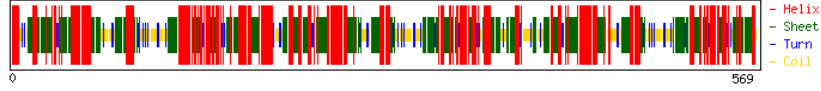
Name of the sequence is *LGUL_SOLLC*

*MASEAKESPANNPGLSTVRDEATKGYIMQQTMFVRVKDPKASLDFYSRVLG
 MSLKRLDFS.*

Sequence consists of 125 amino acids.

Target Sequence:

EMKFSLYFMG YEDTASAPSD PVERTAWTFS QKSTLELTHN WGTESDPNFT GYHNGNSEPR GFGHIGVTVD
 DVYKACERFE SLGVEFVKKP LDGKMKGIAF IKDPDGYWIE IFDTHKIKDA AGSAS

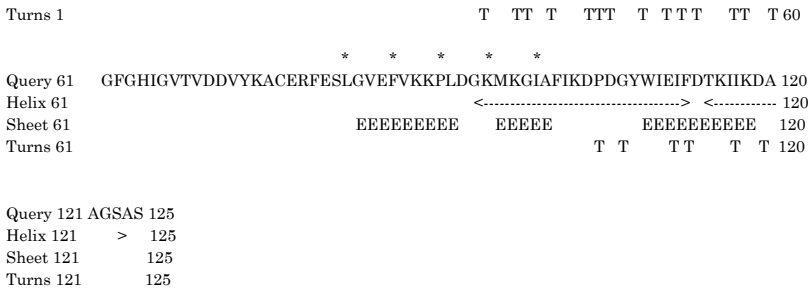


Secondary Structure:

```

      *   *   *   *   *
Query 1  EMKFSLYFMGYEDTASAPSDPVERTAWTFSQKSTLELTHNWGTESDPNFTGYHNGNSEPR 60
Helix 1                                <-----> <-----> 60
Sheet 1                                EEEEEEEEEEE EEEEEEE EEEEE 60
    
```

Uzma Jabeen, Arif Zubair, Waseem Ahmed, Bushra Nazir, Sikandar Khan Sherwani–
Bioinformatics Study of the Sequence Analysis and Secondary Structure Prediction of *Avicennia marina glyoxalase I* protein



Total Residues: H: 77 E: 44 T: 20
 Percent: H: 61.6 E: 35.2 T: 16.0

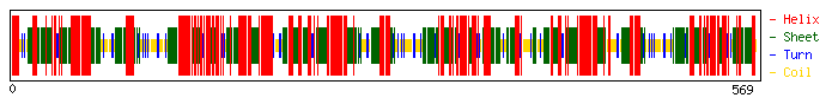
FIGURE 8. Secondary structure prediction of salt tolerant plant *Solanum lycopersicum* (LGUL_SOLL) glyoxalase protein.

Name of the sequence is *LGUL_BRAJU*
MASEAKESPANNPGLSTVRDEATKGYIMQQTmFRVKDPKASLDFYSRVLG
MSLLKRLDFS.

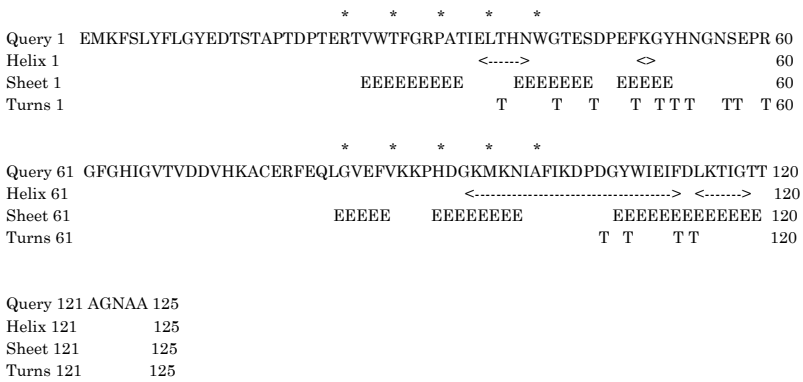
Sequence consists of 125 amino acids.

Target Sequence:

EMKFSLYFLG YEDTSTAPTDP TERTVWTFGR PATIELTHN WGTESDPEFK GYHNGNSEPR GFGHIGVTVD
 DVHKACERFE QLGVEFVKKP HDGKMKNI AF IKDPDGYWIE IPDLKTIGTT AGNAA



Secondary Structure:



Total Residues: H: 58 E: 47 T: 14
 Percent: H: 46.4 E: 37.6 T: 11.2

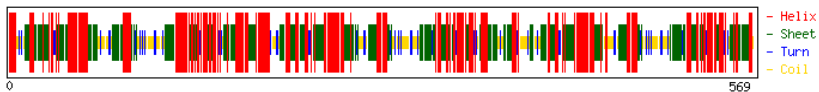
FIGURE 9. Secondary structure prediction of salt tolerant plant *Brassica juncea* (LGUL_BRAJU) glyoxalase protein.

Name of the sequence is *LGUL_ARATH*
MASEAKESPANNPGLSTVRDEATKGYIMQQTMFRVKDPKASLDFYSRVLG
MSLLKRLDFS.

Sequence consists of 125 amino acids.

Target Sequence:

EMKFSLYFLG YEDTTTAPTDP TERTVWTFG QPATIELTHN WGTESDPEFK GYHNGNSEPR GFGHIGVTVD
 DVHKACERFE ELGVEFAKKP NDGKMKNI AF IKDPDGYWIE IFDLKTIGTT TVNAA



Secondary Structure:

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                *   *   *   *   *
Query 1  EMKFSLYFLGYEDTTTAPTDP TERTVWTFGQPATIELTHNWGTESDPEFKGYHNGNSEPR 60
Helix 1  <-----> <-----> 60
Sheet 1  EEEEEEEEEEEEEEE EEEEEEEEE EEEEE 60
Turns 1  T T T T TTT TT T 60

                *   *   *   *   *
Query 61  GFGHIGVTVD DVHKACERFEELGVEFAKKPNDGKMKNI AF IKDPDGYWIEIFDLKTIGTT 120
Helix 61  <-----> <-----> <-----> 120
Sheet 61  EEEEE EEEEEEEEEEEEEEE 120
Turns 61  TT T TT 120

Query 121 TVNAA 125
Helix 121 125
Sheet 121 EE 125
Turns 121 125
    
```

Total Residues: H: 62 E: 49 T: 15
 Percent: H: 49.6 E: 39.2 T: 12.0

FIGURE 10. Secondary structure prediction of salt tolerant plant *Arabidopsis thaliana* (LGUL_ARATH) glyoxalase protein.

Table 1: Percentage of amino acids sequence forming secondary structure in Chou-Fasman Secondary Structure Prediction (CFSSP) Server.

S.NO.	Name of species	Number of amino acid	α -helix (H) (%)	β -sheet (E) (%)	Turns (T) (%)
1	<i>Avicennia marina</i> (Q9AXH1_AVIMR)	184	67.4	54.9	14.7
2	<i>Cicer arietinum</i> (LGUL_CICAR)	186	59.1	61.8	15.6
3	<i>Glycine max</i> (LGUL_SOYBN)	185	64.9	60.5	13.5
4	<i>Solanum lycopersicum</i> (LGUL_SOLLC)	125	61.6	35.2	16.0
5	<i>Brassica juncea</i> (LGUL_BRAJU)	125	46.4	37.6	11.2
6	<i>Arabidopsis thaliana</i> (LGUL_ARATH)	125	49.6	39.2	12.0

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