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# The Amino Acid Sequence of the Adult Sumatran Tiger (*Panthera tigris*, Carnivora) Hemoglobins

## Comments

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## Carnivora: The Amino-Acid Sequence of the Adult Sumatran Tiger (*Panthera tigris sumatrae*) Hemoglobins.\*

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**Summary:** The complete amino-acid sequences of the hemoglobins from the adult Sumatran tiger (*Panthera tigris sumatrae*) have been determined on automatic liquid- and gas-phase sequenators. The globin chains were isolated by reverse phase HPLC on a column of Nucleosil-C4. *N*-Acetylserine was detected by

FAB-mass spectroscopy as *N*-terminal amino-acid residue of the  $\beta$ I chain. Comparing the sequences of the globin chains of the tiger with that of human Hb-A, 23 substitutions were recognized in the  $\alpha$ , 29 in  $\beta$ I and 28 in the  $\beta$ II chain.

*Carnivora: Die Aminosäuresequenz der Hämoglobine vom adulten Sumatra-Tiger (Panthera tigris sumatrae)*

**Zusammenfassung:** Die vollständigen Sequenzen der Hämoglobine des adulten Sumatra Tigers (*Panthera tigris sumatrae*) wurden mit Flüssig- und Gasphasen-Sequenatoren bestimmt. Die Globinketten wurden mit "reverse phase HPLC" über eine Nucleosil-C4-Säule isoliert. *N*-Acetyl-

serin wurde mittels FAB-Massenspektroskopie am N-terminalen Ende der  $\beta$ I-Kette nachgewiesen. Der Vergleich der Sequenzen der Globinketten von Tiger- und Human-Hb-A ergibt 23 Substitutionen in der  $\alpha$ -, 29 in der  $\beta$ I- und 28 in der  $\beta$ II-Kette.

**Key words:** Sumatran tiger, carnivora, hemoglobin, primary structure.

The tiger is placed within the order Carnivora, family Felidae, genus *Panthera*. We have already studied the primary structure of hemoglobins from a few members of the family Felidae; jaguar, leopard, lion and cat<sup>[2–5]</sup>. This communication continues our comparative study on Carnivora hemoglobins. The amino-acid sequence of tiger (*Panthera tigris sumatrae*) hemoglobin may be considered as a compilation

of work on the primary structure of hemoglobins from the genus *Panthera*.

### Materials and Methods

#### Preparation of hemolysate

Blood from a Sumatran tiger was collected in heparinized tubes at Zoological Garden, Berlin. The erythrocytes were washed three times with physiological saline and

#### Enzyme:

Trypsin (EC 3.4.21.4).

#### Abbreviations:

TosPheCH<sub>2</sub>Cl = (*N*-tosyl-L-phenylanyl)chloromethane; Quadrol = *N,N,N',N'*-tetrakis(2-hydroxypropyl)ethylene-diamine; reagent IV = trisodium 7-(isothiocyanato)naphthalene-1,3,5-trisulfonate; FAB = fast-atom bombardment; RP-HPLC = reverse phase high performance chromatography; TFA = trifluoroacetic acid.

\* 136th Communication on hemoglobin, for 134th and 135th communication see ref.<sup>[1,2]</sup>. Extract from Ph. D. thesis of M. Jahan, Faculty of Science, University of Karachi, 1988.

lysed with distilled water in the cold<sup>[6]</sup>. The hemolysate was checked for heterogeneity by polyacrylamide gel disc electrophoresis in Tris/glycine buffer at pH 8.3<sup>[7]</sup>, as well as under dissociating conditions in the presence of Triton X-100 and urea<sup>[8]</sup>.

#### Globin chain separation

The globin chains were separated by RP-HPLC on Nucleosil-C4 column using a Gradient Liquid Chromatograph 334, controller 421 (Beckman). Elution of chains was carried out with 0.1% aqueous trifluoroacetic acid (TFA) with a linear gradient of 0–35% acetonitrile within 2 min, followed by 35–60% in 60 min.

