THE PARTIAL AMINO ACID SEQUENCE OF LEAF-NOSED VIPER (*ERISTICOPHIS MACMAHONII*) SNAKE HEMOGLOBIN.

H. Waheed[1, 2], S. K. Doman[3] and A. Ahmed [3*]

[1]H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan; [2]Dow University of Health Sciences, Karachi-74200, Pakistan
[3]Chapman University School of Pharmacy, Irvine, CA 92618, USA
Corresponding author Email: aahmed@chapman.edu

ABSTRACT

The hemoglobin from Leaf-nosed Viper (*Eristocophis macmahonii)* was analyzed to better understand interspecies relationship among various snakes at the molecular level. After isolation, the hemoglobin from erythrocytes was analyzed by cation-exchange chromatography. The chains from hemoglobin were separated by reversed phase-high performance liquid chromatography. The N-terminal amino acid sequences of globin chains were obtained by the process of Edman degradation in an automated protein sequencer using an online Phenylthiohydantoin (PTH) analyzer. The obtained sequences were compared using online pairwise sequence alignment tool and Clustal Omega. The N-terminal protein sequence results revealed that two hemoglobin components, comprised of α^A, β^I and β^{II} -globin chains are expressed in Leaf-nosed viper. The α^A-globin chain showed highest similarity with Sindhi krait, β^I with Texas indigo snake and β^{II}-with Indian cobra and interestingly with Blue-lipped sea krait (sea snake). N-terminal sequence alignment studies also showed some important amino acid substitutions that may affect iso-Hb composition and function.

Keywords: *Eristocophis macmahonii*; hemoglobin characterization; evolution; amino acid sequence; snakes; Leaf-nosed viper.

Abbreviations: RP-HPLC, reversed phase-high performance liquid chromatography; IEX, ion-exchange; Em, *Eristocophis macmahonii*; TFA, trifluoroacetic acid; PTH, phenylthiohydantoin.

INTRODUCTION

Throughout the evolution, hemoglobin has played an important physiological role of transporting oxygen and carbon dioxide. They were evolved from stem lineage of jawed vertebrates (gnathostomes) because of two genome duplication events (Stroz, 2016). The globin proteins retain their specific function in oxygen transport and storage to aid physiological division of labor during developmental stages of larger vertebrates. Hemoglobin had been selected evolutionary to function as oxygen transport protein due to its allosteric and cooperative oxygen delivery and uptake (Stroz *et al.*, 2013; Burmester and Hankeln, 2014). It serves as a bi-directional respiratory carrier by; binding oxygen in lungs or other respiratory organs and unloading at metabolizing sites and returning carbon dioxide to the respiratory organs (Marengo-Rowe, 2006). This universal oxygen transport protein is a tetramer composed of a pair of identical α- and β-globin chains that arise from duplications of an ancestral hemoglobin gene into two different paralogous gene families. These paralogous genes further duplicate to give α-and β- globin to form isomeric Hbs (iso-Hbs). The expression of different iso-Hb is the adaptation of vertebrates to fulfill energy demands during various developmental stages. Their differential expression is regulated by various physiological and ontogenetic factors (Lukin and Ho, 2004; Hardison, 2012). The difference in their primary structure has resulted in altering the ligand affinity and sensitivity towards allosteric molecule, thus altering the function. This expression versatility enables the fulfillment of oxygen at the various developmental stages of an organism (Brittain, 2002).

Reptiles (snakes, lizards, and amphisbaenians) constitute phylogenetically diverse group with more than 9400 extant species (Wiens *et al.*, 2012; Pyron *et al.*, 2013). Due to their characteristic physiological system, the reptiles in the snake family with 3070 extant species, serve as an important model to better understand its behavior and molecular evolution (Vidal and Hedges, 2009). Snakes being ectothermic, experience variation in oxygen affinity. Also, their oxygen affinity is affected by the changes in other environmental conditions and physiological states (Herman and Ingermann, 1996; Amiel *et al.*, 2011; Bovo *et al.*, 2015). The present goal of the study was to characterize the hemoglobin of Leaf-nosed viper (*Eristicophis macmahonii*), member of family Viperidae. The snake is found in the deserts of Afghanistan, Iran and Pakistan. In Pakistan, it is mainly found in Nushki, Kharan and other desert areas of Balochistan (Khan, 2002). These snakes experience high summer temperature (40-50 °C) along with diurnal temperature variation. Various physiological and

