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In silico characterization of physiochemical properties of camel (*Camelus dromedarius*) toll like receptor 4 to 10

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Abstract
Toll-like receptors (TLRs) are important for raising innate immune responses in both invertebrates and vertebrates. TLRs in fishes, birds and mammals have been evolving under positive selection. However, relatively lack of knowledge for the dromedary camel TLRs, compared to that available for the human or other animal. It is essential to understand the structural and functional aspect of camel TLRs. The present work deals with the use of bioinformatics to describe the physiochemical properties of TLR4-TLR10 from obtained sequences which were based on gene bank of NCBI. GRAVY (grand average of hydropathicity) demonstrated the hydrophilic nature of these proteins. The computed instability index represented the stable nature of the proteins of TLR 5, 6, 7 and 10. All proteins were acidic in nature except TLR 9. Our findings provide a chemical and structural basis for optimizing the adjuvant design to elicit broad-based antibody and T cell responses with protein antigens.

Keywords: Dromedary camel, physiochemical properties, toll-like receptors, instability index

Introduction
Toll-like receptors were first identified, most well characterized PRRs (Pattern recognition receptos) (Kawai and Akira, 2011) [9] and expressed on the surface of immune cells (Mukherjee *et al*., 2014) [11]. TLRs belong to the type I transmembrane glycoprotein receptor family from insect to vertebrate present on the cell surface or membrane compartments of immune and non-immune cells (e.g., epithelial cells) (Silverman and Maniatis, 2001) [14]. TLRs recognize wide variety of PAMPs, present in bacteria, virus and fungi, in which include glycolipids such as bacterial lipopolysaccharides (LPS), proteins such as bacterial flagellin and viral nucleic acids (Girling and Hedger, 2007) [4]. Cell surface TLRs (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10), which recognize cell membrane and cell wall component of microbes and help in their phagocytosis and (ii) Intracellular TLRs (TLR3, TLR7, TLR8, TLR9, TLR11, TLR12, and TLR13) which are localized in the intracellular vesicle such as endosome, endoplasmic reticulum (ER), lysosomes, and endo-lysosomes (Yu *et al*., 2008) [16] which binds nucleic acid of microorganism (Dunne and O·Neill, 2005) [1]. Though TLRs share common structural similarities and properties, each TLR is specific for particular ligand (Sen and Sarkar, 2005) [13]. Because of their ability to modulate adaptive immunity, toll-like receptors represent strategic therapeutic targets for diseases that involve in appropriate adaptive immune responses, such as sepsis, autoimmune disorders, cancer and allergy (Lawton and Ghosh, 2003) [10]. Multiple TLR-ligand interactions are required to induce effective host resistance to pathogens, which has important implications for designing improved strategies for vaccination and immunotherapy against infectious diseases (Duthie *et al*., 2011) [11].
The basis of such hypothesis is yet to be explored since not much work has been carried out on the immunogenetics of camels. Although in dromedary camels there is lack of information about most of TLRs. Keeping in mind the present study was conducted for physiochemical characterization of camel TLR4-10 genes.

Materials and Method
Ethics committee approval
The research protocol was approved by the Institutional Animal Ethics Committee (IAEC) with authorization number i.e. CVAS/IAEC/CPCSEA-2044/ GO/Re/SL/18/2019/05.

Collection of samples, total RNA extraction and synthesis of cDNA

Blood samples of camel were collected from the jugular vein in EDTA vial for RNA isolation, from TVCC, Bikaner. A Gene Jet Whole blood RNA purification kit was used for total RNA extraction, following the manufacturer's instructions; An aliquot of total RNA was reverse transcribed by RevertAid™ First Strand cDNA Synthesis kit (Thermo scientific) as per manufacturer protocol.

Amplification of cDNA

cDNA of TLR4-10 was amplified by simplex PCR using specific primers designed by Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/ primer3 _www.cgi) on the basis of predicted sequence of dromedary camel TLRs. They were synthesized at Integrated DNA Technologies (IDT), India.

Sequence analysis and evaluation of physicochemical properties of the TLR4-10 protein

The sequencing of PCR products was done at the sequencing facility of Delhi University (South Campus). The Contig Assembly program (CAP) of BioEdit software was used to create contigs in order to make complete sequence assembly of camel TLR4-10. Nucleotide sequences were translated into amino acid through the Expert protein analysis system (ExPASY) software. To determine the properties of the designed novel protein, we have computed the physiochemical parameters of the same. The analysis was done with the help of open access ExPasy server- ProtParam (https:// web.expasy.org/protparam/) which is compute the variety of parameters from the primary sequence of a protein, such as molecular weight, instability index, aliphatic index, GRAVY (Grand average hydropathy value), extinction coefficient, theoretical pI and the half-life of the protein.

