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# Preliminary Survey of Carnivore Hemoglobin Compositions

## Conclusions

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**Summary.** The amino acid differences among 12 $\beta$ chains and 10 $\alpha$ chains of carnivore hemoglobins are given. These hemoglobins conform to the substitution rate found for hemoglobins in general. A table compares the differences among known $\alpha$-chain sequences when deduced from tryptic peptide compositions and when taken from actual sequences. Among the carnivore hemoglobins studies, tryptic peptide compositions are about 14 % low in giving the number of sequence differences.

**Key words:** Carnivore hemoglobins – tryptic peptide compositions

A comparative study of carnivore hemoglobins was begun by Seal (1969), who examined them by gel electrophoresis. Since 6 families of the Canoidea all had a major hemoglobin component of identical mobility, he concluded that the hemoglobins had not diversified since the Eocene. The studies of tryptic peptide compositions reported in the preceding pages show that such diversity does indeed exist and that hemoglobins of different carnivore species contain many amino acid differences which do not involve changes in electrophoretic mobility. For example, in comparison to dog hemoglobin, the tryptic peptide composition of raccoon hemoglobin shows 26 amino acid substitutions and polar bear shows 20, yet no charge changes are involved. The carnivore hemoglobins have a much higher proportion of such substitutions than was originally estimated from electrophoretic studies of enzymes; first suggested was a ratio of three substitutions with no charge difference to every one with a charge difference (Smith, 1972). The high rate of occurrence of enzyme variants with no charge difference in natural populations has been demonstrated by Bernstein et al. (1973) by heat stability studies.

In Tables 1 and 2 are compared probable sequences for 12 carnivore $\beta$-chains and 10 $\alpha$-chains deduced from their tryptic peptide compositions compared to dog hemoglobin I. Only the dog $\alpha$- and $\beta$-chains (Brimhall et al., preceding paper) and badger

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**Table 1.** Amino acid differences between hemoglobin $\beta$-chains of dog and other carnivores as deduced from tryptic peptide compositions. Dog $\alpha$ and $\beta$ chains (Brimhall et al., 1976) and badger $\beta$ chains (Hombrados et al., 1976) have been completely sequenced. Residue positions which contain the same amino acid in all the carnivores listed have been omitted. The space under a residue number is left blank when the amino acid is the same as for dog.

Carnivore beta chains

| Residue number | Diff. | 1 | 2 | 4 | 5 | 6 | 9 | 10 | 12 | 13 | 16 | 21 | 33 | 41 | 43 | 50 | 51 | 54 | 56 | 58 | 69 | 73 | 80 | 81 | 84 | 87 | 104 | 120 | 123 | 125 | 130 | 139 | 144 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dog | 0 | V | H | T | A | E | S | L | S | G | G | D | I | F | D | T | P | V | S | A | N | D | N | L | T | K | K | K | T | Q | Y | N | K |
| Coyote | 0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fox | 2 | | | | | | | | T | | | | | | | | | | G | | | | | | | | | | | | | | |
| Polar bear | 6 | | | | G | | | | T | | | | V | | | S | | I | N | | | | | | | | | | | | | | |
| Badger | 8 | | | | | | | A | T | S | | | V | Y | | | | | G | P | | E | | | | | | | | | | | |
| Raccoon | 16 | | | | | D | T | A | T | T | | E | V | | E | S | A | I | G | P | Q | | | | | | R | | | P | | | |
| *Coati mundi* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **N. Nasua** | 14 | | | | G | | T | A | T | A | N | | V | | E | S | | I | G | P | Q | | | | | | R | | | | | | |
| **N. Narica (F)** | 15 | | | | G | | T | A | T | S | A | | V | | E | S | | I | G | P | Q | | | | | Q | R | | | | | | |
| **N. Narica (S)** | 14 | | | | G | | K | A | T | S | | | V | | E | S | | I | G | P | Q | | | | | Q | R | | | | | | |
| Cat (F) | 20 AC | S | F | S | | | N | | G | | | | V | | E | S | S | I | | | | | D | I | A | | R | N | N | H | F | S | R |
| Cat (S) | 18 | G | F | S | | | N | | G | | | | V | | E | S | S | I | | | | | D | I | A | | R | N | N | H | F | | |
| Lion | 19 AC | S | F | S | | | N | | | | | | V | | E | S | A | I | | | | | D | I | A | | R | Q | N | H | F | S | R |