behavioral thermoregulatory mechanisms have been adopted by them to modulate their body temperature to meet their metabolic needs (Girons, 1980; Seebacher and Franklin, 2005). The only globin chain sequence reported from Viper snakes is from *Vipera aspis* describing the sequence of α-chain of hemoglobin (Duguet *et al*., 1974). Here we describe the preliminary studies on hemoglobin of Leaf-nosed viper.

MATERIALS AND METHODS

Blood Sample Collection and Hemolysate Preparation: Leaf-nosed viper (*Eristicophis macmahonii*) specimens were collected from Nushki, Balochistan, Pakistan. The blood was collected from the anesthetized snake by cardiac puncture in the presence of heparin as an anticoagulant. The blood samples were centrifuged to separate erythrocytes from the plasma. The erythrocytes were then washed with cold 0.9% NaCl solution (saline). The washed cells were subjected to hypotonic lysis. The hemolysate (supernatant) after dialysis against deionized water was lyophilized and stored (Riggs, 1981).

Isolation of Hemoglobin Components: Cation exchange column (Mono S-5/50 GL) was equilibrated with 20mM sodium phosphate buffer, pH 6.0 containing 0.01% KCN. The crude hemolysate was subjected to the column and components were eluted using a gradient of 0-0.5 M NaCl in 15 min. at a flow rate of 1 mL/min. The absorbance of the eluent was recorded at 280 and 415 nm.

Globin Chain Separation: The globin was precipitated in cold acidified acetone. The pellets were then washed with cold acetone, solubilized and dialyzed against deionized water and lyophilized (Rossi-Fanelli and Antonini, 1958). The globin chain separation was carried out by using a reversed phase column (Nucleosil C4-250 x 4.6 mm) equilibrated in solvent A (0.1% TFA in water). Globin (1mg/mL), dissolved in solvent A was loaded to the column. Globin chains were eluted with solvent B (0.1% TFA in acetonitrile) using a linear gradient of 37-45% in 60 minutes, with a flow rate of 1mL/min. The absorbance recorded at 280 nm.

Amino Acid Sequence: The N-terminal amino acid sequence of purified globin chains was deduced by Edman degradation in an automated sequencer; model Procise 491 (Applied Biosystems). The partial amino acid sequence of LNV globin chains established up to 30 residues, were compared with other reported snakes globin sequences using online Pairwise sequence alignment tool (http://www.ebi.ac.uk/Tools/psa/) and the multiple sequence alignment by Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/).

RESULTS

Purification of Hemoglobin: Adult Leaf-nosed viper (*E. macmahonii)* hemolysate that was analyzed by cation exchange chromatography resolved into two peaks. The major (P1) and minor (P2) peaks were observed in the ratio of 1:0.3 (Fig. 1). The crude globin was resolved into 3 major peaks by RP-HPLC (Fig. 2). The RP-HPLC peaks eluted at 32, 51, 55 and 56 min were annotated as β^{II}, β^{I}, α^{A} and α^{A} globin chains respectively based on N-terminal amino sequence (Fig. 2). Based on these observations, it is expected that at least two iso-Hb expressed in adult leaf-nosed viper and the major iso-Hb is adult type i.e. HbA.

N-terminal Amino Acid Sequence

α^{A} *globin chain:* The N-terminal amino acid sequence of intact α^{A} globin chains of Leaf-nosed viper (*E. macmahonii*) was successfully established up to thirty amino acid residues by Edman sequencing. Based on N-terminal sequence analysis, Leaf-nosed viper α^{A} chain showed maximum sequence similarity (93.3%) with α^{A} chain from Sindhi krait (*Bungarus sindanus sindanus*), followed by Indian cobra (*Naja naja*), Texas indigo snake (*Drymarchon melanurus erebennus*) and Black-banded sea krait (*Laticauda semifasciata*) where 90% similarity was observed. Blue-lipped sea krait (*Laticauda laticaudata*) showed 86.7%, Small-headed sea snake (*Microcephalophis gracilis*) and Eastern diamondback rattlesnake (*Crotalus adamanteus*) showed 83.3% similarity. Aspic viper (*Vipera aspis*) that belongs to viperidae family showed least similarity i.e. 78.8% as shown in Fig: 3.

