Molecular cloning and cDNA characterization of *Camelus dromedarius* putative cytochrome P450s 1A, 2C, and 3A

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**ABSTRACT.** The domesticated one-humped Arabian camel, *Camelus dromedarius*, is one of the most important animals in the Arabian Peninsula. Most of its life, this animal is exposed to both intrinsic and extrinsic genotoxic factors that are known to cause gross metabolic alterations in many organisms. This study determined the full length coding sequence of 3 cytochrome P450s cDNAs; namely, CYP450 1A1, CYP450 2C and CYP450 3A using reverse transcription polymerase chain reaction. The *C. dromedarius* CYP450s 1A1, 2C, and 3A have open reading frames of 1563, 1473, and 1566 bp and cDNAs that encode proteins of 520, 490, and 521 amino acid residues, respectively. The molecular weights
calculated for CYP1A1, 2C, and 3A were found to be 58.651, 56.03, and 58.594 kDa, while the predicted calculated isoelectric points using a computer algorithm were 7.315, 6.579, and 9.46. The deduced amino acid sequences of these CYPs showed the membrane anchored signal peptide, the conserved proline-rich amino terminus and the characteristic heme-binding signature localized near the carboxy terminus of the protein.

**Key words:** Arabian camel; *Camelus dromedarius*; Cytochrome P450; Xenobiotics; Reverse transcription polymerase chain reaction; Mixed-functions oxidases

**INTRODUCTION**

The Arabian one-humped camel (*Camelus dromedarius*) belongs to the family Camelidae, which has 6 camel-like animals (1-hump and 2-hump camels, llamas, alpaca, guanaco, and vicuna). This camel is found in the Arabian deserts and arid and semi-arid areas of the Middle East (Thomas et al., 1985; Hussein et al., 1992; Al-Khedhairy, 2004). The Arabian camel has unique physiological modifications and is able to live in harsh desert conditions, such as high temperature, direct exposure to sunlight and lack of water resources, with this animal being able to survive without drinking water for weeks (Hussein et al., 1992).

While there has been an increasing number of studies on camels in recent years, pharmacological and biomolecular research of camels remains limited. The Arabian camel genome consists of 74 chromosomes. The DNA sequence of camels exhibits wide variations with other species, including human; however, functional similarities exist. Therefore, studies of camel protein represent a potentially viable target for human applications (Al-Khedhairy, 2004).

Like other living organisms, the Arabian camel is continuously exposed to intrinsic and extrinsic agents that, if not treated properly, may result in mutation and cell death. Many enzymes are involved in the clearance of such compounds such as Phase I and Phase II drug metabolizing enzymes. The clearance of xenobiotics involves both activation (Phase I) and detoxification (Phase II) reactions. Cytochrome P450 monoxygenases (CYPs or P450) are heme-containing proteins belonging to Phase I drug metabolizing enzymes. These enzymes use molecular oxygen and the hydride donor, NADPH, to effect the overall oxidative insertion of one oxygen atom into the substrate (Jung et al., 2011; Nelson, 2011). Cytochrome P450s are involved in the metabolism of a broad range of substrates, and catalyze a variety of chemical reactions, including the conversion of both endogenous and exogenous compounds to more soluble hydrophilic metabolites that are easily removed from the body (Nelson and Strobel, 1987; Nelson et al., 1993). Cytochrome P450s are mixed-functions oxidases that represent one of the body’s most important defenses against chemical-induced toxicity. However, in some situations, the metabolism of some chemicals by P450s is undesirable, and may lead to toxic or reactive intermediates, resulting in target organ toxicity and/or carcinogenic insult (Rooney et al., 2004; Nebert and Dalton, 2006; Stiborova et al., 2011).

Most CYP enzymes are expressed constitutively in a variety of mammalian tissues, such as liver, kidney, lung, adrenal gland, and gonads (Seliskar and Rozman, 2007). Many other CYPs are inducible enzymes, and their expression levels increase markedly when exposed to various chemicals, such as ethanol and polychlorinated dibenzo-p-dioxins (Uppstad et al., 2010). P450 proteins are conveniently arranged into families and subfamilies, based...
on the percentage of amino acid sequence identity. For instance, enzymes that share ≥40% identity are assigned to a particular family designated by an Arabic numeral, whereas those sharing ≥55% identity are assigned to a particular subfamily designated by a letter (Hasler et al., 1999). With the advent of sequencing techniques and the sequencing of genomes of many different organisms, the number of sequenced P450s is rising. At present, the P450 superfamily consists of 12,456 named sequences, with about 6000 more known but not yet named.