Result and discussion

In our study we report the full-length cDNAs sequences of the camel TLR4-10. These sequences were submitted to GeneBank at NCBI along with different accession no. with the help of Bankit tool. The compositions of above mentioned TLRs were determined by the ExPasy software. They were made up of 20 types of amino acids with different composition. After that, computed the physicochemical parameters of the selected TLRs (4-10) protein which are mention in table.1. The molecular weight of TLR7, 8, and 9 was greater than TLR4, 5, 6 and 10 because of long open reading frame. Subcellular localization, interactions and solubility depend upon isoelectric point and number of positively and negatively charged residues. pI is the pH value at which proteins carry no charge or the sum of negatively and positively charges is equal. pI value more than 7 was observed for TLR9, remaining had the pI value less than 7. It was indicated that all proteins were acidic in nature identified by hypothetical pI values except TLR 9. This study showed that TLRs can be either acidic or basic in nature. pI proteins are stable and compact (Gangadhar *et al.*, 2016). It will be useful for developing buffer system for purification of the recombinant proteins by the isoelectric focusing method.

Instability index (II) indicates about the protein stability under both in-vivo and in-vitro conditions. Proteins with instability index > 40 are referred to as unstable (Guruprasad *et al*.,1990) [6]. Instability index of TLR 5,6, 7 and 10 were found to be less than 40 indicating their stable nature whereas, TLR 4, 8, and 10 had stability index of more than 40, indicating them to be unstable proteins. Apart from this, aliphatic index (AI) of the globular protein structure represents the thermostability of the protein. The increased AI (>100) of the TLRs protein denotes that they are thermostable in nature. TLR4-10 had higher values (>100) of AI indicating that they were thermostable. The aliphatic index should be higher (Oladipo *et al.*, 2020) [12]. For a protein, AI can be defined as the relative volume captured by aliphatic side chains of amino acids like A (alanine), V (valine), L (leucine) and I (isoleucine). Earlier a good correlation was established between AI and thermostability of proteins by researchers (Ikai, 1980) [7]. Apart from studying above, its hydrophobic or hydrophilic character is also analysed with the GRAVY score. It is calculated for particular protein as the sum of hydropathy values of all amino acids present in the protein, divided by the number of residues in that protein. It was obtained in negative values, demonstrated the hydrophilic nature of studied TLRs proteins. Its value lies between -2 to +2 where; the positive value of GRAVY displays the hydrophobic nature of the protein sequence while the greater negative value demonstrates the hydrophilic in nature with good solubility and vice-versa (Gupta *et al*., 2019; kaur *et al*.,2020) [8]. If a protein has more than 0.4 GRAVY score, suggest its hydrophobic nature and difficult to detect on 2-D gels (Wilkins *et al.*, 1998) [15]. Moreover, the half-life was assessed to be 30 hours in mammalian reticulocytes, *in vitro*; more than 20 hours in yeast and more than 10 hours in *E. coli*, *in vivo*. It denotes how much time the protein will take to disappear after the formation in the body.

Table 1: Physicochemical parameters of camel TLRs4-10

S. No.	Physiochemical properties	TLR 4	TLR 5	TLR 6	TLR 7	TLR 8	TLR 9	TLR 10
1.	Molecular weight (kDa)	962.93	997.53	959.86	1210.99	1189.30	1484.27	942.47
2.	Theoretical pI	6.14	6.15	6.04	6.20	6.14	8.88	6.90
3.	Instability index	41.19	38.98	36.83	37.61	40.40	45.16	38.97
5.	GRAVY (Grand average of hydropathicity)	-0.019	-0.044	-0.020	-0.141	-0.124	-0.054	-0.053
6.	Aliphatic index	104.20	102.06	103.03	102.49	102.05	101.69	103.69

Our findings provide a chemical and structural basis for optimizing the adjuvant design to elicit broad-based antibody and T cell responses with protein antigens. The importance of TLRs as therapeutic targets is widely accepted. While TLR agonists have been extensively explored as vaccine adjuvants, their use in TLR monotherapy to induce innate immune activation has been less thoroughly explored to enhance host resistance to infection by a variety of pathogens.

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