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CLONING AND BIOINFORMATICS REVEALED MOLECULAR CHARACTERISATION OF CYP2E1 GENE FROM BACTRIAN CAMEL (*Camelus bactrianus*)

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ABSTRACT

In the present study, the CYP2E1 gene from Bactrian camel (*Camelus bactrianus*) was cloned, sequenced and explored for its structural and functional aspect. The full-length sequence of CYP2E1 gene was 1754bp long, contained a coding region of 1488bp open reading frame (ORF), which was predicted to encoding 495 amino acid residues. The comparison between CYP2E1 from Bactrian camel (*Camelus bactrianus*) and other animal species showed that nucleotide and encoded amino acid sequences of the Bactrian camel CYP2E1 gene exhibited high similarity with the wild Bactrian camel (*Camelus ferus*), and the phylogenetic analysis revealed that Bactrian camel (*Camelus bactrianus*) CYP2E1 was grouped with that of wild Bactrian camel (*Camelus ferus*). The CYP2E1 protein contained a transmembrane domain which extended from 2-23 amimo acids, while 24-495 amino acids were outside the cell, and no signal peptide was found. The prediction of tshe secondary structure revealed that there had 44.04% alpha helix, 7.07% beta fold, and 48.89% random coils. Our research may be valuable for exploring the function and detoxification molecular mechanisms underlying the molecule.

Key words: Bactrian camel, bioinformatics, CYP2E1 gene

Cytochrome P450 (CYP450) is an isoenzyme encoded by superfamily genes that are closely related to structure and function in an organism. It is essential to catalyse the metabolic conversion, activation and inactivation of a wide variety of endogenous, including steroid hormones, vitamins, and fatty acid derivatives, and exogenous substances, such as drugs, xenobiotics, protoxicants, and chemicals toxicity, and carcinogens (Ohmori *et al,* 1993; Nelson, 2011). In the Bactrian camel genome there are about 17 families and 38 subfamilies, including about 63 CYP gene copies. Within them, 9 multi-gene families were found and there are more CYP2J and CYP3A copies, which may contribute to the distinct biological characteristics and metabolic pathways of Bactrian camels for adaptation to the harsh environments (Jirimutu *et al,* 2012; Hasi *et al,* 2018). CYP2E1 is an important member of the CYP450 enzyme system, and mainly located in the membrance of the endoplasmic recticulum but has also been detected in other cellular compartments such as the plasma membrane and the mitochondria (Loeper *et al,* 1990; Neve and Ingelman-Sundberg, 2001). The special importance of CYP2E1 focused on metabolising xenobiotics, high production chemicals, industrial waste as ethanol, solvents, carbon tetrachloride, benzene, and drugs such as acetaminophen, chlorzoxazone and theophyline (Song *et al,* 1986; Park *et al,* 1993; Alanazi and Saeed, 2012).

Like other mammals, several studies have focused on the camel in the CYP2E1 gene in the liver and extrahepatic tissues such as kidney, lung, spleen, tongue, and the hump (Sheikh *et al,* 1991; Raza *et al,* 2004; Alanazi *et al,* 2010). Furthermore, Mohammad *et al,* cloned the full-length CYP2E1 gene sequence from dromedary (Alanazi and Saeed, 2012). Mahmoud *et al*, determined the camel CYP2E1 and detected its evolution rate and its power to bind with various chemicals, protoxins, procarcinogens, industrial toxins and drugs were isolated (Kandeel *et al,* 2016). In the present study, Bactrian camel full-length CYP2E1 DNA were isolated and cloned and sequenced for the first time, furthermore to analyse the bioinformatics characteristics.

Materials and Methods

Experimental sample collection

The liver tissue was obtained immediately after slaughter from male domestic Bactrian camel (*Camelus*

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bactrianus) at the Alxa, Inner Mongolia abattoir under the supervision of a skilled veterinarian.