The human genome contains 57 genes encoding P450 enzymes and 58 pseudogenes that are distributed in 18 P450 families. Seven of these genes are Type I enzymes, which are found in the mitochondria, while the other 50 genes encode Type II enzymes, which are found in the endoplasmic reticulum (Guengerich, 2004). One-fourth of the human P450s is not well characterized and are termed orphans (Nelson, 2011). The Arabian camel exhibits P450-dependent activities in hepatic and extrahepatic tissues, such as the kidney, lung, spleen, tongue, and the hump (el Sheikh et al., 1991; Raza et al., 1998, 2004; Jung et al., 2011). The present study aimed to isolate and characterize the full-length coding sequence cDNAs of C. dromedarius cytochrome P450 1A, 2C and 3A and to assess the degree of similarity of the deduced proteins with those of other ungulates.

MATERIAL AND METHODS

Chemicals

All of the chemicals used in this study were of analytical reagent, molecular biology, or chromatographic grade, as appropriate. Water was de-ionized and distilled.

Animals and tissue preparation

Liver samples from 3 adult male Arabian one-humped camels (C. dromedarius) were obtained from the local slaughterhouse, after the animals were killed under the observation of a skilled veterinarian. The tissue samples selected for RNA preparation and analysis were immediately submerged in RNAlater® solution (Qiagen, Ambion, Courtabeuf, France) to avoid RNA degradation. The samples were stored at 4°C for 24 h, and then stored at 20°C until use.

RNA isolation and cDNA synthesis

Samples of 30-60 mg of the preserved tissues were homogenized in RLT lysis buffer (Qiagen) supplemented with 1% 2-mercaptoethanol, using a rotor-stator homogenizer (MEDIC TOOLS, Switzerland). Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany), with a DNase digestion step, following manufacturer protocols. Elution was performed with 50 µL nuclease-free water. Concentration, purity, and quality of the isolated RNA were determined by the Agilent 2100 Bioanalyzer System and Agilent Small RNA analysis kit, following manufacturer protocols (Agilent Technologies, Waldbronn, Germany). Total RNA in aliquots of 2 µg was retro-transcribed into single-stranded cDNA by the ImProm-II Reverse Transcription System (A3800, Promega, Madison, USA). Complementary DNA was synthesized by reverse transcription, and used as a template for the amplification of the camel CYPs being studied.
PCR

PCR was carried out in a final volume of 50 µL, containing 25 µL 2X high-fidelity PCR master mix (GE Healthcare, USA), 5 µL c-DNA, 3 µL 30 pM of each primer, CYP1A forward: 5'- GGATCCATGTCTCTGTTGGACTCTCC-3' and reverse: 5'- GCGGCCGCCTAAGAGCGCATATGCACCT-3'; CYP3A forward: 5'- ATGGACCTGATCCCAAGCTTTTCCTT-3' and reverse: 5'- GTCTTACGGAGAAGTCAGGGCTTCATT-3'; and CYP2C forward: 5'- ATGGATCTCTTCATAGTCCTGGTG-3' and reverse: 5'- GACTGGGAATGAAACAGACCTCATAGA-3'. The PCR condition was 1 cycle at 95°C for 5 min, followed by 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. The final extension step was carried out at 72°C for 5 min. The PCR products were analyzed on 1% agarose gel stained with ethidium bromide, at a concentration of 0.5 µg/mL.

Cloning and sequencing of the PCR products

To ligate the PCR products generated onto pGEM*-T Easy vector (Promega Co. Cat No. A1360), 2 µL of the product was placed in a clean 0.5-mL tube, to which 1 µL pGEM*-T-Easy vector (50 ng) and 1 µL 10X ligase buffer were added, followed by the addition of 2 U ligase enzyme. The final volume of the ligation reaction was adjusted to 10 µL, by the addition of nuclease-free water. The tube was incubated at 16°C for 16 h. Transformation of Escherichia coli JM109 competent cells was carried out according to Sambrook et al. (1989). The recombinant E. coli JM109 harboring the pGEM*-T-Easy vector was screened on selective LB/IPTG/X-gal/Ampicillin/agar plates. Plasmids were prepared from positive clones by the PureYield Plasmid Miniprep System (Cat No. A1222, Promega, Madison, WI, USA).

