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Hemoglobins of Reptiles

# The Primary Structures of the $\alpha^I$- and $\beta^I$-Chains of Common Iguana *(Iguana iguana)* Hemoglobin*

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**Summary:** The primary structures of $\alpha^I$- and $\beta^I$-chains from the hemoglobins of the Common Iguana *(Iguana iguana)* are presented. The globin chains were separated on CM-cellulose in 8M urea buffer. The amino-acid sequences were established by automatic Edman degradation of the native chains, the tryptic peptides and a peptide obtained by cyanogen bromide cleavage.

The sequences are compared with human hemoglobin. Amino-acid replacements at positions critical for structure and function of the hemoglobin are discussed. The requirements for binding of ATP and also of DPG as allosteric effectors at the β-chains seem to be fullfilled.

Comparison of the α-chains with those of the Viper *(Vipera aspis)* shows 66 amino-acid substitutions. This number is in the same order of magnitude as the ones found by comparison with α-chains of crocodiles and mammals as well as with $\alpha^A$-chains of a turtle and birds. This result points towards a period of independent evolution of the reptile lines leading to the Common Iguana on one hand and to the Viper on the other. This time span is comparable to the one separating mammals from reptiles.

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*Hämoglobine der Reptilien: Die Primärstrukturen der $\alpha^I$- und $\beta^I$-Ketten des Hämoglobins des Grünen Leguans* (Iguana iguana)

**Zusammenfassung:** Die Primärstrukturen der $\alpha^I$- und $\beta^I$-Ketten der Hämoglobine des Grünen Leguans *(Iguana iguana)* werden beschrieben. Die Globinketten wurden chromatographisch an CM-Cellulose in 8M Harnstoffpuffer getrennt. Die Aminosäure-Sequenzen wurden durch automatischen Edman-Abbau der nativen Ketten, der tryptischen Peptide und eines Bromcyan-Peptids ermittelt.

Die Sequenzen werden mit Human-Hämoglobin verglichen. Aminosäure-Austausche an für Struktur und Funktion des Hämoglobins entscheidenden Positionen werden diskutiert.

Die Voraussetzungen für die Bindung von ATP wie auch von DPG als allosterische Effektoren an den β-Ketten sind erfüllt. Beim Vergleich der α-Ketten mit denen der Viper *(Vipera aspis)* zeigen sich 66 Austausche. Diese Zahl ist von derselben Größenordnung wie diejenigen, die beim Vergleich mit α-Ketten von Krokodilen und Säugern sowie mit $\alpha^A$-Ketten von Vögeln und Schildkröten gefunden werden. Dieses Ergebnis deutet auf eine Zeitspanne unabhängiger Evolution der Reptilienzweige, die zum Grünen Leguan einerseits und zur Viper andererseits führten, vergleichbar derjenigen die Säuger von Reptilien trennt.

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*Abbreviations:*
CM- = Carboxymethyl-; Quadrol = *N,N,N',N'*-tetrakis(2-hydroxypropyl)ethylendiamine; Reagent I = sodium 1-(isothiocyanato)benzene-4-sulfonate; Reagent IV = trisodium 7-(isothiocyanato)naphthalene-1,2,5-trisulfonate; DPG = 2,3-bisphosphoglycerate; IPP = inositol-1,3,4,5,6-pentaphosphate; TosPheCH$_2$Cl = (*N*-tosyl-L-phenylalanyl)chloromethane; Ig = *Iguana iguana*; Hu = human; Hb = hemoglobin; HPLC = high-performance liquid chromatography.

* 132th Communication on hemoglobins; for 129th, 130th and 131th communication see ref.[1–3].

** Extract from thesis of K.P. Rücknagel, Fakultät für Chemie und Pharmazie, Ludwig-Maximilians-Universität München, 1985.

*Key words:* Reptiles, Common Iguana, hemoglobin, primary structure, evolution.

In the broad collection of hemoglobin sequence data one of the biggest orders of tetrapods, the Squamata, is represented by only one complete sequence of the α-globin chains from the Viper[4] (a member of the suborder of Serpentes).

In this paper we present the sequences of α- and β-globin chains of the Common Iguana (*Iguana iguana*, a member of the suborder of Sauria).

From this contribution, together with following ones, insights into functional developments of the hemoglobin molecule might be gained.

