Pakistan J. Zool., vol. 47(4), pp. 1015-1023, 2015.

Molecular Modeling and Phylogeny of the Manganese Superoxide Dismutase from the Camel, *Camelus dromedarius*

Dalia Fouad[1,2]

[1]*Department of Zoology, College of Science, King Saud University. P.O. Box 22452, Riyadh 11459, Saudi Arabia*

[2]*Department of Zoology and Entomology, Faculty of Science, Helwan University, Ein Helwan, Cairo, Egypt*

Abstract.- The manganese superoxide dismutase (MnSOD or SOD2) is an important antioxidant enzyme in mammals as it eliminates the reactive oxygen species (ROS) produced by the aerobic reactions. The one-humped camel (*Camelus dromedarius*) is adapted to live in the widely varying arid climate and in many intrinsic and extrinsic ROS producing agents. Studying the MnSOD in *C. dromedarius* could help understand the impact of exposure to such factors on the health status of camel. The coding sequence of *MnSOD* of *C. dromedarius* (*cSOD2*) was amplified by reverse transcription PCR from the liver. The cDNA sequencing revealed an open reading frame of 666 nucleotides encoding a protein of 222 amino acids which is comparable to the *SOD2* genes from many eukaryotic organisms. The calculated molecular weight and isoelectric point of cSOD2 were 24.6 kDa and 8.15, respectively. The amino acid sequence analysis revealed the presence of the mitochondria targeting sequence at the N-terminus, the conservation of the characteristic MnSOD motif signature and the four manganese binding sites in cSOD2. The alignment and phylogenetic analysis of the cSOD2 with sequences from 18 organisms indicated that cSOD2 groups with mammals which took late evolutionary line different from SOD2 from birds, amphibians, fish and invertebrates.

Keywords: One-humped camel, superoxide dismutase, manganese superoxide dismutase, reactive oxygen species, anti-oxidant enzyme.

INTRODUCTION

During the normal cell activity, the molecular oxygen (O_2) is reduced to superoxide anion (O_2^-) when absorbs excited electron released from the metabolic reactions. O_2^- causes severe damage to the cellular components as it denatures or inactivates enzymes (Fouad, 2015; Gardner *et al.*, 1995), oxidizes lipids and damages the DNA (Alscher *et al.*, 2002). Superoxide dismutase enzymes (SODs) represent the first line of defense against oxidative stress through the catalysis of O_2^- radical conversion into either O_2 or to less harmful hydrogen peroxide (H_2O_2) by adding or removing an electron from the O_2^- (Turrens, 2003; Fridovich, 1997) in a reaction requiring a metal ion in the enzyme's active site capable of changing its oxidation state in oxidation-reduction reaction.

There are three types of mammalian SODs classified according to their cellular localization, protein folding and the catalytically important bound metal cofactor. The cytosolic and extracellular SOD (SOD1 and SOD3, respectively) contain copper and zinc, while the mitochondrial (SOD2) contains manganese (Zelko *et al.*, 2002).

The general reaction outline can be summarized as follows:

$$M^{(n+1)+}\text{-SOD} + O_2^- \rightarrow M^{n+}\text{-SOD} + O_2$$
$$M^{n+}\text{-SOD} + O_2^- + 2H^+ \rightarrow M^{(n+1)+}\text{-SOD} + H_2O_2.$$

where M = Cu (n=1) ; Mn, or Ni (n=2)

Mammalian SOD2 is mitochondrial enzyme consists of a tetramer harboring one manganese in each monomer. Its presence in mitochondria protects the cell against the large amount of superoxide produced during the oxidative reactions in Kreps cycle. In case of low level of SOD2, the superoxide inactivates the aconitase enzyme of the TCA cycle, ceases the energy metabolism, and releases the potentially toxic iron from aconitase (Gardner *et al.*, 1995).

* Correspondence author: dibrahim@ksu.edu.sa
0030-9923/2015/0004-1015 $ 8.00/0

Abbreviations: MnSOD, manganese superoxide dismutase; TCA cycle, Tri Carboxylic acid cycle; ROS, reactive oxygen species.

Lacking SOD2 causes mice death several days after birth (Li *et al.*, 1995). Mutation and/or polymorphisms in SOD2 gene is associated with DNA damage (Van Remmen *et al.*, 2003), infertility (Mruk *et al.*, 2002), premature aging (Muller *et al.*, 2007), lipid peroxidation (Strassburger *et al.*, 2005), carotid atherosclerosis (Kakko *et al.*, 2003), and many other diseases. A new trend is emerging for using of SOD2 expression level as a biomarker for breast cancer prediction and prognosis but it is still under investigation (Becuwe *et al.*, 2014).