Sequencing of the PCR products cloned onto pGEM*-T-Easy vector (4 different clones) was carried out according to Sanger et al. (1977) by the MegaBACE 1000 DNA Sequencing System (Pharmacia/Amersham Co., USA). The chain termination sequencing reaction was conducted by the DYEnamic ET terminator kit, as an integral part of the MegaBACE 1000 DNA sequencing system. The sequencing reaction products were purified by the DyeEx 2.0 Spin Kit (Qiagen, Cat No. 63206), and applied to the MegaBace 1000 Sequencing machine. The cDNA and amino acid sequences of the camel CYP1A, CYP3A, and CYP2C were aligned with those of other mammals using the ClustalW and DNAStar programs.

Cloning and expression of C. dromedarius CYP1A into the pET28a(+) vector

pGEM*-T-Easy plasmid that carries the C. dromedarius CYP1A cDNA insert was digested with BamHI and NotI restriction enzymes to release the cloned insert. After agarose gel electrophoresis, the CYP1A fragment was purified from the agarose gel and subcloned into the pET-28a(+) expression vector. The pET-28a(+) (Novagen Co.) plasmid carries an N-terminal His-Tag/thrombin/T7 configuration. The expression of the cloned CYP1A cDNA in pET28a(+) is under the control of the T7 promoter. The pET-28a(+) was digested with BamHI and NotI restriction enzymes, and treated with shrimp alkaline phosphatase (Promega), according to the method of Sambrook et al. (1989). Ligation was carried out in a 10-µL final volume that contained 2 µL pET-28a(+) plasmid (20 ng), 2 µL CYP1A cDNA insert (20 ng), 1 µL 10X ligase buffer, and 1 µL 2 U ligase enzyme. The final volume of the ligation reaction was adjusted to 10 µL by the addition of...
nuclease-free distilled water. The tube was incubated at 16°C overnight. Transformation of *E. coli* BL21(DE3) pLysS (Promega) competent cells was carried out according to the method of Sambrook et al. (1989). The recombinant *E. coli* BL21(DE3) pLysS harboring the pET-28a(+) vector was screened on selective LB/IPTG/X-gal/Kanamycin/chloramphenicol/agar plates, and by using the colony PCR strategy utilizing CYP1A gene specific primers. Plasmids were prepared from positive clones using the PureYield Plasmid Miniprep System (Promega, Cat No. A1222).

**Expression of recombinant *C. dromedarius* CYP1A in *E. coli* BL21(DE3) pLysS**

A 1-L Erlenmeyer flask containing 200 mL of terrific broth supplemented with kanamycin and chloramphenicol (Sigma/Aldrich Co., USA) at concentrations of 50 and 35 µg/mL, respectively, were inoculated with 2 mL overnight culture of *E. coli* BL21(DE3) pLysS pET-28a(+) carrying the CYP1A gene. The flasks were shaken at 250 rpm at 37°C until the absorbance at 600 nm was 0.2, at which point 0.5 mM δ-aminolevulinic acid was added to the culture medium. The incubation was continued until the absorbance at 600 nm reached 0.8. Then, 1 mM IPTG was added to the culture and the temperature was adjusted to 30°C and 150 rpm for 20 h. After the incubation period, the cells were harvested by centrifugation at 8000 rpm at 4°C for 20 min, washed with 50 mM potassium phosphate buffer (pH 7.5, containing 5.0 mM MgCl₂), and pelleted for a second time. The cells were then resuspended in 10 mL 50 mM potassium phosphate buffer (pH 7.5, containing 5.0 mM MgCl₂, 0.2 mM DTT, and 10% glycerol), and sonicated using 4X 15 s pulses. After the first pulse, 1 mM PMSF, 0.1 µg/mL leupeptin, and 0.04 U/mL aprotinin were added to the tubes and then sonication was continued. Cells debris was removed by centrifugation at 12,000 rpm at 4°C for 10 min after which the supernatant was collected for protein assays and Western blotting.