Since the knowledge about origin and interrelationship of reptiles is still incomplete, a greater set of molecular data might contribute to a better understanding of reptile evolution pathways.

## Material and Methods

*Hemoglobin preparation*

Blood was taken from the caudal vein of an anestesized Common Iguana (female, 880 g) with a heparinized syringe. Red blood cells, obtained by centrifugation, were washed with isotonic saline and lysed with distilled water in the cold. Lipids were removed by extraction with 1/10 volume of carbon tetrachloride. The hemolysate was dialysed against distilled water or against 0.02M Tris/HCl (pH 8.5) containing 1 mmol dithioerythrol. The hemoglobin composition was analysed by polyacrylamid gel electrophoresis under alkaline[5] and dissociating conditions[6] (Fig. 1).

*Chain separation*

Globin was prepared by precipitation in cold acidic acetone according to Rossi-Fanelli[7]. The globin chains were separated by ion-exchange chromatography on carboxymethyl cellulose[8]. Freeze-dried globin was dissolved in 8M urea and reduced with dithioerythrol for 4 h under nitrogen. The pH was adjusted to 5.0 and the sample was applied to a column of carboxymethyl cellulose (Whatman/CM-52 preswollen, 2.5 x 17 cm) equilibrated with 0.02M sodium acetate buffer in 8M urea, pH 5.0, containing 0.2% mercaptoethanol. The chromatography was developed with a logarithmic gradient of 0 to 0.13M sodium chloride followed by a logarithmic gradient to a final concentration of 0.3M sodium chloride (Fig. 2). The chains were checked by polyacrylamide gel electrophoresis under denaturating conditions in the presence of Triton X-100[6].

*Tryptic peptides*

The globin chains were briefly oxidized with performic acid in a cooling bath. Oxidized chains were digested with TosPhe$CH_2$Cl-treated trypsin at pH 10.5 to 8.4 for 4 and 6 h, respectively, at room temperature (enzyme/substrate ratio 5 : 100). The enzyme was added in portions. At pH 4.8 Tp11/12a (pos. 93–118) from the α-chains was precipitated. It was purified by reversed-

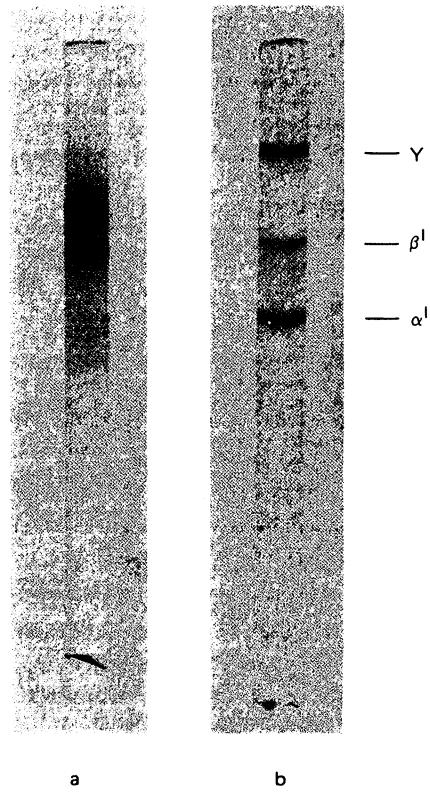


Fig. 1. Polyacrylamide gel electrophoresis of Common Iguana hemolysate.

a) Gel: 10% polyacrylamide in 0.4M Tris/HCl, pH 8.9; Buffer: 57mM glycine, 7.4mM Tris, pH 8.3; Current: 4.5 mA per tube.

b) Denaturating electrophoresis in presence of Triton X-100 and urea.

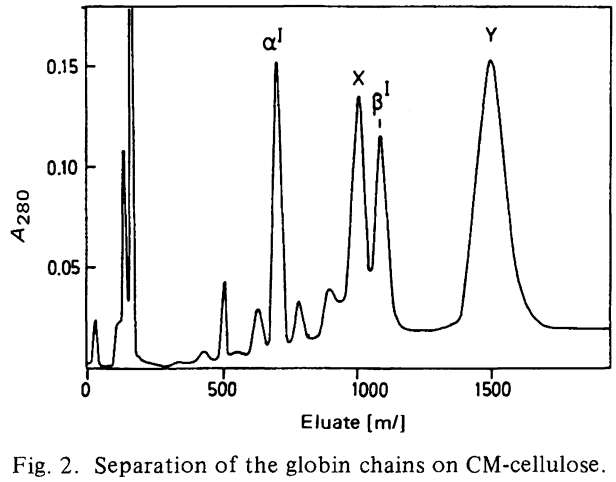


Fig. 2. Separation of the globin chains on CM-cellulose.