RNA extraction

The total RNA was extracted from Bactrian camel liver tissue using RNAiso Plus (TaKaRa) kit and quantified by spectrophotometry on the NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Integrity of RNA sample was assessed using formaldehyde agarose gel (1%) electrophoresis.

Primer design and synthesis

All primers used in this study were shown in Table 1, and all primers were synthesised by Sangon Biotech, Co., Ltd. Shanghai, China.

The first strand of cDNA synthesis

The reaction volume of 25μl comprised of 12.5μL of 2×GC buffer, 0.5μL of both forward and reverse primers (10μM), 0.2μL of dNTP mixture (10mM), 10.0μL of ddH_2O, 1μL cDNA template, and 0.2μL of Taq enzyme (5U/μL). The PCR reaction included an initial denaturation cycle of 3min at 95°C, followed by 33 cyclys of denaturation for 30s at 94°C, annealing for 30s at 58°C, and an extension for 90s at 72°C, with a final extension of 7 min at 72°C. The PCR products were excised from a 1.0% agarose gel, purified using Gel Extraction Kit (Sangon Biotech, B518131).

The amplification of 3′RACE and 5′RACE

The first round of 3′RACE PCR was carried out using both RC543-F1 and 5.3′ outer primers. Then, the second round of nested PCR was performed with primers RC543-F2 and 5.3′ inner using the first round of PCR product as a template. The PCR products were excised from a 1.0% agarose gel, purified using Gel Extraction Kit (Sangon Biotech, B518131). The reaction component and PCR cycling were provided in Table 2 and Table 3.

Table 2. The detail reaction component of amplification of 3′RACE.

Reaction Component	The first round (μl)	The first round (μl)
2X GC Buffer I	12.5	25
F (10 μM)	0.5 (RC543-F1)	1(RC543-F2)
R (10 μM)	0.5 (5.3′outer)	1 (5.3′inner)
dNTP (2.5 mM)	4	8
ddH20	6.3	12.5
Template	1 (cDNA)	1 (first round of PCR product)
Taq enzyme (5 U/μl)	0.2	0.5
Total	**25**	**50**

Table 3. The information PCR cycling of 3′RACE.

	The first round		The second round	
Initial denaturation	95°C	3min	95°C	3 min
Denaturation	94°C	30s	94°C	30s
Annealing	58°C	30s	58°C	30s
Extension	72°C	60s	72°C	60s
Final extension	72°C	7min	72°C	7min
Cycling	33C		33C	

According to the in vitrogen 5′RACE system manual, both RC543-RT1 and RC543-RT2 primers were used to obtain the first strand cDNA; then the first round of 5′RACE PCR was carried out

Table 1. Primers for CYP2E1 gene cloning.

Primer name	Primer sequence
Adaptor primers	5′adaptor GCTGTCAACGATACGCTACGTAACGGCATGACAGTGCCCCCCCCCCCCCCC
	3′adaptor GCTGTCAACGATACGCTACGTAACGGCATGACAGTGTTTTTTTTTTTTTTTT
	5.3′outer GCTGTCAACGATACGCTACGTAAC
	5.3′inner GCTACGTAACGGCATGACAGTG
Amplification primer	RC543-92F TGGCTGTCTTGGGCATCAC
	RC543-1559R TTGGCAAACCCAGTCGCA
Specific primer of 3′RACE	RC543-F1 GTGATTCCGACACTGGACTCCGTCTT
	RC543-F2 CCAGAGAAGTTTAAGCCGGAGCACTTTC
Specific primer of 5′RACE	RC543-R2 GAACACTGGCCCGAACCGCTCT
	RC543-R1 GCGAGCCCAGGTACAGCGTGAA
	RC543-RT2 AGAGGATGTCGGAGATGA
	RC543-RT1 TAGTCAGAGTGCTTGTGGAA

Adaptor primers5′adaptor RACE: Rapid-amplification of cDNA ends

using both 5′adaptor and RC543-R1 primers, and the second nested PCR was performed with 5.3′outer and RC543-R2 primers. The reaction component and PCR cycling were provided in Table 4 and 5. The PCR products were excised from a 1.0% agarose gel, purified using Gel Extraction Kit (Sangon Biotech, B518131).

