# The myoglobin of rodents *Proechimys guairae* (casiragua) and *Mus musculus* (house mouse)

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Received 8 July 1985; revised version received 24 July 1985

The amino acid sequences of the myoglobins of two rodents, the casiragua and the house mouse, have been determined. The myoglobin of casiragua differs from that of viscacha (another hystricomorph) at 6 positions. Mouse myoglobin differs from that of mole-rat (another myomorph) at 17 positions, whereas casiragua and mouse differ at 22 positions. Mouse myoglobin possesses several features unique among all known myoglobins (Gly 31, Cys 66, Thr 74 and Glu 113) and one substitution unique among known mammalian myoglobins (Glu 53).

*Amino acid sequence* *Myoglobin* *Rodent* *Casiragua* *Mouse* *Phylogeny*

## 1. INTRODUCTION

Out of about 1600 species of rodents only two rodent myoglobin sequences have been published, those of viscacha (a hystricomorph) and mole-rat (a myomorph) [1]. The amino acid sequences of the myoglobins of casiragua (*Proechimys guairae*) and of house mouse (*Mus musculus*) are presented here. Casiragua is a member of the Echimyidae (spiny rats), a family among the hystricomorph rodents. Mouse is a member of the Muridae, a family among the myomorph rodents. Mouse myoglobin is of particular interest owing to its use in studies on the antigenic determinants of the myoglobin molecule [2,3].

## 2. MATERIALS AND METHODS

The casiragua was provided in 1976 by Drs I.W. Rowlands and Barbara J. Weir from their breeding colony in Cambridge. The mouse myoglobin was prepared from a mixed sample derived from about 65 laboratory animals of various strains, including some which had been bred by Dr M. Wallace from wild-caught South American stock.

The suppliers of enzymes, reagents and chromatography media were the same as those given in Gurnett et al. [1].

The rodent myoglobins were each prepared by ammonium sulphate precipitation of muscle extracts, gel filtration through a column of Sephadex G-100 and then ion exchange chromatography on a column of DEAE-cellulose (Whatman DE52) equilibrated with 10 mM Tris-HCl/2 mM KCN (pH 8.6). The proteins were eluted with a linear gradient to 40 mM NaCl in the starting buffer. After this step the haem was removed from the casiragua myoglobin by acid/acetone precipitation. Before the removal of the haem group from mouse myoglobin it was further purified by ion exchange chromatography on DEAE-cellulose in 10 mM imidazole-HCl/2 mM KCN (pH 6.9).

The enzymatic digestion of the myoglobins and their fragments, the separation of the resulting peptides by HPLC and the determination of their

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*Published by Elsevier Science Publishers B.V. (Biomedical Division)*

sequences were performed as reported previously [1,4].

The complete sequences were established by alignment of overlapping peptides and homology with other myoglobin sequences.

## 3. RESULTS AND DISCUSSION

The myoglobin content of casiragua muscle was determined to be 0.03% wet wt. A total of 16 mg apomyoglobin were prepared from 78 g muscle.