Sample: 100 mg Common Iguana globin in 10 ml starting buffer, pH 5.0; Column: Carboxymethyl cellulose CM-52, 2.5 x 17 cm; Starting buffer: 20mM sodium acetate, 8M urea, 0.2% 2-mercaptoethanol, pH 5.0; Gradient: logarithmic, 0–0.13M NaCl (600/800 ml) followed by a logarithmic gradient generated by the addition of 500 ml buffer containing 0.3M NaCl; Flow-rate: 40 ml/h; Fractions: 10 ml/15 min.

phase high-performance liquid chromatography on LiChrosorb RP 2 (7 μm) column (4.6 x 250 mm) with 0.05M ammonium acetate buffer, pH 6, using a gradient from 0 to 60% acetonitrile. Peptides from the soluble fractions were isolated by reversed-phase HPLC on LiChrosorb RP 2 columns (as above) or on Vydac RP 18 TP columns with 0.1% aqueous trifluoroacetic acid and gradients from 0 to 60% acetonitrile. Coeluting peptides were separated by preparative thin-layer electrophoresis.

*Cyanogen bromide cleavage*[9]

Reduced α-chains (15 mg) were cleaved with cyanogen bromide (1 g) in 70% formic acid for 18 h at room temperature in the dark. Excess reagent was removed by evaporation. The peptide CBpII (pos. 126–141) was isolated on a Sephadex G-50 fine column in 0.1M acetic acid.

*Amino-acid analysis*

Globin chains and peptides were hydrolysed in 5.7M HCl at 110 °C for 20 h and 200 h, respectively, and analysed on a Biotronic Model LC 5000 amino-acid analyser. Cysteine and methionine were determined after performic acid oxidation[10], tryptophan in the presence of 6% thioglycolic acid[11].

*Sequence determination*

The sequences of the chains were determined by automated Edman degradation in liquid phase sequenators[12] (Beckman Instruments, models 890B and 890C). A Quadrol program (0.25M Quadrol)[13] was applied for degradation of native intact chains and of lysine peptides which had been reacted with reagent IV[14]. Arginine peptides, the C-terminal cyanogen bromide peptide and large hydrophobic lysine peptides (reacted with reagent I[15]) were sequenced using a 3-(diethylamino)propyne program[16]. Phenylthiohydantoine derivatives were identified by reversed-phase high performance liquid chromatography[17] or by thin-layer chromatography[13,15].

## Results and Discussion

The red blood cells of the Common Iguana contain at least two hemoglobin components. Polyacrylamide-gel electrophoresis of the hemolysate reveals two very diffuse bands (Fig. 1a). Efforts to separate the components on cation as well as on anion-exchanger columns yielded inhomogeneous protein fractions with a high tendency for precipitation (data not shown). As indicated by these results the hemoglobins of the Common Iguana show very low stability. Denaturating Triton X-100 polyacrylamide gel electrophoresis of fresh hemolysate (Fig. 1b) resolved $\alpha^I$- and $\beta^I$-chains. The third band assigned Y has not been characterized. An additional diffuse zone migrated between $\alpha^I$- and $\beta^I$-chains. Pure $\alpha^I$- and $\beta^I$-chains were obtained by ion exchange chromatography under denaturating conditions (Fig. 2). The material in peak X gave an inhomogeneous zone in Triton electrophoresis. It aggregates on aging. Its N-terminal sequence is of β-type. Peak Y consists of aggregates made of α- and β-type globin chains (as established by N-terminal Edman degradation of 15 residues). In Triton electrophoresis only a portion of this material enters the gel giving rise to band Y, while the greater part sticks to the top of the gel. It has not been further analysed. The complete primary structures of the $\alpha^I$-chains and the $\beta^I$-chains were determined by Edman degradation of the first 42 N-terminal residues of the native intact chains and their tryptic peptides, which were aligned by homology. The C-terminal region of the $\alpha^I$-chains was overlapped by sequencing the corresponding cyanogen bromide peptide. The amino-acid compositions are given in Tables 2 and 3 of Supplementary Material. The complete primary structures of $\alpha^I$- and $\beta^I$-chains are presented in Fig. 3.

