Proteomics of old world camelid (Camelus dromedarius): Better understanding the interplay between homeostasis and desert environment

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ABSTRACT

Life is the interplay between structural–functional integrity of biological systems and the influence of the external environment. To understand this interplay, it is useful to examine an animal model that competes with harsh environment. The dromedary camel is the best model that thrives under severe environment with considerable durability. The current proteomic study

Abbreviations: 2D, two-dimensional; MS, mass spectrometry; CHAPS, 3-(3-cholamidopropyl)-dimethylammoniopropane sulfonate; pI, isoelectric point; IPG, immobilized pH gradient; DTT, dithiothreitol; SDS, sodium dodecylsulfate; PAGE, polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid; MALDI, matrix assisted laser desorption ionization; CHCA, 2-cyano-4-signal-to-noise; ACTH, adrenocorticotropic hormone; PMF, peptide mass fingerprinting; PDB, protein database; TOF, time of flight; hsp, heat shock protein; MAPK, map kinase; Dvl, dishevelled: scaffold protein involved in the regulation of the Wnt signaling pathway; DAPLE, Dvl-associating protein with a high frequency of leucine residues.

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Peer review under responsibility of Cairo University.
Introduction

One humped camel (Camelus dromedarius) is a unique creature belonging to old world camelid that is adapted for desert life. These camels are found mainly in the Middle East with extension into tropical and subtropical areas. With drought becoming an increasingly common global threat, the peculiar nature of the camel to cope with hot and arid conditions makes it a strategically important animal. For 14 centuries, the dromedary has been referred to as a creature of wonder [1] having a special ability to both conserve and store water. The camel can survive long periods even after more than 40% loss of its body hydration. Moreover, camels can drink as much as 57 l of water in a short period of time; such rapid rehydration is capable of causing death to other mammals.

The camel shows a true rumination pattern of digestion, expected for a ruminating ungulates; however, based on anatomical and physiological issues, it is considered as pseudoruminant. The camel also has the highest blood glucose level among all ruminants with similarly high glucagon levels [2].

Dromedary red blood cells have an unusual elliptical shape, possibly to facilitate their flow in the dehydrated animal. These cells are also showing less osmotic fragility than red cells in other mammals [3]. Thus, the camel’s red blood cells can withstand high osmotic variation without rupturing, even during rapid rehydration. This may result from altered membrane phospholipids distribution in its red blood cells [4]. Interestingly, as a result of having very efficient kidneys, the camel urine is as thick syrup and feces are so dry that they can fuel fires [5].

Sporadic research has led to discoveries of the uniqueness of dromedary, but our understanding of this domestic ruminant is still in its infancy. For example, camelids have an unusual immune system, where part of the antibody repertoire is devoid of light chains [6]. The role of the camel’s immune system to their resistance to hot arid environments is currently unknown. The current systemic study attempts to elucidate the molecular basis for the adaptive changes required for the camel’s survival in an arid environment. The peculiarity of dromedary camel among mammals turns our eyes to study on dromedary organs explains a number of cellular mysteries providing functional correlates to arid living. Proteome profiling of camel organs suggests a marked increased expression of various cytoskeleton proteins that promote intracellular trafficking and communication. The comparative overexpression of α-actinin of dromedary heart when compared with rat heart suggests an adaptive peculiarity to sustain hemoconcentration–hemodilution episodes associated with alternative drought-rehydration periods. Moreover, increased expression of the small heat shock protein, α B-crystallin facilitates protein folding and cellular regenerative capacity in dromedary heart. The observed unbalanced expression of different energy related dependent mitochondrial enzymes suggests the possibility of mitochondrial uncoupling in the heart in this species. The evidence of increased expression of H+-ATPase subunit in camel brain guarantees a rapidly usable energy supply. Interestingly, the guanidinoacetate methyltransferase in camel liver has a renovation effect on high energy phosphate with possible concomitant intercession of ion homeostasis. Surprisingly, both hump fat tissue and kidney proteomes share the altered physical distribution of proteins that favor cellular acidosis. Furthermore, the study suggests a vibrant nature for adipose tissue of camel hump by the up-regulation of vimentin in adipocytes, augmenting lipoprotein translocation, blood glucose trapping, and challenging external physical extra-stress. The results obtained provide new evidence of homeostasis in the arid habitat suitable for this mammal.
Table 1  Identified heart proteins in NCBI database search GI; NCBI gene bank ID, Mw; molecular weight, pi; isoelectric point, ΔC/R: relative change (camel/rat%).

| Spot no. | Identified AA sequence (MS/MS) | MATCHED protein | NCBI acc no. | Score | Mr/pl | Seq.cov | ΔC/R |
|----------|---------------------------------|-----------------|--------------|-------|-------|---------|------|
| C2       | R.KPLVIAEDVDGEALSTLVR.L R.AVEEIGVLLGSCALLR.C | Heat shock protein 65 (Mus musculus) | 51455 | 103 | 60903/5.48 | 7 | 49 |
| C3       | K.APIQWEER.N K.TPYTVNIVTR.E R.IAEIFEYAR.N | NAD+ isocitrate dehydrogenase, alpha subunit (Macaca fascicularis) | 1182011 | 183 | 3677/5.72 | 8 | 201 |
| C4       | MS, 11 PEPTIDE MATCHED FROM 65 | 3-Hydroxy-3-methylglutaryl Co-enzyme A reductase | 2648815 | 64 | 47116/7.65 | 7 | 213 |
| C5       | K.IWHHTFYNELR.Y K.SYLPDGQVITIGNER.F | Gamma non-muscle actin (Oryctolagus cuniculus) | 1703 | 128 | 41729/5.30 | 7 | 35 |
| C6       | K.IWHHTMYNELR.Y R.GYSVTVAER.E K.SYLPDGQVITIGNER.F | Muscle actin (Styela clava) | 10111 | 121 | 42040/5.29 | 9 | 280 |
| C7       | MS, 6 PEPTIDE MAT FROM TOTLA 65 | Histone deacetylase HDAC3 (Oryza sativa) | 50906299 | 49 | 56469/5.54 | 7 | 34 |
| C8       | K.AHGGYSVFAGYGER.T R.VALTGLTVAEYFR.D R.DQEQGQVLLFINDIFR.F | ATP synthase beta subunit (Oncorhynchus mykiss) | 76362315 | 215 | 18719/4.87 | 24 | 29 |
| C11      | R.VGWELLLITIAR.T K.GITQEQMNFR.A R.ASFHDFDR.R R.EATADTDSEAQVIASFR.I R.ILASDKPYILAEER.R | Actinin, alpha 2 (Homo sapiens) | 4501893 | 229 | 103788/5.31 | 6 | 984 |
| C12, 13  | K.IEFTPEQIEEFKEAFMLFDRT.K I.GTTQGCDVLR.A R.ALGQNPQVEAEVR.V K.NKDTGYEDFAYGLR.V K.DTGYEDFVEGLR.V | PREDICTED: similar to myosin light polypeptide 3 | 57101266 | 445 | 22355/5.02 | 29 | 286 |
| C5       | R.RPFFPFSHSPSL.R R.APSWDTGLSEMRL.R R.IPADVPLATSSLSSDGVL | Alpha B-crystallin chain | 7954784 | 212 | 20054/6.76 | 28 | 773 |
| C16      | R.RPFFPFSHPSL.R R.APSWDTGLSEMRL.R R.IPADVPLATSSLSSDGVL | Crystallin, alpha polypeptide 2-;Hsps | 27805849 | 183 | 20024/6.76 | 28 | 321 |
| C18      | R.RPFFPFSHPSL.R R.APSWDTGLSEMRL.M | Alpha B-crystallin polypeptide 2 (Rattus rattus) | 57580 | 99 | 19945/6.84 | 13 | 320 |

its proteome in comparison with rat. The choice of rat as a generally accepted central point mammalian model expands our scope of comparison beyond the limited frame of ungulates. Proteomic differences between different organs in the camel and the rat are examined by two-dimensional (2D) mass spectrometry (MS/MS)-enabled 2D electrophoresis. This study affords a better understanding of the interplay between mammalian homeostasis and a harsh environment.