β^{I} *globin chain:* The β^{I} globin chain sequence of Leaf-nosed viper showed highest similarity (93.3%) with β^{I} chain of Texas indigo snake (*Drymarchon melanurus erebennus*). It also showed 90% similarity with Eastern diamondback rattlesnake (*Crotalus adamanteus*), 80% with Blue-lipped sea krait (*Laticauda laticaudata*) and Water snake (*Liophis miliaris*). Least similarity was observed with Black-banded sea krait (*Laticauda semifasciata*) and Indian cobra (*Naja naja*) i.e. 73.3% and 70% respectively (Fig. 4).

β^{II} *globin chain:* The β^{II} globin chain of Leaf-nosed viper showed maximum sequence similarity (93.3%) with β^{I} chain sequence from Indian cobra (*Naja naja*) and Blue-lipped sea krait (*Laticauda laticaudata*). Similarity of 86.7% was observed with both Small-headed sea snake (*Microcephalophis gracilis*) and Eastern diamondback rattlesnake (*Crotalus adamanteus*). Least similarity of 83.3% was observed with Black-banded sea krait (*Laticauda semifasciata*) β^{II} globin chain (Fig. 5).

Multiple Sequence Alignment

α^A **globin chain:** Multiple sequence alignment of the of α^A globin chain of Leaf-nosed viper is shown in Fig. 3. The sequence comparison showed some important amino acid substitutions in Leaf-nosed viper α^A globin chain that includes the presence of Ser at position 3, in the LNV while majority of other species have Thr. At position 8, Asn is present in LNV while in most species compared, showed the presence of Ala or Ser or Thr. At position 13, Ala is present in Leaf-nosed viper, and few other snakes, while Ser is present in some snake species. Further, it was observed that Ser at position 15 in LNV is replaced by Ala, Val or Gly in other species. The residue at position 18 in LNV identified is Gly, while in others snake Ser is present. At position 21, Pro is observed in Leaf-nosed viper and Aspic viper, while in all other species Ala is present. The highest sequence similarity in first 30 amino acid residues is 93.3% with Sindhi Krait and least similarity 78.8% with Aspic viper.

β^I **globin chain:** Multiple sequence alignment of β^I globin chain of Leaf-nosed viper is shown in Fig. 4. At position 9, Ser is present in Leaf-nosed viper and some other species while in others Asn is present. In Leaf-nosed viper and Eastern diamondback rattlesnake Thr is at position 10, which is replaced with Ala or Ile in other snakes. Thr or Ser at 12th position mostly is replaced with

Asn in Eastern diamondback rattlesnake and Indian cobra. Ala at position 13 in Leaf-nosed viper, Eastern diamondback rattlesnake and Water snake is replaced mostly with Ser in others. At position 21, Pro is present in Leaf-nosed viper, Eastern diamondback rattlesnake and Texas indigo snake is replaced with Ala or Gly in others. Ala at position 22 is replaced with Gln in Blue-lipped sea krait, His in Black-banded sea krait and Glu in Indian cobra. Ser is present at position 29 is replaced with different amino acids i.e. Ala, Gly, Cys or Lys. Asn is present at 30th position in LNV, while it is also replaced by Arg except Gln in Eastern diamondback rattlesnake and Leu in Indian cobra. In contrast to α chain, the β^I-chain of Leaf-nosed viper has highest similarity 93.3% with Texas indigo snake and least similarity 70% with Indian cobra.

