Full Length Research Paper

Molecular cloning and characterization of a putative OGG_N domain from the camel, *Camelus dromedarius*

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Reactive oxygen species (ROS) oxidize the guanine base in the DNA to 8-oxoguanine (8-oxoG). This lesion, if left unrepaired, causes the transversion of G:C pair to T:A following replication. 8-oxoG is targeted by one of the DNA glycosylases, namely OGG1. Arabian camel (one humped camel, *Camelus dromedarius*) is adapted to live in desert climate conditions under direct exposure to endogenous and exogenous ROS-producing conditions, among of them the sunlight. In the recent study, partial sequence of camel *OGG-1* gene was cloned and analyzed for the first time. A DNA fragment of 567 bases was amplified by reverse transcription PCR. It is equivalent to about 55% from the coding region of the known transcript of many organisms. The level of expression of *OGG-1* in different camel tissues (liver, kidney, spleen, lung and testis) was examined using real time-PCR. The highest level of *OGG-1* transcript was found in the camel liver (represented as 100%) followed by testis (85%), spleen (78%), kidney (37%) and lung (3%) using 18S ribosomal subunit as endogenous control. The obtained cDNA sequence of *OGG-1* showed high similarity with *Ailuropoda melanoleuca* (86%), *Sus scrofa* (86%), *Canis familiaris* (85%), *Bos taurus* (85%), *Macaca mulatta* (85%), *Homo sapiens* (84%), *Pan troglodytes* (84%) and *Pongo abelii* (82%).

Key words: Camelus dromedarius, cloning, OGG1, gene expression, DNA glycosylase.

INTRODUCTION

Domesticated Arabian camel (one-humped camel, *Camelus dromedarius*) is the most important animal in the Arabian desert, as it represents the main source of meat and milk, and for its high cultural and economical values. This animal, like other living organisms, is continuously exposed to deleterious endogenous and exogenous factors that if not treated properly, may result in mutations and cell death. The most life-threatening compounds are the damaging reactive oxygen species (ROS). They affect mainly the DNA causing the

ROS induce the production of various kinds of oxidative DNA damage. The most affected is the guanine base

Abbreviations: ROS, Reactive oxygen species; **8-oxoG,** 8-oxoguanine.

generation of oxidized bases with high frequency. The presence of such abnormal bases makes gross DNA alterations, for example, single- and double-strand breaks and DNA base modifications. Under normal physiological conditions the mammalian DNA is damaged between 10⁴ and 10⁵ times per cell daily and this number can be increased substantially by stress (Mullart et al., 1999; Lindahl 1993). If these DNA lesions are not repaired correctly, the cell may die or be subjected to genomic instability, which may lead to aging and initiation or acceleration of the carcinogenic process through mutations in genes controlling these biological processes (Slupphaug et al., 1995; Jaiswal et al.,1998; Pearl, (2000).

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which is oxidized to 8-oxoguanine (8-oxoG). This lesion is a potent mutagen and can generates transversions of G:C pair to T:A, since 8oxoG in DNA allows the incurporation of adenine as well as cytosine opposite the lesion (Moriya et al., 1991; Shibutani et al., 1991). It can be formed spontaneously in the genomic DNA of aerobic organisms since ROS are constantly generated *in vivo* as byproducts of respiration and normal metabolism and/or by the action of ionizing radiation, chemical pollutions, heavy metals, etc. (Marnett, 2000; Floyd et al., 1986).

The damaged DNA is repaired by a number of enzymes that act sequentially to remove damaged bases and replace them with regular ones through the base excision repair (BER) pathway which involves the catalytic activity of DNA glycosylase enzymes (Krokan et al., 2000). DNA glycosylases fall into two major classes, which differ with respect to catalytic mechanism; monofunctional and bifunctional glycosylases. The first one removes the damaged base, generating an abasic site which can be converted to a single-strand break by apurinic/apyrimidinic (AP)—endonuclease, while the second has lyase activity besides its glycosylase activity; hence the name bifunctional glycosylases (Ide and Kotera, 2004).