The one humped camel, *C. dromedarius* lives in harsh arid climatic conditions in the Arabian Gulf region and is continuously exposed to both endogenous and exogenous factors that necessitate powerful enzymatic and non-enzymatic antioxidant defense mechanisms capable of minimizing the impact of ROS. Camel is well adapted to such harsh desert life. So, it is proposed that it could have robust mechanisms for eliminating ROS. To the best of our knowledge, no study has been done on the molecular structure of the camel's *SOD2* gene. In this study, a molecular and modelling approach has been used to identify and predict the phylogeny and structure of camel's SOD2. Comparing camel's SOD2 sequence with well-studied enzymes from many organisms provides an important perspective on SOD2 diversity and potential in the cellular defense against high ROS levels in desert living animals.

MATERIALS AND METHODS

Tissue samples and materials

Liver tissues was collected from three male camels (2 years old), immediately after killing the animal in Western Riyadh slaughterhouse by skilled veterinarian. Tissues were submerged in RNAlater® solution (Qiagen, Ambion, Courtabeuf, France) to avoid RNA degradation and stored until used at −20°C. *E. coli* strains were used and grown in LB medium supplemented with 100 µg/mL ampicillin.

Oligonucleotide design

Primers for PCR were designed from the highly conserved regions of known *SOD2* genes available in the GenBank. The primers were Fwd: 5'-CGGGCGGCGTCCACCAT-3' and Rev:5'-GCTACATTCAGAAACGCTACAACA-3'.

Total RNA preparation and cDNA synthesis

Fifty mg of each tissue in RNAlater were homogenized in RTL lysis buffer (Qiagen) containing 1% 2-mercaptoethanol. Total RNA was extracted using AllPrep DNA/RNA Mini kit (Qiagen, Cat# 80204), following the manufacturers manual and the RNA was eluted with 50 µL nuclease free water. Quantification of the extracted RNA was determined using NanoDrop-8000 and its integrity was assessed by formaldehyde agarose gel (1%) electrophoresis. Two µg of the total RNAs were retrotranscribed into single stranded cDNA using ImProm-II Reverse Transcription System (Promega, Cat # A3800,) according to the manufacturer manual.

PCR amplification SOD2 gene and cloning

Gradient annealing temperature PCR was carried out from 50 to 60°C in a final volume of 50 µL as follow: 25 µL of GoTaq® Green Master Mix (Promega, Cat # M712c), 5 µL of cDNA, 3 µL of each forward and reverse primers (30 pmole) then the final volume was adjusted to 50 µL with nuclease free water. Initial denaturation was carried out at 95°C for 1 min followed by 40 repeated cycles of 94°C for 30 seconds, 50–60°C for 45 seconds and 68°C for 60 seconds. A final extension was carried out at 72°C for 5 min and cooling to 5°C. The amplified product was analyzed by electrophoresis using 1.2% agarose gel in TAE buffer.

The band of amplified DNA of the expected size was cut from the gel after electrophoresis and purified using Wizard SV and PCR Clean-up kit (Promega, Cat # A9282), then cloned into the pGEM®-T Easy vector (Promega, Cat # A1360). Ligation was performed by mixing the gel-purified PCR products (2 µL) with 1 µL pGEM-T Easy vector (50 ng) and 5 µL of 2× rapid ligation buffer. Finally, the reaction was initiated by the addition of 3 units of T4 DNA ligase enzyme. The final volume of the ligation reaction was adjusted to 10 µL by the addition of nuclease free water and the ligation mixture was incubated at 15°C for 16 h. Transformation of *E. coli* JM 109 competent cells was carried out according to Sambrook *et al*. (1989)

and the positive clones were screened in selective LB/IPTG/X-gal/Ampicillin/agar plates. Moreover, colony PCR was conducted to ensure the presence of the recombinant plasmid using the universal T7/SP6 multiple cloning site primers. A small part of each bacterial colony was transferred to a clean sterile Eppendorff tube containing 5µL nuclease free water, mixed well and the rest of the PCR reaction components was added as described earlier. The colony-PCR condition was as follows, 1 cycle at 95°C for 5 min followed by 30 cycles at 94°C for 1 min, 50°C for 1 min and 72°C for 2 min. The PCR products were analyzed by 1.2% agarose gel electrophoresis.

Sequencing of the PCR product and prediction of amino acid sequence

Sequencing of SOD2 gene cloned onto pGEM-T Easy vector was carried out using DNA Analyzer (Applied Biosystems) using chain termination sequencing reaction (Sanger *et al.*, 1977) and the T7 or SP6 primers. The emerged sequences from both directions were analyzed using the Seqman of the DNAstar PROGRAM (2003). The amino acid sequence was predicted by translating the open reading frame of the sequenced DNA and compared with sequences from NCBI Protein Database using the BLASTP algorithm (http://blast.ncbi.nlm.nih.gov/Blast).

