98. *Phylogeny of Primates as Inferred from α -Hemoglobin Sequences*

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Molecular record of amino acid sequence in protein and of nucleotide sequence in nucleic acid is becoming an important means to clarify phylogeny of extant species as well as morphological record of extant species and fossils that has been used up to this time. The phylogenetic trees from the primary structures of cytochrome C and hemoglobin are well-known examples made from molecular record in protein.^{1),2)} These phylogenetic trees are theoretically based on neutral theory³⁾ in the wide sense.

These trees have been made by statistical treatment of numbers of substituted amino acid residues in homologous proteins. This could be called a statistical method. On the other hand, a phylogenetic tree from molecular record can be made visually from inferring the primary structures of the ancestral proteins by detailed comparison of each amino acid residue in homologous proteins, without statistical treatment. If we temporarily call this a visual method, the method seems to be possible, only in the case that the primary structures of homologous proteins from enough numbers of biological species, related closely to each other, have been determined.

The possibility of interference by the investigator's subjectivity in the case of statistical method, seems to be less than that in the case of visual method. On the other hand, this statistical method assumes that the chances of substitutions for all amino acid residues in a protein are quite equal. But, in fact, it is often recognized that amino acid substitutions concentrate on particular amino acid residues. According to the phylogenetic tree made by the visual method, it is possible to infer the kinds and positions of substituted and back mutated amino acids in the course of molecular evolution.

The author planned to make a phylogenetic tree by the comparison of the primary structures of hemoglobins from animals belonging to primates and insectivora, which has the closest relation to primates. Since 1962, he and his associates have determined the primary structures of 54 kinds of polypeptide chains of hemoglobins: 30 kinds of α -polypeptide chains and 24 kinds of β -polypeptide chains from 21 kinds of primates (Orangutan,⁴⁾ Agile gibbon,⁵⁾ White-handed gibbon,⁵⁾ Hanuman langur,⁶⁾ Rhesus monkey,⁷⁾ Japanese monkey,⁸⁾ Bonnet monkey,⁵⁾ Toque monkey,⁵⁾ Assamese monkey,⁵⁾ Stump-tailed monkey,⁵⁾ Savannah monkey,⁹⁾ Patas monkey,¹⁰⁾ Silvery marmoset,¹¹⁾ Cotton-headed tamarin,¹¹⁾ Geoffroy's spider monkey,¹²⁾ Tufted capuchin monkey,¹³⁾ Grand galago,¹⁴⁾ Slender loris,¹⁵⁾ Slow loris,¹⁶⁾ Brown lemur¹⁷⁾ and Common tupaia¹⁸⁾) and two kinds of insectivora (European hedgehog¹⁹⁾ and Musk shrew²⁰⁾).

From the primary structures of extant hemoglobins of animals belonging to primates and insectivora which have been determined by the author and his associates, and those of three kinds of primates (Human,²¹⁾ Chimpanzee,²²⁾ and

Gorilla²²) that have been determined by another group, the author inferred step by step, ascendingly, the primary structures of hemoglobins of genus ancestor, subfamily ancestor, family ancestor, superfamily ancestor, infraorder ancestor, suborder ancestor and order ancestor of primates, and finally, primates-insectivora ancestor. The decision of kind of amino acid at a site in ancestral proteins was made according to the following principles: (1) An amino acid found most frequently in descendant proteins of the ancestor was chosen. (2)

Fig. 1. A phylogenetic tree as inferred from α -polypeptide chains of hemoglobins of animals belonging to primates and insectivora. ①-⑳ show junctions (ancestors) of the phylogenetic tree. The numbers between two junctions and those between a junction and an extant animal show the numbers of amino acid substitutions. Besides I, II and III show polymorphism of α -polypeptide chains of hemoglobins.

An amino acid found most frequently, including the one earlier ancestor's descendant proteins, was chosen, when the amino acids found most frequently are over two kinds in case of (1). On the strength of these results, two kinds of phylogenetic trees of α - and β -polypeptide chains of hemoglobin were made. Both these phylogenetic trees showed similar tendency. And also, these phylogenetic trees were almost similar to that of primates made from traditional palaeontologic studies. Fig. 1 shows a phylogenetic tree from α -polypeptide chains of hemoglobins of animal belonging to primates and insectivora which was made by the visual method.

