

THE MYOGLOBIN OF PRIMATES: *SYMPHALANGUS SYNDACTYLUS* (SIAMANG)

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1. Introduction

There is little agreement regarding the phylogenetic status of the siamang. Traditionally, its anatomical affinities to the anthropoid apes have been recognized by including it in the hominoid family Hylobatidae, together with the gibbon [1]. Simpson went further and concluded that siamang and gibbon were more similar to the greater anthropoid apes and deserved subfamilial status in the family Pongidae [2].

The siamang has a diploid number of 50 chromosomes, whereas gibbons have 44 (except *Hylobates concolor*, which has 52) and other pongids have 48 [3]. From his studies on chromosome numbers and morphology, Chiarelli has suggested that siamang and gibbon are on the colobine branch of a major cercopithecoide dichotomy and thus should not be included in the Hominoidea, but with the Old World monkeys in the Cercopithecoidea [4,5].

Immunological studies demonstrate some affinities of siamang and gibbon to other anthropoid apes. However, the combined results depict orangutan, gorilla, chimpanzee and man as having a common ancestor after the divergence of the hylobatids, and gorilla, chimpanzee and man as sharing more recent common ancestry than any of these with orangutan [6-8]. This finding is reinforced by protein sequence data. For all the apes and man they are available only for fibrinopeptides: chimpanzee, gorilla and man are identical; whereas the percentages difference from man are for the orangutan 7, for siamang 10, the gibbon 17 and macaque 30 [9].

The results of an electrophoretic study of at least 20 loci, using genetic distances for comparison, have shown that orangutan is much more similar to the great apes and man than to gibbon or siamang, and

that gibbon and siamang have many more alleles in common with the great apes than with the Old World monkeys. Also, siamang is much more similar to gibbon than to any other primate and apparently closer to *Hylobates concolor* than to *Hylobates lar* [10].

From previous work in our laboratory, the primary structure of the myoglobin of 15 primates is known, including 5 hominoids, 2 cercopithecoide, 3 ceboids and 5 prosimians (see references [11-15]). With the exception of the orangutan, the hominoids can be distinguished from all other primates by the presence of 110 Cys, 140 Lys, 144 Ser and 145 Asn. Also, position 23, Ser in gibbon and monkeys, is Gly in man and the great apes, with the exception of orangutan. The myoglobin of orangutan has Ser at position 110 (as do the cercopithecoide) and Ser at position 23. Thus the cladogram representing the most parsimonious, although not necessarily the most acceptable, arrangement (fig.1) which takes into

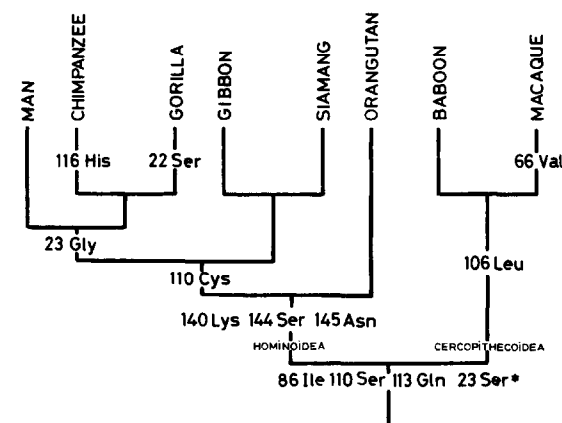


Fig.1. A cladogram representing one of the possible phylogenetic trees for the siamang. For discussion, see the text.

account some thirty other myoglobins known, shows *Pongo* diverging earliest from the hominoid line [14]. Some morphological evidence has been presented that siamang resembles the greater apes, particularly orangutan, more than the gibbon [16]. The present study was undertaken in order to determine whether or not the myoglobin sequence of siamang would resolve its phylogenetic relationships. Specifically, does the myoglobin have the distinctive hominoid residues and, if so, does this give us any evidence regarding siamang's most recent relatives within the Hominoidea? We have found that the siamang myoglobin sequence is identical to that of the gibbon (*Hylobates agilis* [17]). This gives further weight to the close similarity of gibbon and siamang, and the most convincing explanation for this similarity is a recent common ancestry of the two species. Furthermore, the body of sequence data presented here supports the close relationship of gibbon and siamang with the other hominoids and not the suggestion that the hylobatids diverged from within the cercopithecoid radiation. The enigma of the evolutionary position of the orangutan has been discussed elsewhere [14].

