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Carnivora: The Primary Structure of the Beach Marten (*Martes foina*, Mustelidae) Hemoglobin*

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(Received 14 February 1990)

Summary: The primary structures of α- and β-chains from the hemoglobin of the Beach Marten (*Martes foina*, Carnivora) are presented. The globin chains were separated on CM-cellulose in 8M urea buffer. The amino-acid sequences were established by automatic liquid- and gas-phase Edman degradation of the intact chains and the tryptic peptides from oxidized chains.

Comparison of the sequences with human hemoglobin shows 21 exchanges in the α- and 12 in the β-chains. The differences concerning heme and inter-chain contact sites as well as the substitution α77 (EF6)Pro→Ala are discussed. The latter is observed for the first time in a mammalian hemoglobin.

The sequences are compared with those of other Carnivora. The β-chains of *Martes foina* and *Pteronura brasiliensis* (Giant Otter) are found to be identical, but their α-chains differ in 7 positions.

The surprising small numbers of exchanges between the hemoglobin from Beach marten and that from Lesser and Greater Panda are discussed.

Carnivora: Die Primärstruktur des Hämoglobins des Steinmarders (Martes foina, *Mustelidae)*

Zusammenfassung: Die Primärstrukturen der α- und β-Ketten des Hämoglobins des Steinmarders (*Martes foina*, Mustelidae) werden beschrieben. Die Globinketten wurden chromatographisch an CM-Cellulose in 8M Harnstoffpuffer getrennt. Die Aminosäure-Sequenzen wurden durch automatischen Edman-Abbau der intakten Ketten und der tryptischen Peptide von oxidierten Ketten in Flüssig- und Gasphasen-Sequenatoren ermittelt.

Beim Vergleich der Sequenzen mit Human-Hämoglobin werden 21 Austausche in den α-Ketten und 12 in den β-Ketten gefunden. Unterschiede an Häm- und Zwischenketten-Bindungsstellen sowie die Substitution α77 (EF6)Pro→Ala werden diskutiert. Letztere wird hier erstmals bei einem Säuger-Hämoglobin beschrieben.

Die Sequenzen werden mit den bisher bekannten anderer Carnivora verglichen. Die β-Ketten von *Martes foina* und *Pteronura brasiliensis* (Riesenotter) sind identisch, ihre α-Ketten unterscheiden sich jedoch in 7 Positionen.

Die überraschend geringen Austausche zwischen dem Hämoglobin des Steinmarders und dem des kleinen Pandas wie auch des großen Pandas werden diskutiert.

Abbreviations:
CM- = Carboxymethyl-; FPLC = fast protein liquid chromatography; HPLC = high-pressure liquid chromatography; Quadrol = *N,N,N′,N′*-tetrakis(2-hydroxypropyl)ethylenediamine; Reagent I = sodium 1-(isothiocyanato)benzene-4-sulfonate; Reagent IV = trisodium 7-(isothiocyanato)naphthalene-1,2,5-trisulfonate; TFA = trifluoroacetic acid; $TosPheCH_2Cl$ = (*N*-tosyl-L-phenylalanyl)chloromethane.

* 150th Communication on hemoglobins; for 149th communication see ref.[1].
† Deceased on May 27th 1989.

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Key words: Beach Marten, *Martes foina*, Carnivora, hemoglobin, primary structure, evolution.

While there is no doubt about the monophyletic origin of recent Carnivora there are open questions concerning their systematics[2]. Among others the phylogeny of the Pandas still is under discussion. The problem of mono- or diphyletic origin of Pinnipedia, which developed from Arctoidae, is not yet solved.

This contribution is one out of a series of papers that aim to establish a broad collection of hemoglobin sequence data from Carnivora. They will provide a basis for comparative molecular analysis to give a better understanding of hemoglobin evolution. By this way probably conclusions can be drawn which may help to clarify the systematics of Carnivora.

In this paper we present the sequences of α- and β-globin chains of the Beach Marten (*Martes foina*, Mustelidae), a member of the subfamily Mustelinae.

