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Fetal Hemoglobin of the Rhesus Monkey, *Macaca mulatta*: Complete Primary Structure of the γ Chain†

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ABSTRACT: The complete amino acid sequence of the γ chain from the major one of two fetal hemoglobins from the rhesus monkey, *Macaca mulatta*, was determined by automated, stepwise degradation of selected fragments produced by cleavage at methionyl and tryptophanyl residues and at the single aspartylprolyl bond. The minor fetal hemoglobin is similar to human Hb F_1 in relative electrophoretic and chromatographic properties and in the level at which it is found (about 12% of the total Hb F). On these grounds, we assume that this minor component contains, like Hb F_1, γ chains that differ from those of the major component by virtue of acetylation of their amino-terminal glycyl residues. Although the γ chains of most anthropoid primates examined to date are structurally heterogeneous and, hence, appear to be encoded by nonallelic genes, no sign of structural heterogeneity was detected at any position in the major γ chain from *M. mulatta*. Thus, if nonallelic γ-chain genes exist in this species, the chains encoded by them may be identical in sequence. The γ chain from *M. mulatta* is but the sixth primate γ chain whose primary structure has been fully characterized. The slight extent of structural divergence among these chains (the four chains from various species of Old World monkeys differ from one another by no more than two substitutions, while the human and cercopithecoid γ chains differ at no more than five sites) attests to the conservative nature of γ-chain evolution among the higher primates.

Structural heterogeneity of γ chains in the fetal hemoglobins from single animals has served as a clue to the presence of nonallelic γ-chain loci in several species of anthropoid primates. The existence of nonallelic genes for $^{G}\gamma$ (with Gly in position 136) and $^{A}\gamma$ (with Ala in position 136) chains in normal human subjects was first illustrated through structural analysis of mixtures of the chains in question (Schroeder et al., 1968, 1972) and later demonstrated directly by gene mapping (Little et al., 1979; Fritsch et al., 1979; Tuan et al., 1979). In addition, there is compelling evidence, derived from structural analyses of the γ chains from gorillas (*Gorilla gorilla*) and chimpanzees (*Pan troglodytes*), that African apes also harbor nonallelic $^{G}\gamma$ and $^{A}\gamma$ genes (Huisman et al., 1973; De Jong, 1971). Nonallelic γ-chain genes may also exist in orangutans (*Pongo pygmaeus*), the γ chains of which are structurally heterogeneous at positions 75 (Ile/Val) and 135 (Ala/Thr) (Huisman et al., 1973; Schroeder et al., 1978). Among Old World monkeys (Cercopithecoidea), the fully sequenced γ chains of the baboon, *Papio cynocephalus*, are heterogeneous at position 75, where both valyl and isoleucyl residues appear [W. A. Schroeder, unpublished data cited in DeSimone et al. (1979); Nute & Mahoney, 1979a], while products of different γ-chain genes have been detected electrophoretically in lysates of red cells from each of 109 fetal and neonatal pig-tailed macaques (*Macaca nemestrina*) examined to date (Nute & Stamatoyannopoulos, 1971a,b; Nute & Mahoney, 1979b).

Of the primate species in which structurally distinct γ chains have been detected in all members examined, only *M. nemestrina* has two chains that are separable by standard procedures; hence, the majority of structural analyses of primate γ chains has been carried out on mixtures of no less than two different components. While there is no conclusive evidence that the γ chains from nonhuman primates other than those enumerated above are structurally heterogeneous, it must be emphasized that very few detailed analyses of primate fetal hemoglobins have been conducted. Given the presence of nonallelic γ-chain genes in man, as well as the likelihood that such exist in the great apes, pig-tailed macaques, and savannah baboons, it is reasonable to suspect that the γ chains of other higher primates are encoded by genes at more than one locus. In our continuing efforts to determine the extent of structural

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variation among the γ chains of higher primates, and thereby facilitate reconstruction of the evolutionary history of primate fetal hemoglobins, we present the complete amino acid sequence of the γ chain from *Macaca mulatta*.