2. Materials and methods

Muscle, 107 g, from one siamang (*Symphalangus syndactylus*) was obtained, frozen in solid CO₂ for transportation. The preparation and purification of the myoglobin have been described in detail in previous papers [18]. Purified myoglobin, 160 mg, was separated into 55 mg and 105 mg fractions. The 55 mg fraction was aminoethylated prior to tryptic digestion. The insoluble peptides left after tryptic digestion of the nonaminoethylated globin were treated with performic acid [19] and were subsequently digested with pepsin.

The tryptic peptides of aminoethylated and the non-aminoethylated globins and the peptic peptides were separated by two-dimensional high-voltage paper electrophoresis and chromatography on Whatman 3MM [20]. The fingerprints were then developed with ninhydrin and stained for specific amino acids as described elsewhere [21]. All enzymic peptides were eluted from paper using 6 M HCl hydrolysed at 108°C for 24 h, and their composition estimated

in an automatic amino acid analyser. The tryptic peptides, containing residues 103–110 and 17–31 were eluted with 0.5 M NH₄OH for sequential dansyl-Edman degradation. Acid hydrolysis of the whole globin was performed in sealed evacuated tubes at 108°C for 24 h, 48 h and 72 h [21]. The evaluation of tryptophan was achieved by using mercaptoethane–sulphonic acid [22].

3. Results

From our interpretation of amino acid analysis of the whole globin, we have concluded that there are 153 residues (table 1). The fingerprint pattern of the soluble tryptic peptides of the aminoethylated myoglobin is shown in fig.2. The shaded peptides are the outcome of the modification of 110 cysteine to aminoethyl-cysteine. This introduces an additional

Table 1
Amino acid composition of siamang myoglobin

Amino acid	Average values from hydrolysates at 24 h, 48 h and 72 h	
Cysteic acid	1.00	(1) ^a
Aspartic acid	11.29	(11)
Threonine	4.22	(4)
Serine	7.66	(8)
Glutamic acid	20.62	(21)
Proline	5.19	(5)
Glycine	14.18	(14)
Alanine	11.62	(12)
Valine	7.22	(7)
Methionine	3.14	(3)
Isoleucine	7.69	(8)
Leucine	16.88	(17)
Tyrosine	1.94	(2)
Phenylalanine	6.61	(7)
Histidine	8.85	(9)
Lysine	19.88	(20)
Tryptophan	1.92	(2) ^b
Arginine	2.42	(2)
Yield in μmol/one residue	3.73	

^aOxidation of the insoluble tryptic peptides left after tryptic digestion in performic acid

^bThe value for tryptophan was obtained by amino acid analysis after hydrolysis with mercaptoethane sulfonic acid for 24 h