Despite the obvious structural similarity of 8-oxoG to the vastly more abundant G, 8-oxoG is recognized specifically and efficiently by 8-oxoG DNA glycosylases (OGG1). It or its analogue has been proposed to be implicated in repair of ultraviolet A (UVA)-induced DNA lesions (Dahle et al., 2008). It catalyses the removal of 8oxoG through its glycosylase activity and cleaves the DNA sugar backbone through its lyase activity. This incision generates a normal 3'-hydroxyl group and an abasic deoxyribose-5-phosphate, which is processed subsequently by β-polymerase and DNA ligase and several accessory proteins (Roldan-Arjona et al., 1997; Rosenquist et al., 1997). OGG1 genetic polymorphisms and altered gene expressions and/or enzyme activities are associated with oxidative DNA damage (Jensen et al., 2012). Many contradictory results are published regarding the association between OGG1 Ser326Cys polymorphism and the increased risk of breast, colorectal and lung cancer (Gu et al., 2010; Yuan et al., 2010; Li et al., 2011; Zhang et al., 2011).

The sequence of OGG-1 gene and the predicted amino acid sequence are highly conserved from Saccharomyces cerevisiae to humans (Demple and Harrisson, 1994; Hoeijmakers, 1993; Aspinwall et al., 1997). We have used sequence homology of the highly conserved regions as a criterion to identify potential human homolog of the camel OGG-1 gene through the preparation of primers from such conserved regions. Generally, the study of DNA repair genes has facilitated the understanding of carcinogenicity and cancer susceptibility in man (Kolodner, 1995). Therefore, the identification of OGG-1 homolog in camel may provide valuable information on the etiologies of degenerative

diseases in higher organisms exposed to ROS produced by exposure to natural sunlight for long time.

The Arabian camel spends most of its life in direct exposure to sunlight, and to many other factors which end to the production of ROS. So, it is proposed that camel could have robust mechanisms for repairing oxidized DNA lesions. To date, no researches have been done on camel to show how it can resist the direct sun exposure and how it can manipulate supposed UVA-induced DNA lesions.

In this recent study, we sequenced and cloned a partial coding region of *OGG-1* and we studied its expression on the level of the transcript by qPCR in order to identify the tissue of highest *OGG-1* expression which reflects the tissue most affected by DNA damaging agents.

MATERIALS AND METHODS

Unless otherwise stated, all *Escherichia coli* strains were grown in Luria Bertani (LB) medium supplemented with 100 µg/ml ampicillin. Camel tissues were obtained from three different two-year old male adult camel, immediately after killing the animal in Southern Riyadh Main Slaughterhouse. Tissue samples were immediately submerged in RNAlater® solution (Qiagen, Ambion, Courtabeuf, France) to avoid RNA degradation, stored at -20°C till use.

Oligonucleotide design

Two primers were designed from the highly conserved regions of known *OGG-1* genes available in the gene bank. These primers are named OGF (forward, 5'- CATCCCGTGCCCTCGCTCTGA-3') and OGR (reverse, 5'- ATGGCTCGGGCACTGGCACTCA-3'), respectively. These primers were used in RT-PCR for amplification of *OGG-1* cDNA fragment. On the other hand, two new primers were designed to amplify 194 bp for qPCR namely OG1qF 5'-CAACATTGTCCGTATCACTGG-3' and OG1qR 5'-GCTCGGGCACTGGCACTCACG-3', for the forward and reverse respectively.

DNA/RNA extraction and cDNA synthesis

Fifty mg of liver, kidney, spleen, lung or testis in RNAlater were homogenized in radial transmission line (RTL) lysis buffer (Qiagen, Ambion, Courtabeuf, France) supplemented with 1% 2-mercaptoethanol. Genomic DNA and total RNA was extracted using AllPrep DNA/RNA Mini kit (Qiagen, Ambion, Courtabeuf, France) according to the manufacturer's instruction. Elution was performed with 50 μl nuclease free water. Concentrations and integrity of RNA samples were assessed using NanoDrop-8000 and formaldehyde agarose gel (1%) electrophoresis. Two micrograms of the total RNAs were retrotranscribed in single stranded cDNA using ImProm-II Reverse Transcription System (Promega Corporation, Madison, Wisconsin, USA) as recommended by the manufacturer, with the following cycling conditions: 96°C for 1 min, then 40 cycles at 94°C for 30 s, 65°C for 30 s, and 72°C for 1 min.

