The myoglobin of primates: the Night Monkey, *Aotes trivirgatus* (Cebidae, Platyrrhini, Anthropoidea)

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The amino acid sequence of the myoglobin of the South American Night Monkey, *Aotes trivirgatus*, is identical to that of the marmoset (*Callithrix jacchus* [1]) except for residue 21 which is isoleucine in the marmoset, like in all other anthropoids, but valine in *Aotes*. Analysis of a possible pathway of the evolution of *Aotes* myoglobin using 18 known primate myoglobin sequences [2–5] supports the classification of the Night Monkey within Anthropoidea and Platyrrhini but it indicates that this species might be more closely related to the marmoset (family Callitrichidae) than to the family Cebidae as a member of which it is commonly classified.

Aotes trivirgatus *Myoglobin* *Sequential analysis* *Amino acid analysis* *High-performance liquid chromatography of peptides* *Molecular evolution*

1. INTRODUCTION

The small, purely arboreal South American Night Monkey, *Aotes trivirgatus*, is the only nocturnal species in the primate suborder Anthropoidea. Two thirds of the Prosimii, the other primate suborder, are exclusively nocturnal. This resemblance and other similarities of physical and behavioural characteristics of *Aotes* and Prosimii have mostly been considered as convergent adaptations without phylogenetic significance [6]. However, it has been suggested on dermatological grounds that *Aotes* might be a New World prosimian [7]. Previous biomolecular evidence for the phylogeny of the Night Monkey based on immunological comparisons of serum proteins [8,9] and on β-type haemoglobin chain sequences [10] has not been conclusive. We determined the amino acid sequence of the myoglobin of the Night Monkey in order to reconstruct a possible pathway of the evolution of this protein using 18 known primate myoglobin sequences aiming to obtain some insights into the phylogenetic relationships of this primate.

Abbreviations: SDS, sodium dodecyl sulphate; RP–HPLC, reverse phase high-performance liquid chromatography; DABS-Cl, 4-*N,N*-dimethylaminoazobenzene-4′-sulphonyl chloride; DABITC, 4-*N,N*-dimethylaminoazobenzene-4′-isothiocyanate; PITC, phenylisothiocyanate

2. MATERIALS AND METHODS

2.1. *Preparation of apomyoglobin*

Myoglobin was extracted from 80 g of *A. trivirgatus* hind limb muscle with 280 ml of 2 mM NaCn/0.6 mM $K_3Fe(CN)_6$, and the extract was centrifuged. The supernatant was adjusted to 55% ammonium sulphate saturation and the precipitate removed by filtration. The filtrate was concentrated to 10 ml by pressure ultrafiltration (Amicon PM 10 membrane). One hundred ml of 10 mM

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phosphate buffer containing 2 mM NaCN and 0.3% 2-mercaptoethanol (pH 6.35) were added and the volume again reduced to 10 ml by ultrafiltration. This step was repeated 4 times. The sample was applied to a carboxymethyl ion-exchange column (Whatman CM 23) equilibrated with starting buffer and eluted stepwise with 10, 35 and 100 mM phosphate buffer containing 2 mM NaCN and 0.3% 2-mercaptoethanol (pH 6.35). Myoglobin was further purified by gel filtration chromatography (Sephadex G-100, eluted with 50 mM Tris–HCl containing 2 mM NaCN, pH