Material and Methods

Hemoglobin preparation

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 containing 1 mmol dithioerythrol. The hemoglobin composition was analysed by polyacrylamid gel electrophoresis under alkaline[3] and dissociating conditions[4] (Fig. 1).

Chain separation

Globin was prepared by the methyl ethyl ketone method according to Teale[5]. The globin chains were separated by ion exchange chromatography on carboxymethyl cellulose[6]. Freeze-dried globin was dissolved in 8M urea and reduced with dithioerythrol for 4 h under nitrogen. The pH was adjusted to 4.8 and the sample was loaded on a column of carboxymethyl cellulose (Whatman/CM-52 preswollen, 1.6 × 10 cm) equilibrated with 0.05M sodium acetate buffer in 8M urea, pH 5.7, containing 0.01% 1,4-dithiothreitol. The globin chains were eluted by a linear gradient of 0 to 0.08M sodium chloride (350 + 350 m*l*) with a flow rate of 17 m*l*/h (Fig. 2). The chains were checked by polyacrylamide gel electrophoresis under denaturating conditions in the presence of Triton X-100[14].

Tryptic peptides

The globin chains were oxidized briefly with performic acid in a cooling bath. Oxidized and native chains were digested with Tos-PheCH$_2$Cl-treated trypsin at pH 9.5 to 8.4 for 1 and 1.5 h, respectively, at room temperature (enzyme/substrate ratio 10:100). The enzyme was added in portions. Peptides were isolated by reversed phase HPLC on Nucleosil C4 (7 μm) columns (4.6 × 250 mm) with 0.1% aqueous trifluoroacetic acid using a gradient from 0 to 30% B (acetonitrile containing 0.1% TFA) developed during 90 min and continued to 60% B within 30 min at a flowrate of 0.6 m*l*/min. Coeluting peptides were separated on Vydac RP 18TP columns (4.6 × 60 mm) with 0.1% aqueous trifluoroacetic acid and gradients from 0 to 60% acetonitrile (+ 0.1% TFA).

Amino-acid analysis

Peptides were hydrolysed in 5.7M HCl at 110 °C for 20 h and analysed on an Amino-Acid Analyser LC 5000 (Biotronic, D-8039 Puchheim bei München). Cysteine and methionine were determined after performic acid oxidation[7], tryptophan in the presence of 2% thioglycolic acid[8].

Sequence determination

The sequences of the chains were determined by automated Edman degradation[9]. In the liquid phase sequencers (models 890B and 890C, Beckman Instruments, Palo Alto, Cali., U.S.A.) a modified Quadrol program[10] was applied for degradation of native intact chains (0.25M Quadrol) and of Tp 5 (pos. 41–59) from the β-chains (0.15M Quadrol) which had been reacted with reagent IV[11] whereas a 3-(diethylamino)propyne program[12] was used for sequencing Tp 9 (pos. 62–90) and Tp 12 (pos. 100–127) from the α-chains (both reacted with reagent I[13]) in the presence of polybrene. The remaining tryptic peptides were sequenced by the gas phase method[14] in a non-commercial sequenator[15]. Phenylthiohydantoine derivatives were identified by reversed-phase high performance liquid chromatography with isocratic elution[16]. The HPLC system consisted of an Intelligent Sample Processor (Millipore/Waters), a model 114M Solvent Delivery Module (Beckman Instruments), an UV-detector SPD-6A (Shimadzu) and a C-R3A Chromatopac integrator (Shimadzu).