PCR and cloning

Gradient PCR was carried out at annealing temperatures ranged from 50 to 60°C in a final volume of 50 μ I as follow: 25 μ I of FideliTaqTM master mix (USB Corporation, Cleveland, Ohio, USA) 5

µI of either genomic DNA or c-DNA, 3 μI of each forward and reverse primers (30 pmoles) then the final volume was adjusted to 50 μI with nuclease free water. The PCR condition was 1 cycle at 95°C for 45 s followed by 40 cycles at 94°C for 30 s, 50 to 60°C for 45 s and 68°C for 1 min. Final extension was carried out at 72°C for 5 min. The PCR products were analyzed using 1.5% agarose gel by electrophoresis in Tris-acetate-EDTA (TAE) buffer.

The selected PCR fragment of the expected size was cut from the agarose gel after electrophoretic separation and purified using QiAquick gel extraction (Qiagen, Ambion, Courtabeuf, France) then cloned into the pGEM®-T Easy vector (Promega Corporation, Madison, Wisconsin, USA). To ligate the purified PCR products onto pGEM-T vector, 2 µl of each purified PCR products were taken in a clean 0.5 ml tube to which 1 µl pGEM-T- Easy vector (50 ng) and 5 µl of 2X rapid ligation buffer were added followed 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. 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). The recombinant E. coli harboring the recombinant plasmid was screened in selective LB/IPTG/X-gal/Ampicillin/agar plates. Moreover, colonies PCR was conducted to screen recombinant bacteria for ligated DNA insert using T7/SP6 primers.

Studying gene expression by qPCR

The expression of OGG-1 transcripts was studied by real-time quantitative PCR (qPCR). The reaction was performed three times, each contained 10 times diluted cDNA from camel liver, kidney, spleen, lung or testis, 5 pmoles each OG1qF and OG1qR primers and 10 μ I Fast-SYBR Green qPCR Master Mix (Applied Biosystems, Foster city, California, USA) in a final 20 μ I reaction volume as recommended by the manufacturer. The qPCR was performed using Applied Biosystems 7500 Fast real-time PCR system. The following standard conditions was used, initial denaturation at 95°C for 30 s and 60°C for 30 s. The amplified product from these amplification parameters was subjected to SYBR Green I melting analysis by increasing the temperature to 95°C for 15 s followed by 60°C for 1 min and ramping the temperature of the reaction samples from 60 to 95°C.

Sequencing of the PCR products and sequence analyses

Sequencing of the PCR product cloned onto pGEM-T- Easy vector was carried out according to Sanger et al. (1977) using MegaBACE 1000 DNA Sequencing System (Amersham Pharmacia Biotech, Inc., New Jersey, USA). The chain termination sequencing reaction was conducted utilizing the DYEnamic ET terminator kit mix (USB Corporation, Cleveland, Ohio, USA) as an integral part of the MegaBACE 1000 DNA sequencing system. The reaction mixture contained 8 µl DYEnamic ET terminator sequencing premix, 3 µl T7 or SP6 primers and 500-700 ng plasmid and the final volume was adjusted to 20 µl by DNAse-free water. The chain termination PCR condition was as follow; 1 cycle at 95°C for 30 s followed by 30 cycles at 94°C for 30 s, 50°C for 30 s and 60°C for 1 min. The chain termination PCR products were purified using DyeEx 2.0 Spin Kit (GE Healthcare UK Limited. UK), dried by speed vacuum centrifuge and dissolved in 10 µl MegaBACE loading buffer, then applied to MegaBace 1000 Sequencing machine.

The nucleotide sequences were determined in both directions and analyzed using the Genetics Computer Group (GCG, Madison, WI, USA), BioEdit and DNASTAR programs. The prediction

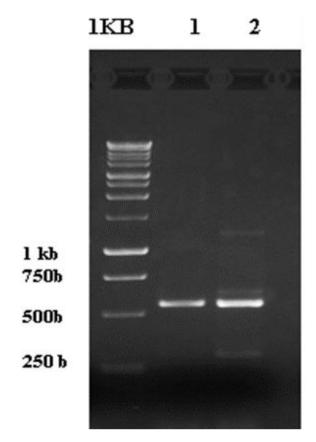


Figure 1. Agarose gel (1.2%) electrophoresis of RT–PCR products of *OGG-1* gene using OGF/OGR primers + cDNA (lane 1) or genomic DNA (lane 2), respectively.

of the secondary structure was done using the PSIPRED protein structure prediction server. The deduced amino acid sequence was compared with sequences obtained from searches in the NCBI Protein Database using the basic local alignment search tool program (BLASTP) algorithm (Altschul et al., 1990). The amino acid sequences were aligned and converted to a phylogenetic tree using MegAlign of the DNASTAR package.