Table 4. The detail reaction component of amplification of 5′RACE.

Reaction Component	The first round (μl)	The second round (μl)
2X GC Buffer I	12.5	25
F (10 μM)	0.5 (5′adaptor)	1 (5.3′outer)
R (10 μM)	0.5 (RC543-R1)	1 (RC543-R2)
dNTP (2.5 mM)	4	8
ddH_2O	6.3	12.5
Template	1 (cDNA)	1 (第一轮PCR稀释产物)
Taq enzyme (5 U/μl)	0.2	0.5
Total	**25**	**50**

Table 5. The information PCR cycling of 5′ RACE.

	The first round		The second round	
Initial denaturation	95°C	3 min	95°C	3 min
Denaturation	94°C	30 s	94°C	30 s
Annealing	68°C	30 s	68°C	30 s
Extension	72°C	60 s	72°C	60 s
Final extension	72°C	7 min	72°C	7 min
Cycling	33C		33C	

Multiple sequence alignment and phylogenetic analysis

Multiple sequence alignment was carried out using the sequence analysis software Lasergene 1 (DNASTAR Inc., Madison, WI, USA) and Clustal X program, version 1.83. A neighbor-joining tree was drawn with MEGA5.0 software (http://www.megasoftware.net), with confidence levels assessed using 1000 bootstrap replications. The sequences of reference CYP2E1 genes concluded in the multiple sequence alignment were obtained from GenBank and summarised in Table 6.

Bioinformatic analysis of CYP2E1

The basic physical and chemical properties of CYP2E1 were analysed using ProtParam online tools (https://web.expasy.org/protparam/). The hydrophobicity of CYP2E1 was predicted using ProtScale online tools (https://web.expasy.org/protscale/). The transmembrane structure of CYP2E1 was predicted using TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/). The subcellular localisation of DQA was predicted using the online software Target P1.1 server (https://www.cbs.dtu.dk//services/ TargetP/). The phosphorylation sites site of CYP2E1 were predicted using the online tools: NetPhos 3.1 server (http://www.cbs.dtu.dk/services/NetPhos/). The amino acid sequence of CYP2E1 was subjected to predict the secondary and 3D structures. The secondary structure was predicted using PredictProtein (http://www.predictprotein.org/), while the 3D conformation was predicted using the Swiss-model server (https://www.swissmodel.expasy.org/) for modeling the homologous structure.

Table 6. Sequence information used in the construction of phylogenetic trees.

Species	Accession Number
Camelus ferus	XM006178829.2
Camelus dromedarius	GU998962.1
Vicugna pacos	XM006207481.2
Sus scrofa	AY581116.1
Bos taurus	AJ001715.1
Ovis aries	NM001245972.1
Equus caballus	EU232117.1
Homo sapiens	J02625.1

Results

Molecular cloning and sequence analysis of CYP2E1 from Camelus bactrianus

Based on the RACE, a full-length CYP2E1 cDNA of 1754-bp was obtained from the camel liver tissue, and consisted of 1488-bp open reading frame (ORF). That encoded a 495-aa (32-1519bp, length: 1488bp) polypeptide of 145604 molecular weight (Fig 1). Total number of atoms was 18186, the atomic formula was C5187H8622N1754O2120S503, theoretical pI was 4.90, and instability index was 53.60, which classified the protein as unstable. Furthermore, protein sequence of CYP2E1 was predicted by EditSeq (DNAStar), which revealed that there were 59 strongly basic (+) (K, R), 56 strongly acidic (-) (D, E), 186 hydrophobic (A, I, L, F, W and V), 107 polar (N, C, Q, S, T and Y) with isolectric point at 8.304 and 5.490 charge at pH 7.0. The gene sequence comprised of 24.19% A (360), 25.47% G (379), 21.57% T (321), 28.78% C (428), 45.77% A+T (681), and 54.23% C+G (807).

