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Ahmed, A., M. Jahan, Z.H. Zaidi, G. Braunitzer, and R. Goeltenboth. 1987. The primary structure of hemoglobins of the adult jaguar (Panthera onco, Carnivora). *Biol. Chem. Hoppe-Seyler* 368: 1385-1390. doi: 10.1515/bchm3.1987.368.2.1385

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Comments
This article was originally published in *Biological Chemistry Hoppe-Seyler*, volume 368, in 1987. DOI: 10.1515/bchm3.1987.368.2.1385

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Biol. Chem. Hoppe-Seyler
Vol. 368, pp. 1385–1390, October 1987

The Primary Structure of the Hemoglobins of the Adult Jaguar (*Panthera onco*, Carnivora)*

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(Received 29 June/2 September 1987)

Summary: The primary structure of the hemoglobins from Jaguar *(Panthera onco)* are presented. Electrophoretic separations without and with a dissociating agent revealed the presence of two hemoglobin components, $\alpha_2\beta_2^{I}$ and $\alpha_2\beta_2^{II}$. The separation of the hemoglobin components was achieved by ion-exchange chromatography. The globin chains were separated by ion-exchange chromatography and also by reversed phase HPLC. The amino-acid sequences of the native chains and peptides were determined by liquid-phase and gas-phase sequencing. N-Acetylserine was detected by FAB-mass spectroscopy as N-terminal group of the βI chain. The sequences are compared with that of human hemoglobin (Hb A).

*Die Primärstruktur der Hämoglobine des adulten Jaguars (*Panthera onco, *Carnivora)*

Zusammenfassung: Die Primärstruktur der Hämoglobine des adulten Jaguar *(Panthera onco)* wird angegeben. Mit elektrophoretischen Methoden wurden zwei Hämoglobinkomponenten, $\alpha_2\beta_2^{I}$ und $\alpha_2\beta_2^{II}$, nachgewiesen. Die Isolierung der Komponenten und Ketten erfolgte mit Ionenaustauschchromatographie, die der Ketten auch mit „reversed phase" HPLC. Die Aminosäuresequenz wurde mit Flüssigphasen- und Gasphasensequenatoren bestimmt. Mit FAB-Massenspektroskopie wurde N-Acetylserin als N-terminale Gruppe der βI-Kette nachgewiesen. Die erarbeiteten Primärstrukturen wurden mit den α- und β-Ketten des menschlichen Hämoglobins (Hb A) verglichen.

Key words: Jaguar, hemoglobin, blocked chain, amino-acid sequence.

A large number of mammalian hemoglobins has been sequenced to date. We have undertaken a study on Jaguar hemoglobins to fill a gap in the existing sequences of Carnivora. Jaguar belongs to the order Carnivora, family Felidae, and genus *Panthera*. 2,3-Bisphosphoglycerate showed only minimal effect on the oxygen affinity of the hemoglobins within this family[2]. The presence of a blocked N-terminal chain was also reported for a hemoglobin of different members of the Felidae family[3].

Materials and Methods

Blood from a Jaguar was collected in heparinised tubes (Zoological Garden, Berlin). Erythrocytes were washed four times with physiological saline and then lysed with

Enzyme:
Trypsin (EC 3.4.21.4).

Abbreviations:
Quadrol, *N,N,N',N'*-Tetrakis(2-hydroxypropyl)ethylenediamine; reagent IV, trisodium 7-(isothiocyanato)-naphthalene-1,3,5-trisulphonate; HPLC, high-performance liquid chromatography; TosPheCH$_2$Cl = (*N*-tosyl-L-phenylalanyl)chloromethane.

* 114th Communication on hemoglobins; for the 112th and 113th communication see ref.[1a,b].

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twice distilled water for 1 h in the cold. Disc electrophoresis was done in 12% polyacrylamide gels in Tris/glycine buffer at pH 8.3[4]. Triton electrophoresis[5] was used to check the purity of the isolated chains. The hemoglobin components were separated by chromatography on a column of DEAE-Sephacel[6].

Globin chains were separated on a column of CM-cellulose in the presence of 8M urea[7]. In addition, HPLC on columns filled with reversed-phase material was used to separate the globin chains. A column of LiChrosorb-RP2 was equilibrated with 50mM ammonium acetate and 12% formic acid. The globin chains were eluted by applying a gradient with increasing concentrations of acetonitrile (35–60%) within 60 min. Globin chains were oxidized as described in ref.[8].