#### Enzymatic cleavage and peptide separation

The globin chains were oxidized with performic acid and digested with trypsin (TosPheCH<sub>2</sub>Cl-treated, Worthington) for 3 h at pH 10.5 and 9.5 with an enzyme/substrate ratio of 5:100<sup>[9–10]</sup>. After 3 h, the hydrolysate was titrated to pH 4 and centrifuged. The soluble peptides were fractionated by gel filtration on Sephadex G-25 fine (2.6 × 150 cm) in 0.1M acetic acid. The pre-fractionated peaks were re-chromatographed by RP-HPLC on a column of LiChrosorb-RP2, (4.6 × 25 mm) equilibrated in 0.05M ammonium acetate<sup>[11]</sup>. Peptides were eluted with a gradient of acetonitrile from 0–60% in 60 min.

#### Sequence determination

Amino acid sequences were determined by automated Edman degradation<sup>[12]</sup> in liquid phase sequencers (models 890B and 890C, Beckman Instruments). A modified Quadrol program<sup>[13]</sup> (0.25M Quadrol) was applied for sequencing of the intact chains and large lysine peptides which had been reacted with reagent IV<sup>[14]</sup>. 3-Diethylamino propyne<sup>[15]</sup> was employed for sequencing of the arginine peptides. Some peptides were sequenced by gas phase method using a non-commercial sequencer<sup>[16,17]</sup>. The thiazolinone derivatives were converted to phenylthiohydantoin derivatives in the presence of 3M TFA at 80 °C, and identified by HPLC<sup>[18]</sup>.

## Results and Discussion

The hemoglobin of tiger consist of two components as verified by polyacrylamide gel disc electrophoresis in approximately equal amounts (Fig. 1a). Electrophoresis under dissociating conditions revealed three bands corresponding to two  $\beta$  and one  $\alpha$  (Fig. 1b).

RP-HPLC of hemolysate on a Nucleosil-C4 column resulted in the separation of three chains;  $\alpha$ ,  $\beta$ I and  $\beta$ II, (Fig. 2) confirming the result of Triton electrophoresis.

The primary structure determination of the globin chains was achieved by sequencing the N-terminal regions of the native chains up to 42 amino-acid residues, followed by sequencing of the tryptic peptides. The complete primary

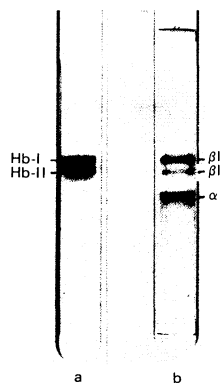


Fig. 1. Electrophoretic pattern of Sumatran tiger hemolysate on polyacrylamide gel.

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

structure of the  $\alpha$  and  $\beta$  chains is presented in Fig. 3 and the amino-acid compositions of all peptides are listed in Supplementary Material (Tables 2–4).

The sequence is aligned with that of human hemoglobin, revealing 23 (16.3%) substitution in  $\alpha$  chain, 29 (19.8%) in  $\beta$ I and 28 (19.1%) in  $\beta$ II chain. The exchanges are distributed over the entire length of the molecule, resulting in the alteration of four  $\alpha$  $\beta$ I contact points:  $\alpha$ 34 Leu/Cys,  $\alpha$ 111 Ala/Cys,  $\beta$ 123 Thr/Asn,  $\beta$ 125 Pro/Gln and one  $\alpha$  $\beta$ II contact point  $\beta$ 43 Glu/Gln. Among the heme contact points, only one

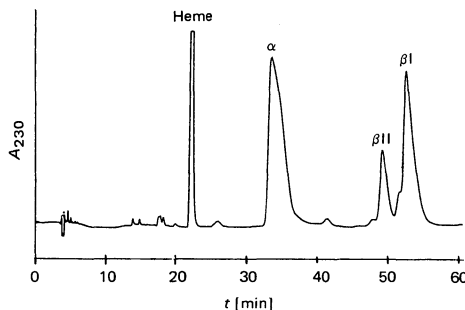


Fig. 2. Separation profile of the whole hemolysate by RP-HPLC on a column of Nucleosil C4 (size: 4.6 × 250 mm).

Sol.A: 0.1% trifluoroacetic acid; gradient 0–35% acetonitrile in 2 min, followed by 35–60% acetonitrile in 60 min; flow rate 1 ml/min.