RESULTS

A cDNA fragment of 567 bp was amplified by reverse transcription PCR (Figure 1) using OGF and OGR primers designed from the highly conserved regions of *OGG-1* sequences as previously explained. The optimum annealing temperature was at 58°C. This fragment represents about 55% of the coding region comparing with the corresponding regions from different organisms (~1035 bp). Our sequence was submitted in the gene bank with the accession number HM369806 http://www.ncbi.nlm.nih.gov/nuccore/HM369806.1. This cDNA fragment encodes part of the OGG1 enzyme of 188 amino acids (Figure 2) which forms the OGG_N domain (accession number ADK35367.1).

1	CCG	TGC	CCT	CGC	TCT	GAG	CTG	CGC	CTG	GAC	CTG	ATT	CTG	GCT	TCT	45
	P	C	P	R	S	E	L	R	L	D	L	I	L	A	S	15
46	GGA	CAG	TCT	TTC	CCG	TgG	ATG	GAG	CAA	AGC	CCT	GCG	CGT	GGG	AGT	90
16	G	Q	S	F	P	W	M	E	Q	S	P	A	R	G	S	30
91	GGC	g t g	CTG	GCG	GAC	CAG	GTA	TGG	ACA	CTG	ACG	CAG	ACT	GAG	GAA	135
31	G	V	L	A	D	Q	V	W	T	L	T	Q	T	E	E	45
136	CAG	CTC	TAC	TGC	ACT	g t g	TAC	CGA	GGG	GAC	AAG	GGC	CAG	G TT	GGC	180
46	Q	L	Y	C	T	V	Y	R	G	D	K	G	Q	V	G	60
181	AAA	GCC	ACA	CCA	GAA	GAG	CTA	AAG	GCC	ATG	CGA	CAG	TAC	TTC	CAG	225
61	K	A	T	P	E	E	L	K	A	M	R	Q	Y	F	Q	75
226	CTG	ATT	g t c	AGC	CTG	GCT	CAA	CTG	TAT	CAC	CAT	TGG	AGT	TCC	ATG	270
76	L	I	V	S	L	A	Q	L	Y	H	H	W	S	S	M	90
271	GAC	CCC	CAC	TTT	CAA	GAG	g t g	GCT	CAG	AAA	TTC	CAA	GGT	g t g	GGA	315
91	D	P	H	F	Q	E	V	A	Q	K	F	Q	G	V	G	105
316	CTC	TTG	CAA	CAG	GAC	CCC	ATC	GAA	TGC	C TT	TTC	TCC	TTC	ATC	TGT	360
106	L	L	Q	Q	D		I	E	C	L	F	S	F	I	C	120
361	TCC	TcC	CAC	AAC	AAC	ATT	g t c	CGT	ATC	ACT	GGC	ATG	g t g	GAG	CGG	405
121	S	S	H	N	N	I	V	R	I	T	G	M	V	E	R	135
406 136	CTC L	TGC C	CAG Q		TTC F			CGG R	CTC L	ATC I	CAG Q	CTT L	GAT D	GAT D	g t c V	450 150
451	ACC	TAC	TAT	GGC	TTC	CCC	AGC	CTG	CAG	GCA	CTG	GCT	GGG	CCG	GAG	495
151	T	Y	Y	G	F	P	S	L	Q	A	L	A	G	P	E	165
496 166																
541 181	TAC Y	g t g V	AGT S	GCC A	AGT S	GCC A	CGA R	GCC A	5	64						

Figure 2. The nucleotide sequence and the deduced amino acids of the cloned fragment of camel *OGG-1*. The sequence was submitted to NCBI GeneBank (accession number HM369806.1 and ADK35367.1).

The comparison between the predicted amino acid sequence of OGG1 and the sequences of OGG1 from different organisms indicated the percentage of similarity with *C. dromedarius* was 86% for panda (Ailuropoda melanoleuca), 86% for pig (Sus scrofa), 85% for dog (Canis lopus familiaris), 85% for cow (Bos taurus), 85%

for Rhesus monkey (Macaca mulatta), 84% for human (Homo sapiens), 84% for chimpanzee (Pan troglodytes) and 82% for orangutan (Pongo abelii) (Table 1). The alignment of amino acid sequences from different organisms is shown in Figure 3.