Oxidized chains were digested with trypsin (TosPhe-CH_2--Cl-treated) for 3 h at 20 °C and pH 10.5 and 9.5 with an enzyme/substrate ratio of 5 : 100[9]. Digested chains were centrifuged at 4000 rpm for 10 min and subjected to chromatography on a column of Sephadex-G25 (2.5 x 150 cm). The peptides were eluted with 0.1M acetic acid. Peaks prefractioned in this way were subjected to HPLC on a column with reversed-phase material[10]. The peptides were eluted from the column of LiChrosorb-RP2 equilibrated with 50mM ammonium acetate by applying a linear gradient of 0–60% acetonitrile in 60 min.

The amino-acid composition of tryptic peptides was determined by the automatic an amino-acid analyser

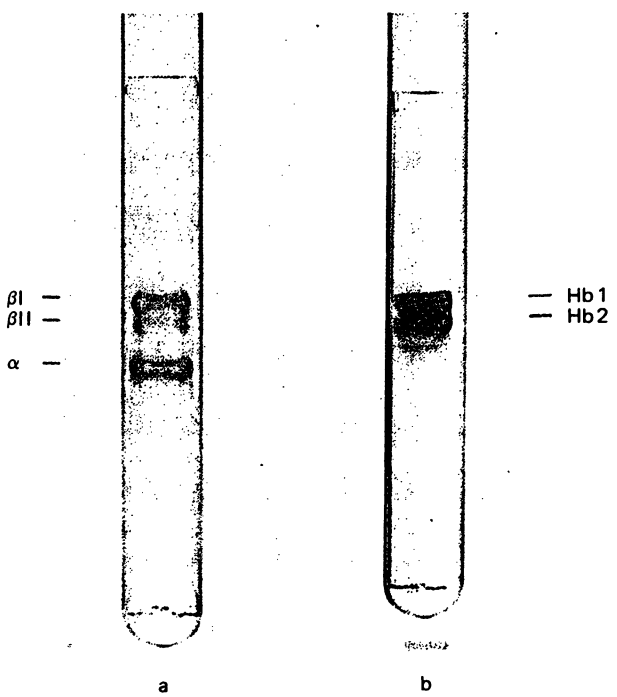

Fig. 1. Electrophoretic pattern of crude hemoglobin of Jaguar on polyacrylamide gel.

a) Under dissociating conditions, in 8M urea and Triton X-100.
b) Disc electrophoresis at pH 8.3.

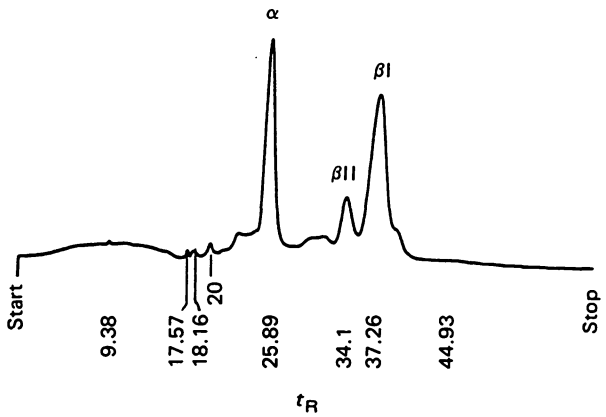

Fig. 2. Elution pattern of the Jaguar globin chains by reversed-phase HPLC on a LiChrosorb RP2 column.

Buffer: 12% formic acid/50mM ammonium acetate; gradient 0–35% acetonitrile in 2 min, followed by 35–60% acetonitrile in 60 min; the flow rate was 1 ml/min.