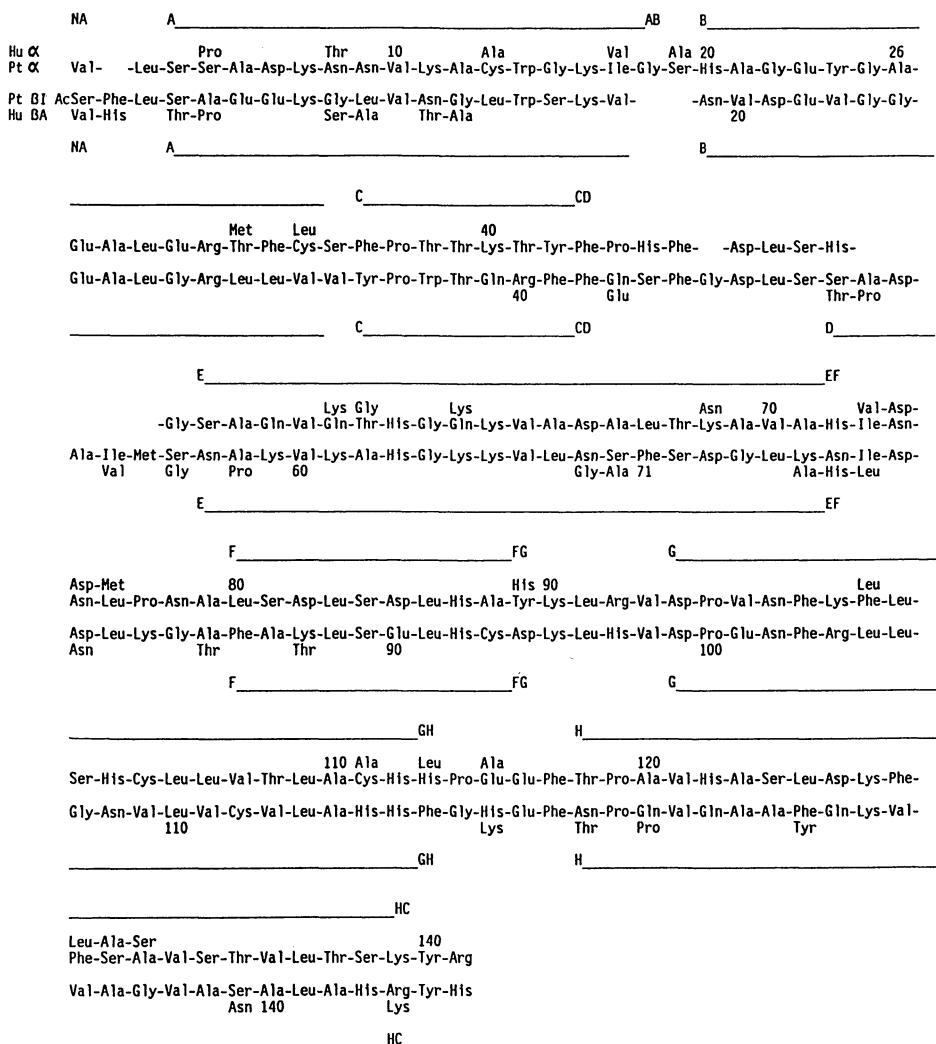


Fig. 3. Amino acid sequence of Tiger (Pt) globin chains in alignment with the corresponding chain of human (Hu). In case of human hemoglobin only the exchanges are given. The Hb-II differs from the Hb-I at the following positions.  $\beta$ 1/ $\beta$ II:  $\beta$ NA1 Ac-Ser/Gly,  $\beta$ HC1 Arg/Lys.

substitution was found at  $\beta$ 70 Ala/Ser. In the 2,3-diphosphoglycerate binding sites, which play an important role in the oxygen affinity of hemoglobin, the following exchanges were detected;  $\beta$ I chain:  $\beta$ 1 Val/Ac-Ser,  $\beta$ 2 His/Phe and in the case of  $\beta$ II chain:  $\beta$ 1 Val/Gly and  $\beta$ 2 His/Phe.