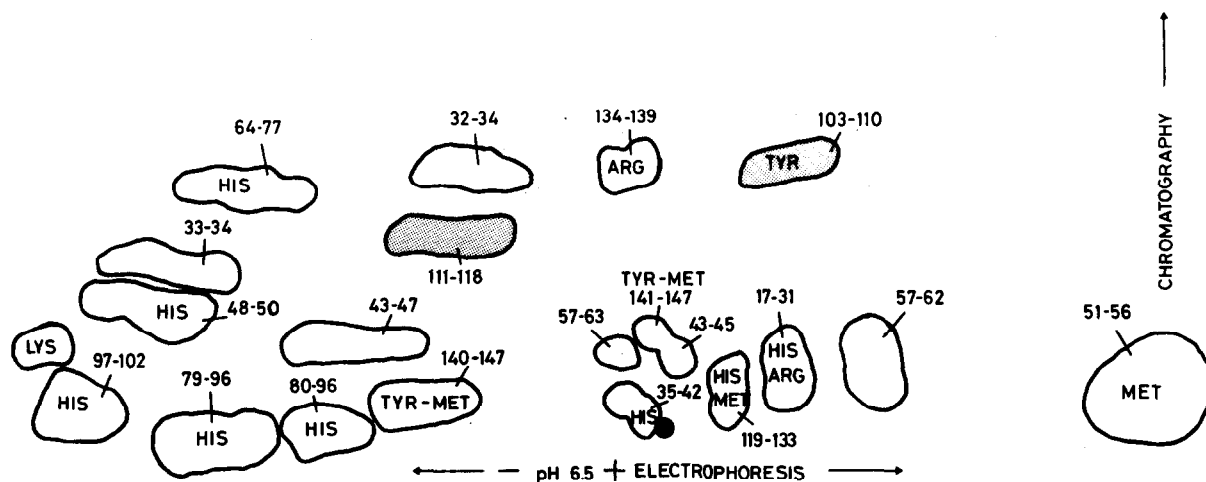


Fig.2. Siamang myoglobin. Fingerprint of the soluble tryptic peptides from aminoethylated globin showing the two new peptides (shaded area) which appear after aminoethylation. The specific staining reactions are indicated. (●) is the point of application.

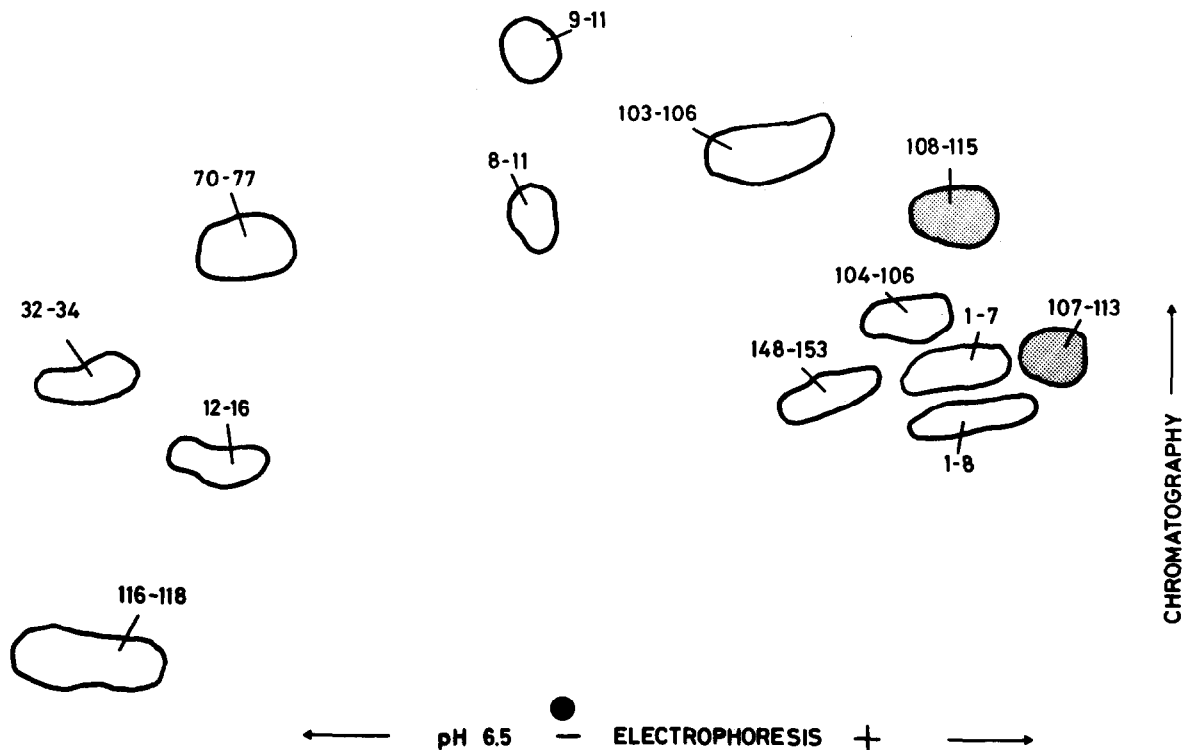


Fig.3. Siamang myoglobin. Fingerprint of the pepsin digest of the insoluble tryptic peptides showing the two new peptides (shaded area) which appear after performic acid oxidation. (●) is the point of application.