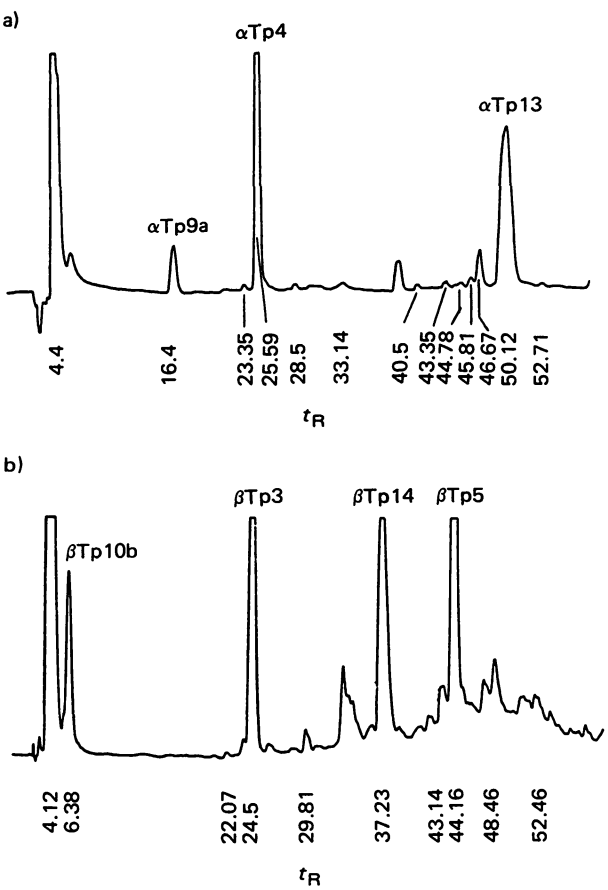

Fig. 3. Rechromatography of the prefractionated peaks of the tryptic peptides from Sephadex-G25 on a LiChrosorb RP2 column.

Buffer: 50mM ammonium acetate; gradient: 0–60% acetonitrile in 60 min; flow rate was 1 ml/min.
a) Peptides from globin α chain.
b) Peptides from globin β chain.

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(Model LC 5000, Biotronic). Amino-acid sequences were determined by Edman degradation[11] in liquid-phase sequencers (Models 890B and 890C, Beckman). A modified quadrol programme[12] was used for the sequencing of the native chains and lysine peptides. The lysine peptides were sequenced after modification by reagent IV[13]. A 3-(dimethylamino)propyne programme[14] was employed for arginine peptides. Some peptides were also sequenced by a gas-phase sequencer[15]. The phenylthiohydantoin derivatives of amino acids were identified by HPLC[16]. The sequence of a peptide with a blocked N-terminus from the βI chain (Tp 1) was established by FAB mass spectroscopy.