The amino acid sequences of camel OGG-1 and other

Table 1. Homology of the translated *OGG1* gene of 9 species with the deduced amino acid sequence of *C. dromedarius OGG1*.

Animal	Scientific name	Accession #	Percentage identity
Panda	Ailuropoda melanoleuca	EFB29741.1	86
Pig	Sus scrofa	XP_001928227.3	86
Dog	Canis lopus familiaris	XP_541781.2	85
Cow	Bos taurus	NP_001073754.2	85
Rhesus monkey	Macaca mulatta	XP_001096322.1	85
Human	Homo sapiens	1N3C_A	84
Chimpanzee	Pan troglodytes	XP_003309654.1	84
Orangutan	Pongo abelii	XP_002813520.1	82

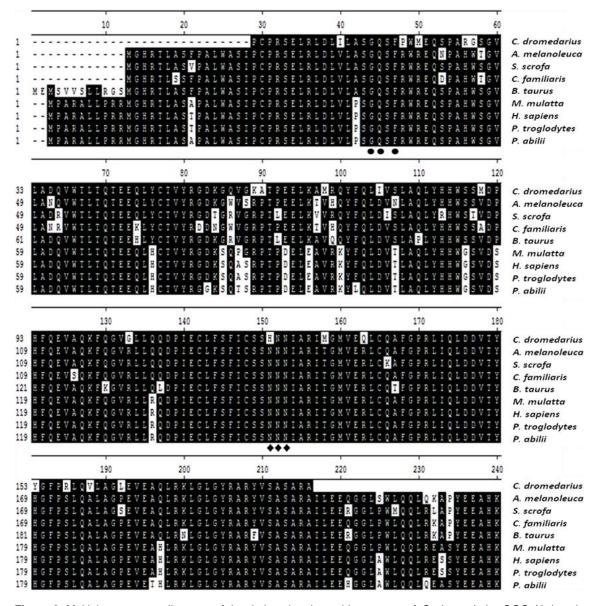


Figure 3. Multiple sequence alignment of the deduced amino acid sequence of *C. dromedarius* OGG_N domain (accession number ADK35367.1) with other similar species from the NCBI database. The alignment was generated with the MegAlign program. The three conserved amino acids which form part of the pocket are marked (●) and the NNN motif labeled (★).

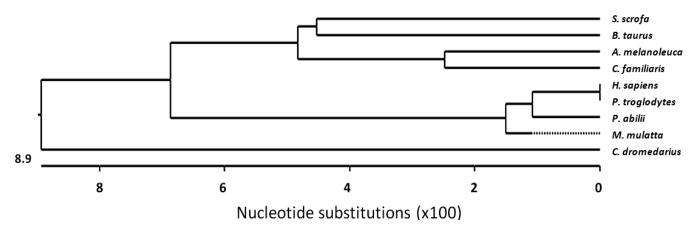


Figure 4. The phylogenetic tree of *C. dromedarius* OGG_N domain region (accession number ADK35367.1) and potentially related proteins. The deduced amino acid sequence of Camel *OGG-1* was compared with other sequences of the GenBankTM data base. The alignment was generated with the MegAlign program.

Table 2. Predicted structure analysis of the cloned fragment of *OGG-1* camel liver using Protean Program.

Parameter	Value
Molecular weight	21.244
Length	188
I microgram =	47.07
Molar extinction coefficient	27910 ± 5%
1 A (280 nm)	0.76 mg/ml
Charged amino acids (RKHYCDE)	52
Acidic amino acids (DE)	18
Basic amino acids (KR)	17
Polar amino acids (NCQSTY)	53
Hydrophobic amino acids (AILFWV)	68

eight mammalian *OGG-1* enzymes were used to construct phylogenetic tree using MegAlign of the DNASTAR package (Figure 4). We found that *OGG-1* from human, chimpanzee, orangutan and Rhesus monkey are grouped together, while panda and dog, pig and cow are grouped in form other two branches, respectively. Surprisingly, camel *OGG-1* takes a different evolutionary line from all the above examined species.