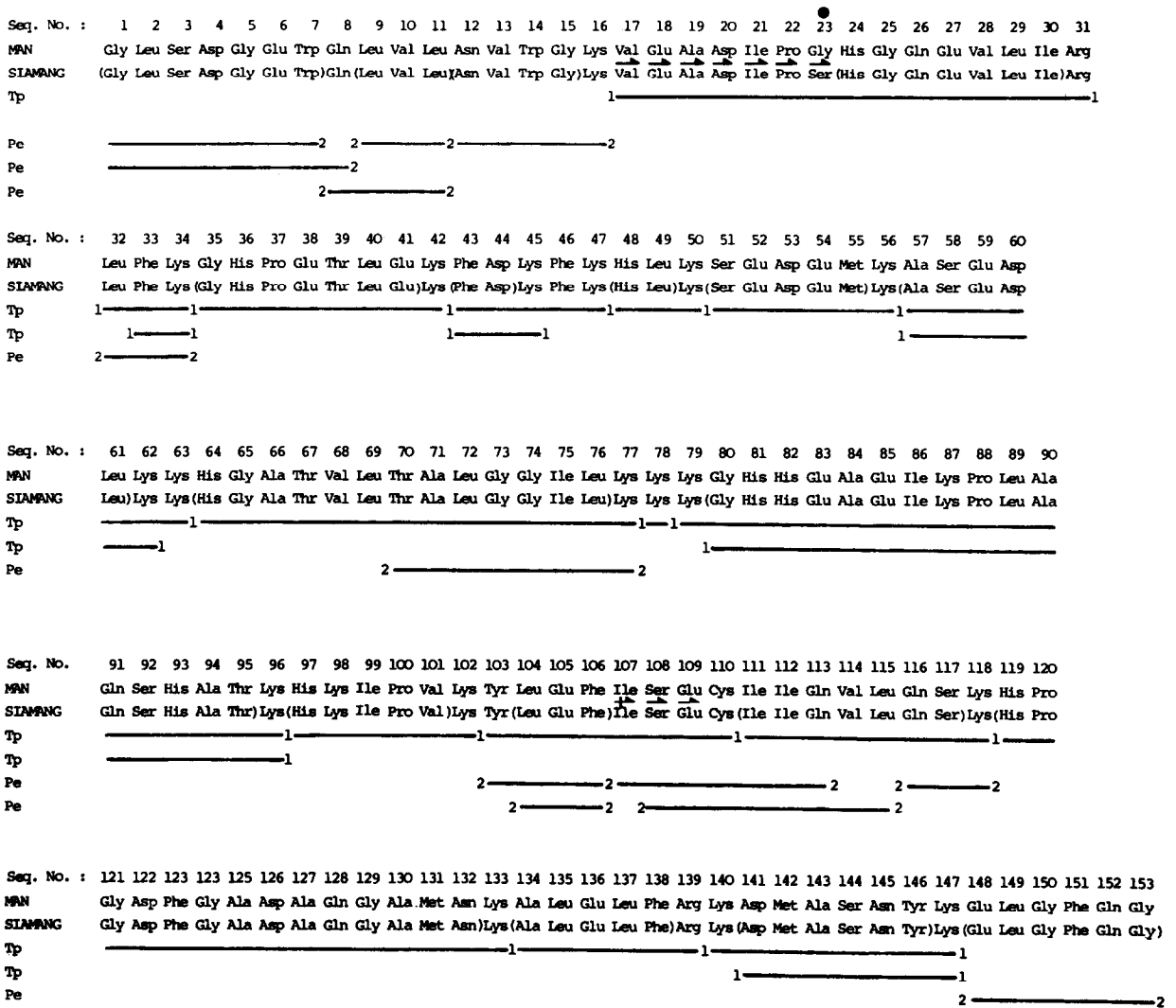


Fig.4. Myoglobin of siamang compared with that of man. (1-1) tryptic peptides; (2-2) peptic peptides; (—>) dansyl-Edman degradation. Peptides which are aligned by homology are in brackets.

point of tryptic hydrolysis and permits formation of the two new soluble tryptic peptides (103-110 and 111-118). Figure 3 shows the fingerprint pattern of the insoluble tryptic peptides digested with pepsin after performic acid oxidation. The two new extra peptides (108-115 and 107-113) shown are shaded and both were found to contain cysteic acid. Thus, two approaches have shown position 110 to be occupied by a residue of cysteine.

The amide and acidic groups were determined by electrophoretic mobility of the relevant peptides.

The amino acid sequence of siamang myoglobin was derived from aligning the amino acids of overlapping tryptic and peptic peptides by homology with the known sequence of human myoglobin and by establishing the nature of certain residues by dansyl-Edman degradation (fig.4).

4. Discussion

It will be seen (fig.4) that the siamang myoglobin sequence is identical to that of the gibbon. Thus, the

Table 2

	MAN	CHIMPANZEE	GORILLA	ORANGUTAN	SIAMANG	GIBBON	BABOON	MACAQUE	WOOLLY MONKEY	SQUIRREL MONKEY	MARMOSET	GALAGO	SLOW LORIS	POTTO	SPORTIVE LEMUR	TREESHREW
MAN	1	1	2	1	1	5	7	16	17	14	23	19	17	22	13	MAN