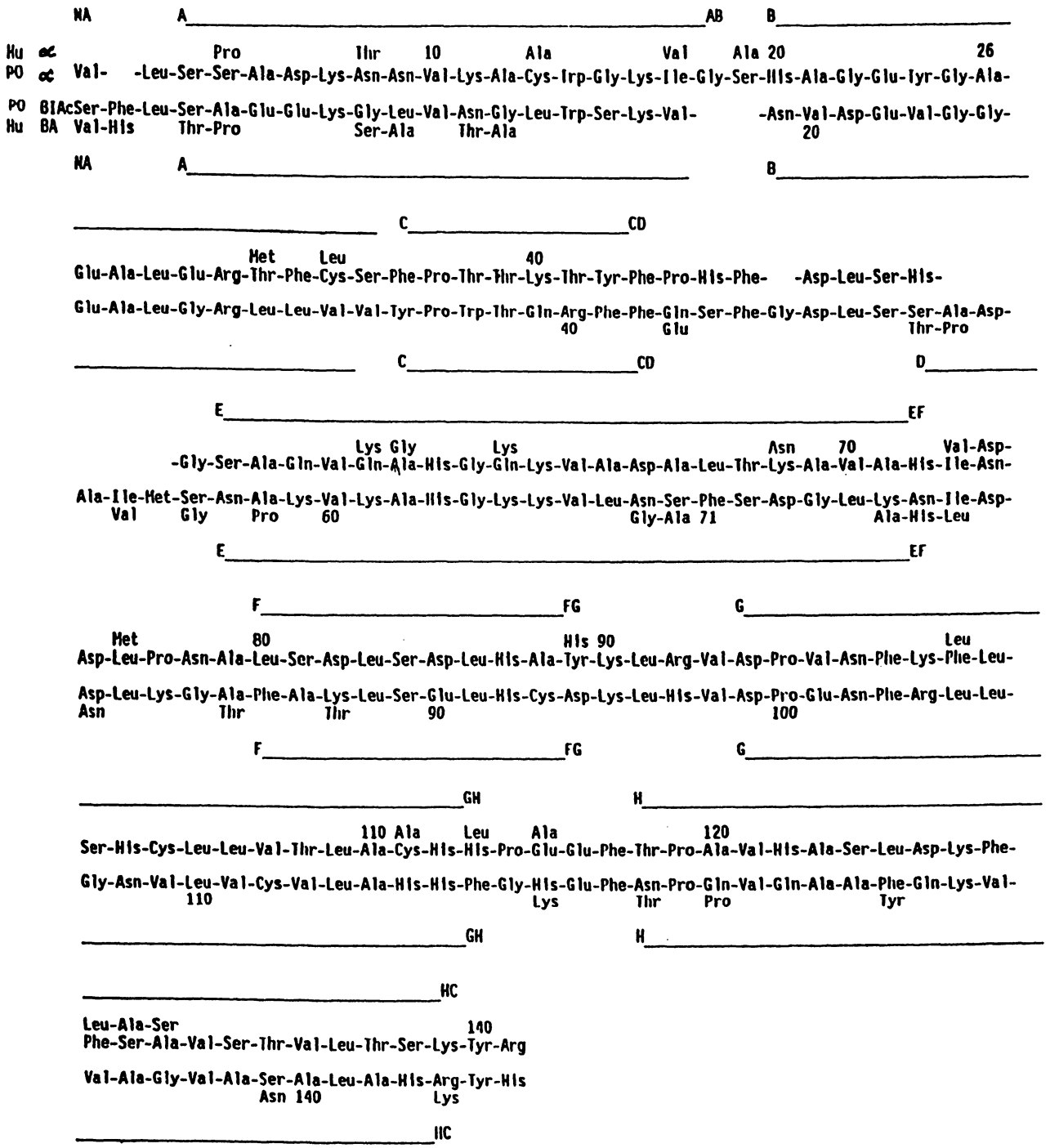

Fig. 4. Amino-acid sequences of Jaguar (PO) globin chains in alignment with the corresponding chains of human (Hu) globin.

In case of human hemoglobin only the exchanges are given. The Hb-II differs from the Hb-I at the following positions βI/βII: βNA1 Ac-Ser/Gly, βHC1 Arg/Lys.

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Results and Discussion

Electrophoresis on disc and triton gel showed two hemoglobin components of similar intensity containing three globin chains, namely α, βI, and βII (Fig. 1). Chromatography on DEAE-Sephacel revealed the presence of two hemoglobin components. Crude globin was separated by chromatography on a CM-Cellulose column to give three peaks. Triton electrophoresis of these peaks showed both, βI and βII, the latter being contaminated with the α chain. Reversed-phase HPLC resulted in the resolution of three purified chains (Fig. 2).

Prefractionation of the tryptic peptides on Sephadex-G25 gave some of the peptides in pure form. Rechromatography of the peptide mixture on reversed-phase HPLC led to pure peptides (Fig. 3), the amino-acid compositions of which are presented as Supplementary Material (Table 2–4). The amino-sequence of blocked β chain as determined by mass spectroscopy showed N-terminal acetylation.

The complete amino-acid sequence of all chains is presented in Fig. 4. The sequence comparison with human globin chains revealed 22 (15.6%) substitutions in the α, 28 (19.1%) in the βI and 27 (18.4%) in the βII chains. Between two the β chains differences were found at (βI/βII): βNA1 Ac-Ser/Gly, βHCl Arg/Lys. The exchanges which are distributed over the entire length of the molecule result in the alteration of four α1β1 contact points: α34(B15)Leu/Cys, α111-(G18)Ala/Cys, β123(H1)Thr/Asn, β125(H3)-Pro/Gln; one α1β2 contact point at β43 (CD2) Glu/Gln and one heme contact point at β70-(E14)Ala/Ser. Exchanges were also observed at binding sites for 2,3-bisphosphoglycerate at β1(NA1)Val/Ac-Ser, and β2(NA2)His/Phe. Substitution of the hydrophilic residue βNA2 (His) with a hydrophobic (Phe) should result in the alteration of the secondary structure[17]. Exchanges at these phosphate-binding sites were also found in cat hemoglobin[18] and in Leopard hemoglobin[19]. The minimum numbers of amino-acid differences found between the known sequences of the α and βI chains of three felidae are presented in Table 1.