β^II **globin chain:** Multiple sequence alignment of β^II globin chain is tabulated in Fig. 5. At position 14, Phe is present in Leaf-nosed viper while in all other compared snakes, Leu is present. At 16th position, Ser in Leaf-nosed viper and Blue-lipped sea krait is replaced with Ala, Gly or Pro in rest of the compared species. At position 21, Pro in Leaf-nosed viper, Indian cobra and Eastern diamondback rattlesnake is replaced with Ala in other snakes. In contrast to β^I chain, theβ^II chain of leaf-nosed viper has highest similarity 93.3% with Indian cobra and least similarity 83.3% with Black-banded sea krait.
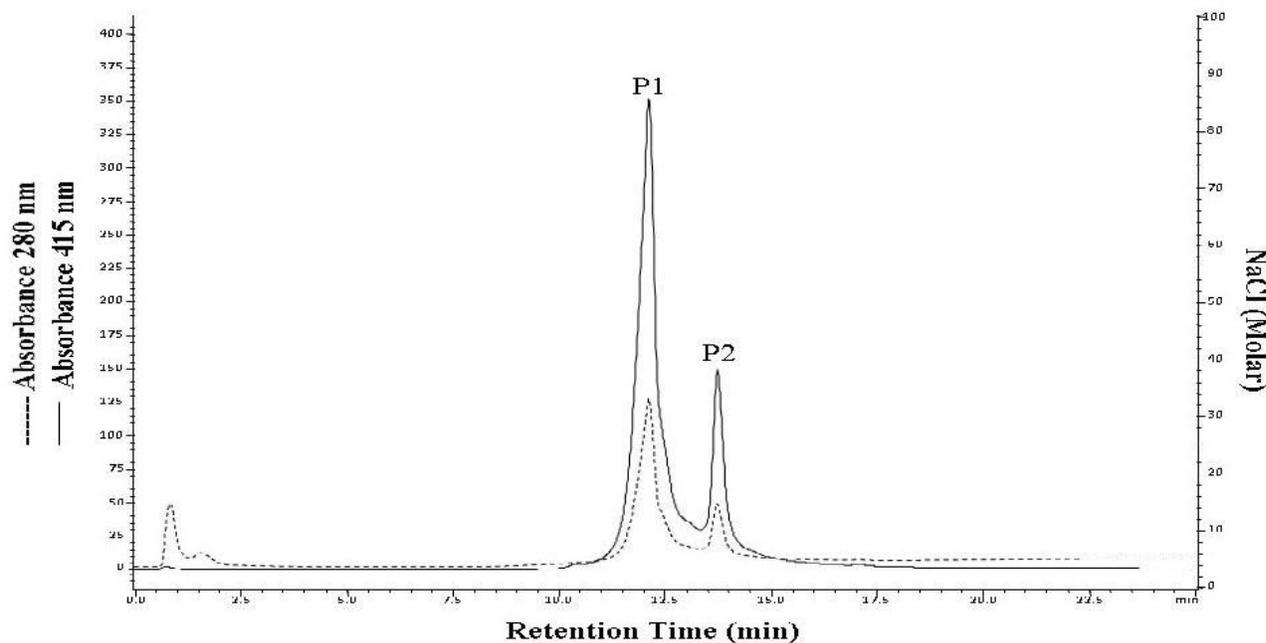

Fig. 1. Separation of Leaf-nosed viper crude hemolysate by FPLC on Mono S (5/50 GL) column. Buffer A, 20 mM phosphate buffer, 0.01% KCN, pH 6.0; Buffer B, 20 mM phosphate buffer, 0.01% KCN, 1M NaCl; gradient from 0-0.5 M NaCl over 30 min; flow rate of 1 mL/min. Absorbance was recorded at 280 and 415 nm.

Fig. 2. Separation profile of Leaf-nosed viper crude globin on Nucleosil (250 x 4.6 mm) RP-C4 column. Elution solvent A, 0.1% TFA water; solvent B, 0.1% TFA-acetonitrile; linear gradient 37-45% B in 60 min; flow rate 1 mL/min. The peaks were labelled as α and β chains based on N-terminal sequence analysis. (* represents peaks with no sequence data).