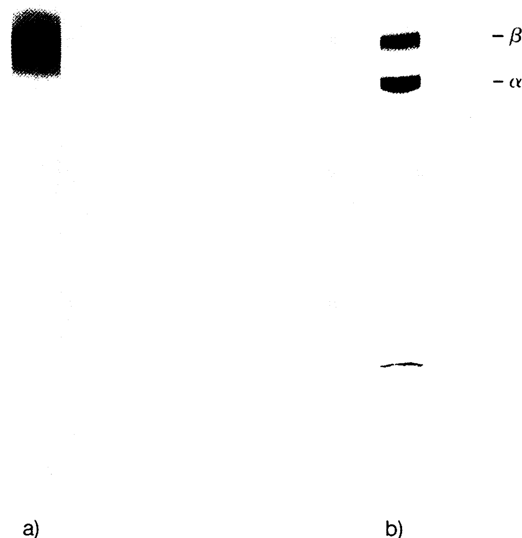

Fig. 1. Polyacrylamide gel electrophoresis of Beach Marten hemolysate.

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.

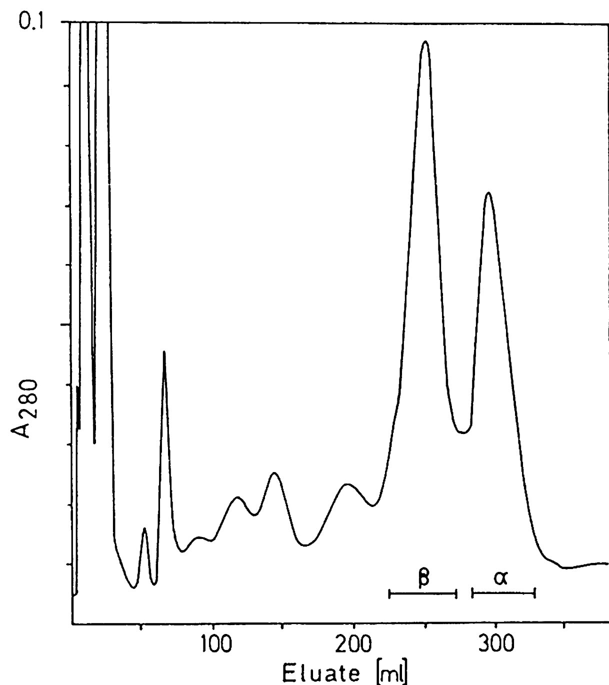

Fig. 2. Separation of the globin chains on CM-cellulose.
Sample: 40 mg Beach Marten globin in 5 m*l* starting buffer, pH 4.8; column: carboxymethyl cellulose CM-52, 1.6 × 10 cm; Starting buffer: 50mM sodium acetate, 8M urea, 0.01% 1,4-dithiothreitol, pH 5.7; gradient: linear 0–0.08M NaCl (350/350 m*l*); flowrate: 17 m*l*/h; fractions: 4.25 m*l*/15 min. Only the fractions designated as α and β contain globin chains.

Results and Discussion

In the erythrocytes of the adult Beach marten only one hemoglobin component is present. This is indicated by the results of gel electrophoresis (one band under alkaline, two bands under dissociating conditions, see Fig. 1) and of FPLC experiments (only one peak, data not shown). The primary structures of the α- and β-chains were determined by Edman degradation of the intact chains (pos. 1–42) and of tryptic peptides from oxidized chains, which were aligned by homology[17] with the corresponding human globin chains. All tryptic peptides could be isolated in good yields from the unfractionated digest of oxidized chains with exception of the tryptophan-containing peptides. These were isolated from the digest of intact chains. The amino-acid compositions of the tryptic peptides are given in Tables 2–3 in Supplementary Material. The primary structures of the α- and β-chains of the Beach Marten are shown in Fig. 3.

Five out of the 33 substitutions (21 in the α- and 12 in the β-chains) found in comparison with human hemoglobin concern contact sites[18,19]. Three α1/β1 contacts are affected: α34 (B15)Leu→Ala, α111 (G18)Ala→Cys and β125 (H3)Pro→Gln; α34 and β125 are interaction partners. β43 (CD2)Glu→Asp is an α1/β2 contact whereas β70 (E14)Ala→Ser is a heme contact site. All of these substitutions are frequently found in the hemoglobins of mammals, especially of carnivores, and their functional influences, if any, are not yet known. One exception has to be mentioned: α77 (EF6)Pro is highly conserved in mammalian hemoglobins. Only in the α-chains of the phylogenetically old Echidna[20] and Platypus[20] Asp is expressed at this external site. Ala in this position, as found in Beach Marten hemoglobin, for so far has been only known from avian, reptilian and amphibian α-chains. It has to be questioned which factors restrict the exchange of this residue in the EF loop region in almost all mammals.

