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Entitled

EVALUATION OF THE GENETIC AND STRUCTURAL VARIATIONS OF CAMEL HEMOGLOBIN

by

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Abstract

The single-humped Arabian camel (Camelus dromedarius) thrives in the hot arid Arabian desert. Many unique adaptations permit it to accomplish this. Camel erythrocytes or red blood cells (RBCs) have a peculiar elliptical shape and are amenable to large variations in physical conditions resulting from dehydration and rehydration cycles. The oxygen transport protein hemoglobin is found abundantly in RBCs and is also believed to behave differently in camels. While several physiological and biochemical studies have been performed on camel hemoglobin, very little is known about genetic and structural adaptions in this protein. The camel genome harbors several unique variations which are being investigated for the treatment of several disorders. In this study, several aspects of camel hemoglobin were investigated from genetic, pharmacological and molecular modeling perspectives. Genetic analysis of camel hemoglobin revealed that camels harbor a unique variation in the region of hemoglobin that harbors a peptide called hemorphin. Hemorphins are endogenous bioactive peptides produced during proteolytic cleavage of hemoglobin and are highly conserved among mammals. Several therapeutic properties of mammalian hemorphins have been reported. However, their precise molecular binding behavior remain elusive. This study extensively investigated the binding behavior and pharmacologic effects of human and camel hemorphins, with angiotensin-converting enzyme (ACE), mu-opioid receptor (MOR), and insulin-regulated aminopeptidase (IRAP) receptor using *in silico* and *in vitro* approaches. Camel hemorphins produced more potent activity, better binding affinity, and more stable interactions with critical residues of ACE, MOR and IRAP receptors when compared to human hemorphins. This study also identified, for the first time, a G protein-coupled receptor angiotensin II type 1 receptor (AT1R) that is a target of hemorphins. *In silico* and *in vitro* data demonstrated that LVV-hemorphin-7 binds to the intracellular side of AT1R and allosterically potentiated the potency of AngII as well as its downstream signaling. Lastly, 1000 ns comparative molecular dynamics (MD) simulations were performed using camel and human hemoglobin protein structures to see how these molecules differ in varying conditions of osmolarity and temperature. Camel hemoglobin demonstrated limited fluctuations, especially near the heme binding regions at higher salt and temperature conditions, compared to human hemoglobin. Additionally, the binding pose, energetics and interaction stability of oxygen-affinity determining energy molecules, adenosine triphosphate (ATP) and 2,3-bisphosphoglycerate (2,3- BPG) were determined *in silico*. 2,3-BPG is abundantly present in erythrocytes and plays a significant role in the unloading of oxygen molecules in peripheral tissues, compared to ATP. In simulations, 2,3-BPG formed more stable interactions with camel hemoglobin at severe dehydrated conditions compared to human hemoglobin. In summary, this study provides insights into the overall stability of camel hemoglobin as well as the binding behavior of ATP and 2,3-BPG at different dehydrated conditions, along with pharmacological and therapeutic activity of the hemoglobin derived hemorphin peptides

Keywords: camel, hemoglobin, hemorphins, molecular docking, molecular dynamics, molecular biological techniques.