Fig. 3. Alignment of N-terminal amino acid sequence of αA-globin chain from Leaf-nosed viper with Sindhi krait (*Bungarus sindanus sindanus*), Indian cobra (*Naja naja naja*), Texas indigo snake (*Drymarchon corais*), Black-banded sea krait (*Laticauda semifasciata*), Blue-lipped sea krait (*Laticauda laticaudata*), Small-headed sea snake (*Hydrophis gracilis*), Eastern diamondback rattlesnake (*Crotalus adamanteus*) and Aspic viper (*Vipera aspis*).

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Name_Globin                            Aminoacid Sequence                    Similarity (%)
Code Chain

                                       10          20          30
                                       |           |           |
Leaf-nosed viper-βI                    VHWTAEEKST  ITAVWSKVDV  PAVGAEALSN
Texas indigo snake-βI                  --------A   --SI-N----  ----S----R    93.3
Eastern diamondback rattlesnake-βI     -Q--------  VN-I------  --I---S-AQ    90.0
Blue-lipped sea krait-βI               --------NA  -SSI------  AQ--G---GR    80.0
Water snake-βI                         --------A   ---I-G----  A-I-G---CR    80.0
Black-banded sea krait -βI             --------NI  NSVI------  GHI-G---KR    73.3
Indian cobra-βI                        --------NA  -NS--V--P-  GE--GA--AL    70.0
                                       * ******    *  ** *    *     *
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Fig. 4. Alignment of N-terminal amino acid sequence of β^I-globin chain from Leaf-nosed viper with Texas indigo snake (*Drymarchon corais*), Eastern diamondback rattlesnake (*Crotalus adamanteus*), Blue-lipped sea krait (*Laticauda laticaudata*), Water snake (*Liophis miliaris*), Black-banded sea krait (*Laticauda semifasciata*) and Indian cobra (*Naja naja naja*).

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Name_Globin                            Aminoacid Sequence                    Similarity (%)
Code Chain

                                       10          20          30
                                       |           |           |
Leaf-nosed viper-βII                   VHWSAEEKQL  ITSFWSKVDV  PEVGGATLGK
Indian cobra-βII                       ----------  ---L-A----  ----A-----    93.3
Blue-lipped sea krait-βII              ----------  ---L-----L  A---------    93.3
Small-headed sea snake-βII             ----------  --GL-G----  A---------    86.7
Eastern diamondback rattlesnake-βII    ---TP-----  --AL-G----  ----A-----    86.7
Black-banded sea krait-βII             ----------  ---L-P---L  A----E--N     83.3
                                       ***  *****  **  * ***   *** * **
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Fig. 5. Alignment of N-terminal amino acid sequence of β^{II}-globin chain from Leaf-nosed viper with Indian cobra (*Naja naja naja*), Blue-lipped sea krait (*Laticauda laticaudata*), Small-headed sea snake (*Hydrophis gracilis*), Eastern diamondback rattlesnake (*Crotalus adamanteus*) and Black-banded sea krait (*Laticauda semifasciata*).

DISCUSSION

Two or more structurally different iso-Hbs are expressed during various developmental stages of squamate reptiles as a regulatory mechanism. The expression and concentration of different iso-Hbs present depend upon their metabolic need of oxygen that differs during different stages of development as well as various physiological states (Storz and Moriyama, 2008; Storz *et al.*, 2011). These iso-Hbs are classified into Hb-D or Hb-A based on type of α-globin (α^D or α^A) present in that specific iso-Hb. Phylogenetic studies of hemoglobin showed that, single copy of α^D or α^A-globin and ß-globin

gene duplicates i.e. β^I and β^{II} are expressed in adult snakes (Bonilla *et al.*, 1994; Gorr *et al.*, 1998; Hoffmann *et al.*, 2010; Storz *et al.*, 2015). Mainly, two hemoglobin components and four globin chains have been observed in snakes (Gorr *et al.*, 1998; Storz *et al.*, 2015). These include: Blue-lipped sea krait (Eguchi and Eguchi, 2002), Indian cobra (Naqvi *et al.*, 1994), Black-banded sea krait (Eguchi and Eguchi, 2003), Small-headed sea snake (Islam *et al.*, 1990), Indigo snake (Stoeckelhuber *et al.*, 2005) and Indian Python (Gorr *et al.*, 1998). Expression of three iso-Hbs were observed in SouthAmerican rattlesnake and Sindhi krait where at least one of the iso-Hbs is expected to be the post-translational modified

form of the major iso-Hb present (Storz *et al.*, 2015; Waheed *et al.*, 2016).