Table 1. The minimum amino-acid differences in α and βI chains of Felidae hemoglobins.

α Chains	Leopard	Cat	β Chains	Leopard	Cat
Jaguar	0	8	Jaguar	3	6
Leopard	–	8	Leopard	–	5

The authors are grateful to Prof. *W. Schäfer* and *Dr. A. Abbasi*, Max-Planck-Institut für Biochemie, Martinsried for performing mass spectra of the blocked peptide and for kind cooperation, respectively. We sincerely thank Ms. *B. Schrank*, Mr. *R. Mentele*, Ms. *R. Gautsch*, Ms. *A. Muhr*, and Ms. *E. Wottawa* for their help in amino-acid analysis and sequence work. A. Ahmed is thankful to the *Deutscher Akademischer Austauschdienst* (DAAD) for providing a fellowship and M. Jahan to the *Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.* for the award of a doctoral fellowship.

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Supplementary Material

Table 2. Amino-acid composition of peptides from α chain of Jaguar.

Pos.	Tp1 1-7	Tp2 8-11	Tp3 12-16	Tp4 17-31	Tp5 32-40	Tp6/7/8 41-61	Tp9a 62-68	Tp9b 69-90	Tp10 91-92	Tp11 93-99	Tp12 100-127	Tp13 128-139	Tp14 140-141
Asx	1.08	1.91	-	-	-	1.14	0.92	4.68	-	1.95	1.00	-	-
Thr	-	-	-	-	2.79	0.89	0.93	-	-	-	2.05	1.93	-
Ser	1.80	-	-	0.89	1.10	1.80	-	1.85	-	-	1.60	2.92	-
Glx	-	-	-	3.13	-	3.19	-	-	-	-	2.05	-	-
Pro	-	-	-	-	1.23	0.89	-	1.06	-	1.16	2.02	-	-
Gly	-	-	1.02	2.85	-	2.03	-	-	-	-	0.72	-	-
Ala	1.00	-	0.97	3.08	-	2.17	1.75	4.05	-	-	2.77	1.04	-
Cys*	-	-	0.97	-	0.95	-	-	-	-	-	2.30(2)	-	-
Val	0.96	0.98	-	-	-	0.98	0.91	1.04	-	1.91	1.75	2.05	-
Ile	-	-	-	0.96	-	-	-	0.90	-	-	-	-	-
Leu	1.08	-	-	1.04	-	1.10	1.45	4.20	1.02	-	4.00	1.05	-
Tyr	-	-	-	0.73	-	0.83	-	0.83	-	-	-	-	0.76
Phe	-	-	-	-	1.91	1.96	-	-	-	0.98	2.30(2)	1.97	-
His	-	-	-	1.26	-	3.09	-	2.31	-	-	4.11	-	-
Trp	-	-	0.81	-	-	-	-	-	-	-	-	-	-
Lys	1.06	1.10	1.03	-	1.00	0.98	1.02	1.02	-	0.99	0.88	0.98	-
Arg	-	-	-	1.01	-	-	-	-	1.2	-	-	-	0.81
Sum	7	4	5	15	9	21	7	22	2	7	28	12	2

* Determined after performic acid oxidation.
Values in brackets are taken from sequence data.

Table 3. Amino-acid composition of peptides from β I chain of Jaguar.