The sequence analysis for the coding region of *Camelus bactrianus* CYP2E1 gene indicated a high similarity with other ungulates. We found that camel CYP2E1 showed the highest similarity with

CCCACGAGTCAGCAGACACTCAGAAGGCACC

ATG GCT GTC TTG GGC ATC ACG ATC GCT CTG CTG GTG TGG ATG GCC ACC CTG CTG CTC ATC TCC ATC TGG AAG CAC ATC TAC AGC AGC

M A V L G I T I A L L V W M A T L L L I S I W K H I Y S S

TGG AAA CTG CCC CCT GGC CCT TTC CCA CTG CCC ATC ATT GGG AAT CTT TTC CAG CTG GAT ATT AAG GAT ATT CCC AAA TCC TTA ACC

W K L P P G P F P L P I I G N L F Q L D I K D I P K S L T

AGG CTG GCA GAG CGG TTC GGG CCA GTG TTC ACG CTG TAC CTG GGC TCG CGG CGC ATC GTG GTC CTG CAT GGC TAC AAG GCC GTC

R L A E R F G P V F T L Y L G S R R I V V L H G Y K A V

AAG GAG GTC CTG CTG GAC TAC AAG AAC GAG TTT TCT GGC AGA GGA GAT AAC CCG GCA TTC CAG GTG CAC AAG AAC AAA GGA ATC

K E V L L D Y K N E F S G R G D N P A F Q V H K N K G I

ATT TTC AAC AAT GGA CCG ACC TGG AGG GAC ACC CGG AGG CTT TCC CTG ACC GTC CTC CGT GAC CTC GGG ATG GGG AAA CGG GGC

I F N N G P T W R D T R R L S L T V L R D L G M G K R G

AAC GAG GAG CGG ATC CAG AGG GAG GTC CCC TTC CTG CTG GAG GCA CTC AGG AAG ACC CAG GGC CAG CCC TTT GAC CCC ACC TTC

N E E R I Q R E V P F L L E A L R K T Q G Q P F D P T F

GTC ATT GGC TTC GCG CCC TAC AAT GTC ATC TCC GAC ATC CTC TTC CAC AAG CAC TCT GAC TAC AGT GAT AAG ACG GGC CTG AGG CTG

V I G F A P Y N V I S D I L F H K H S D Y S D K T G L R L

ATG TAT CTG TTC AAC GAG AAC TTC TAC CTG CTC AGC ACG CCC TGG ATC CAG CTT TAT AAT AAT TTC TCA AGC TGT CTA CAA TAC CTG

M Y L F N E N F Y L L S T P W I Q L Y N N F S S C L Q Y L

CCA GGA AGC CAT AGA AAA CTA TTA AAA AAT GTG TCC GAA ATA AAA GAT TAT GCT TCA GCA AGA GTG AAG GAA CAC CAG GAG TCA

P G S H R K L L K N V S E I K D Y A S A R V K E H Q E S

CTG GAC CCC AGC TGC CCC CAA GAC TTC ATA GAC AGC CTG CTG GTG GAA ATG GAG AAG GGA AAG CAC AGT GCA CAG CCT GTG CAC

L D P S C P Q D F I D S L L V E M E K G K H S A Q P V H

ACC TCG GAG GGC ATC GCC GTG ACC GTC GCT GAC CTG CTC TTC GCA GGG ACG GAG ACC ACC AGC ACC ACC CTG AGA TAT GGG CTC

T S E G I A V T V A D L L F A G T E T T S T T L R Y G L

CTG ATT CTC ATG AAA CAC CCG GAG GTC GAA GAG AAG CTT CAT GAA GAA ATT GAC AGG GTG ATC GGG CCG AGC CGA GTC CCT GCT

L I L M K H P E V E E K L H E E I D R V I G P S R V P A

ATC AAG GAC AGG CTA GAC ATG CCC TAC CTG GAT GCC GTG GTG CAC GAG ATT CAG CGA TTC ATC GAC CTC TTG CCC TCC AAC CTG