Our results showed that at least two types of hemoglobin components are expressed in Leaf-nosed viper. One of the two components are expected to be adult type hemoglobin *i.e.* Hb-A. The other component could be the isoform of the adult type and may be a physiological adaptation of this snake to its environment. In RP-HPLC chromatogram, a small peak was also observed at a retention time of 40-45 min., which showed the mix type of sequence (data not shown). It is already being reported that several snake hemoglobin, at high pH, undergo oxygenation-linked dissociation into αβ-dimers in the absence of ATP (organic phosphate) (Storz *et al.*, 2015). The mixed type of sequence that was observed could be possibly due to the dimeric form of hemoglobin. However, these were our initial results and further experimental authentication is in progress.

Conclusion: From our initial experimental observations, we conclude that two hemoglobin components that are composed of αA, βI and βII-globin chains, are expressed in Leaf-nosed viper. The globin chains were designated as αA, βI and βII based on their N-terminal sequence alignment analysis. Multiple sequence alignment studies showed some interesting substitutions that may influence its iso-Hb composition and function. Further studies on hemoglobin from this snake are in progress and will be reported elsewhere. Currently, only the complete amino acid sequence of an α-chain of Viper (*Vipera aspis*) a member of family Viperidae hemoglobin is available (Girons, 1980). The knowledge gained from this study will provide an insight into the understanding of the evolutionary relationships and molecular mechanisms of physiological adaptation among various snakes.

Conflict of Interest: The authors declare that they do not have any conflicts of interest with the contents of this article.

REFERENCES

Amiel, J.J., B. Chua, R.J. Wassersug, and D.R. Jones (2011). Temperature-dependent regulation of blood distribution in snakes. The J. Experimental Biology. 214:1458-1462.

Bonilla, G.O., A. Jr. Focesi, C. Bonaventura, J. Bonaventura, and R.E. Cashon (1994). Functional properties of the hemoglobin from the South American snake *Mastigodryas bifossatus.* Comp. Biochem. Physiol. A.109: 1085-1095.

Bovo, R.P., A. Fuga, M.A. Micheli-Campbell, J.E. Carvalho, and D.V. Andrade (2015). Blood oxygen affinity increases during digestion in the South American rattlesnake, *Crotalus durissusterrificus*. Comp. Biochem. Physiol. A.186: 75-82.

Brittain, T. (2002). Molecular aspects of embryonic hemoglobin function. Mol. Aspects Med. 23: 293-342.

Burmester, T. and T. Hankeln (2014). Function and evolution of vertebrate globins. Acta Physiologica. 211: 501-514.

Duguet, M., J.P. Chauvet, and R. Acher (1974). Phylogeny of hemoglobins: The complete amino acid sequence of an α-chain of Viper *(Vipera aspis)* hemoglobin. FEBS Letters.47: 333-337.

Eguchi, Y. and T. Eguchi (2002). Amino acid sequence of the alpha and beta globin chains of the Hiroo sea snake (*Laticauda laticaudata*). J. Protein Chemistry. 21: 215-221.

Eguchi, Y. and T. Eguchi (2003). Amino acid sequence of the alpha and beta globin chains of the Erabu sea snake (*Laticauda semifasciata*). J. Protein Chemistry. 22: 489-497.

Girons, H.S. (1980). Thermoregulation in reptiles with special reference to the Tuatara and its ecophysiology. Tuatara.24: 56-79.

Gorr, T.A., B.K. Mable, and T. Kleinschmidt (1998). Phylogenetic analysis of reptilian hemoglobins: Trees, rates, and divergences. J. Molecular Evolution. 47: 471-485.

Hardison, R.C. (2012). Evolution of hemoglobin and its genes. Cold Spring Harb. Perspect. Med. 2(12): a011627.