**Table 2.** Alpha chain differences.

Carnivore alpha chains

| Residue number | Diff. | 3 | 4 | 8 | 10 | 12 | 13 | 15 | 19 | 23 | 26 | 30 | 34 | 50 | 56 | 60 | 68 | 70 | 71 | 73 | 76 | 77 | 78 | 80 | 82 | 89 | 100 | 111 | 115 | 120 | 130 | 131 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dog 1 | 0 | S | P | T | I | S | T | D | G | D | G | D | Q | G | K | K | T | V | A | L | M | P | G | L | A | Y | L | C | T | A | A | A |
| Dog 2 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | T | |
| Coyote | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | T | |
| Fox | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | N | | T | |
| Polar bear | 14 | | | S | V | A | | | S | E | | E | A | | | | | G | | | | A | | | | L | F | S | A | | | S |
| Raccoon | 10 | | | A | | A | | | | E | | E | A | | | | L | | G | | | | | | | | | | A | | T | S |
| *Coati mundi* | 8+ | | | | | | | | S | E | A | E | A | | | | N | | | | | | | | | | | [– | – | –] | S | S |
| Cat | 21 | | A | S | V | A | C | G | S | E | A | E | C | H | | Q | Q | | | M | L | | T | M | | | F | | A | | | S |
| Lion (F) and (S) | 19+ | T | A | N | V | A | C | G | S | E | A | E | C | H | Q | Q [– | – | – | – | – | – | – | – | – | – | –] | F | | A | E | | S |

See legend for Table 1. The αT-12B and 12C peptides of coati mundi and the αT-9 peptides of lion were not found. F and S refer to electrophoretic mobility of the hemoglobin β-chain (Hombrados et al., 1976) have been completely sequenced. Limited sequencing has been done on some of the others as detailed in the preceding articles. It should be emphasized that a few changes in positions of the substituents may be required when complete sequences are known. The α-chain of coatimundi and 2 α-chains of lion are included in the table although they are not completed. The one-letter symbols for amino acids are those used by Dayhoff (1972).

It has become increasingly evident that the functional protein molecule and not the individual amino acid residue is the unit on which evolutionary processes act. Each amino acid replacement has the capability of affecting one or more functions of the molecule of which it is a part. Zuckerkandl (1976) has synthesized an overall picture of the evolving molecule: "On account of a competitition between different component functions for individual sites in polypeptide chains, each protein molecule represents a functional compromise, with some functions optimized, but the overall state of the molecule suboptimal . . . . . Genetic sufficency is considered a notion more adequate than genetic optimality for describing biological fitness." According to his model, most amino acid substitutions are considered evolutionary noise compatible with selection.

Romero-Herrara and Lehmann (1973) compared 18 mammalian myoglobin sequences and found an average fixation rate of one per 4.2 million years. Their work showed that ". . . Periods of apparently low rates of fixed mutation are often followed

**Table 3.** Actual amino acid sequence differences (lower rows of figures) and minimum differences determined by tryptic peptide compositions (upper rows of figures) among vertebrate hemoglobin alpha chains. Asterisks indicate one, two and three additional minimum differences when the number of Asn plus Gln are known for each tryptic peptide

| | Cow | Horse | Canine | Rabbit | Kangaroo | Opossum | Echidna | Chicken | Viper | Newt | Carp |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Human | 15 | 13 | 23 | 24 | 22 | 31 | 29 | 27 | 42* | 51* | 52 |
| | 17 | 18 | 23 | 25 | 27 | 40 | 37 | 35 | 50 | 65 | 68 |
| Cow | | 15 | 27 | 23 | 20* | 33 | 36 | 31 | 44 | 53** | 52 |
| | | 18 | 28 | 25 | 26 | 43 | 43 | 38 | 52 | 67 | 65 |
| Horse | | | 25 | 18 | 21* | 34 | 34 | 32** | 42* | 55** | 48 |
| | | | 27 | 25 | 29 | 42 | 42 | 40 | 52 | 69 | 67 |
| Canine | | | | 24 | 28 | 35* | 35 | 36* | 44* | 57* | 52 |
| | | | | 27 | 33 | 46 | 42 | 44 | 54 | 68 | 67 |
| Rabbit | | | | | 24* | 35* | 33 | 35* | 44* | 55* | 54 |
| | | | | | 37 | 50 | 48 | 43 | 52 | 72 | 71 |
| Kangaroo | | | | | | 32 | 37 | 29* | 42 | 53* | 51 |
| | | | | | | 42 | 49 | 41 | 54 | 70 | 71 |
| Opossum | | | | | | | 43 | 44* | 53** | 56** | 53 |
| | | | | | | | 60 | 60 | 70 | 72 | 77 |
| Echidna | | | | | | | | 37 | 50 | 57* | 57 |
| | | | | | | | | 48 | 66 | 74 | 75 |
| Chicken | | | | | | | | | 43* | 50* | 51** |
| | | | | | | | | | 57 | 66 | 72 |
| Viper | | | | | | | | | | 50* | 51** |
| | | | | | | | | | | 74 | 72 |
| Newt | | | | | | | | | | | 55*** |
| | | | | | | | | | | | 74 |

by apparently high rate episodes and vice versa". They conclude that the term "average rate" is a more applicable term than "constant" rate.