*Structural features*

Several of the salt bridges stabilizing the tertiary structure of human HbA are absent in the globin chains of the Common Iguana. These are in the α-chains the bonds between α20 (B1)His and α23 (B4)Glu and between α30 (B11)Glu and α50 (CD8)His. In Iguana we find uncharged residues: α20 (B1)Asn, α23 (B4)Ala, α30 (B11)-Thr and α50 (CD8)Gln. In the β-chains the bonds between β21 (B3)Asp and β61 (E5)Lys and between β26 (B8)Glu and β116 (G18)His are affected. In Iguana β21 (B3)Ala and β116 (G18)Ala cannot be involved in salt bridge formation.

It is known from abnormal human hemoglobins, that substitutions of nonpolar amino-acids with their side chains placed in the hydrophobic core of the molecule against polar or even charged residues destabilize the protein. In the Iguana β-chain β112 (G14)Thr, β134 (H12)Thr and especially β113 (G15)Arg, which has not been found in any other globin β-chain before, might have a similar effect.

As compared to human hemoglobin there is a striking number of differences at α1–β1 binding sites:

8 in the α-chains:
α35 (B16)Ser→Ala, α36 (C1)Phe→Tyr, α114 (GH2)Pro→His, α117 (GH5)Phe→Leu, α118 (H1)Thr→Lys, α119 (H2)Pro→Ala, α122 (H5)His→Ala and α123 (H6)Ala→Leu.

6 in the β-chains:
β30 (B12)Arg→Cys, β55 (D6)Met→Cys, β112 (G14)Cys→Thr, β116 (G18)His→Ala, β125 (H3)Pro→Ala and β127 (H5)Gln→His.