Material and methods

Tissues

Healthy, clinically normal adult male one humped camels (Camelus dromedarius) were used in the study. Animals were kept on rest with food and water ad libitum one week before slaughtering. Liver, heart, brain, kidney, and hump fat from camels were collected and cut into thin slices at an authorized abattoir house (Giza District, Egypt). At least five animals were sampled for each organ. Samples were snap-frozen in liquid nitrogen and stored in –70°C until processing. The collection and use of these samples was approved by the Institutional Review Board of Egyptian Animal Health Affairs. Samples of the same organs were similarly prepared from rat (Rattus norvegicus) maintained at the animal care unit (Medical School – Inje University, Republic of Korea).

2D-gel electrophoresis and proteomics

Protein samples from camel organs were examined in parallel with rat control organs. Proteins were extracted for 2D gel electrophoresis using a 2D Quant kit (GE Healthcare) as
Camel and rat heart proteins. (A) Enlarged three-dimensional electrophoresis spots images showing the 10 overexpressed and 10 under expressed protein spots. (B) Histograms quantify these protein spots. (The error bars represent the SEM of mean of at least three independent experiments, $p < 0.05$ vs control) (pH range: 3–10; with 10–225 MW range).

**Image analysis**

Silver-stained gels were scanned on a flatbed scanner (Umax PowerLook 1100; Fremont, CA, USA), and the resulting digitized images were analyzed using ImageMaster 2D Platinum software (GE Healthcare). At least three separate gels of the same organ from different animals were independently analyzed to increase experimental certainty. Further gel analysis was performed as previously described [7] and described in Supplementary data sheet 1.

**Protein mass analysis and identification**

The selected stained spots were excised, destained, reduced and digested with trypsin. Peptides were analyzed with matrix-assisted laser desorption ionization (MALDI) TOF/TOF mass spectrometer, 4700 Proteomics Analyzer (Applied Biosystems, Framingham, MA) for protein identification [7,8]. Resulting data were analyzed by GPS ExplorerTM 3.5 (Applied Biosystems) software. The proteins were identified by using MASCOT 2.0 search algorithm (Matrix Science, London) to search rodent subset of the National Center for Biotechnology Information (NCBI) protein databases.

**Results**

**Data handling**

The logical evaluation of the camel proteome is complicated by the absence of previously published genomic and proteomic data. Since rat (*Rattus norvegicus*) is a well known mammalian model with many Protein Data Bank (PDB) entries, the proteome of corresponding rat organs was used as the reference control. The protein levels in various camel organs were visualized on 2D electrophoresis gels. Based on an automated spot-counting algorithm (Image Master 2D Platinum), means of 1325 ± 95 protein spots were detected in the gel of the heart, liver, adipose tissue, kidney, and brain. All spots were distributed in the region of pI 4–9 and had relative molecular weights (MW) between 15 and 200 KDa. The protein spots in both camel and control gels were then excised from the gel and incubated with trypsin to digest the proteins in the gel, which were then analyzed by MALDI-time of flight (TOF) MS/MS.

**Camel heart proteome**

The camel heart proteome showed a well matched proteomic image to that of the rate heart control (Fig. 1 and Table 1). It is clear that actinin and alpha B-crystallin were markedly overexpressed in camel compared to that of the control (Fig. 2). In the 2D electrophoresis-MS/MS data, alpha B-crystallin in camel heart showed peptides (Fig. 3A) that covered both conserved domains of bovine alpha B-crystallin [*Bos taurus*] as well as the intervening peptides (57–69 amino acid residues). These results demonstrate a strong identity between camel and bovine alpha B-crystallin with possible two sites for phosphorylation. Despite a twofold increase in the expression of NAD+-dependent isocitrate dehydrogenase in camel heart when compared to the rat heart, there was a parallel down regulation of ATP synthase expression. Moreover, all the overexpressed proteins had acidic pIs.

**Physical distribution of the camel proteome**

Camel heart proteomic data closely matched its counterpart rat proteome. To amplify the differences in proteomic data from the remaining organs, each gel was divided into four quarters and proteins separated based on MW and pI. The relative abundance of proteins in each group was estimated from the total number spots, and the percent area in each quarter gel occupied by proteins as revealed by gel imaging. These data were then compared to the corresponding quarters in rat control for liver adipose tissue and kidney (Fig. 4A–C). Interestingly, both adipose tissue and kidney proteomes shared a higher density of acidic proteins (pI < 7). While these acidic proteins are concentrated in the low molecular weight range in hump adipose tissue, in the kidney proteome, these acidic proteins displayed a wide range of molecular weights.

**Camel liver proteome**

The camel liver proteome was dissimilar to the rat liver control. An area of well defined dimensions (pH and MW) was selected in that showed marked similarity by visual and digital inspection (Fig. 5A). The protein spots within these clearly defined boundaries were then analyzed by MALDI-TOF MS or MS/MS. The proteins identified in camel proteome with no corresponding counterpart in the rat control are representative of overexpressed proteins. To determine the amino acid sequence of proteins of camel proteome that does not match with the known proteome MS database, the MS/MS was then performed.
Fig. 3  Comparative analysis of sequence data obtained from the camel proteome. (A) α-B-crystallin belonged to bovine [Bos Taurus: gi:117384; top] showing the MS/MS-derived matched sequences in camel α-B-crystallin (red-marked with blue boxes sequences; bottom). The reported sequences in camel cover both conserved domains of bovine crystallins, α-B with marked identity to bovine one and possible two serine phosphorylation sites (indicated by arrows). (B) Cytochrome b5 sequence of rabbit [Oryctolagus cuniculus] (top) and the shared amino acids sequence residues with that indicated by MS/MS data for camel adipose tissue (bottom). The shared sequences of camel with that of rabbit cytochrome b5 (gi:164785) are red-marked in blue boxes. The matched sequences carry different motives that responsible for the final enzyme activity. (C) Galectin-1 belonging to sheep [Ovis aries]. The sequence shown in red with blue framed box is the MS/MS-reported matched sequences in camel galectin-1. The specific region of interest in reported sequence of sheep (69–75 amino acid residues; WGAEQRE gi:3122339) is included with the matched camel sequence. (D) Bovine [Bos Taurus] phosphatidylethanolamine binding protein (gi:1352725) sequence. The MS/MS-matched amino acid residues in camel brain proteome (shown in red in blue framed box) are proved to have interspecies similarities in Beta-strand region (Res. # 62–70); hydrogen bonded turns (Res. # 71–72 and Res. # 94–95); helical region (97–99), and second Beta-strand region (Res. # 100–104).
The results of proteins were identified by MS, MS/MS for liver of camel and rat, respectively (Tables 2 and 3). The amino acid sequence from MS/MS of the guanidinoacetate methyltransferase (Fig. 6) matched this same protein in the corresponding NCBI peptide database. Among the determined set of liver proteins, a total 13 proteins were identified by MS to be over 70 (Mowse score) and/or over 34 in MS/MS peptide sequencing. The liver proteome showed differential expression of metabolic enzymes and cytoskeleton proteins. In contrast to the large number of metabolic enzymes identified in rat liver within the circled area, few of these were observed in the camel proteome (Fig. 5A). The MS/MS data show the similarity of camel metabolic enzymes to those of other species.