Experimental Procedures

Erythrocytes from 11 fetal *M. mulatta* (of approximately 145 days gestational age) were pooled, washed 3 times with normal saline, and lysed by mixing with 3 volumes of cold, deionized water. After centrifugation to remove cellular debris, an aliquot of the clear supernate was subjected to starch–gel electrophoresis in Tris–EDTA–borate buffer, pH 8.6 (Smithies, 1965). The resultant pattern contained a minor, anodal band of adult hemoglobin followed by a broad band of fetal hemoglobin and was similar in appearance to that produced by electrophoresis of hemoglobins from normal human fetuses.

Ion-Exchange Chromatography. The fetal hemoglobin was isolated from the dialyzed hemolysate in a 2.5 × 45 cm column of DEAE-Sephadex (A-50) employing a gradient formed by mixing 800 mL of 0.03 M Tris-HCl, pH 7.8, with an equal volume of 0.08 M Tris-HCl, pH 6.5. Both buffers were 0.001 M in KCN. After examination by starch–gel electrophoresis, the contents of the two Hb F-containing peaks (see Results) were pooled, concentrated by ultrafiltration, and dialyzed against deionized water. Following precipitation of globin in cold acid–acetone (Rossi-Fanelli et al., 1958), α and γ chains were separated by chromatography in a 2 × 12 cm column of carboxymethylcellulose (Whatman CM-23, Advanced Fibrous) using phosphate buffers 8 M in urea (Clegg et al., 1968). The contents of fractions constituting the major γ-chain peak were pooled, concentrated by ultrafiltration, desalted by gel filtration (Nute & Mahoney, 1979b), and lyophilized.

Reduction and S-Pyridylethylation. Purified γ chains (70 mg) were dissolved in 6 M guanidine hydrochloride (Pierce, "Sequanal" grade), 0.13 M in Tris and 0.3 mM in EDTA, pH 7.6, to a concentration of 10 mg/mL. Following addition of 10 mg of dithioerythritol, the solution was stored at room temperature for 18 h, at which point 20 μL of vacuum-redistilled 4-vinylpyridine was added. After 90 min, the reaction products were applied to a 2.5 × 45 cm column of Sephadex G-25 (fine) and desalted by using 9% formic acid as the eluant. The *S*-pyridylethyl γ chains were recovered by lyophilization.

Chemical Cleavages and Isolation of Products. *S*-Pyridylethyl γ chains were cleaved at the single aspartylpropyl linkage by a modification of the procedure of Jauregui-Adell & Marti (1975). The chains (14 mg) were dissolved in 2 mL of 70% formic acid, 7 M in guanidine hydrochloride and containing 2 mg of dithioerythritol, and incubated at 40 °C for 48 h. The reaction was stopped by adding 2 mL of cold, glass-distilled water, and the resultant solution was applied directly to a 2.5 × 90 cm column of Sephadex G-50 (superfine), which was then developed with 30% acetic acid.

Cleavage at the tryptophanyl residues in an additional 20 mg of pyridylethylated γ chains was accomplished with *o*-iodosobenzoic acid (Mahoney & Hermodson, 1979, 1980). In brief, 40 mg of *o*-iodosobenzoic acid (Pierce) was dissolved in 2 mL of 80% acetic acid, 4 M in guanidine hydrochloride. After addition of 0.1 mol of *p*-cresol per mol of *o*-iodosobenzoic acid, the solution was placed in the dark for 2 h. The γ chains were then added, and the solution was placed in the dark at room temperature for 24 h. Introduction of 5 mg of dithioerythritol terminated the reaction and, after dilution with 2 mL of water, the solution was applied to a 2.5 × 90 cm column of Sephadex G-50 (superfine), from which the γ-chain fragments were eluted with 9% formic acid. The largest fragment (containing residues 38–130) was recovered by lyophilization and dissolved in 2 mL of 10% acetic acid, 0.2 M in mercaptoacetic acid (Aldrich), pH 2.6. This solution was incubated at 37 °C for 24 h, thus effecting reduction of the methionine sulfoxide formed during cleavage. After being dried under a stream of purified nitrogen, the residue was dissolved in 10% acetic acid and lyophilized.