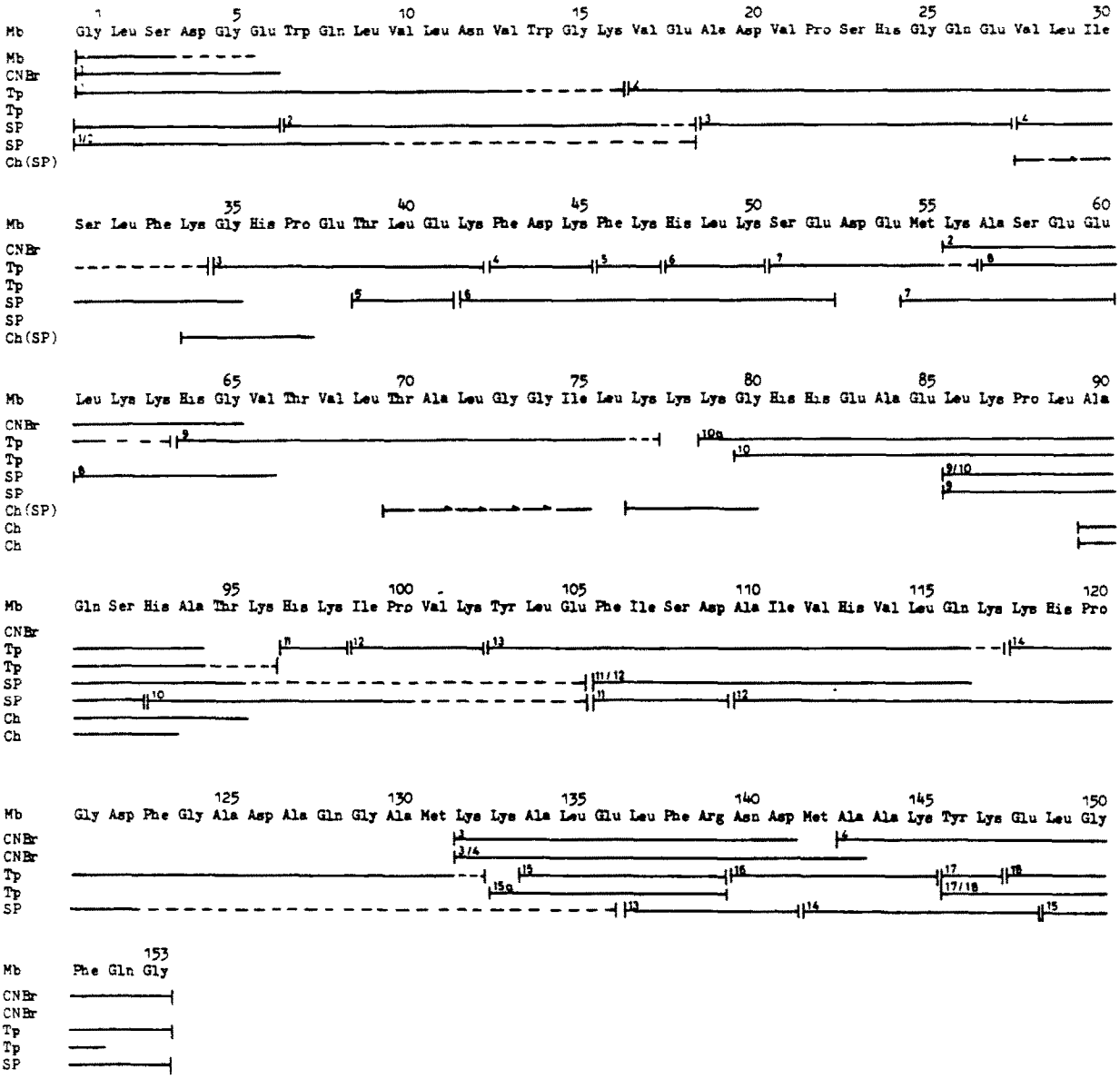

Fig.1. The complete amino acid sequence of *Aotes trivirgatus* apomyoglobin. Alignment of peptide sequences (CNBr, cyanogen bromide peptide; Tp, tryptic peptide; SP, *Staphylococcus aureus* protease peptide; Ch, chymotryptic peptide). (——) Amino acid sequences determined by the DABITC/PITC double coupling method. (– – –) Amino acid residues deduced from the amino acid composition analysis. (— → —) Edman degradation; arrowhead indicates that the respective residue was not identified.