Comparison of the hemoglobin from Beach Marten with other Carnivora hemoglobins poses several problems. There seems to be a trend within the superfamily Arctoidae that the β-chains evolve more slowly than the α-chains (the only exception being Procyon lotor). The comparison of *Martes foina* with *Pteronura brasiliensis* gives a drastic example of this trend: They have identical β-chains but their α-chains differ in 7 positions. Compared to *Lutra lutra* there is a difference of 1 and 8, to *Melivora capiensis* of 1 and 10 replacements in the β- and α-chains, respectively. Lack of amino-acid exchanges usually is explained by functional constraints. In avian hemoglobins the phosphate-binding capacity of the β-chains has been made responsible for their reduced mutation rate[21]. It can be doubted that this explanation holds true in our case, because then a smaller substitution frequency for the β-chains should be observed also among Primates or Chiroptera (just to mention two examples) where the phosphate binding sites are present as well. But there the β-chains have more exchanges. It also can be suspected that some jet unknown functional constraint on the α-chains is released in Arctoidae hemoglobins.

These irregularities in the frequencies of substitutions extremely complicate the interpretation of phylogenetic trees created on the basis of molecular data from hemoglobins.

The taxonomic classification of the pandas has been subject of extensive discussion in the last few years[22–25]. A surprising sequence similarity of the hemoglobins from *Lutra lutra* and *Ailurus fulgens* has been reported recently[28]. An even smaller number of substitutions is found when we compare *Martes foina* with *Ailurus fulgens*: 3 exchanges in the α- and 2 in the β-chains. This is the smallest difference between *Ailurus fulgens* and any other investigated Carnivora hemoglobin and taken together it is also smaller than the differences between members of the family Mustelidae. The difference between *Martes foina* and

```
       NA         A_____________________________________________________ AB  B_____________

Hu α    1                                    10    Ala-Ala    Gly     Val     Ala  20
Mf α   Val-   -Leu-Ser-Pro-Ala-Asp-Lys-Thr-Asn-Val-Lys-Ser-Thr-Trp-Asp-Lys-Ile-Gly-Gly-His-Ala-Gly-Glu-Tyr-
Mf β   Val-His-Leu-Thr-Gly-Glu-Glu-Lys-Ala-Ala-Val-Thr-Ala-Leu-Trp-Gly-Lys-Val-       -Asn-Val-Asp-Glu-Val-
Hu β    1           Pro          Ser  10                                               20
       NA         A___________________________________________________       B_____________


     ________________________________________ C_________________________ CD