Cleavage of 20 mg of pyridylethylated γ chains at methionyl residues was accomplished by using cyanogen bromide as described previously (Nute & Mahoney, 1979b). Following lyophilization of the reaction products, the fragments were isolated by passage through a 2 × 195 cm column of Sephadex G-50 (superfine) using 9% formic acid as the eluant.

Designation of Fragments. Fragments produced by each method of cleavage are numbered consecutively, from those containing the amino-terminal sequence to those containing the carboxyl-terminal sequence of the intact γ chain. Thus, cleavage at methionyl residues yielded fragments CB-1 (residues 1–55), CB-2 (56–133), and CB-3 (134–146). Fragments Trp-1 (residues 1–15), Trp-2 (16–37), Trp-3 (38–130), and Trp-4 (131–146) were the products anticipated upon cleavage with *o*-iodosobenzoic acid. Finally, cleavage at the aspartylprolyl bond was expected to generate fragments AP-1 (residues 1–99) and AP-2 (100–146).

Amino Acid Analysis. Intact, *S*-pyridylethyl γ chains and selected fragments were hydrolyzed in glass-distilled, 6 N HCl in vacuo at 110 °C for 24 h (fragments) or for 24, 48, 72, 96, and 120 h (whole chains). All analyses were performed on a Durrum D-500 amino acid analyzer according to the manufacturer's instructions. The color value for *S*-(pyridylethyl)cysteine was 1.02 times that for leucine (Friedman et al., 1970).

Sequence Analysis. Solvents used in the sequencer (benzene, 1-chlorobutane, and heptane) were of "pesticide" grade (Fisher) and were not further purified. *N,N*-Dimethylbenzylamine (Pierce), containing 5 g of phthalic anhydride per 100 mL, was purified by vacuum distillation through a 30-cm Snyder column; only the constant-boiling fraction was recovered. "Sequanal" grade (Pierce) 1-propanol was redistilled through a 30-cm Vigreux column, and only that fraction yielding a negative Tollens' reaction (Edman & Begg, 1967) was saved for further use. *n*-Heptafluorobutyric acid (Pierce or 3M) was exhaustively oxidized by refluxing for 24 h with CrO_3 (15 g/kg of acid) and redistilled through a 30-cm Snyder column; only the constant-boiling fraction (120–121 °C) was collected. Phenyl isothiocyanate (Pierce) was prepared as described by Edman & Begg (1967).

The automated, PITC[1]-degradative procedure of Edman & Begg (1967), as modified by Hermodson et al. (1972), was employed, using a Beckman 890 C sequencer and the "peptide program" of Hermodson et al. (1977). Products generated by the sequencer were dried, at 60 °C, under a stream of purified nitrogen. Conversion to Pth amino acids was accomplished by adding 2 mL of 1 N HCl to the dried residue and heating at 80 °C for 10 min. Ethyl acetate-soluble Pth derivatives were identified by high-performance liquid chromatography using a 0.46 × 25 cm Zorbax ODS 5-μm column (Du Pont) and either a Varian Model 5000 (Nute & Mahoney, 1980) or a Bioanalytical Systems Model CS-1 liquid chromatograph (Mahoney & Nute, 1979).

Decomposition of Pth-histidine, Pth-*S*-(pyridylethyl)cysteine, and Pth-arginine, which remain in the aqueous phase after extraction with ethyl acetate, occurs with prolonged exposure

[1] Abbreviations used: PITC, phenyl isothiocyanate; Pth, phenylthiohydantoin; 2,3-DPG, 2,3-diphosphoglyceric acid.