The comparison of the amino-acid sequence of the  $\alpha$ ,  $\beta$ I and  $\beta$ II chains with the members of the

genus *Panthera*, lion, jaguar and leopard (Table 1) revealed that both  $\beta$  chains have identical primary structure. In the case of  $\alpha$  chain three differences were found when compared with lion, at position  $\alpha$ 57 Thr/Ala,  $\alpha$ 71 Ala/Val and  $\alpha$ 75 Asn/Asp, whereas in the case of jaguar and leopard only two exchanges have been observed, at  $\alpha$ 57 Thr/Ala and  $\alpha$ 75 Asn/Asp. All of these exchanges are not involved in the functionally

Table 1a. Amino-acid differences between globin chains of tiger and other carnivora. Sequences are compared to the reference sequence (tiger) in the top line. Only differences are marked by the one-letter-code designation of the respective amino acids. Ferret  $\alpha$ -chain has not been reported. References for data cited: see text.

$\alpha$ -chains	
Residue No:	4 8 10 12 13 15 17 19 23 26 30 34 36 50 56 57 60 68 70
Tiger	S N V A C G I S E A E C F H Q T Q K V
Lion	
Jaguar	
Cat	
Persian leopard	
Lesser panda	P T S T D L G G A P K A K L
Giant panda	P T T D G G A A K K T T
Asiatic black bear	P S T D G G A A K K T T A
Polar bear	P S T D G G A A K K T T A
Malayan bear	P S T D G G A A P K A K T T
Domestic dog	P T I S T D G D G D Q Q P K A K T T
Coyote	P T I S T D G D G D Q Q P K A K T T
Badger	P A I T D G G A K G K N
Raccoon	P A I T D G G A P K A K L
Harbor seal	P T T D G G T A K A K L

71 72 74 75 78 80 82 89 100 101 111 115 129 130 diff.	
A V I N N D Q H D D G A H L L S S A A T 0	
G L D D D G A H L L S S A A T 3	
G L D D D G A H L L S S A A T 2	
G L D D D G A H L L S S A A T 7	
G L D D D G A H L L S S A A T 2	
G L D D D G A H L L S S A A T 25	
G L D D D G A H L L S S A A T 24	
G L D D D G A H L L S S A A T 25	
G L D D D G A H L L S S A A T 25	
L L D D D G A A L A L A T 24	
L L D D D G A A L A L A T 23	
G L D D D G A H L L A D T 20	
M D D G A H L L A D T 23	
	21

important regions, hence it may not effect the functions of the hemoglobin molecule.

A comparison of primary structures of  $\alpha$  and  $\beta$  chains from 16 carnivora hemoglobins as shown in Table 1a and 1b entails the following six families of the order Carnivora. Felidae (cat and allies), Canidae (dogs), Mustelidae (badgers), Phocidae (true seals), Procyonidae (racoons) and Ursidae (bears).

From Table 1a the following positions can be seen in the  $\alpha$ -chain which appear to contain amino-acid residues characteristic of the Felidae: position  $\alpha$ 4 Pro/Ser,  $\alpha$ 13 Thr/Cys,  $\alpha$ 15 Asp/Gly,  $\alpha$ 19 Gly/Ser,  $\alpha$ 26 Gly/Ala,  $\alpha$ 56 Lys/Gln,  $\alpha$ 60 Lys/Gln, and  $\alpha$ 82 Ala/Asp. At position  $\alpha$ 23 and  $\alpha$ 30 all carnivora have Glu, as do most other animals, but dog and coyote<sup>[19,20]</sup>, have Asp in these two positions. Furthermore residue  $\alpha$ 36 and  $\alpha$ 111 are also interesting, as these two positions are involved in  $\alpha$ 1 $\beta$ 1 contacts. In Harbor seal<sup>[21]</sup>  $\alpha$ 36 is replaced by Ala whereas other

carnivora have Phe. The residue  $\alpha$ 111 is substituted by Ser in Ursidae (lesser panda, giant panda, Asiatic black bear, polar bear, Malayan bear)<sup>[22-24]</sup> but in other carnivores this position is occupied by Cys. Regarding the two heme binding sites ( $\alpha$ 80 and 101) at  $\alpha$ 80, Leu is found in all carnivora except in coyote where this position is occupied by Met, and  $\alpha$ 101 is occupied by Leu in all carnivora families, except in Ursidae where Leu is substituted by Ser.