0.65		2	3	2	2	7	8	17	18	15	24	20	17	21	14	CHIMPANZEE
0.65	1.31		3	2	2	7	8	17	18	15	23	19	16	22	13	GORILLA
1.31	1.96	1.96		1	1	4	5	15	16	13	24	20	17	23	14	ORANGUTAN
0.65	1.31	1.31	0.65		0	5	6	15	16	13	24	20	17	23	14	SIAMANG
0.65	1.31	1.31	0.65	0.00		5	6	15	16	13	24	20	17	23	14	GIBBON
3.28	4.58	4.58	2.61	3.27	3.27		1	13	12	11	22	18	15	21	12	BABOON
4.58	5.23	5.23	3.27	3.92	3.92	0.65		12	12	10	21	17	14	21	17	MACAQUE
10.46	11.11	11.11	9.80	9.80	9.80	8.50	7.84		4	4	21	19	20	19	17	WOOLLY MONKEY
11.11	11.76	11.76	10.46	10.46	10.46	7.84	7.84	2.61		4	23	21	22	17	17	SQUIRREL MONKEY
9.15	9.80	9.80	8.50	8.50	8.50	7.19	6.53	2.61	2.61		23	29	18	17	17	MARMOSET
15.03	15.69	15.03	15.69	15.69	15.69	14.38	13.73	13.73	15.03	15.03		11	12	21	16	GALAGO
12.42	13.07	12.42	13.07	13.07	13.07	11.77	11.11	12.42	13.73	18.95	7.19		3	17	12	SLOW LORIS
11.11	11.11	10.46	11.11	11.11	11.11	9.80	9.15	13.07	14.38	11.76	7.84	1.96		18	13	POTTO
14.38	13.73	14.38	15.03	15.03	15.03	13.73	13.73	12.42	11.11	11.11	13.73	11.11	11.76		14	SPORTIVE LEMUR
8.50	9.15	8.50	9.15	9.15	9.15	7.84	11.11	11.11	11.11	11.11	10.46	7.84	8.50	9.15		TREESHREW

Matrix showing the number of amino acid differences among the myoglobins of the sixteen primates, for which the myoglobin sequences are known. The lower matrix provides the same information in terms of percentage.