I K D R L D M P Y L D A V V H E I Q R F I D L L P S N L

CTC CAC GAA GCC ACC CAG GAC ACA GTG TTC AGA GGA TAC GTC ATC CCC AAG GGC ACG CTC GTG ATT CCG ACA CTG GAC TCC GTC

L H E A T Q D T V F R G Y V I P K G T L V I P T L D S V

TTG TAT GAC AAC CAA GAA TTC CCC GAG CCA GAG AAG TTT AAG CCG GAG CAC TTT CTG AAC GAA CAT GGG AAG TTC AAG TAC AGT

L Y D N Q E F P E P E K F K P E H F L N E H G K F K Y S

GAC TAT TTC AAG CCA TTT TCC GCA GGA AAG CGA GTG TGC GTT GGA GAA GGC CTG GCG CGC ATG GAA TTG TTT CTG TTC TTG GCC

D Y F K P F S A G K R V C V G E G L A R M E L F L F L A

GCC ATC TTG CAG CAT TTT AAC TTG AAG TCT CTC GTT GAC CCC AAG GAT ATT GAC CTC AGC CCC ATT GCG ACT GGG TTT GCC AAG ATT

A I L Q H F N L K S L V D P K D I D L S P I A T G F A K I

CCC CCC CGT TAC AAA TTC TGT GTC ATT CCC CGC TCT CAA GCG TGA

P P R Y K F C V I P R S Q A *

GGCACACGCGCTCTGAAGACCCCCGGGCACTTGATGCTGCCTGGCGGCTTCCTGCCCCACACCGTCCCCTCCGGTGGGCAGCCAGGGCTCC

ACAGGGGATCAGCTGGGCCAGGGAGGCTGCTTCCGAGTCACATTCTTCAGACAGAATTTGAAAGCAAAATCCAAAAAAAAATTGTGTCAAA

CTAATTAAACTGAATAAAGTGAACTGTGACTTCCAAAAAAAAAAAAAAAAAAAA

Fig 1. The nucleotide and deduced amino acid sequence of Bactrian camel CYP2E1. The deduced amino acid sequence is reported in one-letter code, and the stop coden is showed with asterisks.

the aminal of Camelidae family, which were wild Bactrian camel (*Camelus ferus*), alpaca (*Vicugna pacos*), and dromedary (*Camelus dromedarius*), (99.9%, 98.5% and 98.4%); followed by goat (*Ovis aries*) and cattle (*Bos taurus*) (87.8% and 87.4%); the similarity with pig (*Scrofa microsomal*) and horse (*Equus caballus*) were 87.0% and 85.8%, respectively. The deduced amino acid sequences similarities ranging from 79.9% to 94.7% as compared to other ungulates, such as 94.7% with dromedary (*Camelus dromedarius*), 87.3% with goat (*Ovis aries*), 85.5% with cattle (*Bos taurus*), 83.4% with pig (*Sus scrofa*), and 79.9% with horse (*Equus caballus*). Furthermore, the phylogenetic tree was constructed using neighbor-joining method, and selected 1000 bootstrap repetition (Fig 2). The result showed that *Camelus bactrianus* and *Camelus ferus* formed one monophyletic clade, meanwhile that from other species except *Equus caballus* formed a

separate clade. The *Camelus bactrianus* CYP2E1 gene was grouped closely with that of *Vicugna pacos* and *Camelus dromedarius* (Fig 2).