Among the Felidae members  $\alpha$ 71 is occupied by Ala, only lion has Val at this position. Residue  $\alpha$ 75 is also interesting because in all carnivora this position is occupied by Asp whereas in tiger, a non polar residue Asn is found.

Comparison of the sequences of the  $\beta$ -chain in Table 1b shows the following residues which appear to be characteristic of the panthera. Residues  $\beta$ 78 Leu/Ile,  $\beta$ 80 Asn/Asp,  $\beta$ 84 Thr/Ala,  $\beta$ 90 Glu/Asp,  $\beta$ 120 Lys/His,  $\beta$ 123 Thr/Asn,  $\beta$ 130 Tyr/Phe,  $\beta$ 139 Asn/Ser and  $\beta$ 144 Lys/Arg.

Table 1b. Carnivora  $\beta$ -chains. See legend for Table 1a.

Residue No.	1	2	4	5	6	9	10	12	13	16	21	27	33	41	43	47	50	51	54	56
	Ac-S	F	S	A	E	G	L	N	G	S	D	A	V	F	Q	E	S	A	I	S
Tiger																				
Lion																				
Jaguar																				
Persian leopard																				
Cat	G	T								G						E	D			
Lesser panda	V	H	T	G		A	A	T								D	D		P	V
Giant panda	V	H	T	G		A	A	T								D	D	T	P	V
Asiatic black bear	V	H	T	G		S		T								D	D			V
Polar bear	V	H	T	G		S		T		G						D	D			V
Malayan bear	V	H	T	G		S		T		G						D	D			V
Domestic dog	V	H	T	G		S		S		G						D	D	T	P	V
Coyote	V	H	T	G		S		S		G			I			D	D	T	P	V
Badger	V	H	T	G		S	A	S	A	G		T		Y		D	D	T	P	V
Raccoon	V	H	T	G		D	T	A	T	T	G	E				E	D			V
Ferret	V	H	T	G		A	A	T	A	G			T			D	D		P	V
Harbor seal	V	H	T	G		S	A	T	A	G						D	D		P	V

	58	73	78	80	84	90	104	120	121	123	130	139	144	diff.
	A	D	I	D	A	D	R	H	E	N	F	S	R	0
														0
														0
														9
P	E	L	N	T	E	E	K	K	D	T	Y	N	K	25
P	E	L	N	T	E	E	K	K		T	Y	N	K	24
P	D	L	N	T	E	E	K	K		T	Y	N	K	23
P	D	L	N	T	E	E	K	K		T	Y	N	K	23
P	D	L	N	T	E	E	K	K		T	Y	N	K	23
		L	N	T	E	E	K	K		T	Y	N	K	22
		L	N	T	E	E	K	K		T	Y	N	K	22
P	E	L	N	T	E	E	K	K		T	Y	N	K	29
P	E	L	N		E		K	K		T	Y	N	K	23
P	E	L	N	T	E		K	K		T	Y	N	K	26
P	E	L	N	T	E		K	K		T	Y	N	K	26

Table 1b shows changes in  $\beta$  chains depicting that most of the carnivora have N-terminal Val in  $\beta$ I except the Felidae members where the lion, tiger, jaguar, and leopard have Ac-Ser and in  $\beta$ II free Gly, but it is interesting to note that in cat, belonging to the same family,  $\beta$ I has free N-terminal Gly, whereas  $\beta$ II is blocked (Ac-Ser). His is found at position  $\beta$ 2 in all carnivora except in Felidae where only cat has Thr, but all other Felidae have Phe.

Residue  $\beta$ 41, which is involved in heme-binding and  $\alpha$ 1 $\beta$ 2 contact, is occupied by Phe in all carnivora except in badger<sup>[25]</sup> where Tyr is found. In position  $\beta$ 123, which is an  $\alpha$ 1 $\beta$ 1 contact point, all Felidae have Asn as compared to other carnivora. An Asp at position  $\beta$ 43 is found in all carnivora except in lion, tiger, jaguar and leopard, where Asp is altered into Gln, while in cat and racoon<sup>[26]</sup> it is altered into Glu.