4 residues common to all hominoids (except orangutan, as previously mentioned) were found in the siamang sequence. A matrix of the myoglobin amino acid differences, for all the primates for which the myoglobin sequence is known, clearly shows that the siamang and the gibbon myoglobin is very similar to that of the other hominoids (upper matrix, table 2).

This result is consistent with the hypothesis that gibbon and siamang have shared a common ancestor more recently than either has with any other primate and that both are closely related to the other hominoids. The myoglobin sequence for a colobine is as yet unknown, therefore the hypothesis that siamang and gibbon may be part of this evolutionary group

cannot be refuted. However, since the difference between myoglobins of the gibbon and siamang and that of man is only 1 amino acid it is doubtful that they could be more similar (i.e. identical) to a colobine. The myoglobin sequence of a colobine would either provide evidence against this hypothesis or would shed little light on the question.

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References

- [1] Straus, W. L. (1949) *Quart. Rev. Biol.* 24, 200–223.
- [2] Simpson, G. G. (1945) *Bull. Amer. Mus. Nat. Hist.* 85, 1–350.
- [3] Napier, J. R. and Napier, P. H. (1967) in: *A Handbook of Living Primates* pp. 409–410, Academic Press, London, New York.
- [4] Chiarelli, A. B. (1968) in: *Taxonomy and Phylogeny of The Old World Primates* (Chiarelli, A. B. ed) pp. 151–186.
- [5] Chiarelli, A. B. (1973) in: *Evolution of the Primates*, pp. 181–186, Academic Press, London, New York.
- [6] Goodman, M. and Moore, G. W. (1971) *Syst. Zool.* 20, 19–62.
- [7] Sarich, V. M. and Wilson, A. C. (1966) *Science* 154, 1563–1566.
- [8] Sarich, V. M. (1968) in: *Perspectives on human evolution* (Washburn, S. L. and Jay, P. C. eds) pp. 97–121, Holt, Rhinehart and Winston, New York.
- [9] Dayhoff, M. O. (1972) *Atlas of Protein Sequence and Structure*, 5, The National Biomedical Research Foundation, Silver Spring, Maryland.
- [10] Bruce, E. J. and Ayala, F. J., unpublished results.
- [11] Romero-Herrera, A. E., Lehmann, H., Joysey, K. A. and Friday, A. E. (1973) *Nature* 246, 389–395.
- [12] Romero-Herrera, A. E. and Lehmann, H. (1974) *Biochim. Biophys. Acta* 359, 236–241.
- [13] Romero-Herrera, A. E., Lehmann, H. and Fossey, D. (1975) *Biochim. Biophys. Acta* 393, 383–388.
- [14] Romero-Herrera, A. E., Lehmann, H., Castillo, O., Joysey, K. A. and Friday, A. E. (1976) *Nature* 261, 162–164.
- [15] Romero-Herrera, A. E., Lehmann, H. and Castillo, O. (1976) *Biochim. Biophys. Acta* 420, 387–396.
- [16] Schultz, A. H. (1973) in: *Gibbon and Siamang* (Rumbaugh, D. M. ed) 2, 1–55 Karger, Basel.
- [17] Romero-Herrera, A. E. and Lehmann, H. (1971) *Biochim. Biophys. Acta* 251, 482–488.
- [18] Romero-Herrera, A. E. and Lehmann, H. (1972) *Biochim. Biophys. Acta* 278, 465–481.
- [19] Hirs, C. H. W. (1956) *J. Biol. Chem.* 219, 611–621.
- [20] Ingram, V. M. (1958) *Biochim. Biophys. Acta* 28, 539–545.
- [21] Romero-Herrera, A. E. and Lehmann, H. (1974) *Proc. R. Soc. Lond. B.* 186, 249–279.
- [22] Penke, B., Ferenier, R. and Han, K. (1976) *Biochim. Biophys. Acta* 420, 376–386.