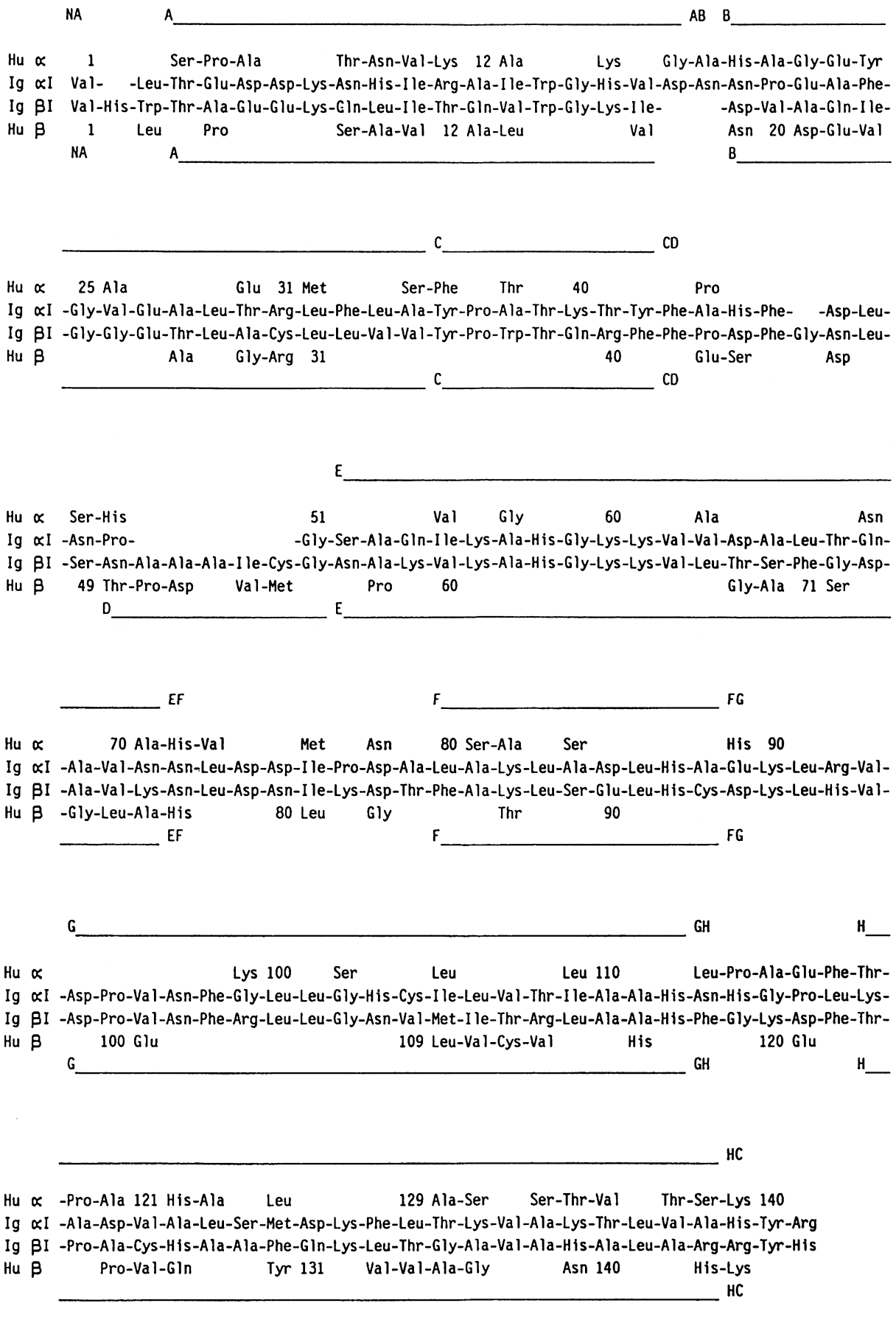


Fig. 3. Amino-acid sequences of the $\alpha^{I}$- and $\beta^{I}$-chains from Common Iguana *(Iguana iguana)* hemoglobin (Ig). The sequences are homologously aligned with those of adult human hemoglobin (Hu) showing only substituted residues from the latter. Bars indicate helical regions.

The large number and the nature of these substitutions show clearly that the system of bonds and contacts which stabilizes the $\alpha 1-\beta 1$ dimers in Iguana hemoglobin differs greatly from that in the human hemoglobin model.

Substitutions at the $\alpha 1-\beta 2$ interface have a larger influence on the oxygen-binding characteristics of hemoglobin. Two amino-acid substitutions at $\alpha 1-\beta 2$ binding sites are found in the globin chains of the Common Iguana:

$\alpha 38$ (C3)Thr→Ala, $\alpha 44$ (CD2)Pro→Ala, $\beta 42$ (CD2)Glu→Pro and $\beta 101$ (G3)Glu→Val.

In the human hemoglobin model $\alpha 38$ Thr makes one hydrogen bond to the $\beta 2$ subunit in the deoxy structure but two hydrogen bonds in the oxy structure: Replacement of the polar threonine by alanine should lead to a relative destabilisation of the high-affinity form of the hemoglobin. $\beta 43$ (CD2)Glu forms a salt bridge with $\alpha 92$ (FG4)Arg in human hemoglobin. $\beta 43$ Pro therefore, cannot contribute to the stabilisation of the $\alpha 1-\beta 2$ interaction in the hemoglobin of the Common Iguana.

It has been shown on abnormal human hemoglobins that substitution of $\beta 101$ Glu results in altered functional properties due to the different charge or size of the side chain of the substituent[18].

Two of the histidine residues which have been reported to be involved in the alkaline Bohr effect[19,20] in human Hb are replaced in the globin chains of the Common Iguana: $\alpha 122$ (H5)His→Ala and $\beta 143$ (H21)His→Arg. It is unclear which amino acids are responsible for an alkaline Bohr effect of the hemoglobin of the Common Iguana[21] which is comparable to that of human HbA. The temperature independence of the alkaline Bohr effect of pig hemoglobin has been explained by a decrease of the p$K$-value of $\alpha 1$ (NA1)Val due to the vicinity of $\alpha 131$ (H14)Asn (in contrast to $\alpha 131$ (H14)Ser in human Hb) in the deoxyform which prevents the oxygenation-dependent chloride binding between the $\alpha 1$ and $\alpha 2$ subunits[22]. $\alpha 131$ (H14)Lys in the hemoglobin of the Common Iguana should decrease the p$K$ of $\beta 1$ Val to an even greater extent and might thus be responsible for the temperature-independent alkaline Bohr effect observed in this hemoglobin[21].