Herman, J.K., and R.L. Ingermann (1996). Effects of hypoxia and hyperoxia on oxygen-transfer properties of the blood of a viviparous snake. The J. Experimental Biology. 199: 2061-2070.

Hoffmann, F.G., J.F. Storz, T.A. Gorr, and J.C. Opazo (2010). Lineage-specific patterns of functional diversification in the α- and β-globin gene families of tetrapod vertebrates. Molecular Biology and Evolution. 27: 1126-1138.

Islam, A., B. Persson, Z.H. Zaidi, and H. Jörnvall (1990). Sea snake (*Microcephalophis gracilis*) hemoglobin: Primary structure and relationships to other forms. J. Protein Chem. 9: 533-541.

Khan, M.S. (2002). A guide to the snakes of Pakistan. Andreas S. Brahm: Frankfurt am Main.

Lukin, J.A. and C. Ho (2004). The structure-function relationship of hemoglobin in solution at atomic resolution. Chemical Reviews. 104: 1219-1230.

Marengo-Rowe, A.J. (2006). Structure-function relations of human hemoglobins. Baylor University Medical Center Proceedings. 19: 239-245.

Naqvi, S., A. Abbasi, and Z.H. Zaidi (1994). Primary structure of hemoglobin from cobra *Naja naja naja*. J. Protein Chemistry. 13: 669-679.

Pyron, R.A., F.T. Burbrink, and J.J. Wiens (2013). A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evolutionary Biology. 13: 1-53.

Riggs, A. (1981). Preparation of blood hemoglobins of vertebrates. Methods in Enzymology. 76: 5-29.

Rossi-Fanelli, A. and E. Antonini (1958). Studies on the oxygen and carbon monoxide equilibria of human myoglobin. Archives of Biochemistry and Biophysics. 77: 478-492.

Seebacher, F. and C.E. Franklin (2005). Physiological mechanism of thermoregulation in reptiles: A review. J. Comparative Physiology B.175: 533-541.

Stoeckelhuber, M., T. Gorr, and T. Kleinschmidt (2005). The primary structure of three hemoglobin chains from the Indigo snake (*Drymarchon coraiserebennus*, Serpentes): First evidence for α^D chains and two β chain types in snakes. Biological Chemistry. 383: 1907-1916.

Storz, J.F. and H. Moriyama (2008). Mechanisms of hemoglobin adaptation to high altitude hypoxia. High Altitude Medicine and Biology.9: 148-157.

Storz, J.F., F.G. Hoffmann, J.C. Opazo, T.J. Sanger, and H. Moriyama (2011). Developmental regulation of hemoglobin synthesis in the green anole lizard *Anolis carolinensis*. The J. Experimental Biology. 214: 575-581.

Storz, J.F., J.C. Opazo, and F.G. Hoffmann (2013). Gene duplication, genome duplication, and the functional diversification of vertebrate globins. Mol. Phylogenet. Evol. 66(2): 469-478.

Storz, J.F., C. Natarajan, H. Moriyama, F.G. Hoffmann, T. Wang, A. Fago, H. Malte, J. Overgaard, and R.E. Weber (2015). Oxygenation properties and isoform diversity of snake hemoglobins. Am. J. Physiol. Regul. Integr. Comp. Physiol. 309(9): 1178-1191.

Storz, J.F. (2016). Gene duplication and evolutionary innovations in hemoglobin-oxygen transport. Physiology. 31(3): 223-232.

Vidal, N. and S.B. Hedges (2009). The molecular evolutionary tree of lizards, snakes, and amphisbaenians. Comptes. Rendus. Biologies. 332: 129-139.

Waheed, H., H. Friedman, S.F. Moin, S. Zarina, and A. Ahmed (2016). The primary structure of β(I)-chain of hemoglobin from snake Sindhi krait (*Bungarus sindanus sindanus*). Protein J. 35: 193-201.

Wiens, J.J., C.R. Hutter, D.G. Mulcahy, B.P. Noonan, T.M. Townsend, J.W. Sites, and T.W. Reeder (2012). Resolving the phylogeny of lizards and snakes (Squamata) with extensive sampling of genes and species. Biol. Lett. 8(6): 1043-1046.