Phylogenetic analysis and multiple sequence alignment of SOD2

The deduced amino acid sequence of camel SOD2 was used to identify similar sequences of other living organisms using the PSI-BLAST facility of the NCBI website (www.ncbi.nlm.nih.gov). Homologous sequences from 18 different organisms were aligned with camel SOD2 sequence by ClustalW and the phylogenetic tree for these sequences was built using BLOSUM62 (MAFT program 2011, Jalview program 2011) from MAFFT Multiple Sequence Alignment.

Prediction of the secondary structure of cSOD2, antigenicity and hydrophilicity

The secondary structure of camel SOD2 was predicted using PSIPRED program (2008). The subcellular localization was determined by ProtComp 9.0 program (http://linux1.softberry.com/berry.phtml?topic=protcompan&group=programs&subgroup=proloc). The antigenic determinant in the cSOD2 were predicted according to the methods of Kolaskar and Tongaonkar method (1990) and the antigenicity score of more than 1.0 for at least six amino acid residues was considered. The hydrophilicity of cSOD2 was calculated according to the method of Parker *et al*. (1986).

RESULTS AND DISCUSSION

cSOD2 gene

The full length *cSOD2* obtained by PCR-based technique showed a cDNA fragment of 789 bp (Fig. 1). Figure 2 shows the sequence of SOD2 gene. It covers the full coding region preceded by 15 bases from the 5'side and 105 bases after the stop codon. cSOD2 sequence was given accession number KR023951 in the GenBank database. The open reading frame consists of 666 bases which gives deduced amino acid sequence of 222 amino acid residues (Fig. 2).

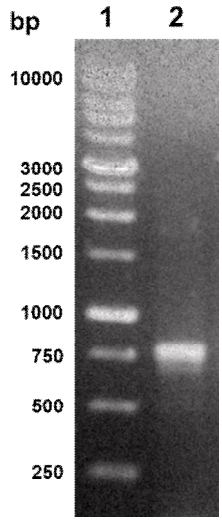

Fig. 1. Electrophoretic separation of the PCR product of camel's *SOD2* (Lane 2). Amplification was performed at 58°C annealing temperature. Lane 1 contains 1 kb ladder.

The high percentage of sequence identity between cSOD2 and other compared species

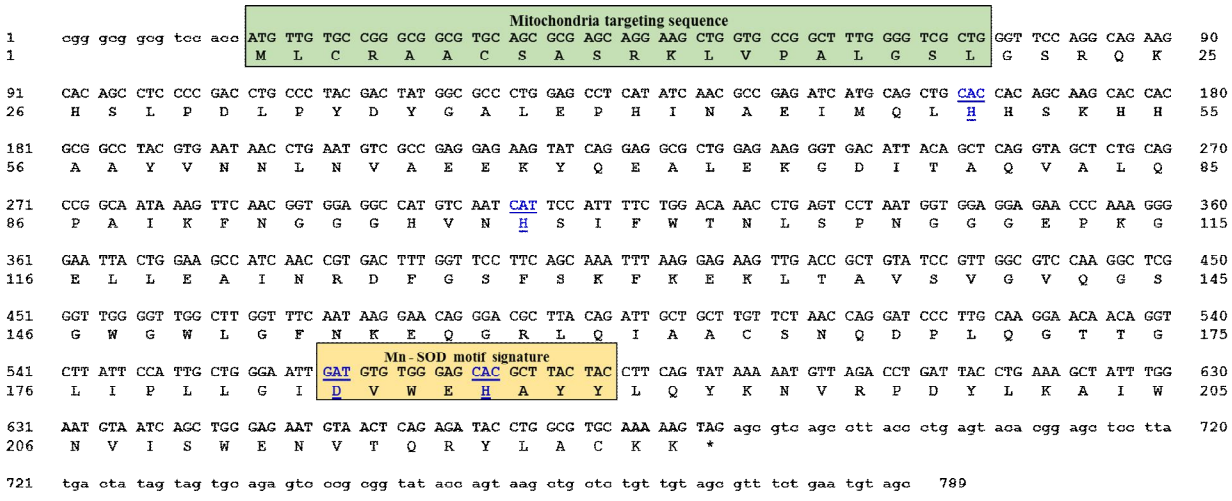

Fig. 2. The nucleotide and the deduced amino acids sequences of the cSOD2. The sequences were submitted to NCBI GenBank (accession number KR023951). The mitochondrial targeting sequence and the MnSOD motif signature were highlighted. The metal binding residues and their corresponding codons are labelled in blue colour.

indicate that the gene encoding this protein originates from common ancestor that maintains high conservancy during evolution (Figs. 3, 4). The Mn-SOD motif signature (DVWEHAYY starting from 186 to 193 on the graph) and the four manganese binding sites (H54, H102, D187 and H191 on the graph) are conserved in cSOD2 and in all compared organisms including mammals, amphibians, birds, fish and arthropods. The identification of both the metal-binding residues and the signature sequence support that our cMnSOD belongs to the MnSOD family.