to the acid in this phase. Even when analysis, by high-performance liquid chromatography, of derivatives in the aqueous phase can be accomplished immediately upon their conversion, Pth-histidine and Pth-arginine elute from C_{18} columns as broad peaks and, hence, are difficult to quantitate. Thus, yields of Pth amino acids from the aqueous phase were not estimated. Pth-arginine was identified by using phenanthrenequinone (Yamada & Itano, 1966). In brief, the residue remaining after drying of the aqueous phase under a stream of purified nitrogen was dissolved in 25 μL of methanol and a 10-μL aliquot of this solution was dried on a strip of Whatman No. 3 chromatographic paper. The staining solution was prepared by dissolving 5 mg of phenanthrenequinone in 25 mL of absolute ethanol; 2 mL of this solution was agitated on a vortex mixer while an equal volume of 10% (w/v) NaOH in 60% (v/v) ethanol was added. The paper strip was then dipped in the freshly prepared, alkaline solution of phenanthrenequinone and air-dried for 20 min. A bright, light-blue fluorescence, visible under long-wave ultraviolet light, indicated the presence of as little as 1 nmol of Pth-arginine. Pth-histidine was identified by the Pauly test as previously described (Nute & Mahoney, 1979b), while Pth-*S*-(pyridylethyl)cysteine was identified by thin-layer chromatography (Hermodson et al., 1972).

Since there was no proportionality between amounts of serine and threonine in the sample under analysis and the yields estimated by liquid chromatography, owing to partial decomposition of these residues during conversion to their Pth derivatives, no attempt was made to estimate yields of these Pth amino acids. Serine and threonine were identified primarily as their dithioerythritol adducts, that of Pth-dehydroserine eluting about 0.25 min before Pth-alanine and that of Pth-dehydrothreonine eluting approximately 2 min before Pth-valine from both of the chromatographic systems employed.

Identification of the Carboxyl-Terminal Residue. The first 12 residues of fragment CB-3 were identified as described above. Following cleavage of the phenylthiocarbamoyl derivative of the penultimate residue and before the carboxyl-terminal residue could be coupled with PITC, the 12th cycle was stopped. Material remaining in the cup of the sequencer was taken up in 50% acetic acid and identified, without prior hydrolysis, by amino acid analysis.

Quantitation of Pth Amino Acids by High-Performance Liquid Chromatography. Yields of all ethyl acetate-soluble Pth amino acids, save Pth-serine and Pth-threonine, were estimated by comparing the response of the chromatographic system to each derivative generated in the course of stepwise degradation with that to a known amount of the appropriate Pth amino acid standard (Pierce). From the yields of those Pth derivatives that can be estimated in this way, it was calculated that at least 70% of the expected amount (based on the size and quantity of the fragment degraded) of each derivative was recovered after each cycle. When the ratio of peak to background fell below 3, no further identifications were attempted. Stepwise yields ranged from 94 to 96% and, in all instances, a single sequence was observed.

Results

Ion-Exchange Chromatography. The elution profile produced by DEAE-Sephadex chromatography of pooled, red-cell lysates contained three peaks (Figure 1), the relative positions of which differed little from those of human Hb's A, F, and F_1 when isolated by similar procedures (Huisman & Dozy, 1965; Dozy et al., 1968). In addition, the components constituting peaks II and III comprised 88 and 12% of the total fetal hemoglobin, proportions comparable to those of Hb F

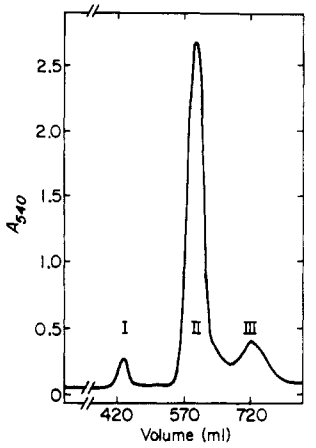

FIGURE 1: Elution profile produced by column chromatography, using DEAE-Sephadex, of the hemoglobins from fetal *M. mulatta*. Peak I, adult hemoglobin; peak II, major fetal hemoglobin; peak III, minor fetal hemoglobin (see text for details).