Molecular Characterisation of CYP2E1 gene from Camelus bactrianus

Analysis of Hydrophilic and Hydrophobic

The hydrophilicity and hydrophobicity were analysed of CYP2E1 protein based on the protscale, and Hphob./Kyte & Doolittle were selected as the prediction standards. The highest score was 3.211, at position 451Leu (L), represented maximal hydrophobicity; while the lowest score was -2.678, at position 190Asp (D), represented strongest hydrophilicity. Since more than half of the amino acid sequence were hydrophilic residues, the entire polypeptide chain was considered to be a hydrophilic residue (Fig 3).

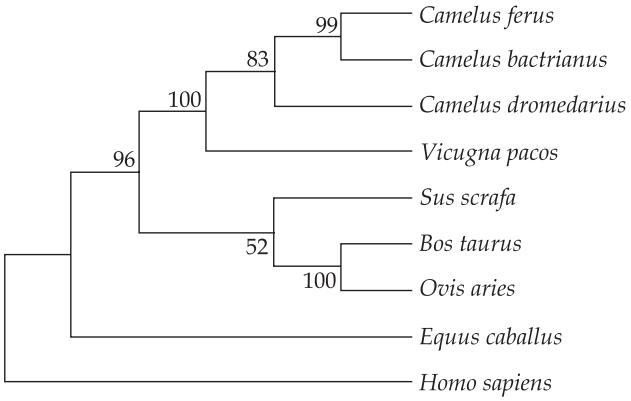

Fig 2. CYP2E1 genetic phylogenetic tree between bactrian camel and other species. The bootstraping value at the nodes and the bold refered to sequencing of this study

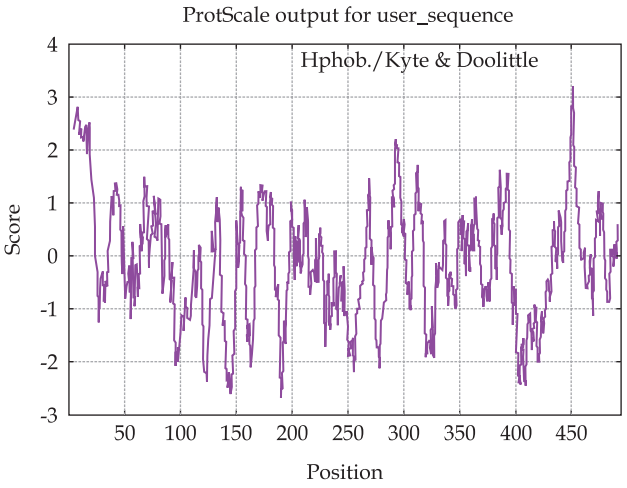

Fig 3. The hydrophilicity profile of *Camelus bactrianus* CYP2E1 protein. Y-axis displays the hydrophilic index: a positive number indicates hydrophobicity, the greater the value, the greater the hydrophobicity; negative numbers indicate hydrophilicity, smaller values indicate stronger hydrophilicity. The x-axis displays the position of CYP2E1 amino acids.

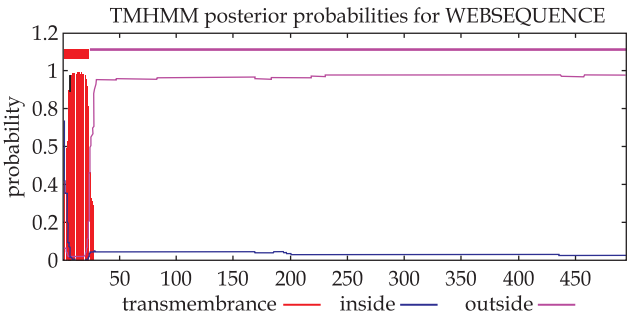

Fig 4. Prediction of transmembrance region of CYP2E1 protein from *Camelus bactrianus*.