*Allosteric control*

In most vertebrates the oxygen affinity of hemoglobin is modulated and controlled by organic phosphate compounds[23,24]. In Squamata usually ATP is found as the major intraerythrocytic phosphate. Only in Crocodiles hydrogen-carbonate ions act as allosteric effectors instead of phosphates[25,26]. As can be seen from Table 1, in the $\beta$-chain of the Common Iguana all the prerequisits for the binding of ATP are fullfilled. $\beta 2$ (NA2)His can function as hydrogen bond acceptor, contributing to ATP-binding, and in contrast to Glu, to the binding of DPG as well. We suppose that the oxygen affinity of the hemoglobin of the Common Iguana is allosterically controlled by ATP and expect that DPG, if present in the erythrocytes, will create a similar effect.

Table 1. Binding-sites of heterotropic allosteric effectors[25,27–29].

| Effektor | NA1 $\beta 1$ | NA2 $\beta 2$ | EF6 $\beta 82$ | H13 $\beta 135$ | H17 $\beta 139$ | H21 $\beta 143$ | H22 $\beta 144$ |
|---|---|---|---|---|---|---|---|
| $HCO_3$ | Ac-Ala | – | Lys | – | – | – | Glu |
| 2,3-DPG | Val | His | Lys | – | – | His | – |
| IPP | Val | His | Lys | Arg | His | Arg | – |
| ATP, GTP | Val | Glu | Lys | – | – | Arg | – |
| Iguana | Val | His | Lys | Gly | His | Arg | Arg |

*Evolutionary aspects*

Comparison of the sequence data from Common Iguana hemoglobin with those of other reptiles gives some results of interest. From our present knowledge of phylogenetic relationships we would have expected a significant homology with snake hemoglobin since Sauria and Serpentes both belong to the order of Squamata. However the $\alpha$-globin chains of the Common Iguana only show 53% identity with those of the viper[4]. This value does not differ very much from the values found when comparing Common Iguana $\alpha$-globin chains with those of turtles[30] (49–52% identity) and crocodiles[31] (53–55% identity). Almost the same degree of identity exists between Iguana $\alpha$-chains and those of mammals or birds[32]. Unfortunately up to now no sequences of $\beta$-globin chains from snakes are available. Compared with other $\beta$-chains those of the Common Iguana show more similarity with turtle and bird $\beta$-chains than with those of mammals or crocodiles. It is unclear whether this reflects the manifestation of similar functional properties of the hemoglobin molecule rather than closer phylogenetic relationships. More sequences of hemoglobins from Sauria and Serpentes will be needed in order to decide whether the large difference mentioned above can be interpreted as an indication towards a very early branching of Sauria and Serpentes, and if the assumption of a monophyletic origin of the two suborders is correct.

We would like to express our thanks to Mrs. *E. Mueller*, Mr. *A. Stangl*, Mrs. *B. Schrank*, Mrs. *R. Gautsch* and Mr. *C. Krombach* for their help in amino-acid analysis and sequenator work.

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## Supplementary Material

Table 2. Iguana iguana: $\alpha^{I}$-chains. a) Amino acid composition of tryptic peptides:

| Pos. | Tp1 1-7 | Tp2 8-11 | Tp3/4 12-31 | Tp5 32-40 | Tp6 41-56 | Tp7 57-60 | Tp8 61 | Tp9a (*) 62-82 |
|---|---|---|---|---|---|---|---|---|
| Asx | 2.09 | 1.04 | 2.81 | | 2.03 | | | 5.66 (6) |
| Thr | 0.89 | | 0.94 | 0.97 | 0.93 | | | 1.02 |
| Ser | | | | | 0.84 | | | |
| Glx | 1.03 | | 1.96 | | 1.04 | | | 1.08 |
| Pro | | | 0.92 | 1.04 | 0.97 | | | 1.07 |
| Gly | | | 2.50 (2) | | 1.14 | 1.14 | | |
| Ala | | | 2.77 (3) | 1.99 | 2.02 | 0.84 | | 3.84 |
| Val | 0.79 | | 1.67 (2) | | | | | 2.30 (3) |
| Met | | | | | | | | |
| Ile | | 0.92 | 0.84 | | 0.91 | | | 0.94 |
| Leu | 1.16 | | 1.02 | 2.05 | 1.11 | | | 3.25 |
| Tyr | | | | 0.93 | 0.92 | | | |
| Phe | | | 1.12 | 1.03 | 1.91 | | | |
| Lys | 1.04 | | | 1.04 | 1.09 | 1.01 | 1.00 | 1.12 |
| His | | 1.01 | 1.20 | | 1.10 | 1.02 | | |
| Arg | | 1.03 | 1.26 | | | | | |
| Cys | | | | | | | | |
| Trp | | | (1) | | | | | |
| Sum | 7 | 4 | 20 | 9 | 16 | 4 | 1 | 21 |

