HEMOGLOBINS OF ADULT MACACA SPECIOSA: AN AMINO ACID INTERCHANGE (α 15(gly→asp))

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Several species of monkeys are known to have more than one hemoglobin type. The existence of two hemoglobins in all Macaca speciosa (stumptail macaques) examined has been reported. The two hemoglobins differ in their electrophoretic mobilities; Hb-Fast is more negatively charged at pH 8.6; Hb-Slow resembles human Hb-A in electrophoretic mobility. Hb-Fast and Hb-Slow of the Macaca speciosa have been shown to have alpha chains which differ in only one position, the tryptophan-containing peptide, α TPIII (1).

In the present paper, the amino acid composition of the tryptic peptides III from the two alpha chains of Macaca speciosa is reported. The glycine residue in α^{slow} (α^{s}) has been replaced by an aspartic acid residue in α^{fast} (α^{f}). This substitution would explain the difference in electrophoretic mobilities for the two hemoglobins.

MATERIALS AND METHODS

Blood was obtained from an adult female Macaca speciosa having a ratio of fast ($\alpha_2^f\beta_2$) to slow ($\alpha_2^s\beta_2$) hemoglobin of 60 to 40. The red blood cells were separated from the plasma, washed three times and lysed (1). Globin was prepared from the hemoglobin solution by precipitation with acid-acetone (2). The three polypeptide chains, α^s, α^f, and β, were separated by column chromatography on carboxymethyl cellulose at pH 6.8 according to the method of Clegg, et al. (3). Purity was established by electrophoresis at pH 8.0 in

6M urea-starch gel according to Chernoff and Pettit (2). Samples consisting of either 100-135 mg of the α^f or α^s chains were digested with trypsin and the soluble α^f and α^s tryptic peptides were further fractionated into subgroups by elution from a Sephadex G-15 column, 0.9 x 70 cm, with 0.2M NH_4HCO_3. The resulting fractions were characterized by one-dimensional high voltage electrophoresis at pH 4.8 in 1.25% pyridine, 1.25% acetic acid (4). Ehrlich reagent was used to locate the tryptophan peptides (5). The tryptic peptide III from the two alpha chains was isolated from the appropriate gel filtration subgroups by preparative, high voltage, one-dimensional electrophoresis on paper. This was done by redissolving the lyophilized tryptophan-containing fraction obtained from gel filtration of each alpha chain in 0.5 ml of 0.2M NH_4HCO_3, and applying this solution in a 25 cm streak along the origin line. Electrophoresis was performed at pH 4.8 for 1 hour at 2000 volts. Ehrlich reagent was used for location of the α^f and α^s TPIII peptides which were eluted from the paper with 0.2M NH_4HCO_3. The eluted peptides were lyophilized and acid hydrolyzed in 6N HCl for 20 hours at 105°C. Amino acid analysis was done on a Spinco model 120C amino acid analyser, according to the method of Spackman, Moore, and Stein (6).

RESULTS AND DISCUSSION

The amino acid composition of the tryptic peptides III obtained from the α^f and α^s are shown in Table 1. The glycine residue of α^s has been replaced by an aspartic acid residue in α^f. The substitution of aspartic acid for glycine would explain the difference in electrophoretic mobility between α^f and α^s and hence between Hb-Fast and Hb-Slow.

Since all of the *Macaca speciosa* which have been examined, including individuals resulting from breeding in captivity, have two hemoglobins, and the presence of only one hemoglobin in these monkeys has never been demonstrated (1), it is most probable that the two alpha chains are not products of the same allele. Recent genetic evidence from controlled breedings is consistent with two structural loci for the alpha chains and a single locus

for the beta chain (8). A possible explanation for the two alpha chains could be duplication of the single alpha cistron, followed by a mutation in one of the resulting alpha cistrons. A single nucleotide base change would yield the substitution of aspartic acid for glycine. Duplication of the alpha cistron has previously been proposed to explain the appearance of two alpha chains in certain Macaca irus (9).

The presence of several hemoglobins within an individual monkey or among monkeys of the same species has been reported in other primate species (9, 10, 11, 12, 13, 14, 15). The difference between the several hemoglobins within a monkey or monkey species, is not limited to one of the polypeptide chain types. The hemoglobins may have either different alpha or beta chains, with single or multiple peptide changes. The hemoglobin heterogeneity within monkey erythrocytes may be due to the occurrence of either multi-alleles, resulting perhaps from duplication, or mutation of alleles. Both types of mutation have been reported as the genetic basis of monkey hemoglobin heterogeneity. Several examples of multi-hemoglobins in monkeys resemble the A/A_2 hemoglobin types of man. However, the three alleles, responsible for the production of the various polypeptide chains, may result from the duplication of either the alpha or the non-alpha cistron (9, 12). In other monkeys, the heterogeneity is genetically similar to the human polymorphic hemoglobins, such as A/S, A/C, etc. (10, 11, 13, 14, 15). In some Macaca irus, the heterogeneity of hemoglobins seems to result from both non-allelic and allelic mutations. Three hemoglobin components are present--two major ones, and a minor one. They all appear to share a common beta chain, but have different alpha chains. The alpha chains of Hb-Fast[1] and Hb-Slow[1] appear to be products of alleles, but the alpha chain of Hb-Minor Component[1] is not a product of the alleles for either of the other alpha chains. The alpha chains from Hb-Fast