SOD2 is a nuclear gene that is translated in the cytoplasm and exported to the mitochondria where it confers protection from ROS induced oxidative damage. The analysis of the cSOD2 aminoacid sequence using MitProt website (http://ihg.gsf.de/ihg/mitoprot.html) revealed the presence of putative N-terminal region of 20 aminoacids (MLCRAACSASRKLVPALGSL) that strongly supports a mitochondrial targeting with high probability (0.9907) (Claros and Vincens, 1996). Most mitochondrial MnSODs contain signal peptides which would be essential for translocation into the mitochondria (Fukuhara *et al.*, 2002). The multiple sequence alignment indicated that this sequence is poorly conserved in the compared sequences (Fig. 3). The first 20 amino acids of cSOD2 contains the major characteristics of the mitochondrial targeting sequence like the abundance of the positively charged (lysine and arginine) and the hydroxylated residues (mostly serine). These residues together with some hydrophobic residues form an amphipathic α-helices which is thought to be important for the translocation through the mitochondrial outer and inner membranes (von Heijne 1986; Roise and Schatz, 1988).

Amino acid composition and protein secondary structure

The bioinformatics analysis of the 222-amino acid sequence of cSOD2 using the program PROTEAN (2003) showed that it has a calculated molecular weight of 24.6 KDa and isoelectric point (pI) of 8.15. The predicted protein contains 64 charged amino acids (28.8%), 79 hydrophobic (35.6%), 20 acidic (9%), 22 basic (9.9%) and 59 polar amino acids (26.6%). The detailed amino acid analysis of the predicted protein is given in Table I.

Multiple sequence alignment

The PSI-protein blast comparison between the predicted amino acid sequence of cSOD2 and sequences from the GenBank of the best-characterized representatives of SOD2 from different organisms was carried out. The cSOD2