The carnivora chains compared in this study support the early observation<sup>[27]</sup>, that the family Felidae is a distantly derived lineage of the order carnivora. This work is part of study on molec-

ular and genetic characterization of carnivora at hemoglobin level.

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## Supplementary Material

Table 2. Amino-acid composition of peptides from  $\alpha$  chain of Tiger.

Peptide: Position:	Ip1 1-7	Ip2 8-11	Ip3 12-16	Ip4 17-31	Ip5 32-40	Ip6/7/8 41-61	Ip9a 62-68	Ip9b 69-90	Ip10 91-92	Ip11 93-99	Ip12 100-127	Ip13 128-139	Ip14 140-141
Asx	1.04	2.20	-	-	-	1.04	0.94	4.73(5)	-	1.94	1.21	-	-
Thr	-	-	-	-	2.61(3)	2.02	0.88	-	-	-	2.02	2.05	-
Ser	2.01	-	-	0.97	1.19	2.21	-	1.89	-	-	2.00	2.84	-
Glx	-	-	-	3.14	-	2.64(3)	-	-	-	-	2.35	-	-
Pro	-	-	-	-	1.20	0.87	-	1.21	-	1.00	2.20	-	-
Gly	-	-	1.20	2.77(3)	-	1.66(2)	-	-	-	-	-	-	-
Ala	0.98	-	0.89	3.13	-	1.38(1)	2.05	3.52(4)	-	-	2.84	1.21	-
Cys*	-	-	1.21	-	0.93	-	-	-	-	-	1.96	-	-
Val	0.99	1.11	-	-	-	1.40(1)	0.85	1.09	-	1.92	1.75(2)	2.12	-
Ile	-	-	-	1.03	-	-	-	0.90	-	-	-	-	-
Leu	1.01	-	-	0.89	-	1.24	0.92	4.03	0.98	-	4.83	1.11	-
Tyr	-	-	-	0.88	-	0.70(1)	-	1.17	-	-	-	-	0.98
Phe	-	-	-	-	1.87	1.97	-	-	-	1.03	1.87	2.02	-
Hts	-	-	-	1.20	-	3.25	-	2.10	-	-	3.60(4)	-	-
Trp	-	-	0.79	-	-	-	-	-	-	-	-	-	-
Lys	1.11	0.99	1.02	-	1.14	1.23	1.24	1.16	-	1.09	1.20	0.63(1)	-
Arg	-	-	-	0.98	-	-	-	-	1.01	-	-	-	1.07
Sum	7	4	5	15	9	21	7	22	2	7	28	12	2

\* Determined after performic acid oxidation.  
 Numbers in parentheses denote amino acid residue found during sequencing.



Table 3. Amino-acid composition of peptides from  $\beta$ I chain of Tiger.

Pos.	1p1 1-8	1p2 9-17	1p3 18-30	1p4 31-40	1p5 41-59	1p6 60-61	1p7 62-65	1p8 66	1p9a 67-76	1p9b 77-82	1p10a 83-87	1p10b 88-95	1p11 96-104	1p12-13 105-132	1p14 133-144	1p15 145-146
Asx	-	1.11	2.00	-	3.12	-	-	-	2.13	2.88	-	1.02	1.70	2.10	-	-
Thr	-	-	-	1.12	-	-	-	-	-	-	-	-	-	-	-	-
Ser	1.66(2)	0.99	-	-	3.60(4)	-	-	-	1.72	-	-	0.82	-	-	1.07	-
Glx	2.22	-	1.98	1.22	1.03	-	-	-	-	-	-	1.07	0.70	3.98	-	-
Pro	-	-	-	1.37(1)	-	-	-	-	-	-	-	-	1.12	1.23	-	-
Gly	-	1.89	2.96	-	1.15	-	1.20	-	1.10	-	0.97	-	-	2.16	1.32(1)	-
Ala	0.89	-	1.01	-	3.08	-	0.89	-	-	1.96	-	-	-	3.06	4.11	-
Cys*	-	-	-	-	-	-	-	-	-	-	-	1.16	-	0.85	-	-
Val	-	1.11	2.95	1.77	-	0.91	-	-	0.86	-	-	-	0.79	3.85	2.00(3)	-
Met*	-	-	-	-	1.01	-	-	-	-	-	-	-	-	-	-	-
Ile	-	-	-	-	0.79	-	-	-	-	1.00	-	-	-	-	-	-
Leu	1.00	2.22	0.99	2.11	1.43(1)	-	-	-	2.15	1.08	-	1.96	1.34(1)	4.01	1.43(1)	-
Tyr	-	-	-	1.00	-	-	-	-	-	-	-	-	-	-	-	0.88
Phe	0.98	-	-	-	2.77	-	-	-	1.00	-	0.99	-	1.20	2.94	-	-
His	-	-	-	-	-	-	1.11	-	-	-	-	1.27	1.27	3.07	1.04	1.22
Trp	-	0.88	-	0.86	-	-	-	-	-	-	-	-	-	-	-	-
Lys	1.01	0.89	-	-	1.13	0.87	1.00	0.90	1.07	1.06	1.08	0.98	-	0.94	-	-
Arg	-	-	1.09	1.22	-	-	-	-	-	-	-	-	0.87	-	1.00	-
Sum	8	9	13	10	19	2	4	1	10	6	5	8	9	28	12	2