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[1] In the author's nomenclature, Hb-Fast is Hb-P^{mi}; Hb-Slow is Hb-A^{mi}; and Hb-MC is $\alpha_2^{Xmi}\beta_2^{Ami}$ (10).

TABLE 1 - Experimental results[1] of acid hydrolysis of the tryptic peptides III from α^S and α^f of Macaca speciosa and literature values of the molar ratios obtained from the α TPIII of human hemoglobins (A and J_{OXF}) having similar amino acid compositions (7).

	Micromoles		Molar ratio-monkey		Molar ratio-human	
	α^f-TPIII	α^S-TPIII	α^f-TPIII	α^S-TPIII	$\alpha^{J_{OXF}}$TPIII	α^A-TPIII
Aspartic acid	.0909	<.01	.95	<.01	1.0	<0.1
Glycine	<.015	.0935	<.2	1.16	<0.1	0.9
Alanine	.1884	.1676	1.98	2.08	2.2	2.0
Lysine	.0954	.0804	1.00	1.00	0.9	1.1
Tryptophan	+	+	+	+	+	+

[1]The sum of the micromoles of amino acids reported is 85% of the total. None of the minor unreported amino acid components were present at a concentration greater than 0.015 micromoles/.45 micromoles.

and Hb-MC differ, in part, from the alpha chain of Hb-Slow in their third tryptic peptide (9, 10). In the peptide map of the tryptic peptides of α^f, there has been found a new tryptophan-containing peptide moving closer to the anode than the corresponding tryptophan-containing peptide from α^S (10). The change in mobility between the two peptides of Macaca irus bears the same relationship as that of the tryptophan-containing peptides of α^S and α^f of Macaca speciosa. However, Hb-Fast of Macaca speciosa does not appear to polymerize upon standing as does Hb-Fast of Macaca irus.

The behavior of the soluble alpha tryptic peptides of hemoglobin from a number of different monkey species has been compared to those of man by paper and column chromatographic techniques. For the majority of species examined, the αIII tryptic peptides have characteristics identical to α TPIII of Hb-A (16).

The amino acid composition of the α TPIII of the predominating hemoglobin component from several species is identical to the amino acid content of the homologous peptide from α^A of humans (17, 18).

Therefore, there seems to be an identical composition of α TPIII from man and many monkeys. The composition found for the TPIII from α^s of the Macaca speciosa is also identical to that from Hb-A. It is noteworthy that an abnormal human hemoglobin, $J_{\alpha\ OXFORD}$[1], has the same amino acid substitution in its α TPIII, as is found in $\alpha^{f\ 2}$ of the Macaca speciosa (7).

In heterozygotes having J_{OXFORD}, the ratio of abnormal (Fast) to normal (Slow) hemoglobin is 20 to 80 (7) whereas, in the individual monkey examined the reversed ratio is observed--60 to 40. However, there exist individuals, within the Macaca speciosa population examined, that have a ratio of Hb-Fast/Hb-Slow of 40 to 60 or 50 to 50 (1). The difference in Fast/Slow ratio between the former Macaca speciosa and human J_{OXFORD} suggests that the factor(s) responsible for the unequal amounts of the two hemoglobins in peripheral blood of man and this monkey are not the same. Preliminary work in this laboratory involving the in vitro synthesis of hemoglobins by both marrow and peripheral cells derived from Macaca speciosa suggests that preferential destruction of the slow hemoglobin is not responsible for unequal amounts of hemoglobins in peripheral blood of the monkey.

SUMMARY

The amino acid composition of the third tryptic peptides from α^f and α^s of Macaca speciosa has been determined. The glycine residue of α^s has been replaced by aspartic acid in α^f. The remaining residues of the two peptides are identical in α^s and α^f. The substitution observed explains the difference in the electrophoretic mobility of the two hemoglobins.

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[1] Hb $J_{\alpha\ OXFORD}$ may be represented by the short-hand notation of Hb J_{OXF}: α 15 (gly→asp).

[2] The notation of Hb-Fast of Macaca speciosa by analogy with human hemoglobins might be called Hb-Fast: α 15 (gly→asp).

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