**Total protein determination**

The total protein concentration was assayed by the method of Bradford (1976). A calibration curve was established using bovine serum albumin as a standard at a concentration of 0.5 mg/mL.

**Western immunoblotting analysis of recombinant *C. dromedarius* CYP1A**

Recombinant fusion *C. dromedarius* CYP1A protein expressed in *E. coli* BL21(DE3) pLysS was detected by western blotting using a rabbit polyclonal antibody CYP1A1 (H-70, SC-20772, Santa Cruz Biotechnology, INC., USA) raised against amino acids 246-315 mapped onto an internal region of CYP1A1 of human origin. Total proteins were determined, and 50 µg was used for the immunoblotting analysis. Proteins were separated by electrophoresis on 12% SDS-polyacrylamide gel, and transferred to a nitrocellulose membrane (Laemmli, 1970; Towbin et al., 1979). After transfer, the membrane was blocked for 1 h with 50 mL 1X blocking buffer (Sigma-Aldrich, USA, Cat. No. B6429). Then, the membrane was first incubated with the primary anti-CYP1A1 antibody (1:500 dilution) for 8 h, and then washed for 30 min with 1X Tris buffer saline (1X TBS) containing 0.05% Tween-20. After a final wash with 1X TBS buffer, the membrane was incubated for 1 h with anti-rabbit secondary alkaline phosphatase labeled IgG antibody (1:2000 dilution). The membrane was washed again, and was finally developed using (nitro-blue tetrazo-ium salt/bromo-chloroindolyl-phosphate substrates. The protein bands were photographed by the Alpha Imager System (Alpha Innotech.Version: 2.0.0.9).
Structure modeling

Sequencing data were analyzed using BLASTn, and multiple sequence alignment was carried out using the ClustalW, BioEdit, and DNAStar programs. The CYP protein sequence was obtained by translating the sequenced DNA fragment using the Translation tool at the ExPasy server. The protein sequence was then submitted to the Swiss model server for structure prediction, and the structural data were analyzed by the PDB viewer program. Protein 3D structure models were built based on multiple-threading alignments by LOMET and iterative TASSER assembly simulation (Ortiz et al., 2002; Roy et al., 2010).

RESULTS

Cytochrome P450 1A, 2C, and 3A cDNA isolation and sequence analysis

A previous study reported that C. dromedarius expresses multiple P450 isoforms in hepatic and extrahepatic tissues and that most of these enzymes are constitutively expressed. In the previous study, the maximum expression levels of P450 proteins in the camel liver were in the order of P450 2E1 > 1A1 > 3A > 2B1/2. Moreover, detectable levels of the cytochrome P450 side-chain cholesterol cleavage and P450 21-hydroxylase were only observed in the camel testis (Alanazi et al., 2010). In the current study, full length camel P450 1A, P450 2C, and P450 3A were obtained by RT-PCR using gene specific primers designed from the available EST camel genome project data base at http://camel.kacst.edu.sa/. Utilizing these primers, specific PCR products were obtained that corresponded to 1563, 1473, and 1566 bp for P450 1A, 2C and 3A, respectively (Figure 1). The PCR products were cloned into pGEM-T-Easy vector and the cDNA inserts were then sequenced. Nucleotide sequences corresponding to 1563, 1473, and 1566 bp were then compared with the nucleotide sequences deposited in the GenBank database using the Blastn program on the NCBI Blast server. The isolated cytochrome P450s showed high statistically significant similarity scores to numerous CYP450s from other species. The full-length cDNAs of C. dromedarius CYPs were deposited in the GenBank data base under the accession numbers of JQ619653 for CYP1A, JQ619655 for CYP3A, and JQ619654 for CYP2C. Alignment of the deduced amino acid sequences of C. dromedarius CYP 1A, 3A, and 2C with other species is shown in Figures 2-4. In the comparison of C. dromedarius CYP1A with other ungulates and human, the percentage identity was 83% for Sus scrofa, 82% for Bos taurus, 79% for Macaca mulatta, 78% for Homo sapiens, and 75% for Mus musculus (Figures 2-4 and Table 1). Meanwhile, C. dromedarius CYP3A showed 75% identity with S. scrofa, 72% with H. sapiens, and 68% with M. musculus. Moreover, C. dromedarius CYP2C showed 80% percentage identity with B. taurus, 74% with M. mulatta, 70% with S. scrofa and 68% with H. sapiens. The open reading frames of C. dromedarius CYP1A, 3A and 2C contained coding regions of 1563, 1566 and 1473 nucleotides, and the deduced amino acid sequences represented 520, 521 and 490 amino acids residues with predicted calculated molecular weights of 58.651, 58.594 and 56.030 kDa, respectively (Figures 5-7). Based on the amino acids composition, the predicted calculated isoelectric points using a computer algorithm were found to be 7.315 for CYP1A, 9.46 for CYP3A and 6.579 for CYP2C.
Figure 1. Agarose gel (1.0%) electrophoresis of PCR products for Camelus dromedarius CYP1A (lanes 2 and 3), CYP3A (lanes 4 and 5) and CYP 2C (lanes 6 and 7) cDNA. Lanes 1 and 8 represent 1- and 0.5-kbp DNA molecular weight markers.