Camel hump fat proteome

The proteome of hump adipose tissue was analyzed in comparison with adipose tissue of rat similar to that of liver (Fig 5B; Tables 4 and 5). Hump fat adipose tissue displayed many more protein spots than that of rat adipose tissue. Unlike the rat control, the proteome of camel adipose tissue contains cytoskeleton proteins together with heat shock proteins, including hsp 27, hsp 70, and vimentin (see insert circled area in Fig. 5B). These data clearly confirm the presence of actin and tubulin cytoskeletal proteins and high abundance of vimentin, suggesting the overexpression of cytoskeleton proteins in fat cells.

Camel brain proteome

A number of proteins are uniquely expressed in camel brain with no corresponding protein spots in the equivalent areas of the control. These proteins (Fig. 5C and Table 6) are either uniquely expressed or highly expressed in the brain of camel. The camel brain uniquely expresses or overexpresses chaperonin 10, chaperonin-like beta-synuclein, phosphatidylethanolamine binding protein showing marked homology to bovine brains and cytoskeleton tubulin 5-beta (Fig. 3 D).

Camel kidney proteome

Camel kidney revealed only one unique, identifiable spot belonging to calbindin family of proteins (Fig. 5D and Table 7). Many protein spots failed to match the NCBI peptide MS or MS/MS database.

Discussion

The one humped camel has a unique tolerance for extremely hot and arid conditions. The observed climate change with projected environmental increase in global warming and desertization makes the dromedary camel an economically and logistically strategic animal. The absence of genomic data and a defined proteome makes understanding this important species quite challenging. Proteomic data, even in the absence of a defined genome, should lead to improved understanding of the phenotypic acclimatization of this unique mammal. The current study describes a novel approach to understand the interplay between proteome – homeostasis in the dromedary camel.
Energy balance and structural integrity are indispensable elements for the optimal performance of camel heart in an arid environment. Both isocitrate dehydrogenase and ATP synthase considerably impact mitochondrial energizing of the camel heart. The relative increase in isocitrate dehydrogenase parallels a decrease in ATP synthase and represents evidence for proton leakage in camel cardiac muscle. The wide range of body temperature fluctuation accompanied by variable respiratory frequency and different level of exhaled water in desert camel [10] require a greater flexibility of camel mitochondria to move between respiratory states. Further investigation is required on camel mitochondria decoupling proteins to confirm this hypothesis.

Cardiac myocytes contain intracellular cytoskeleton scaffolds that provide for structural support, compartmentalization of intracellular components, protein synthesis, intracellular trafficking, organelle transport within the cell, and second messenger signaling pathway modulation [11,12]. The observed overexpression of cytoskeleton proteins in camel heart greatly reduces cellular stress by offering rapid and durable tool for direct cellular communication [13].

Fig. 4  The relative abundance of proteins (spot numbers and total area) in each quarter of gel that represents the proteomic images. Camel liver (4-A); hump fat (4-B); and kidney (4-C) were estimated from both total number of spots and % volume of occupied proteins (as revealed by the 3D imaging of the gels) in each quarter. The migrated proteins were, therefore, parted according to their MW and pl. The data were then compared with corresponding quarters in rat control. Both adipose tissue and kidney proteomes shared higher clusters of acid tolerable proteins (pl < 7). (Error bars are SEM, p < 0.05; n = 3 at least).

Camel heart proteome

Energy balance and structural integrity are indispensable elements for the optimal performance of camel heart in an arid environment. Both isocitrate dehydrogenase and ATP synthase considerably impact mitochondrial energizing of the camel heart. The relative increase in isocitrate dehydrogenase parallels a decrease in ATP synthase and represents evidence for proton leakage in camel cardiac muscle. The wide range of body temperature fluctuation accompanied by variable respiratory frequency and different level of exhaled water in desert camel [10] require a greater flexibility of camel mitochondria to move between respiratory states. Further investigation is required on camel mitochondria decoupling proteins to confirm this hypothesis.

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Surprisingly, a marked up-regulation of α-actinin2 expression was observed in camel heart compared to that of the control. Alpha-actinin2 is a cytoskeleton protein belonging to the spectrin gene superfamily. This family has a wide range of cytoskeletal proteins, including the α- and β-spectrins and dystrophins. Alpha-actinin2 is an actin-binding protein with various activities in different cell types. Recent evidence also shows the involvement of α-actinin2 in molecular coupling of a Ca²⁺-activated K⁺ channel to L-Type Ca²⁺ channels giving better ion channels modulation [14]. This may result in an improved tolerance for abrupt ionic imbalance.
with enhanced extra-osmoregulatory capacitance of camel cardiomyocytes.

A sevenfold increase in the expression of alpha B-crystallin fits well with the protection of surrounding structural integrity.
Table 2  Identified camel liver proteins in NCBI database search GI; NCBI gene bank ID, Mw; molecular weight, pI; isoelectric point.

| Camel liver | Identified AA sequence (MS/MS) | MATCHED protein | NCBI acc no. | Score | Mr/pI | Seq.cov |
|------------|--------------------------------|-----------------|--------------|-------|-------|---------|
| C1         | R.AVFPSIVGRPR.H, K.YPIEHGIVTNWEDMEK.I, K.IWHTFYNELR.V, R.VAEEHPVLLTEAPLNPK.T, R.GYSFTTAE.R, K.SYELPDGQVITIGNER.F, K.DLYANTVLSGTTMYPGIADR.M | Hypothetical protein XP_533132 [Canis familiaris] (Actin like protein) | 73964667 | 114 | 42053/5.24 | 27 |
| C2         | K.ELFPIAAQVDK.E, R.ASSTANLIFEDCR.I, K.IAMQTLDLDMGR.I, R.ITEIYEGTSEIQ.R.L, K.LVIAGHLL.R | Acyl-CoA dehydrogenase (EC 1.3.99.3) precursor, short-chain-specific | 111334 | 74 | 44654/8.42 | 13 |
| C4         | K.LAEQAERYDEMVESMK.K, K.KVAGMDVLTVEER.N, K.KVAGMDVLTVEER.N, R.NLSVAYK.N, R.YLAEFATGNDR.K, R.YLAEFATGNDR.E, K.AASDIAMTELPPTHPR.L, K.AASDIAMTELPPTHPR.L | PREDICTED: similar to 14-3-3 protein epsilon (14-3-3E) (Mitochondrial import stimulation factor L subunit) (MSF L) isoform 1 [Canis familiaris] | 73960520 | 103 | 26785/4.73 | 28 |
| C6,7       | K.GAGTDEGCLIEILASR.T, R.ISQTQYQOQR.G, R.SLEDIRSDTSFIMFQ.R.V, R.SDTSFIMFQ.R.V, R.YLVSLASAGR.D, K.SMKGLGTDDNTLIR.V, R.AEIDMLDIR.A, R.AEIDMLDIR.A Oxidation (M) | PREDICTED: annexin IV isoform 5 [Pan troglodytes] | 114577902 | 79 | 36258/5.84 | 23 |
| C8         | R.LHDDVDFYK.A, K.HQLKQDFEQV.Y, K.SLDLQINVVRLQG.LER.D, R.ELEAEHQLQR.D, R.DLTQKPVTVHT.R.T, R.KAEDELEK.V, K.GYEELHAAHK.T.E, R.SSPTPAEVLTEA.V | PREDICTED: similar to DVL-binding protein DAPLE [Canis familiaris] | 73964395 | 71 | 266905/5.87 | 5 |
with improved regeneration. The small heat shock protein alpha B-crystallin is a molecular chaperon, which stabilizes proteins that are partially or totally undergo unfolding as a result of inflammatory stress [15]. Alpha B-crystallin, belonging to the family of ATP-independent chaperones, utilizes minimum energy to prevent misfolded target proteins from aggregating and precipitating. Cardiac crystallin is recently proved to contribute in a localized structural or protective role [16]. Furthermore, MAPK kinase MKK6-dependent phosphorylation of alpha B-crystallin shows cytoprotective effects on cardiac myocytes when they are exposed to cellular stress [17]. The overexpression of alpha B-crystallin in camel heart supports this mechanism and suggests an extra protective role against dehydrating and sudden rehydration stress in arid environments. A high level of identity was observed between bovine in both conservative domains of bovine alpha B-crystallin \([\text{Bos taurus}]\) and the intervening peptides (57–69 aa). These results afford two possible phosphorylation sites in the three major serine residues (Ser19, Ser45, and Ser59) previously shown to be available for post-translational modification [18,19]. Phosphorylation enhances the chaperone activity of alpha B-crystallin, protecting against two types of protein misfolding, amorphous aggregation, and amyloid fibril assembly in the heart [20].