\* Determined after performic acid oxidation.

Numbers in parentheses denote amino acid residue found during sequencing.

Table 4. Amino-acid composition of peptides from  $\beta$ II chain of Tiger.

Pos.	1p1 1-8	1p2 9-17	1p3 18-30	1p4 31-40	1p5 41-59	1p6 60-61	1p7 62-65	1p8 66	1p9a 67-76	1p9b 77-82	1p10a 83-87	1p10b 88-95	1p11 96-104	1p12-13 105-132	1p14 133-144	1p15 145-146
Asx	-	1.00	1.94	-	3.04	-	-	-	2.02	2.90	-	1.18	1.97	2.22	-	-
Thr	-	-	-	1.06	-	-	-	-	-	-	-	-	-	-	-	-
Ser	1.10	0.99	-	-	3.91	-	-	-	1.60(2)	-	-	0.97	-	-	1.05	-
Glx	1.49(2)	-	2.10	1.17	1.20(1)	-	-	-	-	-	-	0.92	0.96	3.92	-	-
Pro	-	-	-	1.23	-	-	-	-	-	-	-	-	0.80	1.02	-	-
Gly	0.98	1.98	3.10	-	1.52(1)	-	1.05	-	1.12	-	0.95	-	-	2.50(2)	1.32(1)	-
Ala	0.88	-	1.06	-	2.66(3)	-	0.99	-	-	-	2.00	-	-	2.98	3.88	-
Cys*	-	-	-	-	-	-	-	-	-	-	-	0.92	-	0.91	-	-
Val	-	0.99	2.93	1.58(2)	-	0.85	-	-	1.04	-	-	-	1.09	3.77	2.09	-
Met*	-	-	-	-	0.67(1)	-	-	-	-	-	-	-	-	-	-	-
Ile	-	-	-	-	0.77	-	-	-	-	1.01	-	-	-	-	-	-
Leu	1.01	1.97	1.00	2.05	1.07	-	-	-	1.98	1.09	-	1.96	1.19	4.00	1.38(1)	-
Tyr	-	-	-	0.77	-	-	-	-	-	-	-	-	-	-	-	0.78
Phe	1.10	-	-	-	3.08	-	-	-	1.03	-	1.02	-	1.01	2.75	-	-
His	-	-	-	-	-	-	1.03	-	-	-	-	0.99	1.00	3.00	1.09	1.11
Trp	-	0.77	-	0.88	-	-	-	-	-	-	-	-	-	-	-	-
Lys	1.11	1.12	-	-	1.09	0.98	0.97	1.01	1.18	0.98	1.02	1.07	-	0.92	1.17	-
Arg	-	-	0.90	0.88	-	-	-	-	-	-	-	-	0.96	-	-	-
Sum	8	9	13	10	19	2	4	1	10	6	5	8	9	28	12	2

\* Determined after performic acid oxidation.

Numbers in parentheses denote amino acid residue found during sequencing.