Hu α      Ala             30    Met     Leu                         40
Mf α  -Gly-Gly-Glu-Ala-Leu-Glu-Arg-Thr-Phe-Val-Ser-Phe-Pro-Thr-Thr-Lys-Thr-Tyr-Phe-Pro-His-Phe-   -Asp-Leu-
Mf β  -Gly-Gly-Glu-Ala-Leu-Gly-Arg-Leu-Leu-Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe-Phe-Asp-Ser-Phe-Gly-Asp-Leu-
Hu β                          30                                      40        Glu
     ________________________________________ C_________________________ CD


                                             E______________________________________________________

Hu α    49 His                                              Gly         60                       Asn
Mf α  -Ser-Pro-                        -Gly-Ser-Ala-Gln-Val-Lys-Ala-His-Gly-Lys-Lys-Val-Ala-Asp-Ala-Leu-Thr-Leu-
Mf β  -Ser-Ser-Pro-Asp-Ala-Val-Met-Gly-Asn-Pro-Lys-Val-Lys-Ala-His-Gly-Lys-Lys-Val-Leu-Asn-Ser-Phe-Ser-Glu-
Hu β    49 Thr                                        60                          Gly-Ala  71     Asp
          D__________________________ E______________________________________________________


     ___________ EF                         F____________________________ FG

Hu α       70 Ala      Val          Met-Pro-Asn      80                                    90
Mf α  -Ala-Val-Gly-His-Leu-Asp-Asp-Leu-Ala-Gly-Ala-Leu-Ser-Ala-Leu-Ser-Asp-Leu-His-Ala-His-Lys-Leu-Arg-Val-
Mf β  -Gly-Leu-Lys-Asn-Leu-Asp-Asn-Leu-Lys-Gly-Thr-Phe-Ala-Lys-Leu-Ser-Glu-Leu-His-Cys-Asp-Lys-Leu-His-Val-
Hu β            Ala-His          80                      Thr         90
     ___________ EF                         F____________________________ FG


       G__________________________________________________________________ GH             H___

Hu α                          100                                   110 Ala      Leu
Mf α  -Asp-Pro-Val-Asn-Phe-Lys-Leu-Leu-Ser-His-Cys-Leu-Leu-Val-Thr-Leu-Ala-Cys-His-His-Pro-Ala-Glu-Phe-Thr-
Mf β  -Asp-Pro-Glu-Asn-Phe-Lys-Leu-Leu-Gly-Asn-Val-Leu-Val-Cys-Val-Leu-Ala-His-His-Phe-Gly-Lys-Glu-Phe-Thr-
Hu β      100           Arg                  110                                           120
       G__________________________________________________________________ GH             H___


     _______________________________________________________________________ HC

Hu α      120                          128 Leu-Ala-Ser                                     140
Mf α  -Pro-Ala-Val-His-Ala-Ser-Leu-Asp-Lys-Phe-Phe-Ser-Thr-Val-Ser-Thr-Val-Leu-Thr-Ser-Lys-Tyr-Arg
Mf β  -Pro-Gln-Val-Gln-Ala-Ala-Tyr-Gln-Lys-Val-Val-Ala-Gly-Val-Ala-Asn-Ala-Leu-Ala-His-Lys-Tyr-His
Hu β      Pro                 130                                     140
     _______________________________________________________________________ HC
```