Figure 2. Alignment of the deduced amino acid sequences of Camelus dromedarius CYP1A (accession No. JQ619653), Bos taurus (accession No. DAA17555), Homo sapiens (accession No. P04798), Macaca mulatta (accession No. Q6GUR1), Mus musculus (accession No. P00184), and Sus scrofa (accession No. BAB85660).
Figure 3. Alignment of the deduced amino acid sequences of *Camelus dromedarius* CYP3A (accession No. JQ619654), *Homo sapiens* (accession No. AAA35744), *Mus musculus* (accession No. Q64464), and *Sus scrofa* (accession No. AAD04628).

Figure 4. Alignment of the deduced amino acid sequences of *Camelus dromedarius* CYP2C (accession No. JQ619655), *Bos taurus* (accession No. AA153846), *Homo sapiens* (accession No. AAB59426), *Macaca mulatta* (accession No. ABB67189) and *Sus scrofa* (accession No. XP_001924722). Asterisks show the proline-rich regions and red-dashed lines show the conserved heme-binding sites.
<table>
<thead>
<tr>
<th>Animal species</th>
<th>CYP1A Accession No. Identity (%)</th>
<th>CYP3A Accession No. Identity (%)</th>
<th>CYP2C Accession No. Identity (%)</th>
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<tr>
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<td>Mus musculus</td>
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<td>Q064646 72</td>
<td>BAA04555 65</td>
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Table 1. Homology of *Camelus dromedarius* CYPs with other species.

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<thead>
<tr>
<th>Animal species</th>
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<td>Mus musculus</td>
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Figure 5. Nucleotides and deduced amino acid sequences of *Camelus dromedarius* CYP1A. Proline-rich regions and heme-binding sites are underlined in red.
Figure 6. Nucleotides and deduced amino acid sequences of *Camelus dromedarius* CYP3A. Proline-rich regions and heme-binding sites are underlined in red.
Figure 7. Nucleotides and deduced amino acid sequences of *Camelus dromedarius* CYP2C. Proline-rich regions and heme-binding sites are underlined in red.
Predicted 3D structure of *C. dromedarius* CYP1A, 3A and 2C

The Arabian camel CYP1A, 3A and 2C proteins motifs secondary structures annotation prediction showed several common characteristic features for CYPs (Figures 8-10). First, the N-terminal hydrophobic segment or signal anchor segment (amino acid residues 1-38). Second, following the N-terminal signal peptide there is a proline-rich region (4-5 proline residues). Third, there is a highly conserved heme-binding cysteine-containing peptide that is localized near the C-terminal end of the CYP proteins that forms a pocket in the 3D structure of the CYPs. This cysteine-containing pocket contains up to 10 amino acid residues (Figure 11), including the invariant cysteine residue that is involved in the ligation of the heme iron prosthetic group. Figure 5 shows the alignment of *C. dromedarius* CYP1A, 3A and 2C sequences with the CYP sequences of other species around the heme-binding site. Comparing the 3 camels CYP heme-binding signatures revealed the conservation of the invariant amino acid residues (phenylalanine, glycine, and cysteine) in the 3 isolated CYPs cDNA (Figure 11). Proteins with similar amino acid sequences have a tendency to adopt similar 3D structures. Camel 3D CYP1A, 3A, and 2C protein crystal structural models were predicted from amino acid sequences using the I-TASSER server based on multiple-threading alignments by LOMETS and iterative TASSER assembly simulations to obtain the most precise 3D structural prediction. Figure 12 show the overall fold and secondary structure contents of the camel CYP 1A, 3A and 2C as ribbon diagrams. The 3 camel CYPs structures clearly contain the same set of secondary structures elements in very similar folds, with respect to the positions of alpha helices, β-strands, and the highly conserved β-bulge or cysteine-pocket. The heme moiety of the CYPs is covalently bound to the invariant cysteine (Cys^465^ for CYP1A, Cys^451^ for CYP3A, and Cys^435^ for CYP2C), which is found in the β-bulge region called the cysteine pocket.