Pos.	Tp1 1-8	Tp2 9-17	Tp3 18-30	Tp4 31-40	Tp5 41-59	Tp6 60-61	Tp7 62-65	Tp8 66	Tp9a 67-76	Tp9b 77 82	Tp10a 83-87	Tp10b 88-95	Tp11 96-104	Tp12/13 105-132	Tp14 133-144	Tp15 145-146
Asx	-	1.28	2.04	-	3.04	-	-	-	2.07	3.07	-	1.05	2.03	2.10	-	-
Thr	-	-	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-
Ser	1.82	1.00	-	-	3.68	-	-	-	1.77	-	-	0.89	-	-	0.98	-
Glx	2.20	-	2.16	1.03	1.18	-	-	-	-	-	-	1.07	1.14	4.38(4)	-	-
Pro	-	-	-	0.90	-	-	-	-	-	-	-	-	0.95	0.75	-	-
Gly	-	1.71	2.90	-	1.13	-	0.99	-	1.13	-	0.87	-	-	2.12	1.18	-
Ala	0.96	-	1.05	-	3.15	-	1.00	-	-	-	2.06	-	-	3.03	4.14	-
Cys*	-	-	-	-	-	-	-	-	-	-	-	1.02	-	1.04	-	-
Val	-	1.17	2.88	1.53(2)	-	1.09	-	-	1.00	-	-	-	0.96	3.89	2.64	-
Met*	-	-	-	-	1.01	-	-	-	-	-	-	-	-	-	-	-
Ile	-	-	-	-	0.81	-	-	-	-	0.95	-	-	-	-	-	-
Leu	1.02	2.07	1.00	2.13	1.03	-	-	-	2.05	0.98	-	1.99	1.05	3.99	1.15	-
Tyr	-	-	-	0.81	-	-	-	-	-	-	-	-	-	-	-	0.87
Phe	0.96	-	-	-	2.96	-	-	-	0.98	-	1.02	-	0.99	2.89	-	-
His	-	-	-	-	-	-	0.93	-	-	-	-	1.00	0.91	2.95	1.05	1.12
Trp	-	0.73	-	0.87	-	-	-	-	-	-	-	-	-	-	-	-
Lys	1.01	0.85	-	-	0.99	0.92	1.05	1.05	1.00	1.00	0.86	0.96	-	0.95	-	-
Arg	-	-	0.94	1.23	-	-	-	-	-	-	-	-	0.93	-	1.04	-
Sum	8	9	13	10	19	2	4	1	10	6	5	8	9	28	12	2

* Determined after performic acid oxidation.
Values in brackets are taken from sequence data.

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Table 4. Amino-acid composition of peptides from β II chain of Jaguar.

Pos.	Tp1 1-8	Tp2 9-17	Tp3 18-30	Tp4 31-40	Tp5 41-59	Tp6 60-61	Tp7 62-65	Tp8 66	Tp9a 67-76	Tp9b 77-82	Tp10a 83-87	Tp10b 88-95	Tp11 96-104	Tp12/13 105-132	Tp14 133-144	Tp15 145-146
Asx	-	1.26	1.94	-	3.19	-	-	-	1.92	2.66	-	1.02	1.99	2.09	-	-
Thr	-	-	-	0.94	-	-	-	-	-	-	-	-	-	-	-	-
ser	0.99	1.01	-	-	3.67	-	-	-	1.54(2)	-	-	0.91	-	-	1.08	-
Glx	1.94	-	2.10	1.03	1.04	-	-	-	-	-	-	1.14	1.05	3.93	-	-
Pro	-	-	-	0.85	-	-	-	-	-	-	-	-	0.78	1.06	-	-
Gly	1.04	1.89	2.73	-	1.24	-	1.04	-	1.33(1)	-	0.98	-	-	2.14	1.19	-
Ala	0.95	-	1.10	-	2.98	-	1.15	-	-	-	1.92	-	-	2.96	3.73	-
Cys*	-	-	-	-	-	-	-	-	-	-	-	0.85	-	0.96	-	-
Val	-	0.91	2.94	1.62	-	1.04	-	-	1.02	-	-	-	1.05	4.01	2.58	-
Met*	-	-	-	-	0.70	-	-	-	-	-	-	-	-	-	-	-
Ile	-	-	-	-	1.00	-	-	-	-	1.11	-	-	-	-	-	-
Leu	1.09	1.79	1.13	2.14	1.10	-	-	-	2.14	1.16	-	2.03	1.08	4.11	1.28	-
Tyr	-	-	-	0.90	-	-	-	-	-	-	-	-	-	-	-	0.87
Phe	0.96	-	-	-	2.89	-	-	-	1.03	-	1.01	-	0.96	2.75	-	-
His	-	-	-	-	-	-	1.05	-	-	-	-	1.01	1.02	2.96	1.12	1.12
Trp	-	0.82	-	0.79	-	-	-	-	-	-	-	-	-	-	-	-
Lys	0.89	1.18	-	-	1.16	0.98	0.80	1.01	0.98	1.05	1.00	1.00	-	0.98	1.15	-
Arg	-	-	1.02	1.10	-	-	-	-	-	-	-	-	0.92	-	-	-
Sum	8	9	13	10	19	2	4	1	10	6	5	8	9	28	12	2

* Determined after performic acid oxidation.
Values in brackets are taken from sequence data.

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