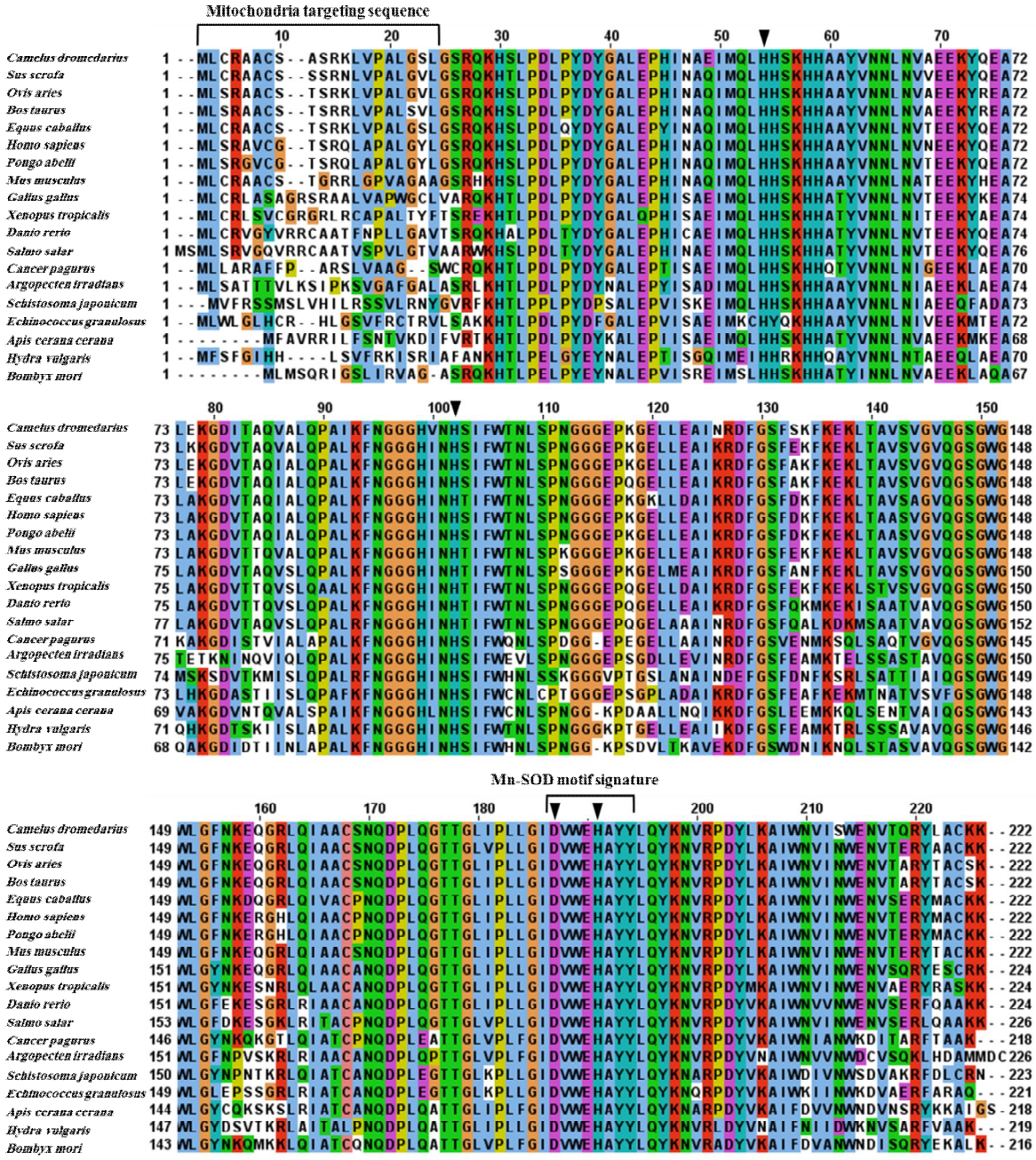

Fig. 3. Amino acid sequence alignment of cSOD2 with 18 different animal proteins using the MAFFT Multiple Sequence Alignment program. Residues are colored according to their conservancy. The mitochondrial targeting sequence and the MnSOD motif signature were marked and the Mn binding residues are labeled by (▼).

sequence was aligned with 18 different organisms belonging to different families in the animal kingdom using ClustalW (*MAFFT* program, version 6.864, 2008 and *Jalview*, version 2.3, 2011) (Fig. 3). The cSOD2 shared high similarity with SOD2 from many mammalian species. The highest identity was found with pig *S. scrofa*, cattle *B. taurus*, horse *E. caballus*, human *H. sapiens*, the monkey Sumatran orangutan *P. abelii*, and house mouse *M. musculus* (93-89%) (Table II, Fig. 3). Such high identity

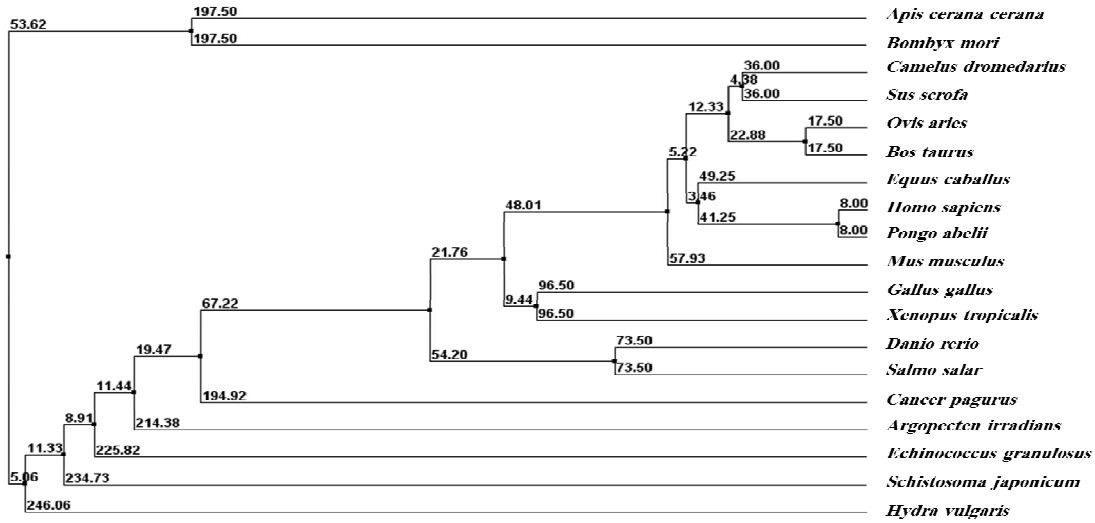

Fig. 4. The phylogeny of the cSOD2 and potentially related proteins. The amino acid sequence of cSOD1 was compared with 18 different sequences of many animal families from the GenBank database. The phylogenetic tree was generated with the BLOSUM62 from MAFFT Multiple Sequence Alignment.

Table I.- Predicted chemical composition of the camel's SOD2 using Protean program.