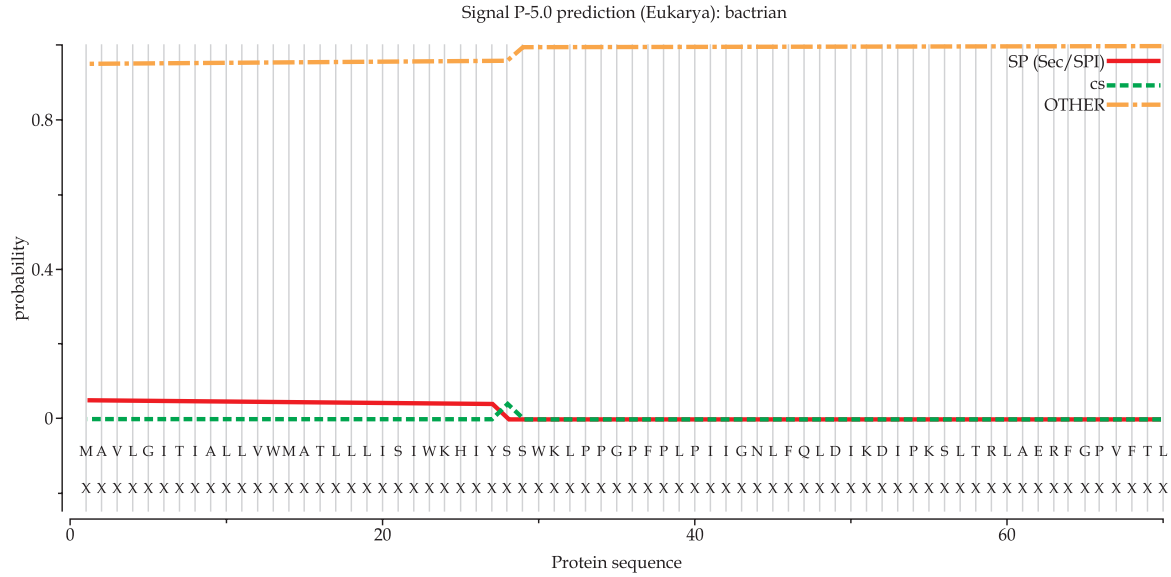

Fig 5. Prediction of signal peptide of CYP2E1 protein from *Camelus bactrianus*.

Analysis of Transmembrane Helical Structure and Signal Peptide

The transmembrane helical structure was predicted of CYP2E1 protein based on the TMHMM 2.0 software (Fig 4). The protein contained a transmembrane domain with extended from 2-23 amimo acids, while 24-495 amino acids were outside the cell. Furthermore, signalP 4.1 server was used to predict the signal pepetide of CYP2E1 protein (Fig 5), and no signal peptide was found in this protein, and it did not have signal peptide recognition function.

Prediction of Phosphorylation site

NetPhos 3.1 server predicted the potential phosphorylation site of CYP2E1 from *Camelus bactrianus* (Fig 6), including 15 Thr (threonines), 11 Tyr (tyrosines), and 28 Ser (serines) above 0.5 threshold.

Subcellular Localisation and Domain Prediction

The Target P1.1 server result showed a signaling pepetide (SP) of CYP2E1 gene from *Camelus bactrianus* (Fig 7); SMART program predicted that 2-23 aa was the transmembrance region.

Advanced structure prediction of CYP2E1 protein

Predict Protein software was used to predict the secondary structure of Bactrian camel CYP2E1. The result showed that the CYP2E1 protein was composed of 44.04% alpha helix, 7.07% beta fold, and 48.89% random coils. Furthermore, the tertiary structure of the DQA protein was predicted using the SWISS-MODEL Server, that indicated that the alpha helix, beta folding, and random crimp was in agreement with the predicted secondary structure of CYP2E1 protein (Fig 8).