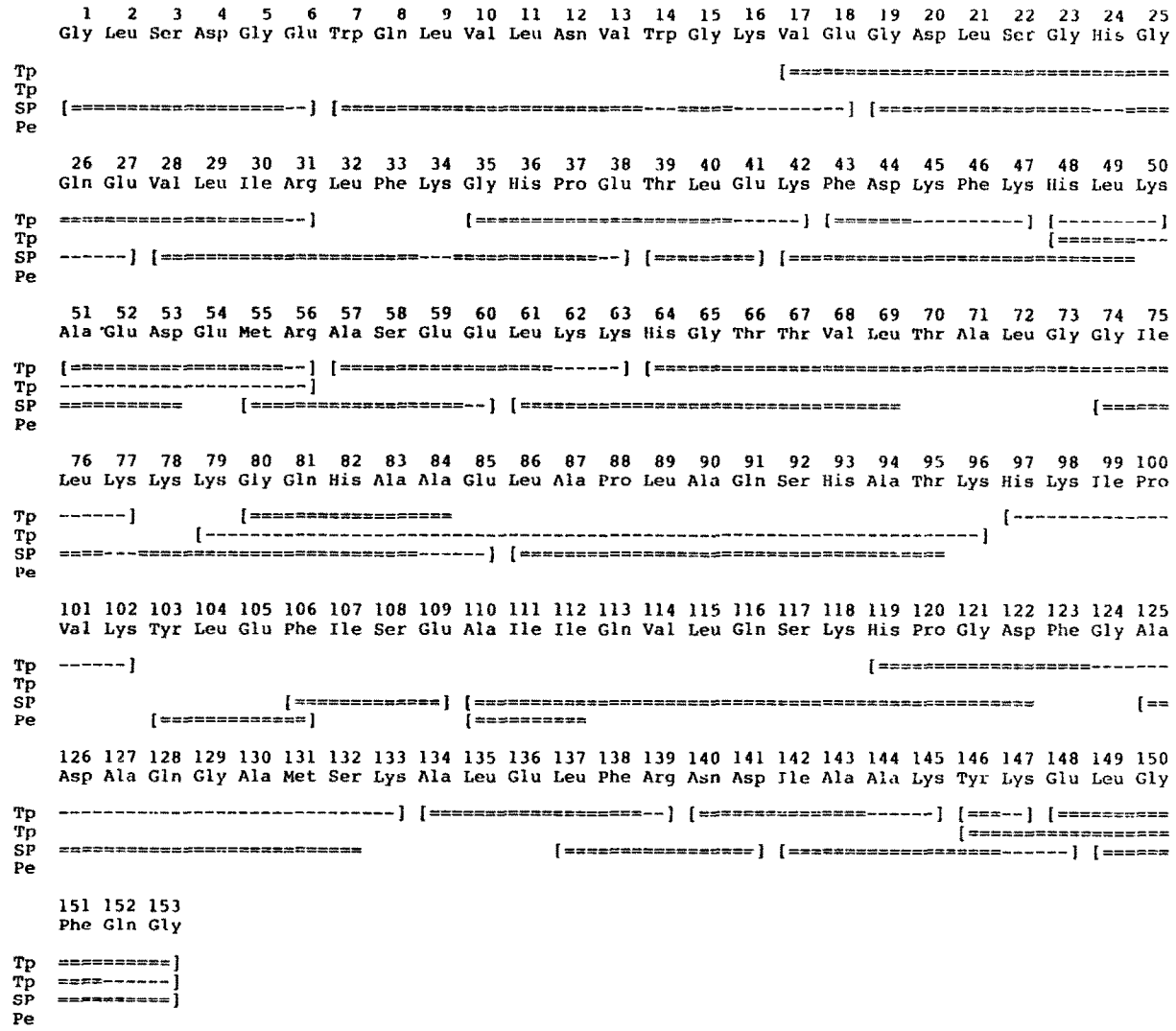


Fig.1. Amino acid sequence of casiragua myoglobin. The peptides isolated from a tryptic digest (Tp), an *S. aureus* V8 protease (SP) digest and from a peptic digest of the tryptic core (Pe) are shown. (===) Amino acid sequences determined by the DABITC/PITC double coupling method of Chang et al. [5]. (---) Amino acid sequences identified by amino acid analysis. The amino terminal sequences of two peptides obtained in the *S. aureus* V8 digest indicated that in addition to cleavage at Gly 73 and Gly 124, cleavage had also occurred to some extent at the carboxyl terminal sides of Gly 65 and Gly 80.

The ion exchange chromatography of mouse myoglobin on DEAE-cellulose at pH 6.9 removed a contaminating protein which co-eluted with myoglobin in the gel filtration step and also during ion exchange at pH 8.6. The myoglobin content of mouse muscle was 0.037% wet wt. 7 mg apomyoglobin were obtained from 70 g tissue.

The amino acid sequence of casiragua myoglobin is shown in fig.1, and that of mouse myoglobin in fig.2. The residues that differ in the known rodent myoglobins and the numbers of differences are shown in tables 1 and 2, respectively.