Fig. 3. Amino-acid sequences of the α- and β-chains from Beach marten *(Martes foina)* hemoglobin (Mf).

The sequences are homologously aligned with those of adult human hemoglobin (Hu) showing only substituted residues from the latter. Bars indicate helical regions.

Table 1. Comparison of Carnivora hemoglobin sequences.
Substitutions in the α- and β-chains are shown. If not otherwise mentioned the sequences are depicted from ref.[20].

Mf	Mp	Mc	Mm	Ll	Pb	Pr	Af	Am	Ut	To	Cc	Cl	Cf	Pp	Po	Pl	Pv	Lw	Or		
	8	10	11	8	7	7	3	5	9	10	20	11	19	22	22	22	10	10	8	α	Martes foina (Mf)
	2	1	5	1	0	10	2	4	7	5	18	10	21	23	24	24	4	7	3	β	Martes foina
		9	8	2	5	10	7	8	12	11	19	10	12	16	16	17	7	9	8	α	Mustela putorius (Mp) [26]
		3	7	3	2	12	4	6	9	7	20	12	23	25	26	26	6	9	5	β	Mustela putorius
			9	9	5	9	11	10	13	12	19	14	17	19	19	20	12	12	9	α	Melivora capensis (Mc) [27]
			4	2	1	9	3	5	8	6	17	9	20	22	23	23	5	6	2	β	Melivora capensis
				8	8	6	11	10	13	12	13	12	17	20	20	21	11	13	11	α	Meles meles (Mm)
				4	5	11	6	6	9	9	18	8	22	24	25	25	7	8	6	β	Meles meles
					5	10	7	8	12	11	18	9	13	17	17	18	6	8	7	α	Lutra lutra (Ll) [28]
					1	10	2	4	7	5	18	10	21	23	24	24	5	8	4	β	Lutra lutra
						8	8	7	11	10	18	11	14	18	18	19	8	9	6	α	Pteronura brasiliensis (Pb) [29]
						10	2	4	7	5	18	10	21	23	24	24	4	7	3	β	Pteronura brasiliensis
							7	7	10	11	15	10	18	21	21	21	12	11	10	α	Procyon lotor (Pr)
							11	13	11	12	17	16	19	22	23	23	9	12	9	β	Procyon lotor
								4	8	9	20	10	18	21	21	21	9	9	7	α	Ailurus fulgens (Af)
								2	7	3	17	10	21	23	22	22	6	9	5	β	Ailurus fulgens
									4	5	19	10	17	20	20	20	7	8	8	α	Ailuropoda melanoleuca (Am)
									7	3	19	9	22	24	23	23	8	10	7	β	Ailuropoda melanoleuca
										1	20	14	15	19	19	19	11	12	12	α	Ursus thibetanus (Ut)
										4	14	8	16	18	19	19	3	7	8	β	Ursus thibetanus
											19	13	14	18	18	19	10	12	11	α	Tremarctos ornatus (To) [24]
											16	10	19	21	20	20	7	11	8	β	Tremarctos ornatus
												21	22	24	24	25	19	21	20	α	Crocuta crocuta (Cc) [30]
												17	14	17	15	15	14	17	17	β	Crocuta crocuta
													20	22	22	23	12	11	10	α	Canis lupus familiaris (Cl)
													17	18	20	20	10	9	9	β	Canis lupus familiaris
														10	10	11	15	18	17	α	Catus felis (Cf)
														8	7	7	18	19	20	β	Catus felis
															0	1	19	20	19	α	Panthera pardus orient.(Pp)
															3	3	20	21	22	β	Panthera pardus orientalis
																1	19	20	19	α	Panthera onca (Po) [31]
																0	21	22	23	β	Panthera onca
																	20	20	20	α	Panthera leo (Pl) [32]
																	21	22	23	β	Panthera leo
																		6	9	α	Phoca vitulina (Pv)
																		5	5	β	Phoca vitulina
																			5	α	Leptonychotes weddelli (Lw) [33]
																			6	β	Leptonychotes weddelli
																				α	Odobenus rosmarus (Or) [34]
																				β	Odobenus rosmarus

Ailuropoda melanoleuca with 5 and 4 exchanges is only slightly bigger than between *Ailuropoda melanoleuca* and *Tremarctos ornatus* with 5 and 3 exchanges in α- and β-chains, respectively. As can be seen from Table 1 the hemoglobins of *Ailurus fulgens* and of *Ailuropoda melanoleuca* have more similarity to the hemoglobins of Mustelidae and Ursidae, respectively, than to that of *Procyon lotor* (as representative of Procyonidae).

These results point towards a closer phylogenetic relationship of the Pandas with Mustelidae than with Procyonidae. If future hemoglobin sequences of other Procyonidae will turn out to be more similar to that of Lesser Panda then the phenomenon of convergent evolution of globins will have to be discussed.

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Supplementary Material

Table 2. Amino-acid composition of tryptic peptides from Martes foina α-chains.