The molecular analysis of the 188-amino acid sequence of camel *OGG-1* using the program Protean showed that this protein contains 52 charged amino acid (27.66%), 68 hydrophobic (36.17%), 18 acidic (9.6%), 17 basis (9%) and 53 polar amino acids (28.25%) (Table 2). This fragment is composed of eight alpha helices and five beta sheets in the same manner of the human OGG1 (Figure 5).

The level of OGG-1 expression

The level of expression of OGG-1 in different camel

tissues (liver, kidney, spleen, lung and testis) is examined using qPCR. The primers were designed to amplify 192 base pairs and the experiment condition was adjusted to give only one band and to eliminate the primer dimer, self dimer or hairpin form. The expression of *OGG-1* in liver was taken as reference sample (calibrator) and the expression of camel 18S ribosomal subunit as house-keeping gene (endogenous control). The relative expressions of *OGG-1* in kidney, spleen, lung and testis were compared with that of the liver. The highest expression level was found in liver (represented as 100%) followed by testis (85%), spleen (78%), kidney (37%) and lung (3%) (Figure 6).

DISCUSSION

Arabian camel is the most important animal in the Middle East. Despite its economic and cultural importance, very little biochemical researches are done to elucidate how it can survive in the desert's harsh conditions. Studying the DNA repair genes of C. dromedarius is essential for understanding the impact of exposure to direct sunlight and desert life on the health status of such mammal. 8oxoG is a premutagenic lesion produced by exposure to ROS. It is targeted by OGG1, a bifunctional DNA glycosylase belonging to base excision repair enzymes. OGG1 catalyses the removal of 8-oxoG through its glycosylase activity (EC: 3.2.2.23) and cleaves the DNA sugar backbone through its lyase activity (EC:4.2.99.18) (Roldan-Arjona et al., 1997; Rosenquist et al., 1997). The structure and function of OGG1 is studied in many eukaryotic and prokaryotic organisms. It is composed of two domains; the OGG N in the N terminal and the ENDO3c domain. The OGG N domain contributes to the 8-oxoguanine binding pocket in the enzyme. The ENDO3c domain exists in many DNA repair enzymes

Homo sapiens OGG1

Camelus dromedarius OGG1

20

60

100

180

10

O

130

Conf: }

Pred: CCCCCHHHHHHHHCCCCCHHHHHHHHHHHHC

170

AA: LAGPEVEAQLRKLGLGYRARYVSASARA

- helix Conf:]]] [- confidence of prediction

Pred:

Pred:

Pred:

Legend:

Conf: } Pred: CCCCCCCHHHHHHHCCCCCCCEEEECCCCEEEEE Pred: CCCCCCCCCCCCCCCCEECCEEECCEEEEE AA: PCPRSELRLDLILASGQSFPWMEQSPARGSGVLADQVWTL AA: PCPRSELRLDLVLPSGQSFRWREQSPAHWSGVLADQVWTL 30 10 20 30 40 Conf: } Pred: Pred: EECCCEEEEEEECCCCCCCCCCHHHHHHHHHHHHCCCCCH Pred: EECCCEEEEEECCCCCCCCCCCHHHHHHHHHHHHCCCCCH AA: TQTEEQLYCTVYRGDKGQVGKATPEELKAMRQYFQLIVSL AA: TQTEEQLHCTVYRGDKSQASRPTPDELEAVRKYFQLDVTL 50 60 80 Conf: } Conf: } Pred: AA: AQLYHHWSSMOPHFQEVAQKFQGVGLLQQDPIECLFSFIC AA: AQLYHHWGSVDSHFQEVAQKFQGVRLLRQDPIECLFSFIC 90 100 110 120 Pred: AA: SSHNNIVRITGMVERLCQAFGPRLIQLDDVTYYGFPSLQA AA: SSNNNIARITGMVERLCQAFGPRLIQLDDVTYHGFPSLQA 130 140 150 160 Pred: СССССНИННИНССССИННИНИННИН AA: LAGPEVEAHLRKLGLGYRARYVSASARA 170 180

- strand

Figure 5. The secondary structure annotation sites of the partial OGG-1 sequence of C. dromedarius.

including endonuclease III (DNA-apurinic or apyrimidinic site lyase), alkylbase DNA glycosidases (Alka-family) and other DNA glycosidases (reviewed Bruner et al., 2000).