Interestingly, the camel heart proteome shows a relatively similar pattern of distribution of rat heart regarding the localization based on pI scaling and molecular weight distribution. Proteome interprets the organ uniqueness in liver morphology

Liver is a metabolically active organ contributing in many homeostatic mechanisms. The maintenance of liver activity necessitates the presence of active metabolic and energy saving enzymes, available building blocks, and the safeguarding of the newly formed biomolecules. The hepatic proteome of camel metabolic enzymes indicates a wide range of similarity with other mammals. Energy shuttling enzymes, such as ATP synthase (\(\beta\)-subunit), are similar in the hepatic proteome of camel and other known species. Moreover, energy related and fatty acid regulatory enzymes show a high level of identity to other species. These include citric acid cycle enzymes, NAD-dependent isocitrate dehydrogenase, members of \(\beta\)-oxidation of...
Table 3  Identified rat liver proteins in NCBI database search GI; NCBI gene bank ID, Mw; molecular weight, pI; isoelectric point.

| Rat liver | Spot no. | Identified AA sequence (MS/MS) | MATCHED protein | NCBI acc no. | Score | Mr/pl score | seq.cov |
|----------|---------|--------------------------------|-----------------|--------------|-------|------------|---------|
| Metabolic enzymes and enzyme like proteins | R1,R2 | R1.RIFSSEHDIFR.E R1.IFSSEHDIFR.E K1.FQFQEVIPYHEWEK.A K1.CIGAIAMTEPGAGSDLQGVR.T K1.QDQTAELFEVDR.L R1.LPASALGGEENKGFFYLMQELPQER.L K1.GFYYLMQELPQER.L R1.LIADLAISACFMFEETR.N | Acetyl-coenzyme A dehydrogenase, long-chain [Rattus norvegicus] | 6978431 | 86 | 48242/7.63 | 23 |
| R4 | K1.VADIGLAAWGR.K R1.KALDIAENEMPGLMR.M K1.WSCNIFSTQDHAAIAAK.A K1.GETDEEYLWICETQTLHFK.D K1.HPQLLSGR.G K1.SKFDNLYGCR.E K1.FDNLYGCR.E K1.EGNIFVTTTCGDIILG.H R1.ILLAEGR.L | Chain A, rat liver S-adenosylhomocystein hydrolase | 4139571 | 123 | 47889/6.08 | 25 |
| R6 | R1.DHGDLAFVDVNDSPFQIVK.N K1.ANEQLAAYVAETQK.N K1.DIVYIGLR.D R1.DVDPGEHYIIK.T K1.VMEEFTSYLLGR.K K1.VMEEFTSYLLGR.K Oxidation (M) R1.EGLYITEEYIK.T K1.TGLSSGSLDEVMNTGKTPVEEVT.T R1.EGNHKPEETDYLKPPK. | Chain A, crystal structure Of the H141c arginase variant complexed with products ornithine and urea | 13786702 | 125 | 35096/6.72 | 35 |
| R7,8 | R1.HIDGAYVVYR.N K1.LWSTDHDEMPVMPARL.E R1.SLVGSNFKN.R K1.SLVGSNFKN.R R1.YKPVTFQVYCHPYFTQTK.L R1.NPLWNVSSSPLLKDLELLTLGK.K R1.TQAQVLR.F R1.FVEMILMWSDHPEYPYFDED | Aldo-keto reductase family 1, member D1 [Rattus norvegicus] | 20302063 | 114 | 37639/6.18 | 31 |

(continued on next page)
| Spot no. | Identified AA sequence (MS/MS)                                                                 | MATCHED protein                                                                 | NCBI acc no. | Score | Mr/pl         | seq.cov |
|----------|------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------|-------|---------------|---------|
| R9, 11   | K.CPGVPSGETTLEETPAKR.L K.THLPSLLPQSLIDQK.V K.VKVIY1AR.N K.EWWELR.H R.HTPVFLYEFIKENPK.R K.KILEFLG.R S R.SLPEETVSIVHHTSF.K K.RSLPEETVSIVHHTSFKK.M K.NFTTVQNERFDAHYAK.T | Aryl sulfotransferase [Rattus norvegicus]                                      | 55765        | 134   | 33422/6.41    | 38      |
| R12      | K.JVGSNASQLAHFDP.R V R.UTMVVFEDIGGR K.Oxidation (M) R.KLTEINTQHENVK.Y K.LTEIINTQHENVK.Y K.FCETTIGCKDP.AQGQLK.E K.ELMQTPNFR.I K.ELMQTPNFR.IOxidation (M) R.IYVQVETVIEICGALK.N R.NIVAVGAFCDGLFGDNTK.A R.ELHSITLHGK.G | Glycerol-3-phosphate dehydrogenase 1 (soluble) [Rattus norvegicus]              | 57527919     | 161   | 38112/6.16    | 32      |
| R10      | R.LGGEVSVLVAGTK.C K.VLVAQHDAYK.G K.QSYTHICAGASAFGK.N K.LNVAPSDIEIK.S R.TIYAGNALCTVK.C K.LLVYDADQLHAAAVGASR.A R.AAVDAGFPNMDQVSVGQTGK.J R.ELPMETEIK.K R.VVPMEMTEIK.K Oxidation (M) | Electron transferring flavoprotein, alpha polypeptide [Mus musculus]           | 31981826     | 114   | 35271/8.42    | 33      |
| R13, 15  | K.MKDLHGEODLQPETR.E K.MKDLHGEODLQPETR.E Oxidation (M) K.AGTTWTOEYDMQINGDVQK.C R.NAKDCLVSYYFSS.R.M K.DCLVSYYFSS.R.M K.VLWGSNDVHVK.G K.GWVQVPROHR.I K.FLEKDIYEVN.K.I R.KGMPGDW.K.N K.NYFTVQSEDY'DY.R.R R.KMAGSNITFR.T | Sulfotransferase family 1A, member 2 [Rattus norvegicus]                        | 13929030     | 148   | 35855/6.09    | 39      |
| ID  | Description                                                                 | UniProt ID   | Mowse Score | E-Value | Mowse Cons. |
|-----|------------------------------------------------------------------------------|--------------|-------------|---------|-------------|
| R14,16 | Malate dehydrogenase, cytoplasmic (cytosolic malate dehydrogenase)           | 92087001     | 116         | 36659/6.16 | 35         |
| R19  | Chain A, structure of phosphorylated phenylalanine hydroxylase               | 4930076      | 100         | 49694/5.67 | 21         |
| R20  | Methionine adenosyltransferase I, alpha [Rattus norvegicus]                  | 77157805     | 85          | 44125/5.70 | 21         |
| R21,22 | Enolase I-like, hypothetical protein LOC433182 [Mus musculus]               | 70794816     | 111         | 47453/6.37 | 28         |
| R23,26 | Chain A, crystal structure analysis of rat enoyl-coA hydratase in complex with hexadienoyl-coA | 24159081 | 133 | 28498/6.41 | 41 |
Table 3 (Continued)