![Figure 8. Arabian camel CYP1A protein motifs secondary structure annotation prediction showing the N-terminal signal peptide, proline-rich region, heme-binding site and protein kinase C-phosphorylation sites, along with secondary structure prediction.](image-url)
Figure 9. Arabian camel CYP3A protein motifs secondary structure annotation prediction showing the N-terminal signal peptide, proline-rich region, heme-binding site and protein kinase C-phosphorylation sites along with secondary structure prediction.

Figure 10. Arabian camel CYP2C protein motifs secondary structure annotation prediction showing the N-terminal signal peptide, proline-rich region, heme-binding site and protein kinase C-phosphorylation sites along with secondary structure prediction.
Figure 11. Alignment of the Camelus dromedarius CYP1A, CYP3A, and CYP2C, with the sequences of other species around the heme-binding site. Asterisks indicate conserved invariant residues; dots and semicolons denote variant residues.

Figure 12. Predictive 3D structure models of the Camelus dromedarius CYP1A, CYP3A, and CYP2C, showing the N-terminal signal peptide, proline-rich region and the heme-binding pocket.
Cloning and expression of *C. dromedarius* CYP1A cDNA in *E. coli*

The full length open reading frame of *C. dromedarius* CYP1A cDNA was cloned into the pET28a(+) vector utilizing the *Bam*HI and *Not*I restriction sites, and expressed in *E. coli* (BL21)DE3pLysS cells. A high level of recombinant fusion CYP1A was attained by adding 1.0 mM IPTG which induced the T7 promoter after an 18-h incubation period (Figure 13). The result of Western immunoblotting analysis (Figure 14) indicated that the anti-human CYP1A1 antibody cross reacted with the *C. dromedarius* recombinant CYP1A fusion protein to produce a specific cross reactivity band of about 60.0 kDa, which corresponds to the His tag fusion protein.

![Figure 13. Sodium dodecyl sulfate gel (12%) electrophoresis for recombinant *Camelus dromedarius* cytochrome P450 1A in *Escherichia coli* BL21(DE3)pLysS (pET28a+) induced with 1 mM IPTG (lanes 3-8) and uninduced culture (lane 2). Lane 1 represents Precision Plus pre-stained protein standards (BioRad Cat No. 161-0373).](image)

![Figure 14. Immunoblotting of recombinant *Camelus dromedarius* CYP1A1 with anti-cytochrome P450 1A1 antibody. Lanes 2-7 = different concentrations of recombinant protein from 10 to 60 µg of the induced culture proteins. Lane 1 = 60 µg uninduced culture.](image)
DISCUSSION