8.5). After desalting (Sephadex G-25), removal of the haem group using 1.5% HCl in acetone (−20°C) and lyophilization, 100 mg of a homogenous apomyoglobin preparation (as shown by SDS–polyacrylamide electrophoresis and N-terminal sequential analysis) were obtained.

2.2. *Sequential analysis*

Ten-mg portions of apomyoglobin were cleaved by treatment with CNBr and by digestion with trypsin, chymotrypsin, and *Staphylococcus aureus* strain V8 protease [11], respectively. Peptides obtained from 50 to 100 nmol apomyoglobin were separated by RP–HPLC (Varian 5060 Liquid Chromatograph) on analytical C_{18} columns (Varian MicroPak MCH-5 and MCH-10, 30 × 0.4 cm) using linear gradients of 0.1% (v/v) aqueous trifluoroacetic acid in acetonitrile [12]. Four pairs of tryptic peptides coeluting in this solvent system were resolved using linear gradients of 10 mM ammonium acetate buffer, pH 6.05 or 6.55, in acetonitrile. The amino acid composition of peptide hydrolysates was determined by pre-column derivatization of amino acids with DABS-Cl followed by separation of the derivatives by RP–HPLC using a gradient of 50 mM sodium acetate buffer (pH 4.15) in acetonitrile [13]. The amino acid composition of apomyoglobin was determined on a conventional amino acid analyser. Amino acid sequences of peptides were determined by the manual DABITC/PITC double coupling method [14] using reduced reagent volumes and reaction times [15]. Leucine and isoleucine residues were identified from the amino acid composition analyses of peptides or by dansyl-Edman degradation of suitable peptides. The complete sequence was obtained by alignment of overlapping peptide sequences.