| Spot no. | Identified protein | MATCHED protein | NCBI acc no. | Score | Mr/pI | seq.cov |
|----------|-------------------|-----------------|--------------|-------|-------|---------|
| R24      | -MAEVGEIIEGCRLPVL.R OXidation (M) | PREDICTED: HIV-1 Tat interactive protein, 60 kDa isoform 4 [Macaca mulatta] | 109105458 | 75 | 50824/8.74 | 23 |
| R27      | M.PGILLLDGEAPNFEEANTTIGHIR.F | Peroxiredoxin 6 [Rattus norvegicus] | 16758348 | 188 | 24860/5.64 | 56 |
| R29      | R.YVQQNAKPGDPQSVLEAIDTYCTQK.E | Chain, catechol O-methyltransferase | 1633081 | 161 | 24960/5.11 | 57 |
| R28      | Phosphatidylethanolamine binding protein [Rattus norvegicus] | | 8393910 | 134 | 20902/5.48 | 62 |
Other ubiquitous protein

R3

K.LYTLVLTPDPADPS.R.K
K.FREWHHFLVVMK.G
K.FREWHHFLVVMK.G Oxidation (M)
K.GNDISSGTVLSEYVGSPPK.D
K.GNDISSGTVLSEYVGSPPK.D
R.YYWLVYEQEQLCDEPLSNK.S
K.FKVESFR.K
K.YHLGAPVAGTCFQAEMWDSPPK.L

F alloantigen,
4-hydroxyphenylpyruvic acid dioxygenase
[\textit{Rattus norvegicus}]

R5

K.HGDGVKAIDEVEVCEHVQK.A
K.FAVLQTYGDTHTTLVEK.I
R.FWSVDDTQVHTEYSSL.R
R.SIVVANYESIK.M
R.SIVVANYESIKMPINEPAPGR.K
K.MPINEPAPGR.K
K.SQIEYVDYNGGAVGQHIALR.T
R.GMEFLAVPSSYR.L
R.GMEFLAVPSSYR.L Oxidation (M)
R.HNHQFGAGNFSF.LK.A

F alloantigen,
4-hydroxyphenylpyruvic acid dioxygenase
[\textit{Rattus norvegicus}]

Cytoskeleton

R18

.K.PR.AVFPSIVGR.S
R.AVFPSIVGR.S
K.IWHHTFYNL.R.V
R.VAEEEHPVLLTEAPLNPK.A
R.DLTDYLMK.I
R.GYSFIIITAER.E
K.SYELPDQVITIGNER.F
K.DLYANTVLSGTMTYGGIADR.M
K.IKIAPP.K.R
K.IIAPPR.K.Y

Put. beta-actin (aa 27-375) [\textit{Mus musculus}]

Proteomics of \textit{Camelus dromedarius}
Adipocytes play a central role in energy balance by serving as a major site of storage and energy expenditure [27]. The relatively high abundance of low molecular weight and low pI proteins in hump fat suggests an enhanced tolerance toward acidity and a prominent involvement in cellular events. Camel hump fat displayed more proteins than rat adipose tissue, suggesting a more metabolically active tissue. In addition to a well-developed cytoskeleton, containing actin and tubulin, the data confirm the presence and the high levels of vimentin. Moreover, vimentin’s essential role in the signal transduction pathway from ss3AR to the activation ERK and its contribution to lipolysis [28] makes vimentin an early marker of adipogenesis. Vimentin regulates lipid droplet content during differentiation [29,30] and controls the key signaling components of lipid raft processing [31]. Moreover, the higher level of glucagon in camel with consequent elevated basal blood glucose [2] is consistent with the proposed role of vimentin in GLUT-induced adipocyte glucose transport [32]. These data suggest a possible modulating role of adipocyte vimentin for the tolerance of high blood glucose levels in camel. Vimentin might operate as an inducer of a cellular trap for glucose in behalf of adipocyte energy storage. Furthermore, the dynamic nature of vimentin could offer the flexibility of fat cells. Since vimentin provides cells with resilience during mechanical stress in vivo, it may response for maintaining cell shape, integrity of its cytoplasm, and stabilizing cytoskeletal interactions. The observed high abundance of vimentin in camel adipocytes could promote the morphology of the hump of the well-nourished camel and can be considered as an adaptive correlate beneficial for living in arid conditions.

Cytchrome b5, a component of dromedary hump tissue, is a membrane bound hemoprotein, which functions as an electron carrier for several membrane bound oxygenases. Its presence in camel fat indicates a well-developed enzymatic system contributing to the detoxification of xenobiotics. Moreover, the conserved domains of cytochrome b5, with rabbit sharing a common sequence (40–89 amino acids residues), supports the extensive homology of this ortholog gene.

Galectin-1, β-galactoside-binding soluble 1 (L-14-I), is a component of dromedary adipocyte. Galectin-1 is widely expressed in epithelial and immune cells, contributing to the control of basic cellular processes, such as proliferation, apoptosis, and signal transduction, and immune modulation [33]. The present investigation suggests a similar role in camel adipocyte metabolism as described for other mammals [34]. The region of interest (residues 69–75) matches carbohydrate-recognition domain. Since the identity of galectin from different mammalian species is 80–90% [35], it is likely that galectin-1 also functions similarly.