Amino Acid	Number count	% by weight	% by frequency
Ala (A)	20	5.78	9.01
Cys (C)	4	1.68	1.80
Asp (D)	7	3.28	3.15
Glu (E)	13	6.82	5.86
Phe (F)	6	3.59	2.70
Gly (G)	21	4.87	9.46
His (H)	9	5.02	4.05
Ile (I)	11	5.06	4.95
Lys (K)	15	7.82	6.76
Leu (L)	24	11.04	10.81
Met (M)	2	1.07	0.90
Asn (N)	14	6.49	6.31
Pro (P)	10	3.95	4.50
Gln (Q)	12	6.25	5.41
Arg (R)	7	4.44	3.15
Ser (S)	14	4.96	6.31
Thr (T)	6	2.47	2.70
Val (V)	12	4.84	5.41
Trp (W)	6	4.54	2.70
Tyr (Y)	9	5.97	4.05
Charged amino acids (RKHYCDE)	64	35.03	28.83
Acidic (DE)	20	10.10	9.01
Basic (KR)	22	12.26	9.91
Polar (NCQSTY)	59	27.82	26.58
Hydrophobic (AILFWV)	79	34.85	35.59

proposed a close evolutionary relationship which best figured by drawing the phylogenetic tree of the examined proteins. Figure 4 indicates that cSOD2 groups with *S. scrofa* and other mammals which took late evolutionary line different from birds, amphibians, fish and invertebrates.

The secondary structure of cSOD2 was predicted using PSIPRED program (2008) (Fig. 5) which indicated that the cSOD2 is composed of 10 major alpha helices and only 2 small β-sheets.

It was also predicted that most of the sequence of cSOD2 is hydrophilic and, as a result, it is highly antigenic as the majority of the protein surface is exposed to the aqueous medium. There are at least 8 potential antigenic peptides (Fig. 6) according to the method of Kolaskar and Tongaonkar (1990) http://emboss.bioinformatics.nl/cgi-bin/emboss/ antigenic. The predicted antigenic sequences and their positions are presented in Table III.

CONCLUSIONS

Camel's MnSOD share the same structure characteristics of mammalian SOD2. Its predicted amino acid sequence and the alignment with sequences from many candidates from the animal kingdom helped in building and determine the phylogenecity and antigenicity of the cSOD2.

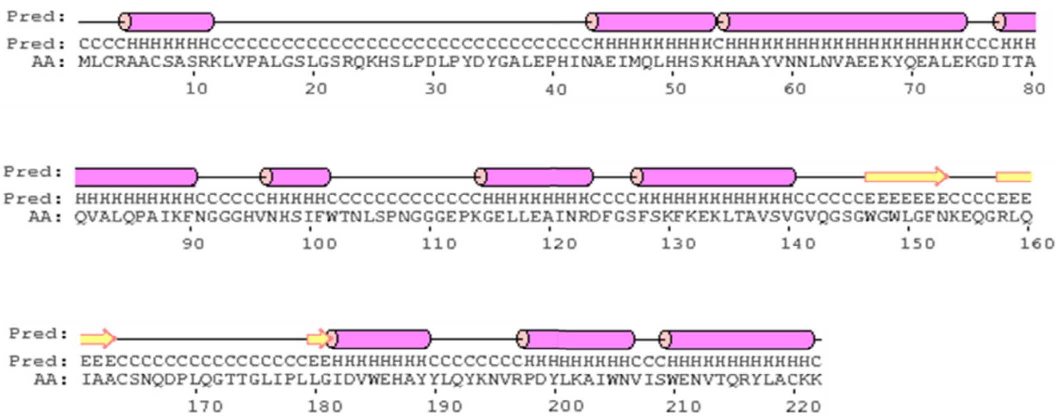

Fig. 5. The predicted secondary structure of the cSOD2 sequence using PSIPRED program. Pink cylinders indicated alpha helix and yellow arrows indicated β-sheets.

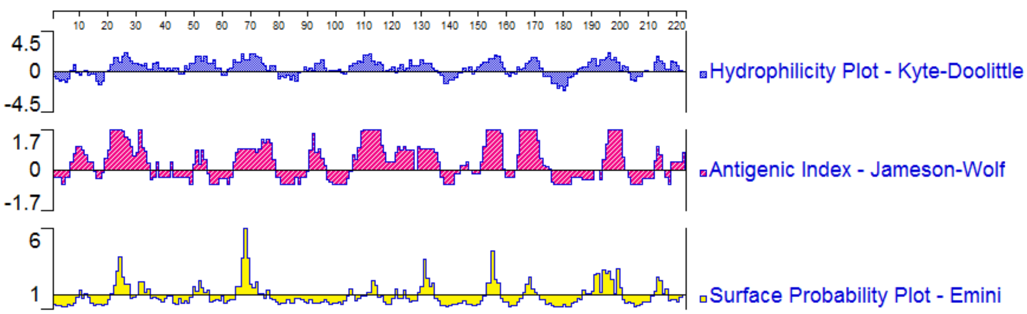

Fig. 6. Predicted hydrophilicity and antigenic properties of cSOD2 using the method of Parker *et al.*, (1986) and Kolaskar and Tongaonkar method (1990), respectively.