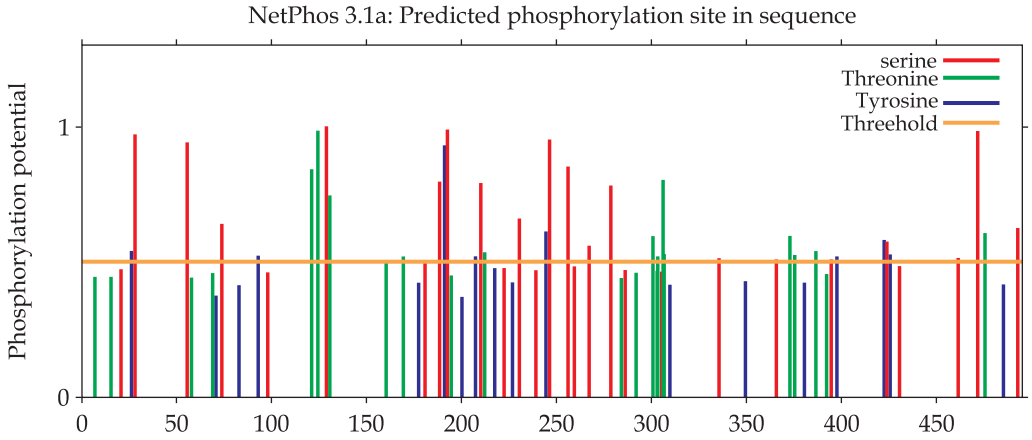

Fig 6. Prediction of phosphorylation sites of CYP2E1 protein from *Camelus bactrianus*.

targetp v1.1 prediction results
Number of query sequences : 1
Cleavage site predictions not included.
Using NON-PLANT networks.

Name	Len	mTP	SP	other	Loc	RC
Sequence	495	0.015	0.993	0.021	S	1
cutoff		0.000	0.000	0.000		

Fig 7. The subcellular location analyses of *Camelus bactrianus* CYP2E1.

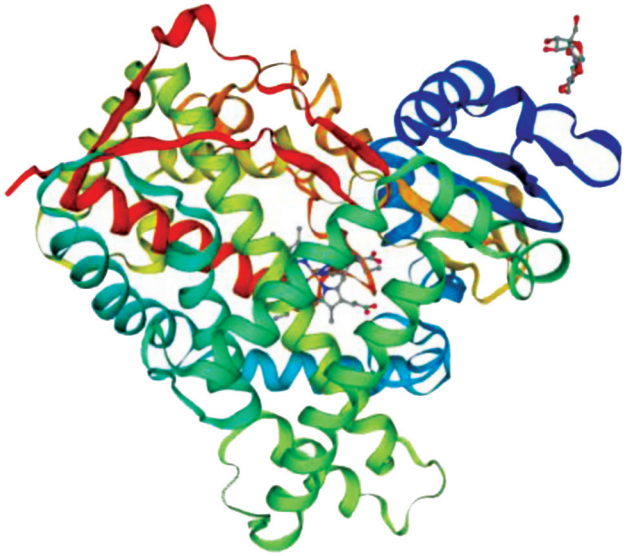

Fig 8. Prediction of the tertiary structure of CYP2E1 protein.

Conclusion

The *Camelus bactrianus* CYP2E1 has an open reading frame of 1488bp, and the cDNA encodes a protein of 495 amino acid residues with a molecular weight of 145.6kDa. The deduced amino acid sequence of Bactrian camel CYP2E1 showed the highest identity with *Camelus dromedarius* (94.7%), *Ovis aries* (87.3%), *Bos taurus* (85.5%), *Sus scrofa* (83.4%), and *Equus caballus* (79.9%). the phylogenetic analysis revealed that Bactrian camel (*Camelus bactrianus*) CYP2E1 was grouped with that of wild Bactrian camel (*Camelus ferus*). The CYP2E1 protein contained a transmembrane domain which extended from 2-23 amimo acids, while 24-495 amino acids were outside the cell, and no signal peptide was found.

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