**Brain proteome**

β-Synuclein has been shown to act as chaperonin inhibiting the fibrillation of α-synuclein [36]. The overexpression of β-synuclein in camel brain suggests an additional mechanism to prevent neurodegeneration in brain under intensive environmental stress.
| Spot no. | Identified AA sequence (MS/MS) | MATCHED protein | NCBI acc no. | Score | Mr/pI | Seq.cov |
|---------|-------------------------------|-----------------|--------------|-------|-------|--------|
| C5      | K.SYELPDGQVITIGNER.F K.DLYANYTVLSGGTTMYPGIADR.M K.QEYDES6PSVHR.K | Gamma non-muscle actin (*Oryctolagus cuniculus*) | 1703 | 324 | 41729/5.30 | 13 |
|         |                 | Mostly gamma non-muscle actin and/or actin in many different species | 49868 | 324 | 39161 | |
|         |                 |                | 63007 | 324 | 41809 | |
|         |                 |                | 71620 | 324 | 41724 | |
| C11     | R.VAPEEHVLLTEAPLNPK.A R.TTGIVMSDGVDGVTPIPYYEGYALPHAIL | Putative beta-actin (amino acid 27–375) (*Mus musculus*) | 49868 | 123 | 39161/5.78 | 13 |
|         |                 | Actin beta rat | 309090 | 123 | 41667 | |
|         |                 | Gamma actin (*Mus musculus*) | 1335823 | 123 | 41740 | |
| C30     | R.AILVDLEPGTMDSVR.S | Unnamed protein product (*Rattus norvegicus*); beta tubulin | 57429 | 84 | 49931/4.79 | 7 |
|         | K.GHYTEGAELVDSVLDVR.K | Beta tubulin (*Homo sapiens*); beta tubulin | 158743 | 84 | 49812 | |
|         |                 | Beta 3 tubulin (*Gallus gallus*) | 1297274 | 84 | 50485 | |
|         |                 | Predicted similar to tubulin, beta 3 isoform 2 (*Canis familiaris*) | 73956775 | 84 | 50648 | |
| C1      | K.EVGVGFATR.K | Adipocyte lipid-binding protein (*Oryctolagus cuniculus*) | 4887137 | 290 | 12528/7.71 | 25 |
|         | K.NTEISFKLGQEFDEVTDDDR.K | Adipose-type fatty acid binding protein (*Spermophilus tridecemlineatus*) | 12802820 | 290 | 14756 | |
|         |                 | Predicted: similar to fatty acid binding protein, adipocyte | 73997350 | 290 | 14678 | |
| C2      | MS, 29 peptide Matched from 65 | Predicted: similar to centromere protein F | 76638067 | 52 | 353257/5.01 | |
| C3      | MS, 12 peptide matched from 65 | mKIAA0421 protein (*Mus musculus*) | 37359936 | 49 | 76263/7.96 | |
| C4      | MS, 11 peptide matched from 65 | Predicted: similar to ATP-binding cassette subfamily A member 3, partial (*Danio rerio*) | 68424078 | 61 | 55936/6.36 | |
| C6      | MS, 12 peptide matched from 65 | Predicted: similar to type I hair keratin KA27 (*Bos Taurus*) | 76649749 | 47 | 52545/4.78 | |
| C7      | K.NTEISFKLGQEFDEVTDDDR.K | Adipocyte lipid-binding protein (*Oryctolagus cuniculus*) | 4887137 | 142 | 12528/7.71 | 17 |
|         |                 | Predicted: similar to fatty acid binding protein, adipocyte | 12802820 | 142 | 14756 | |
| C8      | MS, 14 peptide matched from 65 | Predicted: similar to CG31643-PA isoform 1 (*Bos Taurus*) | 73997350, 142 | 14687 | |
|         |                 |                | 76677435 | 51 | 96302/8.31 | |

(continued on next page)
| Spot no. | Identified AA sequence (MS/MS) | MATCHED protein | NCBI acc no. | Score | Mr/pI | Seq.cov |
|---------|-------------------------------|-----------------|--------------|-------|-------|---------|
| C10     | MS, 15 peptide matched from 65 | Predicted: similar to protein transport protein Sec2 | 73952947     | 44    | 111485/6.87 |
| C12     | MS, 12 peptide matched from 65 | Predicted: similar to aldolase reductase | 76662094     | 52    | 34311/5.34  |
| C13     | K.DGGAWSEQRF.E K.LPDGYEFK.F   | Galectin-1 (beta-galactoside-binding lectin 1-14-1) lactose binding lectin 1 | 3122339      | 120   | 14694/5.37  | 13      |
| C14     | K.LISWYDNEFGYSNR.V            | glyceraldehydes-3-phosphate dehydrogenase (Drosophila hydei) | 11178       | 118   | 35539/8.20  | 4       |
|         |                               | Glyceraldehydes-3-phosphate dehydrogenase (Canis familiaris) | 50978862    | 118   | 35838       |
|         |                               | Predicted: glyceraldehydes-3-phosphate dehydrogenase (Pan troglodytes) | 55637711    | 118   | 36030       |
| C16     | MS, 10 peptide matched from 65 | Apoptosis inhibitor ch-IAP (Gallus gallus) | 11991646    | 46    | 36543/6.36  |
| C20     | MS, 5 peptide matched from 65 | Predicted: similar to mitochondrial ribosomal protein | 68437845    | 51    | 7433/11.19  |
| C22     | MS, 18 peptide matched from 65 | Novel protein similar to vertebrate adenylate cyclase | 56207901    | 54    | 86625/8.60  |
| C24     | MS, 9 peptide matched from 65 | Predicted: similar to ku70-binding protein 3 | 72014818    | 50    | 22290/6.03  |
| C27     | K.FLEEHPGGEEVLR.E R.EQAGGDATENFEDVGHSTDR.E K.TFIELHLPD.R.S | Cytochrome b5(sequence coverage 34%, sequence homology in the center of 134 amino acids polypeptide) | 471150      | 156   | 11226       |
|         |                               | Proteins matching the same peptides | 837345      | 156   | 9453        |
| C28     | MS, 10 peptide matched from 65 | Heat shock protein hsp70-related protein (NADP) | 74005287    | 49    | 46777/6.13  |
| C29     | MS, 20 peptide matched from 65 | F box and leucine rich repeat protein 10 isoform b | 54112380    | 46    | 144676/8.74 |
|         |                               | Soluble cytochrome b5 (Oryctolagus cuniculus) | 204665      | 52    | 22879/6.12  | 7       |
|         |                               | Peditoxin, pedin = cytochrome b-like heme protein (Toxopneustes pileolus; sea urchin) | 545503      | 52    | 12981       |
|         |                               | Heat shock protein 27 (Rattus norvegicus) | 545503      | 52    | 12981       |
|         |                               | Heat shock protein 1 (Mus musculus) | 7305173     | 52    | 22887       |
| C17     | R.EMEENFAEAANYQDTIGRL.R.ISLPNFSSSLNR.E | Vimentin (Homo sapiens) | 37852       | 106   | 53653/5.06  | 7       |
| C18     | MS, 10 peptide matched from 65 | Heat shock protein ksp70-related protein (Homo sapiens) | 6563208     | 49    | 54744/5.41  |
| C23     | R.EMEENFAEAANYQDTIGRL.R.EYQDLLNVK.M R.ISLPNFSSSLNR.E R.DGQVNETSQHDDLE | Vimentin | 340234     | 99    | 35032/4.70  | 19      |
|         | Proteins matching the same peptides | Vimentin (Pan troglodytes) | 56342340    | 336   | 53615       |
|         |                               | Vimentin (Homo sapiens) | 62414289    | 336   | 53619       |
| C25     | R.EMEENFAEAANYQDTIGRL.R.EYQDLLNVK.M R.ISLPNFSSSLNR.E R.DGQVNETSQHDDLE | Vimentin | 340234     | 86    | 35032/4.70  | 19      |
|         | Proteins matching the same peptides | Vimentin (Pan troglodytes) | 56342340    | 314   | 53615       |
|         |                               | Vimentin (Homo sapiens) | 62414289    | 314   | 53619       |
Phosphatidylethanolamine binding protein is a lipid-binding protein that enhances acetylcholine synthesis with additional inhibitory action on MEK- and ERK-signaling pathways. Phosphatidylethanolamine binding protein in camel brain shows high homology to the bovine protein. Furthermore, the larger amounts found in camel brain when compared to rat brain do not show the same proteome spot, suggesting enhanced ERK signaling in camel brain, warranting further study.

Camel proteome and heat shock proteins

Despite a preliminary investigation on a 73 kDa heat shock protein (hsp 73) in camel [37], there are no recent reports on hsp in camel. In the current study, proteins with extensive homology to the hsp 65, hsp 27, and chaperonin 10 were found in various camel organs. Heat shock proteins defense against dehydration or thermal stress in arid environments.