Table II.- Comparison of cSOD2 and other SOD2 enzymes from different organisms including mammals, birds, amphibians, fish, and invertebrates. The comparison included number of amino acid sequence, percent identity and E-value.

Organism	(NCBI Ref.)	a.a. Residues	Total score	Identity (%)	Positive (%)	Gap (residue)	E-Value
Camelus dromedarius	KR023951	222	---	100	100	0	---
Sus scrofa	NP_999292	222	440	93	97	0	9e-155
Bos taurus	NP_963285	222	435	92	96	0	7e-153
Equus caballus	NP_001075986	222	429	90	95	0	2e-150
Homo sapiens	CAA42066	222	424	90	94	0	1e-148
Pongo abelii	NP_001127035	222	424	89	93	0	2e-148
Mus musculus	NP_038699	222	422	89	92	0	8e-148
Gallus gallus	NP_989542	224	398	83	91	2	3e-138
Xenopus tropicalis	NP_001005694	224	382	79	88	2	8e-132
Danio rerio	NP_956270	226	380	78	88	6	5e-131
Salmo salar	ACN10263	226	370	76	86	6	3e-127
Cancer pagurus	CAR82596	218	325	70	81	3	2e-109
Schistosoma japonicum	AAW26480	223	307	62	78	1	2e-102
Apis cerana cerana	AET7406	218	299	70	82	1	3e-99
Bombyx mori	NP_001037299	216	291	67	80	1	5e-96
Hydra vulgaris	XP_002160626	219	292	67	81	0	2e-96

Table III.- The list of the predicted antigenic amino acid sequences and their positions.

No.	Sequence	Position		Score	Max. score pos.	Length
		Start	End			
1	KLTAVSVGVQ	134	143	1.159	140	10
2	RAACSASRKLVPALGSL	4	20	1.155	5	17
3	TAQVALQPAI	79	88	1.142	10	85
4	GLIPLLGIDVWEHAYYLQYK NVRPDYLKAIWNV	175	207	1.142	33	179
5	RLQIAACSNQ	158	167	1.138	10	162
6	MQLHHSKHHAAYVNNL	47	62	1.116	16	56
7	KHSLPDLPYDYGALEPH	25	41	1.095	17	30
8	GELLEA	115	120	1.042	6	118

ACKNOWLEDGMENTS

This research project was supported by a grant from the "Research Center of the Female Scientific and Medical Colleges", Deanship of Scientific Research, King Saud University.

REFERENCES

ALSCHER, R.G., ERTURK, N. AND HEATH, L.S., 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J. exp. Bot.*, **53:** 1331–1341.

ATAYA, F. S., AL-JAFARI, A. A., DAOUD, M.S., AL-HAZZANI, A.A., SHEHATA, A.I., SAEED, H.M. AND FOUAD, D., 2014. Genomics, phylogeny and in silico analysis of mitochondrial glutathione S-transferase-kappa from the camel *Camelus dromedarius*. *Res. Vet .Sci.*, **97:** 46-54.

ATAYA, F.S., FOUAD, D., AL-OLAYAN, E. AND MALIK, A., 2012. Molecular cloning, characterization and predicted structure of a putative copper-zinc SOD from the camel, *Camelus dromedarius*. *Int. J. mol. Sci.*, **13:** 879-900.

BECUWE, P., ENNEN, M., KLOTZ, R., BARBIEUX, C. AND GRANDEMANGE, S., 2014. Manganese superoxide dismutase in breast cancer: from molecular mechanisms of gene regulation to biological and clinical significance. *Free Radic. Biol. Med.*, **77:** 139-151

CLAROS, M.G. AND VINCENS, P., 1996. Computational method to predict mitochondrially imported proteins and their targeting sequences. *Eur. J. Biochem.*, **241:** 770-786.

FOUAD, D., 2015. Antioxidant and modulatory effect of melatonin on hepatotoxicity and oxidative stress induced by orange yellow s in male rats. *Pakistan J. Zool.*, **47:** *383-391.*

FRIDOVICH, I., 1997. Superoxide anion radical ($O_2^{\cdot-}$), superoxide dismutases, and related matters. *J. biol. Chem.*, **272:** 18515–18517.