It has been estimated from the available sequence data that hemoglobins incorporate approximately one substitution per hemoglobin every 2.3 million years (Dayhoff, 1972; p. 50); i.e., 15 amino acid differences per 100 residues per 100 million years. On this basis, since polar bear and raccoon families diverged approximately 25 million years ago, about 22 differences would be expected between their hemoglobins (25/2.3 = 11 for each of 2 divergent lines). Actually a minimum of 24 differences was found. Again, comparing cat and dog which diverged 40–50 million years ago, 36–44 differences would be expected; at least 39 were found. This would indicate that these carnivore hemoglobins conform to the substitution rate found for hemoglobins in general.

Until such time as complete sequences are available, certain observations may be made on the basis of the exploratory data from tryptic peptide compositions. The rationale for such limited studies in structure comparisons has been discussed by Ambler (1974) for bacterial cytochromes.

A lower limit for the number of amino acid sequence differences between a known sequence and one for which tryptic peptide compositions are known may be computed by alignment of homologous peptides. The minimum number of sequence differences so derived for 66 comparisons of vertebrate hemoglobin α-chains appears in Table 3.

**Table 4.** Relation of differences found from tryptic peptide compositions to actual sequence differences between vertebrate α-chains.

| Number of actual sequence differences | Fraction detected from tryptic peptide compositions |
|---|---|
| 17–27 | .86 |
| 28–42 | .78 |
| 42–49 | .79 |
| 50–65 | .78 |
| 65–70 | .77 |
| 71–77 | .73 |

**Table 5.** Minimum number of sequence differences among β-chain hemoglobins of certain carnivores as determined by tryptic peptide compositions.

| | Fox | Polar bear | Badger | Raccoon | N. nasua | N. narica (F) | N. narica (S) | Cat (F) | Cat (S) | Lion |
|---|---|---|---|---|---|---|---|---|---|---|
| Dog-Coyote | 2 | 6 | 8 | 16 | 14 | 15 | 14 | 20 | 18 | 19 |
| Fox | | 5 | 6 | 14 | 12 | 13 | 12 | 21 | 19 | 21 |
| Polar bear | | | 9 | 13 | 9 | 10 | 9 | 19 | 16 | 19 |
| Badger | | | | 13 | 11 | 11 | 10 | 25 | 23 | 25 |
| Raccoon | | | | | 7 | 8 | 8 | 22 | 20 | 21 |
| N. nasua | | | | | | 3 | 4 | 22 | 20 | 22 |
| N. narica (F) | | | | | | | 2 | 23 | 21 | 23 |
| N. narica (S) | | | | | | | | 22 | 20 | 22 |
| Cat (F) | | | | | | | | | 3 | 3 |
| Cat (S) | | | | | | | | | | 6 |

The accuracy of tryptic peptide composition-derived estimates of amino acid sequence differences for 6 ranges of actual variability between α chains is shown in Table 4. Thus, for differences of 27 residues or less per chain, the number of residues found by tryptic peptide compositions is 14 % low. The carnivore chains in this study differ from one another by 25 or less residues, as shown in Table 5 which gives minimum β chain differences among 10 carnivores.

Comparison of the probable sequences of the hemoglobins in Tables 1 and 2 with those of other animals (Dayhoff, 1972, Alignments 17 and 19) shows 3 residues which appear to be characteristic of the carnivores. One of these is Res. 32 of the α chain; other mammals have methionine, but the carnivore and 2 known marsupial sequences, kangaroo and opossum, have threonine. A second characteristic position, α 89, is occupied by histidine in other animals, but in all the carnivores studied except polar bear, it is occupied by tyrosine. At a third position, α 50, there is histidine for other animals and for cat and lion, but proline in the other carnivores listed.

From Table 2 it can also be seen that most of the carnivores have glutamic acid at α 23 and α 30, as do most other animals, but the dog and its more recently diverged relatives, the coyote and fox, have aspartic acid in these 2 positions.

Of interest are 4 carnivores, each of which has two major hemoglobins. In one of these, the coatimundi (*Nasua narica*), there are only two differences in tryptic peptide composition between its two hemoglobins; in the domestic cat (*Felis catus*) there are 3 differences. in the lion (*Panthera leo*) only one was found; in the dog there is one difference. In coatimundi and cat it is only the β chains which differ; in dog and lion it is only the α chains. The small number of differences suggests that the two hemoglobins in each case diverged in fairly recent times.

The differences in tryptic peptide compositions found in this study are in approximate agreement with the evolutionary development of the carnivores derived from fossil records (Romer, 1966) although the construction of an evolutionary tree for these hemoglobins should properly await data from the complete sequences.

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