Though there is controversy as to whether tupaiformes belongs to primates or insectivora, according to this phylogenetic tree, it is suggested that tupaiformes is a singular animal which evolved in a different direction from primates and insectivora lineages just after their junction. Besides, the following facts were suggested. For example, lemuriformes belonging to prosimiae seems to be an animal evolving in a singular direction which differs from other prosimiae. There are many crossings between various species belonging to macaca genus in catarrhini. It seems that orangutan branched off from human lineage earlier than gorilla and chimpanzee.

In this phylogenetic tree as inferred from α -polypeptide chains of hemoglobins, evolutionary rates of most primates seem to slow down compared with those of insectivora and common tupaia. It may be assumed that there are definite limitations, maintaining the distinctive tertiary structure of hemoglobin, imposed on the degree which the primary structure of each protein can vary. In general, any mutation that exceeds that extent of this structural limitation will be rejected by natural selection. The degree of the limitation will be denoted "the evolutionally structural free degree" of the protein.²³⁾ Each protein has its own free degree. It is presumed that the slow rates of molecular evolution of primates are the result of sudden decreasing of the free degree by the substitutions of particular amino acid residues rather than the results of functional adaptation of hemoglobin. At that time, the evolution has a specific direction (molecular orthogenesis). Statistical method and visual method have both advantages and disadvantages. In the future, better phylogenetic trees will be made in considerations of both these methods.

References

- 1) Fitch, W. M., and Margoliash, E.: Science, **155**, 279-284 (1967).
- 2) Goodman, M. *et al.*: Nature, **253**, 603-608 (1975).
- 3) Kimura, M.: The Neutral Theory of Molecular Evolution. Cambridge Univ. Press, Cambridge (1983).
- 4) Maita, T. *et al.*: Hoppe-Seyler's Z. Physiol. Chem., **359**, 129-132 (1978).
- 5) Maita, T. *et al.*: (in preparation).
- 6) Matsuda, G. *et al.*: Int. J. Peptide Protein Res., **5**, 423-425 (1973).
- 7) Matsuda, G. *et al.*: J. Biochem., **63**, 136-137 (1968).
- 8) Matsuda, G. *et al.*: Int. J. Protein Res., **3**, 53-55 (1971).
- 9) Matsuda, G. *et al.*: Hoppe-Seyler's Z. Physiol. Chem., **354**, 1153-1155 (1973).
- 10) Maita, T. *et al.*: J. Chem. Soc. Pak., **1**, 5-13 (1979).
- 11) Maita, T. *et al.*: J. Biochem., **95**, 805-813 (1984).
- 12) Matsuda, G. *et al.*: Hoppe-Seyler's Z. Physiol. Chem., **354**, 1517-1520 (1973).
- 13) Matsuda, G. *et al.*: *ibid.*, **354**, 1513-1516 (1973).
- 14) Watanabe, B. *et al.*: Biol. Chem. Hoppe-Seyler, **366**, 265-269 (1985).

- 15) Maita, T. *et al.*: J. Biochem., **84**, 377-383 (1978).
- 16) Matsuda, G. *et al.*: Int. J. Peptide Protein Res., **5**, 419-421 (1973).
- 17) Maita, T. *et al.*: J. Biochem., **85**, 755-764 (1979).
- 18) Maita, T. *et al.*: *ibid.*, **82**, 603-605 (1977).
- 19) Maita, T. *et al.*: *ibid.*, **85**, 259-269 (1979).
- 20) Maita, T. *et al.*: Hoppe-Seylers Z. Physiol. Chem., **362**, 1465-1474 (1981).
- 21) Braunitzer, G. *et al.*: *ibid.*, **325**, 283-286 (1961).
- 22) Goodman, M. *et al.*: Nature, **303**, 546-548 (1983).
- 23) Matsuda, G.: Evolution of Protein Molecules. Japan Scientific Societies Press, Tokyo, pp.175-185 (1978).