Despite the economic, cultural, and biological importance of the Arabian camel, information remains limited about this species’ Phase I drug metabolizing enzymes. The molecular characterization of Arabian camel CYP450 enzymes is very important for understanding the impact of exposure to various environmental factors on the health status of this unique animal. RT-PCR and immunoblotting results from a previous study (Alanazi et al., 2010) of C. dromedarius CYPs demonstrated that significant mRNA and protein of CYPs isoforms are found in camel liver and extrahepatic tissues including the lung, kidney, spleen, and testis. The present study focused on the molecular characterization of 3 important cytochrome P450s mainly, CYP1A, 3A and 2C from the Arabian camel. Cytochrome P450 1A1 is a member of a multigene family of xenobiotic metabolizing enzymes, and plays a physiological role in the detoxification of polycyclic aromatic compounds (PAHs). However, the activity of this enzyme may be deleterious, because it generates mutagenic metabolites and active oxygen (Mimura and Fujii-Kuriyama, 2003). In this gene, the induction of the CYP1A1 isoenzyme by certain environmental chemicals such as 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin and polymorphism primarily account for its susceptibility to PAH-induced carcinogenesis, including lung cancer (Shah et al., 2009). CYP3A isoenzymes are the major CYPs expressed in humans, and are involved in the metabolism of majority of the drugs. Besides metabolizing drugs, these enzymes also play an important role in the metabolism of steroids and toxins, including carcinogens (Kirby et al., 1993; Gonzalez and Gelboin, 1994; Evans and Relling, 1999). Although the expression of CYP3A isoenzymes is highest in the liver, their expression has also been demonstrated in extrahepatic tissues (Michael and Doherty, 2005). Variability in the expression of CYP3A enzyme might also affect an individual’s susceptibility to cancers caused by environmental procarcinogens, which are CYP3A substrates (Kirby et al., 1993). The cytochrome P450 2C subfamily is predominantly expressed in the liver, and contains 4 highly homologous genes 2C8, 2C9, 2C18, and 2C19, which are responsible for the metabolism of approximately 20% of all clinically used drugs (Michael and Doherty, 2005).

In the current study, we showed the amplification of C. dromedarius CYP1A, 2C and 3A full-length cDNA using primer sets spanning the entire open reading frame, with cDNA fragments with the anticipated sizes of 1563, 1473, and 1566 bp (Figures 1-4) being exclusively obtained. The cDNA sequences contained 520, 490, and 521 amino acid residues in the proteins of 58.651, 56.03 and 58.594 kDa, with these CYP450 enzymes being matched with several CYPs sequences in the GenBank data base (Figures 2-4). Several observations from the primary structures and from the multiple sequence alignments (Figures 2-10) of camel CYPs are of interest.

First, the primary sequence homology between camel CYPs and other species was greater than 70% (Table 1). Second, the primary structure (Figures 5-10) contained the characteristic N-terminal hydrophobic segment or signal peptide that is rich in hydrophobic amino acid residues, such as valine, leucine, isoleucine, and alanine. This signal peptide is a characteristic feature of P450 cytochromes which are membrane bound proteins with the exception of bacterial enzymes. Microsomal CYP enzymes are tethered to the membrane through this hydrophobic transmembrane helix at the N-terminus of the protein which also serves as a targeting sequence for the signal recognition particle dependent co-translational incorporation of a nascent CYP protein into the endoplasmic reticulum membrane (Bar-Nun et al., 1980; Sakaguchi et al., 1984). About 90% of hepatic cytochrome P450 enzymes are present in micro-
somal membranes, with the remainder being found in the mitochondria (Nelson et al., 2004).

Third, following the N-terminal hydrophobic segment, there is a proline-rich region (Pro\textsuperscript{44, 45, 47, 51} in CYP1A; Pro\textsuperscript{48, 50, 52, 54} in CYP3A, and Pro\textsuperscript{50, 52, 53, 54} in CYP2C) that acts as an alpha helix breaker in the folding of the CYP proteins. Fourth, there is a highly conserved heme-binding cysteine-containing peptide that is localized near the C-terminal end of the proteins (Figures 2-11). This cysteine-containing pocket is a common CYP450 signature (Guengerich, 1997), and this motif contains up to 10 amino acid residues, including the invariant cysteine residue (Cys\textsuperscript{465} in CYP1A, Cys\textsuperscript{451} in CYP3A, and Cys\textsuperscript{435} in CYP2C) which is involved in the ligation of the heme-iron prosthetic group. In general, this signature takes the form of FXXGXXXCXG (Figure 11), where F is phenylalanine, G is glycine, C is cysteine, and X is any amino acid. Fifth, a dilysin motif KKXX, (where K is the lysine residue and X is any other amino acid) at the C-terminal end of the cytoplasmic domain was characterized in camel CYP2C (Lys\textsuperscript{420, 421}) and 3A (Lys\textsuperscript{495, 496}), but it was absent in CYP1A. The dilysin motif, KKXX, facilitates the exclusion of endoplasmic reticulum microsomal CYP enzymes from being transported through the Golgi to other cell compartments, or from exocytosis (Andersson et al., 1999).