| Pos. | Tp9b 83-90 | Tp10 91-92 | Tp11/12a 93-118 | Tp12b 119-127 | Tp13a 128-131 | Tp13b 132-134 | Tp13c/14 135-141 |
|---|---|---|---|---|---|---|---|
| Asx | 1.00 | | 2.87 | 1.94 | | | |
| Thr | | | 0.94 | | 0.94 | | 0.93 |
| Ser | | | | 0.88 | | | |
| Glx | 1.03 | | | | | | |
| Pro | | | 2.29 | | | | |
| Gly | | | 3.03 | | | | |
| Ala | 1.78 | | 2.02 | 1.90 | | 1.01 | 1.07 |
| Val | | | 2.82 | 0.93 | | 0.85 | 1.00 |
| Met | | | | 1.01 | | | |
| Ile | | | 1.85 | | | | |
| Leu | 2.15 | 0.87 | 4.11 | 1.22 | 1.09 | | 1.18 |
| Tyr | | | | | | | 0.88 |
| Phe | | | 1.03 | | 0.93 | | |
| Lys | 1.06 | | 1.09 | 1.13 | 1.04 | 1.14 | |
| His | 0.98 | | 2.87 | | | | 1.03 |
| Arg | | 1.12 | | | | | 0.91 |
| Cys | | | 1.09 | | | | |
| Trp | | | | | | | |
| Sum | 8 | 2 | 26 | 9 | 4 | 3 | 7 |

(*) Val-Val-bonds were not cleaved quantitatively during 20 h hydrolysis.
Met and Cys determined after performic acid oxidation.
Numbers in parentheses denote amino-acid residues found during sequencing.

b) Amino acid composition of the ditryptic peptide Tp8/9a, the cyanogen peptide (CBpII) and the intact chains.

| Pos. | Tp8/9a 61-82 | CBpII 126-141 | Globin 1-141 |
|---|---|---|---|
| Asx | 5.79 (6) | 1.42 (1) | 18.45 (20) |
| Thr | 0.97 | 1.71 | 7.56 ( 8) |
| Ser | | | 2.17 ( 2) |
| Glx | 1.16 | | 6.53 ( 6) |
| Pro | 1.06 | | 5.25 ( 6) |
| Gly | | | 7.39 ( 7) |
| Ala | 3.94 | 2.08 | 18.55 (20) |
| Val | 2.45 (3)(*) | 1.99 | 11.94 (12) |
| Met | | | 0.78 ( 1) |
| Ile | 0.97 | | 5.97 ( 6) |
| Leu | 3.00 | 2.16 | 17.08 (18) |
| Tyr | | 0.86 | 2.84 ( 3) |
| Phe | | 0.98 | 5.66 ( 6) |
| Lys | 2.13 | 2.64 (3) | 11.02 (11) |
| His | | 1.22 | 8.59 ( 9) |
| Arg | | 0.94 | 4.03 ( 4) |
| Cys | | | 1.45 ( 1) |
| Trp | | | 0.62 ( 1) |
| Sum | 22 | 16 | 141 |

(*) Val-Val-bond was not cleaved quantitatively during 20 h hydrolysis.
Met and Cys determined after performic acid oxidation.
Numbers in parentheses denote amino-acid residues found during sequencing.