Casiragua has no unique features in its myoglobin, but the unusual substitutions Ser 22 and Glu 60 may prove useful in assessing its close relationships. It shares a number of residues with viscacha (another hystricomorph) which are not found in mole-rat or mouse and which may therefore prove useful in determining the relationships of hystricomorph rodents (Ala 51, Arg 56

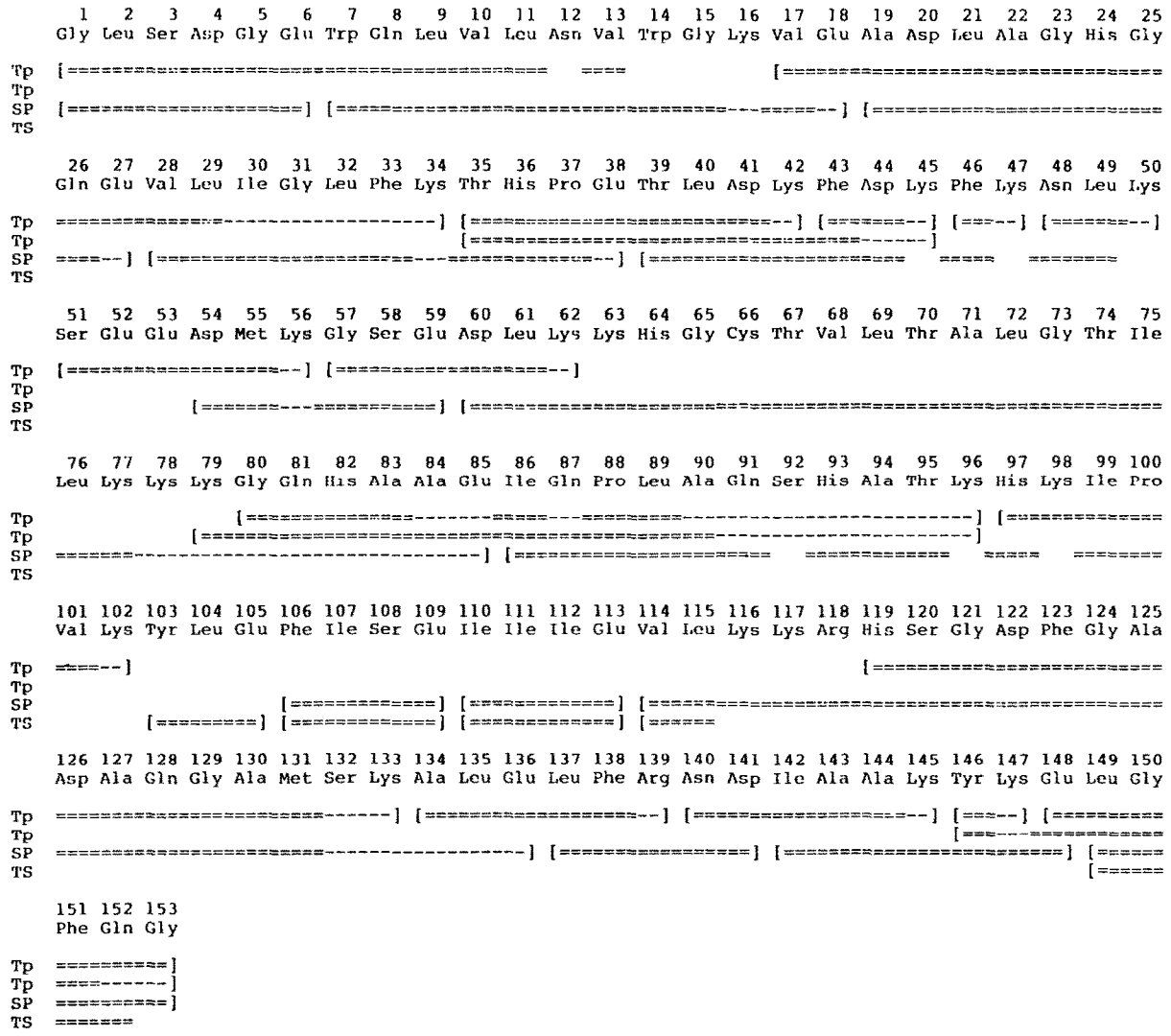


Fig.2. Amino acid sequence of mouse myoglobin. The peptides isolated from a tryptic digest (Tp), an *S. aureus* V8 protease digest (SP) and from a digest of the tryptic core with *S. aureus* V8 protease (TS) are shown. Other codes as in fig.1. Cys at position 66 was identified on the basis of the staining properties of samples of the peptide 60 to 85. In addition to those indicated, a peptide resulting from cleavage at Leu 89 and corresponding to residues 90 to 96 was isolated from the tryptic digest.