The 3D-structure of a protein provides valuable insights about its functions. Ideally, experimental tools and techniques, such as X-ray crystallography nuclear magnetic resonance (NMR) spectroscopy and electron microscopy, are used to determine the 3D-structure of proteins. Unfortunately, these techniques cannot be used on the vast majority of proteins, including microsomal cytochrome P450s, as the proteins are difficult to crystallize, insufficiently soluble, or too large for NMR studies. Alternative methods have been developed to determine the 3D-structures of these proteins, such as comparative (or homology) modeling. It is possible to predict the 3D structure of a protein based solely on knowledge of its amino acid sequences. Hence, we generated a 3D structure of the putative \textit{C. dromedarius} CYP1A, 3A, and 2C, for which the nucleotide and amino acid sequences (but not the 3D structures) are known. The overall secondary structures, folding, and topology are quite similar (Figure 12). Common orientation and anchoring in the membrane by an N-terminal helix was observed for all camel CYPs under study. The heme moiety of the camel CYPs is covalently bound to the invariant Cysteine (Cys\textsuperscript{465} for CYP1A, Cys\textsuperscript{451} for CYP3A, and Cys\textsuperscript{435} for CYP2C) found in the \(\beta\)-bulge region called the Cys-pocket. This \(\beta\)-bulge appears to have the role of enveloping the invariant cysteine residue in a hydrophobic environment (Hasemann et al., 1995). Three residues besides the cysteine (Gly\textsuperscript{461, 467} and Phe\textsuperscript{458} in 1A, Gly\textsuperscript{447, 453} and Phe\textsuperscript{444} in 3A, and Gly\textsuperscript{431, 437} and Phe\textsuperscript{428} in 2C) are very strictly conserved among most of the CYP450 proteins. These 2 glycine residues are involved in the formation of the \(\beta\)-hairpin turn and facilitate the sharp turn from the Cys-pocket into the L-helix, and are involved in determining the proximity to the heme. The phenylalanine side chain completes the hydrophobic enclosure of the proximal heme in combination with other side-chains and the main chain atoms of the Cys-pocket (Kassner, 1973; Yasukochi et al., 1994). Substrates bind to a cavity above the heme surface, because the heme forms the base of the active site cavity, and it must be positioned close to the reactive iron-oxo intermediate for catalysis (Hasemann et al., 1995). The regions that form the outer surfaces of the substrate binding cavity are generally more divergent among enzymes compared to other parts of the proteins, leading to differences in the size, shape, and chemical features of the active sites that provide discrimination for different substrates.

A pilot recombinant protein expression study using \textit{E. coli} BL21(DE3) pLysS harboring pET28a(+) carrying the full length CYP1A cDNA indicated that recombinant fusion camel...
CYP1A protein cross-reacted with the anti-human cytochrome P450 1A antibody (Figure 14). The cross reactivity band was found to be at a molecular weight of 60 kDa, which represents the size of the CYP1A protein in addition to a 1 kDa His-tag fusion peptide localized at the N-terminus of the recombinant fusion protein. Immunoreactivity was not detected in the un-induced E. coli BL21(DE3) pLysS (Figures 13, 14).

CONCLUSIONS

The molecular characterization of the Arabian camel CYP1A, 3A, and 2C revealed a high degree of similarity with other ungulates and human. The data presented in this study provides baseline information on which to develop further studies of camel CYP proteins, such as the characterization of the purified native and recombinant proteins and the role of camel CYPs, especially the CYP3A in the metabolism of xenobiotic compounds and environmental pollutants. Therefore, it would be useful to improve our understanding about the regulation of camel CYP1A, 3A, and 2C gene expression, in relation to the up- or downregulation of appropriate promoter elements in camel cell lines. Moreover, it is important to analyze the substrate specificity of these proteins in greater detail to obtain an appropriate level of functional characterization. Clearly, much effort is required to identify and characterize the isolated C. dromedarius CYPs, and to identify more camel CYPs within the same gene family.

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Conflicts of interest

The authors declare that there is no conflict of interest for this article and there is no financial employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, royalties related to this manuscript.

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