Tp	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Pos.	1-7	8-11	12-16	17-31	32-40	41-56	57-60	61	62-90	91-92	93-99	100-127	128-139	140-141
Asx	1.09	1.05	1.09	-	-	1.35	-	-	4.15	-	2.04	1.13	-	-
Thr	-	0.98	0.86	-	2.91	0.81	-	-	1.01	-	-	2.13	3.07	-
Ser	0.94	-	1.05	-	1.19	1.76	-	-	2.18	-	-	2.21	3.13	-
Glx	-	-	-	2.89	-	0.91	-	-	-	-	-	1.16	-	-
Pro	0.99	-	-	-	1.10	2.11	-	-	-	-	1.13	2.58(2)	-	-
Gly	-	-	-	5.04	-	1.14	1.00	-	1.93	-	-	-	-	-
Ala	0.86	-	-	2.26	0.96	1.09	0.97	-	6.67	-	-	4.04	-	-
Cys	-	-	-	-	-	-	-	-	-	-	-	2.33	-	-
Val	0.50(1)	0.93	-	-	-	0.97	-	-	1.77	-	1.90	1.51(2)	1.43(2)	-
Met	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ile	-	-	-	0.73	-	-	-	-	-	-	-	-	-	-
Leu	0.70(1)	-	-	1.03	-	1.05	-	-	7.05	0.98	-	6.13	1.11	-
Tyr	-	-	-	0.98	-	0.72	-	-	-	-	-	-	-	0.97
Phe	-	-	-	-	1.85	2.01	-	-	-	-	0.89	1.25	2.00	-
His	-	-	-	1.09	-	1.05	0.97	-	3.18	-	-	3.96	-	-
Trp	-	-	(1)	-	-	-	-	-	-	-	-	-	-	-
Lys	1.12	1.04	1.00	-	0.98	1.01	1.05	1.00	1.05	-	1.03	1.15	1.25	-
Arg	-	-	-	0.97	-	-	-	-	-	1.02	-	-	-	1.03
Sum	7	4	5	15	9	16	4	1	29	2	7	28	12	2

Numbers in parentheses denote residues found during sequencing.

Table 3. Amino-acid composition of tryptic peptides from Martes foina β-chains.

Tp	1	2	3	4	5	6	7	8	9a	9b	10a	10b	11	12	13	14	15
Pos	1-8	9-17	18-30	31-40	41-59	60-61	62-65	66	67-76	77-82	83-87	88-95	96-104	105-120	121-132	133-144	145-146
Asx	-	-	2.05	-	4.01	-	-	-	1.11	2.83	-	0.98	2.04	1.18	-	1.04	-
Thr	0.98	0.91	-	0.92	-	-	-	-	-	-	0.91	-	-	-	0.94	-	-
Ser	-	-	-	-	2.76	-	-	-	1.59(1)	-	-	0.94	-	-	-	-	-
Glx	2.13	-	1.88	1.05	-	-	-	-	1.12	-	-	1.08	0.99	-	4.00	-	-
Pro	-	-	-	0.87	2.30	-	-	-	-	-	-	-	0.99	-	1.29	-	-
Gly	1.12	1.12	3.08	-	2.04	-	0.97	-	1.26	-	1.03	-	-	2.20	-	1.11	-
Ala	-	3.10	1.08	-	1.07	-	1.01	-	-	-	1.03	-	-	1.12	1.93	3.75	-
Cys	-	-	-	-	-	-	-	-	-	-	-	0.84	-	0.70	-	-	-
Val	0.79	1.03	2.93	1.55(2)	1.03	0.95	-	-	1.11	-	-	-	1.01	2.04(3)	0.96	2.00(3)	-
Met	-	-	-	-	0.94	-	-	-	-	-	-	-	-	-	-	-	-
Ile	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Leu	1.01	1.08	0.99	2.02	1.10	-	-	-	1.93	2.07	-	1.73	1.01	3.08(4)	-	1.00	-
Tyr	-	-	-	1.11	-	-	-	-	-	-	-	-	-	-	0.92	-	0.76
Phe	-	-	-	-	2.71	-	-	-	0.87	-	0.95	-	1.02	0.83	0.92	-	-
His	1.00	-	-	-	-	-	1.01	-	-	-	-	1.11	1.00	1.83	-	0.97	1.24
Trp	-	0.84	-	0.72	-	-	-	-	-	-	-	-	-	-	-	-	-
Lys	0.97	0.91	-	-	1.01	1.05	-	1.00	1.01	1.10	1.08	1.24	1.02	1.01	1.05	1.14	-
Arg	-	-	0.99	0.87	-	-	1.01	-	-	-	-	-	-	-	-	-	-
Sum	8	9	13	10	19	2	4	1	10	6	5	8	9	16	12	12	2

Numbers in parentheses denote residues found during sequencing.