General outlook and implication of a well-developed cytoskeleton

The entire economy of the cell is a function of the structure of its transport facilities. Cytoskeletal proteins sculpt the structural architecture of cells and are classified into three groups: microfilaments represented in our data finding by actin filaments; intermediate filaments e.g. vimentin as that found in hump fat cells; and microtubules as different kind of β-tubulin. Different cytoskeletal protein monomers can build into a variety of structures based on associated proteins. Actin filaments are dynamic with their length controlled by polymerization driven through nucleotide hydrolysis. Additionally, actin filaments act with microtubules as railroads for motor proteins carrying transport vehicles, unfolded/misfolded proteins, and chromosomes important for cell-cell communication and survival [13]. Widely distributed adherence junctions are self-assembled cadherins interacting with β-catenin, which binds α-catenin and in turn interact with the actin cytoskeleton [38]. Their overexpression in the heart together with actin enhances intracellular communication. In non-muscle cells, the cytoskeletal isoform is found along with microfilament bundles and adherence junctions that bind actin to the membrane. The almost tenfold increase in α-actinin2 expression in camel heart and the presence of β-tubulin as a major energy determent cytoskeleton in the camel brain confers stress adaptation to the camel while guaranteeing more flexibility in ion channel modulation [13] to keep pace with abrupt ionic imbalances associated with dehydration–rehydration cycles.

Cytoskeleton-cellular signaling possible interplay

The DVL-binding protein DAPLE and the marked expression of actins suggest a possible interplay between cytoskeleton and fine tuning of intracellular signaling in camel hepatic cells. DAPLE binds to Dvl and functions as a negative regulator of the Wnt signaling pathway [39]. The Wnt pathway (known as the wingless pathway in Drosophila) has a role in organ development in a number of species [40] with the potential of carcinogenesis development on sudden activation [41]. The inductive properties of Wnt signaling are mediated by setting free actin-bound β-catenin. The accumulated β-catenin is then translocates to the nucleus where it binds to T-cell factors and activates transcription of a
| Spot no. | Identified AA sequence (MS/MS) | MATCHED protein | NCBI acc no. | Score | Mr/pI | Seq. cov |
|---------|--------------------------------|-----------------|--------------|-------|-------|----------|
| **Cytoskeleton** | | | | | | |
| R2 | K.SYELPDGQVITIGNER,F,K.DLYANTVLSGTTMYPGIADR.M,K.DLYANTVLSGTTMYPGIADR.M,K.QEYDEGSPIVHR.K,K.QEYDEGSPIVHR.K | Putative beta-actin (aa 27–375) (*Mus musculus*) | 49868 | 308 | 39161/5.78 | 14 |
| R3 | K.DSNNLCHFNPR.F,K.DDGYWGTGSEQ.R,K.ETAQFPQGSITEVCITFDQADLT.K,K.LNMEAINYMAADGF.R | Beta-galactoside-binding lectin (*Rattus norvegicus*) | 9845261 | 124 | 14847/5.14 | 47 |
| R4 | K.WVYFNKPDIDAWEL.R,K.DVDASAVR.I,K.LNDFASAVR.I | Cytochrome c oxidase, subunit Va (*Rattus norvegicus*) | 2423354 | 129 | 16119 |
| R5 | K.MYVCEYMNAICTR.V | C-fatty acid binding protein (*Rattus norvegicus*) | 546420 | 61 | 15050/6.14 | 10 |
| R6 | R.EGGLGPLNPLADVT.K | Peroxiredoxin 2 (*Rattus norvegicus*) | 34849738 | 74 | 21784/5.34 | 14 |
| R7 | K.LVSSENFDYMK.E,K.LGVEDEITPD.R,K.LGVEDEITPD.R,K.LGVEDEITPD.R,K.LGVEDEITPD.R,K.LGVEDEITPD.R,K.LGVEDEITPD.R,K.LGVEDEITPD.R | Adipocyte fatty acid binding protein (*Rattus norvegicus*) | 1658525 | 133 | 14699/7.71 | 19 |
| R8 | K.FDASSFPGYHPK.Q,R.LLEYSYEAIVDGIINPASL.R,G.R.TNTGTVGVSIGSEASEALS.R.D | Fatty acid synthase (*Rattus norvegicus*) | 57890 | 313 | 272478/5.96 | 2 |
| R9 | K.ITQFCHHFLEG.K,K.NFEVAFDEK.K | Prolyl 4-hydroxylase, beta polypeptide (*Rattus norvegicus*) | 38197382 | 75 | 56916/4.82 | 4 |

**Metabolic enzymes and enzyme like proteins**

| Spot no. | Identified AA sequence (MS/MS) | MATCHED protein | NCBI acc no. | Score | Mr/pI | Seq. cov |
|---------|--------------------------------|-----------------|--------------|-------|-------|----------|
| R3 | K.DSNNLCHFNPR.F,K.DDGYWGTGSEQ.R,K.ETAQFPQGSITEVCITFDQADLT.K,K.LNMEAINYMAADGF.R | Beta-galactoside-binding lectin (*Rattus norvegicus*) | 9845261 | 124 | 14847/5.14 | 47 |
| R4 | K.WVYFNKPDIDAWEL.R,K.DVDASAVR.I,K.LNDFASAVR.I | Cytochrome c oxidase, subunit Va (*Rattus norvegicus*) | 2423354 | 129 | 16119 |
| R5 | K.MYVCEYMNAICTR.V | C-fatty acid binding protein (*Rattus norvegicus*) | 546420 | 61 | 15050/6.14 | 10 |
| R6 | R.EGGLGPLNPLADVT.K | Peroxiredoxin 2 (*Rattus norvegicus*) | 34849738 | 74 | 21784/5.34 | 14 |
| R7 | K.LVSSENFDYMK.E,K.LGVEDEITPD.R,K.LGVEDEITPD.R,K.LGVEDEITPD.R,K.LGVEDEITPD.R,K.LGVEDEITPD.R,K.LGVEDEITPD.R,K.LGVEDEITPD.R | Adipocyte fatty acid binding protein (*Rattus norvegicus*) | 1658525 | 133 | 14699/7.71 | 19 |
| R8 | K.FDASSFPGYHPK.Q,R.LLEYSYEAIVDGIINPASL.R,G,R.TNTGTVGVSIGSEASEALS.R.D | Fatty acid synthase (*Rattus norvegicus*) | 57890 | 313 | 272478/5.96 | 2 |
| R9 | K.ITQFCHHFLEG.K,K.NFEVAFDEK.K | Prolyl 4-hydroxylase, beta polypeptide (*Rattus norvegicus*) | 38197382 | 75 | 56916/4.82 | 4 |