FUKUHARA, R., TEZUKA, T. AND KAGEYAMA, T., 2002. Structure, molecular evolution, and expression of primate superoxide dismutases. *Gene*, **296:** 99e-109.

GARDNER, P.R., RAINERI, I., EPSTEIN, L.B. AND WHITE, C.W., 1995. Superoxide radical and iron modulate aconitase activity in mammalian cells. *J. biol. Chem.*, **270:** 13399–13405.

JALVIEW, 2011. version 2.3; University of Dundee: Scotland, UK.

KAKKO, S., PAIVANSALO, M., KOISTINEN, P., KESANIEMI, Y.A., KINNULA, V.L. AND SAVOLAINEN, M.J., 2003. The signal sequence polymorphism of the MnSOD gene is associated with the degree of carotid atherosclerosis. *Atherosclerosis*, **168:** 147–152.

KOLASKAR, A.S. AND TONGAONKAR, P.C., 1990. A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS Lett.*, **276:** 172–174.

LI, Y., HUANG, T.T., CARLSON, E.J., MELOV, S., URSELL, P.C., OLSON, J.L., NOBLE, L.J., YOSHIMURA, M.P., BERGER, C., CHAN, P.H., WALLACE, D.C. AND EPSTEIN, C.J., 1995. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat. Genet.*, **11:** 376–381.

MAFFT, 2011. version 6.864; Computational Biology Research Center (CBRC): Tokyo, Japan.

MRUK, D.D., SILVESTRINI, B., MO, M. AND CHENGA, C.Y., 2002. Antioxidant superoxide dismutase review: its function, regulation in the testis, and role in male fertility. *Contraception*, **65:** 305e-311.

MULLER, F.L., LUSTGARTEN, M.S., JANG, Y., RICHARDSON, A. AND VAN REMMEN, H., 2007. Trends in oxidative aging theories. *Free Radic. Biol. Med.*, **43:** 477–503.

PARKER, J.M., GUO, D. AND HODGES, R.S., 1986. New hydrophilicity scale derived from high-performance

liquid chromatography peptide retention data: Correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. *Biochemistry*, **25:** 5425–5432.

PROTEAN, 2003. version 5.07; DNASTAR, Inc.: Madison, WI, USA.

PSIPRED, 2008. version 3.0; University College London: London, UK.

ROISE, D. AND SCHATZ, G., 1988. Mitochondrial presequences. *J. biol. Chem.*, **263:** 4509-4511.

SAEED, H.M., ALANAZI, M.S., SHALABY, M.A., ALSHAHRANI, O., ATAYA, F.S., PATHAN, A.A. AND ABDULJALEEL, Z.A., 2014. Molecular cloning and cDNA characterization of Camelus dromedarius putative cytochrome P450s 1A, 2C, and 3A. *Genet. Mol. Res.*, **13:** 2886-2905.

SAMBROOK, J., FRITSCH, E. AND MANAIATIS, T., 1989. *Molecular cloning: a laboratory manual*. 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

SANGER, F., NICKLEN, S. AND COULSON, A.R., 1977. DNA sequencing with chain-terminating inhibitors. *Proc. natl. Acad. Sci. USA*, **74:** 5463–5467.

SEQMAN, 2003. version 5.07; DNASTAR, Inc. Madison, WI, USA.

STRASSBURGER, M., BLOCH, W., SULYOK, S., SCHULLER, J., KEIST, A.F., SCHMIDT, A., WENK, J., PETERS, T., WLASCHEK, M., KRIEG, T., HAFNER, M., KUMIN, A., WERNER, S, MULLER, W. AND SCHARFFETTER-KOCHANEK, K., 2005. Heterozygous deficiency of manganese superoxide dismutase results in severe lipid peroxidation and spontaneous apoptosis in murine myocardium in vivo, *Free Radic. Biol. Med.*, **38:** 1458–1470.

TURRENS, J.F., 2003. Mitochondrial formation of reactive oxygen species. *J. Physiol.*, **15:** 335–344.

VAN REMMEN, H., IKENO, Y., HAMILTON, M., PAHLAVANI, M., WOLF, N., THORPE, S.R., ALDERSON, N.L., BAYNES, J.W., EPSTEIN, C.J., HUANG, T.T., NELSON, J., STRONG, R. AND RICHARDSON, A., 2003. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol. Genomics*, **16:** 29–37.

VON HEIJNE, G., 1986. Mitochondrial targeting sequences may form amphiphilic helices. *EMBO J.*, **5:** 1335-1342.

ZELKO, I.N., MARIANI, T.J. AND FOLZ, R.J., 2002. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radic. Biol. Med.*, **33**: 337-349.

(Received 7 April 2015, revised 11 May 2015)