This present study is the first work to clone and study the chemical composition of the OGG_N domain of the one-humped camel OGG-1 gene. Our results show amplification of a cDNA fragment of 567 bp using a primer set spanning this DNA fragment (Figure 1). The sequencing indicated that this part covers the highly conserved N-terminal domain belonging to OGG-N super family [cl06806] and part of the ENDO3c domain of the known DNA glycosylases.

The comparison between the predicted amino acid sequence of the obtained OGG1 fragment and the sequences of OGG1 from different organisms indicated that the largest identity was found with OGG1 of A. melanoleuca (86%), S. scrofa (86%), C. familiaris (85%), B. taurus (85%), M. mulatta (85%), H. sapiens (84%), P. troglodytes (84%) and Pongo abelii (82%). The alignment of amino acid sequences from different organisms is shown in Figure 3. To date, the three dimensional structure of human OGG1 is the only resolved structure among the previously listed enzymes. Hence we compared the camel OGG_N domain with the human one. The mechanism by which the OGG1 localizes the oxidized guanine (8-oxoG) in the midst of the vast genome containing a huge excess of undamaged guanine bases is an area of interest of many scientists. OGG_N domain contributes to a 8-OxoG pocket on the OGG1 enzyme. The cloned part of the camel OGG1 has three amino acids of the six residues that form that pocket of human OGG1. These residues are Gly42, Gln43, Phe45, which form with Cys253, Gln315 and Phe319 the pocket of 8-oxoG binding on the human OGG1. Gly 42, Gln43 and Phe45 interact with the major groove edge recognizing the protonated N7 of the 8oxoG, whilst the Phe319 and Cys253 pack against opposite faces of 8oxoG (Bjroset al., 2002). Camel OGG1

Pred: predicted secondary structure

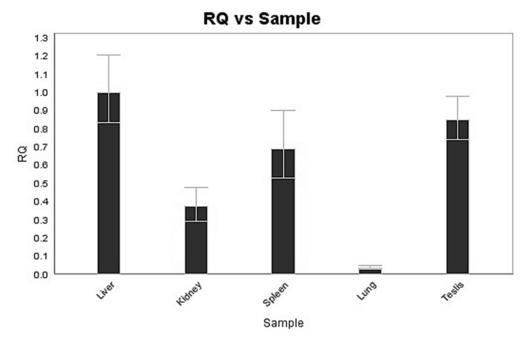


Figure 6. Expression of *OGG-1* using Real time PCR and cDNA from different camel tissues. The results are expressed relative to liver as calibrator and using 18S ribosomal subunit as housekeeping gene

has the HNN motif in positions 149 to 151. This motif differs from hOGG1 that has asparagine in position 149. not histidine. The codon that produces H in camel is CAC while the corresponding codon in human and other compared species is AAC. It seems that this very important codon had changed in the early evolution of camel and it could affect the activity and the structure of the pocket of 8-oxoG binding on the OGG1 enzyme. The function of this motif is to fill the space in the duplex by forming a hydrogen bond through the protruded sidechain amide carbonyl of N149 with the exocyclic N4 amino group of the estranged cytosine base on the distal strand (Bruner et al., 2000; Norman et al., 2003). The Asn residues 150 and 151 form hydrogen bonds back to the protein in the DNA complex and stabilize the cytosinerecognition motif. These two Asn are directed away from the body of the protein. The position occupied around residues 149 and 150 overlaps the site occupied by the phosphodiester backbone on the 5' side of 80xoG in the DNA-bound structure (Bjr-set al., 2002).

The predicted secondary structure of the OGG1 fragment from *C. dromedarius* gave a profile similar to the corresponding region from *H. sapiens*. The only difference is the possibility of formation of a small helix in camel OGG1 by the amino acids 9 to 14. The expression of camel *OGG-1* is studied in different tissues using qPCR technique. Our findings suggest that *OGG-1* is highly expressed in liver and testis. The high level of expression in liver is expected where most of the metabolic processes are performed with the possibility of high ROS production. Also it is important to be present in

the testis and other tissues of active cell division to repair any possible base lesion in the produced germ cells. The activity of *OGG-1* might reflect a link between *OGG-1* and maintenance of cellular integrity in such tissues.

Conclusion

The cloned partial coding region of *OGG-1* represents the first trial of cloning camel *OGG-1*. The expression is higher in the liver and testis where most of the metabolic processes and active cell division are performed with the possibility of high ROS production.

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