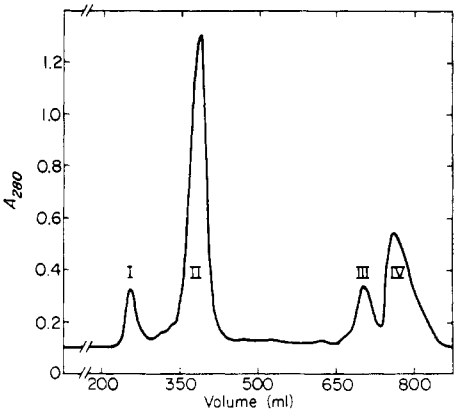

FIGURE 2: Elution profile produced by isolation of α and γ chains from the combined major and minor components of fetal hemoglobin (see Figure 1). Peak I, minor γ chain, suspected of being analogous to the *N*-acetyl γ chain of human Hb F_1; peak II, major γ chain, which was recovered and subjected to further analysis; peaks III and IV, α chains, which were identified as such by amino acid analysis.

and Hb F_1 in erythrocytes from normal human fetuses (Matsuda et al., 1960). Moreover, the relative electrophoretic mobilities of the three macaque components were essentially identical with those of human Hb's A, F, and F_1 when examined in starch gels (Dozy et al., 1968). Finally, isolation of γ chains after combining the two components of macaque fetal hemoglobin yielded two γ-chain peaks (Figure 2), the first and smallest of which contained approximately 10% of the total amount of γ chain eluted from the column. These two peaks stand in much the same relationship to one another as those produced by the γ chains from the normal human Hb's F and F_1 (Stegink et al., 1971). On the basis of the above observations, it was concluded that the minor component of fetal hemoglobin from *M. mulatta* is comparable to human Hb F_1, the γ chains of which differ from those of human Hb F by virtue of acetylation of their amino-terminal glycyl residues (Stegink et al., 1971). For this reason, the γ chain of the minor component was not subjected to further analysis.

Isolation and Identification of Fragments. Elution profiles produced by gel filtration of CB, Trp, and AP fragments appear in Figures 3, 4, and 5, respectively. Amino acid compositions of the complete, *S*-pyridylethyl γ chain and the fragments required for determination of the sequence are presented in Table I.

Sequence Analysis. The strategy employed in analyzing the primary structure of the γ chain is outlined in Figure 6. The

Table I: Amino Acid Compositions of the γ Chain and Selected Fragments[a]

	whole chain[b]	CB-1	CB-2	CB-3	Trp-3	AP-2
residues:	1-146	1-55	56-133	134-146	38-130	100-146
yield (%):		92	73	88	94	50
amino acid						
Asp	13.9 (14)	4.8 (5)	8.9 (9)	0.2 (0)	10.9 (11)	2.3 (2)
Thr	9.0[c] (9)	5.0 (5)	4.2 (4)		4.8 (5)	2.1 (2)
Ser	11.4[c] (11)	4.9 (5)	3.2 (3)	3.0 (3)	6.9 (7)	3.7 (4)
Glu	11.7 (12)	4.8 (5)	6.6 (7)		6.8 (7)	4.9 (5)
Pro	4.0 (4)	1.0 (1)	2.8 (3)		2.6 (3)	1.9 (2)
Gly	12.9 (13)	6.1 (6)	6.1 (6)	1.2 (1)	7.3 (7)	3.3 (3)
Ala	12.0 (12)	4.2 (4)	5.4 (5)	3.0 (3)	6.8 (7)	4.8 (5)
Val	12.7[d] (13)	3.8 (4)	7.3 (7)	1.9 (2)	7.0 (7)	5.7 (6)
Met	1.7 (2)	0.4[e] (1)	0.7[e] (1)		0.8 (1)	0.8 (1)
Ile	3.9[d] (4)	1.9 (2)	1.9 (2)		3.0 (3)	1.0 (1)
Leu	16.9[d] (17)	5.0 (5)	10.6 (11)	1.0 (1)	11.9 (12)	5.1 (5)
Tyr	1.9 (2)	1.0 (1)	0.3 (0)	0.9 (1)		1.1 (1)
Phe	7.8 (8)	4.1 (4)	4.3 (4)		7.1 (7)	3.0 (3)
Lys	11.1 (11)	2.1 (2)	8.6 (9)		7.9 (8)	1.8 (2)
His	5.9 (6)	1.0 (1)	3.6 (4)	0.9 (1)	4.0 (4)	2.1 (2)
Arg	3.7 (4)	2.1 (2)	1.2 (1)	1.0 (1)	1.8 (2)	2.0 (2)
Cys[f]	1.0 (1)		0.7 (1)		0.6 (1)	
Trp[g]	ND (3)	ND (2)	ND (1)	ND (0)	ND (1)	ND (1)