Table 3. Iguana iguana: $\beta^I$-chains.

a) Amino-acid composition of tryptic peptides:

| Pos. | Tp1 1-8 | Tp2 9-17 | Tp3/4 18-40 | Tp5 41-59 | Tp6 60-61 | Tp7 62-65 |
|---|---|---|---|---|---|---|
| Asx | | | 1.36 (1) | 3.88 | | |
| Thr | 1.03 | 0.98 | 1.85 | | | |
| Ser | | | | 0.69 (1) | | |
| Glx | 2.02 | 1.99 | 2.85 | | | |
| Pro | | | 1.13 | 0.97 | | |
| Gly | | 1.19 | 2.27 | 2.13 | | 1.04 |
| Ala | 1.10 | | 2.52 (2) | 3.72 (4) | | 1.00 |
| Val | 0.78 (1) | 0.92 | 2.42 (3) | | 1.03 | |
| Met | | | | | | |
| Ile | | 0.93 | 1.56 (2) | 0.93 | | |
| Leu | | 1.08 | 2.95 | 1.27 | | |
| Tyr | | | 0.80 | | | |
| Phe | | | | 2.82 | | |
| Lys | 1.08 | 1.02 | | 1.15 | 0.97 | 1.02 |
| His | 1.29 (1) | | | | | 0.95 |
| Arg | | | 0.80 | | | |
| Cys | | | 1.12 | 1.16 | | |
| Trp | (1) | (1) | (1) | | | |
| Sum | 8 | 9 | 23 | 19 | 2 | 4 |

| Pos. | Tp8 66 | Tp9a (*) 67-76 | Tp9b 77-82 | Tp10a 83-87 | Tp10/11 88-104 | Tp12a 105-113 |
|---|---|---|---|---|---|---|
| Asx | | 0.98 | 2.84 | 0.86 | 2.95 | 1.08 |
| Thr | | 0.86 | | 1.05 | | 1.03 |
| Ser | | 0.92 | | | 0.83 | |
| Glx | | | | | 0.97 | |
| Pro | | | | | 0.94 | |
| Gly | | 1.29 (1) | | | | 1.05 |
| Ala | | 1.09 | | 1.14 | | |
| Val | | 1.77 | | | 2.16 | 1.06 |
| Met | | | | | | 0.80 |
| Ile | | | 0.96 | | | 0.94 |
| Leu | | 1.05 | 1.06 | | 2.98 | 2.01 |
| Tyr | | | | | | |
| Phe | | 1.04 | | 0.99 | 1.23 | |
| Lys | 1.00 | 1.39 (1) | 1.14 | 0.97 | 1.28 (1) | |
| His | | | | | 1.79 | |
| Arg | | | | | 0.93 | 1.04 |
| Cys | | | | | 0.95 | |
| Trp | | | | | | |
| Sum | 1 | 10 | 6 | 5 | 17 | 9 |

Met and Cys determined after performic acid oxidation.
Numbers in parentheses denote amino-acid residues found during sequencing.

b) Amino acid composition of tryptic peptides (continued) and of the intact chains:

| Pos. | Tp12b 105-113 | Tp13 121-132 | Tp14a 133-143 | Tp14b 144 | Tp15 145-146 | Globin 1-146 |
|---|---|---|---|---|---|---|
| Asx | | 0.96 | | | | 14.87 (15) |
| Thr | | 0.98 | 1.08 | | | 8.85 ( 9) |
| Ser | | | | | | 3.36 ( 3) |
| Glx | | 1.09 | | | | 8.83 ( 9) |
| Pro | | 0.90 | | | | 3.99 ( 4) |
| Gly | 0.99 | | 1.14 | | | 10.10 (10) |
| Ala | 1.90 | 2.85 | 3.72 (4) | | | 18.26 (19) |
| Val | | | 0.98 | | | 11.78 (12) |
| Met | | | | | | 0.98 ( 1) |
| Ile | | | | | | 5.65 ( 6) |
| Leu | 1.07 | | 2.04 | | | 15.20 (15) |
| Tyr | | | | | 1.02 | 2.13 ( 2) |
| Phe | 0.96 | 2.01 | | | | 8.38 ( 9) |
| Lys | 1.03 | 1.12 | | | | 12.58 (12) |
| His | 1.04 | 1.07 | 1.07 | | 0.98 | 9.26 ( 8) |
| Arg | | | 0.95 | 1.00 | | 5.44 ( 5) |
| Cys | | 1.11 | | | | 3.04 ( 4) |
| Trp | | | | | | 2.58 ( 3) |
| Sum | 7 | 12 | 11 | 1 | 2 | 146 |

Met and Cys determined after performic acid oxidation.
Numbers in parentheses denote amino-acid residues found during sequencing.