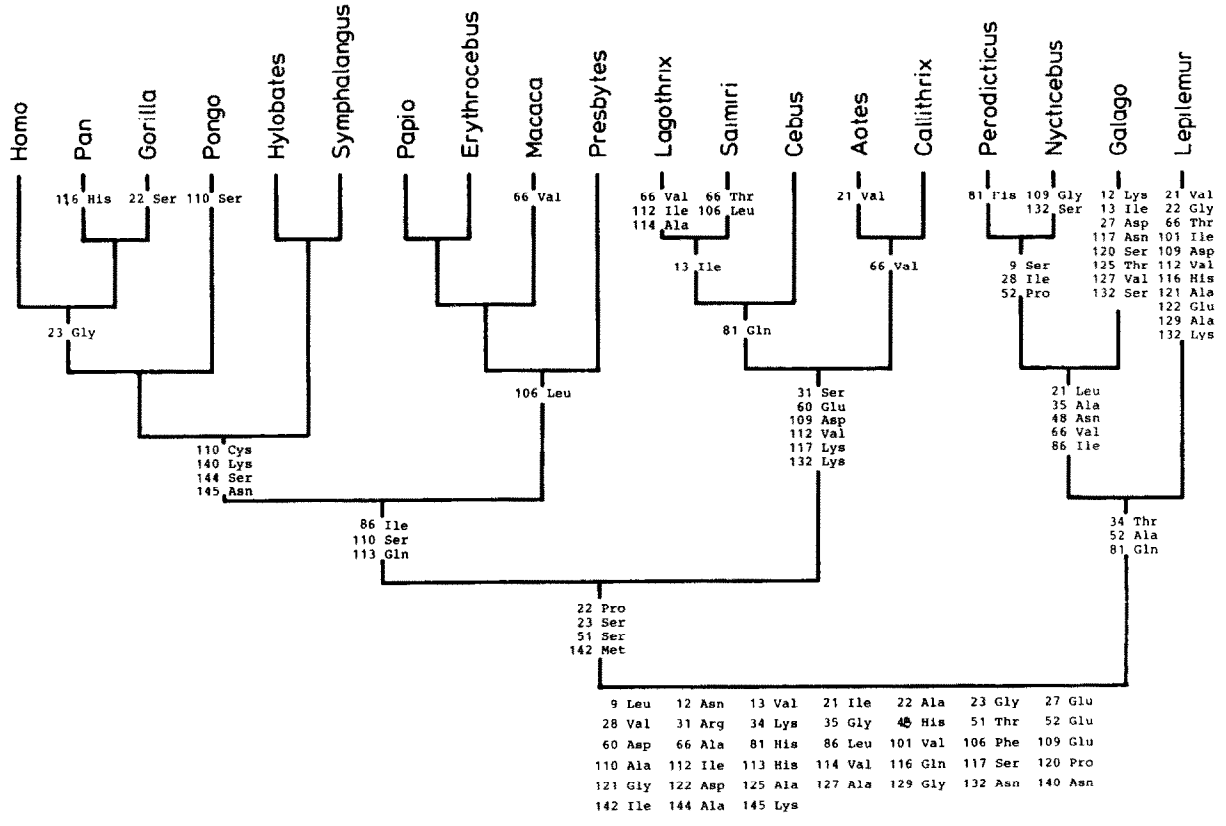

Fig.2. Reconstruction of primate myoglobin evolution [2,5]. The placement of *Aotes* represents the most parsimonious solution.

3. RESULTS AND DISCUSSION

Fig.1 shows the amino acid sequence of *A. trivirgatus* apomyoglobin. Of all known primate myoglobins, *Callithrix jacchus* (marmoset) myoglobin is most similar to *Aotes* myoglobin. Their sequences differ only in position 21 where *Callithrix*, like all other Anthropoidea, has isoleucine whereas valine was found in *Aotes*. *Aotes* myoglobin differs in 3–5 positions from the known myoglobins of Cebidae (*Lagothrix, Saimiri, Cebus*), in 11–16 positions from those of Catarrhini and in 16–23 positions from prosimian myoglobins. Considering possible pathways of the evolution of *Aotes* myoglobin (fig.2), phylogenetic affinity of *Aotes* and the Prosimii appears to be very unlikely because *Aotes* myoglobin shares all 10 residues indicative of common ancestry of Anthropoidea and Platyrrhini. Furthermore, there is no indication of a diphyletic origin of the Platyrrhini [16]. To this extent the conventional systematic classification of the Night Monkey is supported. However, in the most parsimonious tree of the Platyrrhini, *Aotes* is more closely related to the callitrichid *Callithrix* than to the three Cebidae. Shared common ancestry of these cebids is supported by glutamine in position 81. Night Monkey and marmoset have retained the ancestral histidine in this position and share valine in position 66.

Owing to the hypervariability of this position in vertebrate myoglobins, the interpretation of this common residue as a shared derived character could be contested. It cannot be ruled out that the replacement 66 alanine to valine has arisen independently in the lineages leading to *Aotes* and to *Callithrix*. Furthermore, this replacement could have been fixed in the platyrrhine common stem, with a back mutation to alanine in Cebus (which would require two nucleotide replacements). However, an association of *Aotes* and *Callithrix*, as indicated by the most parsimonious reconstruction of mutational events, is in line with Gregory's hypothesis that the Callitrichidae, rather than being the most primitive Anthropoidea, are derived from a primitive cebid resembling *Aotes* [17]. Thus the branching arrangement of the Platyrrhini shown in fig.2 seems to indicate a plausible scheme of New World Monkey relationships.

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DEPOSITED MATERIALS

The following materials have been deposited in the data bank:

(i) amino acid composition analysis of *Aotes trivirgatus* apomyoglobin;
(ii) separation and sequential analyses of CNBr peptides;
(iii) separation, amino acid composition and sequential analyses of tryptic and *Staphylococcus aureus* protease peptides; and
(iv) separation and sequential analyses of chymotryptic peptides.

Details may be obtained from the Publisher, free of charge, quoting:
Data Bank number: FEBS 1071, FLDB/2, 165 (1984) 46–50.

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