Table 1

Differences between the sequences of rodent myoglobins

| | 19 | 22 | 31 | 35 | 41 | 48 | 51 | 53 | 54 | 56 | 57 | 60 | 66 | 74 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Viscacha | Ala | Gly | Arg | Gly | Glu | His | Ala | Asp | Glu | Arg | Ala | Asp | Thr | Gly |
| Casiragua | Gly | Ser | Arg | Gly | Glu | His | Ala | Asp | Glu | Arg | Ala | Glu | Thr | Gly |
| Mole-rat | Gly | Ala | Lys | Asn | Glu | His | Ser | Asp | Glu | Lys | Gly | Asp | Asn | Gly |
| Mouse | Ala | Ala | Gly | Thr | Asp | Asn | Ser | Glu | Asp | Lys | Gly | Asp | Cys | Thr |

| | 77 | 79 | 86 | 87 | 101 | 110 | 113 | 116 | 117 | 118 | 120 | 127 | 129 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Viscacha | Arg Lys | Arg | Leu | Ala | Val | Ala | Gln | Gln | Ser | Lys | Pro | Ala | Ala |
| Casiragua | Lys | Lys | Leu | Ala | Val | Ala | Gln | Gln | Ser | Lys | Pro | Ala | Gly |
| Mole-rat | Lys | Lys | Ile | Gln | Ile | Ala | Gln | Gln | Ser | Lys | Pro | Thr Ala | Gly |
| Mouse | Lys | Lys | Ile | Gln | Val | Ile | Glu | Lys | Lys | Arg | Ser | Ala | Gly |

Two residues were found at position 77 in viscacha and at position 127 in mole-rat [1]

and Ala 87). We have previously drawn attention to the larger number of arginine residues in the myoglobins of diving mammals [6] and in the same context noted Arg 56 as a parallel change in harbour seal and penguin [7]. Following from this, we suggested that the high number of arginine residues found in viscacha may represent an adaptation to living underground [1]. Although casiragua is listed as a solitary, nocturnal, terrestrial animal [8], which does not dig its own burrow, it is known to take refuge by day under logs and the roots of trees, in rocky crevices and in holes dug by armadillo [9].

Gly 19 in casiragua is shared with mole-rat, but not with viscacha and mouse, both of which have Ala 19. Elsewhere among mammals Gly 19 is known only in platypus.

Gly 31, Cys 66, Thr 74 and Glu 113 are unique to mouse among all known myoglobins and Glu 53 is unique to mouse among all known mammalian myoglobins (it is also present in penguin). Comparison of the mouse and mole-rat myoglobin sequences with a putative ancestral sequence indicates that, since their evolutionary divergence, the mouse has incorporated 14 substitutions (5 of which are unique among mammals) and the mole-rat has incorporated 5 substitutions (only 1 of which is unique).

Three substitutions, Gly 57, Ile 86 and Gln 87, are shared by mouse and mole-rat (and not present in casiragua and viscacha).

Mouse myoglobin contains a number of unusual features in the carboxyl terminal half of the G-helix and in the GH region (Ile 110, Glu 113, the Lys-Lys-Arg sequence at 116–118 and Ser 120), none of which is present in other known rodent myoglobins. The structure of sperm whale myoglobin [10] indicates that residue 110 is close to a part of the B-helix where mouse myoglobin contains other uncommon residues (Gly 31 and Thr 35). Some substitutions in mouse myoglobin are of residues involved in side-chain interactions in the sperm whale structure; all of these changes are conservative and none is unique to the mouse pro-

Table 2

The numbers of amino acid differences between rodent myoglobins

| Mole-rat | Casiragua | Viscacha | |
|---|---|---|---|
| 17 | 22 | 23 | Mouse |
| | 12 | 15 | Mole-rat |
| | | 6 | Casiragua |

The values for the mole-rat myoglobin having Thr at position 127 and for the viscacha myoglobin having Arg at position 77 are shown (see table 1)

tein, but the combination of Glu 27 and Arg 118 (Asp 27 and Arg 118 form a salt bridge in sperm whale myoglobin) has been found in only one other species, goose-beaked whale [11].

## ACKNOWLEDGEMENTS

This work was funded by the Medical Research Council and the amino acid analyses were performed by M. Mather on an LKB analyzer provided by the SERC.

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