### Table 5
Identified protein spots of adipose tissue of rat in NCBI database search GI; NCBI gene bank ID, Mw; molecular weight, pl; isoelectric point.
| Protein Name | Species          | Peptides | Start Site | End Site | Net Charge | PPM Score |
|--------------|------------------|----------|------------|----------|------------|-----------|
| Type II peroxiredoxin 1 | *Mus musculus* | R.KEGGLGPLNPIPLLADVTK.S | 1 | 21778/5.20 | 14 |
| Peroxiredoxin 2 | *Mus musculus* | R.QITVNDLPGR.S | 1 | 21770 |
| Perodiredoxin | *Rattus norvegicus* | R.QITVNDLPGR.S | 1 | 21834 |
| Fatty acid synthase | *Rattus norvegicus* | K.LFDHPEVPIPAESV | 1 | 1 |
| Cu/Zn superoxide dismutase (EC 1.15.1.1) | *Rattus norvegicus* | R.VISLSGEHSIHR.T | 1 | 17 |
| Cu/Zn superoxide dismutase | *Rattus norvegicus* | K.GDGPVQGVHIHFQK.A | 1 | 17 |
| Cu/Zn superoxide dismutase | *Rattus norvegicus* | R.VISLSGEHSIHR.T | 1 | 17 |
| Glycerol-3-phosphate dehydrogenase | *Rattus norvegicus* | R.VTMWVFEEDIGGR.K | 1 | 3 |
| Glycerol-3-phosphate dehydrogenase | *Rattus norvegicus* | R.VTMWVFEEDIGGR.K | 1 | 3 |
| Gamma synuclein | *Mus musculus* | K.TVEEAENIVVTGVR | 1 | 13 |
| Alpha fetoprotein | *Mus musculus* | K.DVFLGTFLYEYSR.R | 1 | 12 |
| Albumin | *Rattus norvegicus* | R.LPCVEDYLSAILNL.R | 1 | 7 |

**Cellular signaling related macromolecules**

- Gamma synuclein | *Mus musculus* | R.RHPDYSVSSL.R | 1 | 13 |
- Alpha fetoprotein | *Mus musculus* | K.LGEYFQNAVLV.R | 1 | 12 |

**Other ubiquitous protein**

- Albumin | *Rattus norvegicus* | K.AADKDNCFATEGPNLVAR.S | 1 | 7 |
| Spot no. | Identified AA sequence (MS/MS) | Matched protein | NCBI acc no. | Score | Mr/pI | Seq.cov |
|---------|--------------------------------|----------------|--------------|-------|-------|---------|
| B1      | K.FSPLTSNLINLAENGR.L            | H+-ATPase subunit, OSCP = oligomycin sensitivity conferring protein | 913531 | 51    | 20932/9.76 | 8       |
|         |                               | H+-ATPase subunit, OSCP = oligomycin sensitivity conferring protein | 913531 | 51    | 20932 |   |
|         |                               | [swine, heart, peptide mitochondrial] |            |       |       |         |
|         |                               | Mitochondrial ATP synthase, O subunit [Bos taurus] | 27806307 | 51    | 23449 |   |
|         |                               | Similar to oligomycin-sensitivity conferral protein [Bos taurus] | 28189911 | 51    | 14199 |   |
|         |                               | ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (oligomycin-sensitivity conferring protein) [Bos taurus] | 74268299 | 51    | 23419 |   |
| B2      | K.LYTLVLDTPDAPSR.K 1352725      | Phosphatidylethanolamine binding protein 1 (PEBP-1) (HCNPpp) (Basic cytosolic 21 kDa protein) [contains: Hippocampal cholinergic neurostimulating peptide (HCNP)] | 1352725 | 148   | 21087/6.9 | 18    |
|         | K.GNNISSGTVLSDLVYGSPPK.G       | Chain, phosphatidylethanolamine binding protein from calf brain | 4389366 | 148   | 20828 |   |
|         |                               | Chain A, structure of the phosphatidylethanolamine binding protein from bovine brain | 6729706 | 148   | 20956 |   |
|         |                               | Phosphatidylethanolamine binding protein [Bos taurus] | 75812940 | 148   | 21106 |   |
| B3      | R.IMNTFSVVPSPK.V + Oxidation (M) | Tubulin 5-beta [Homo sapiens] | 35959 | 193   | 50055/4.81 | 9     |
|         | R.AVLVDLEPGTMDSVR.S             | Tubulin 5-beta [Homo sapiens] | 21361322 | 193   | 50010 |   |
|         | R.AVLVDLEPGTMDSVR.S + Oxidation (M) | Tubulin, beta-4, isoform CRA_b [Homo sapiens] | 119589485 | 193   | 54946 |   |
|         | R.AVLVDLEPGTMDSVR.S + Oxidation (M) | Tubulin, beta-4 [Homo sapiens] |            |       |       |         |
|         | R.INVYYNEATGGONYVR.A            | Beta-synuclein (phosphoneuroprotein 14) (PNP 14) (14 kDa brain-specific protein) | 464424 | 67    | 14268/4.4 | 9     |
|         | R.INVYYNEATGGONYVR.A            | Beta-synuclein (phosphoneuroprotein 14) (PNP 14) | 2501106 | 67    | 14495 |   |
|         |                               | Beta-synuclein [Homo sapiens] | 4507111 | 67    | 14279 |   |
| B4      | K.EGVVQGVASVAEKT.T 464424       | Beta-synuclein (phosphoneuroprotein 14) (PNP 14) | 464424 | 67    | 14268/4.4 | 9     |
|         | K.EEVQGVASVAEKT.T                | Beta-synuclein (phosphoneuroprotein 14) (PNP 14) | 2501106 | 67    | 14495 |   |
|         |                               | Beta-synuclein [Homo sapiens] | 4507111 | 67    | 14279 |   |
| B5      | K.AQSELLGAADEATRA.A 11514063    | Chain H, bovine F1-ATPase inhibited by Decd (dicyclohexylcarbodiimide) | 11514063 | 55    | 15056/4.53 | 9     |
|         | K.VLPLEYGGT.K.V                 | ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit precursor [Bos taurus] | 28603800 | 55    | 17601 |   |
| B6      | K.VLQATVAVGSGSK.G               | Chaperonin 10 [Homo sapiens] | 4008131 | 127   | 10576/9.44 | 24    |
number of genes. Overexpressed actin is in accord with the favored homeostasis.

Conclusions

The present investigation tried to shed light on camel proteome as innovative central point to study mammalian evolution. Much of the data obtained for camel cannot fit with proteomics data for other mammals. This mismatch is not an artifact but rather support the peculiarity of the camel and in particular its adaptive nature. This study also confirms the conserved nature of many camel proteins. Thus, the camel proteome corresponds to a remote reference useful in developing a perspective of proteomic evolution among different species.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgement

The authors are grateful for Dr. Moustafa Radwan for helpful assistance of this work.

Table 7  Identified proteins camel kidney in NCBI database search GI; NCBI gene bank ID, Mw; molecular weight, pI; isoelectric point.

| Spot no. | Identified AA sequence (MS/MS) | MATCHED protein | NCBI acc no. | Score | Mr/pI  | Seq. cov |
|----------|--------------------------------|-----------------|--------------|-------|-------|---------|
| K1       | R.TDLALILSAGDN - K.LAEYTDLMLK.L + Oxidation (M) R.LLPVQENFLLLK.F | Calbindin       | 575508       | 145   | 18613/4.6 | 20 |

Proteins matching the same peptides

Unnamed protein product [Mus musculus] 26347157 145 30247
Calbindin-d28 k 203237 143 30225
Calbindin-28 K [Mus musculus] 6753242 143 30203
Cerebellar Ca-binding protein, spot 35 protein [Rattus norvegicus] 14010887 143 30203

Table 8  The ion identification as indicated in spectra panel.

| Ion type | Neutral Mr |
|----------|------------|
| a        | [N] + [M] – CHO |
| a*       | a-NH3 |
| a°       | a-H2O |
| b        | [N] + [M] – H |
| b*       | b-NH3 |
| b°       | b-H2O |
| c        | [N] + [M] + NH2 |
| d        | a – Partial side chain |
| v        | y – Complete side chain |
| w        | z – Partial side chain |
| x        | [C] + [M] + CO – H |
| y        | [C] + [M] + H |
| y*       | y-NH3 |
| y°       | y-H2O |
| z        | [C] + [M] – NH2 |

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jare.2013.03.004.

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