[a] Values in parentheses denote compositions determined from the sequence. [b] Averages over duplicate 24-, 48-, 72-, 96-, and 120-h hydrolyses, except where otherwise noted. [c] Extrapolated to zero time. [d] Averages of duplicate 120-h hydrolyses. [e] Detected as homoserine and homoserine lactone. [f] Detected as *S*-(pyridylethyl)cysteine. [g] ND, not determined.

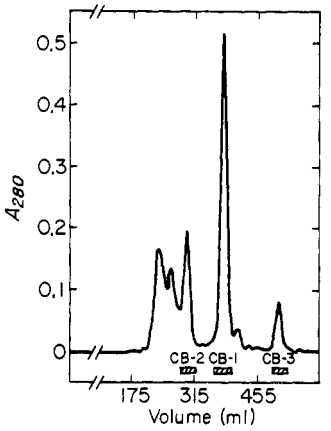

FIGURE 3: Gel filtration of fragments produced by cleavage of intact *S*-pyridylethyl γ chains at methionyl residues.

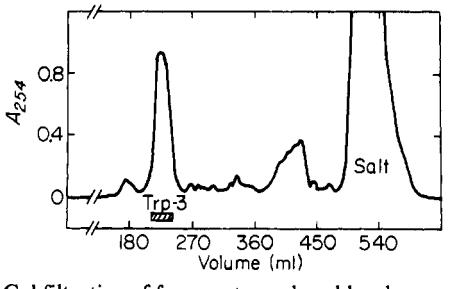

FIGURE 4: Gel filtration of fragments produced by cleavage of intact *S*-pyridylethyl γ chains at tryptophanyl residues. Of the four fragments expected, only the largest (Trp-3) was recovered.

residues occupying positions 1–43, 56–103, and 134–145 were identified by stepwise degradation of fragments CB-1, CB-2, and CB-3, respectively. Subsequent analyses of fragments Trp-3 and AP-2 established the sequences of residues 38–59 and 100–137. Finally, amino acid analysis of the material remaining in the cup of the sequencer following extraction of the anilinothiozolinone derivative of the penultimate residue (tyrosine) in fragment CB-3 yielded only histidine (30.7 nmol) and ammonia (51.0 nmol); the absence of discernible traces of any other amino acids indicated that histidine occupies the

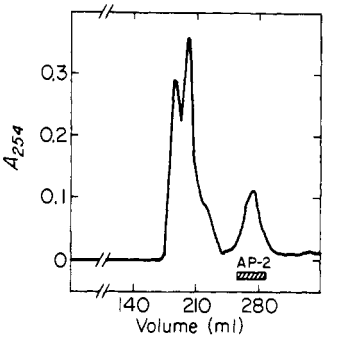

FIGURE 5: Gel filtration of fragments produced by cleavage of intact *S*-pyridylethyl γ chains at the single aspartylprolyl linkage. Of the two products anticipated, only the smaller (AP-2) was recovered.

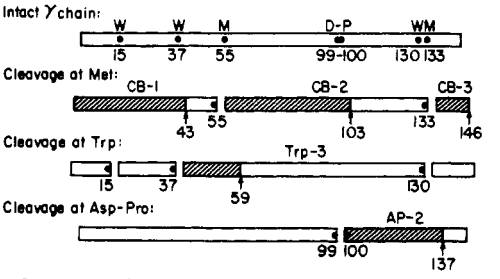

FIGURE 6: Summary of the sequencing strategy. Hatched areas denote those portions of selected fragments that were degraded. Standard, one-letter abbreviations designate those residues of importance to the generation of appropriate fragments.

carboxyl-terminal site of the γ chain. A summary of stepwise yields, as estimated by high-performance liquid chromatography, and the methods employed in identifying the products generated by each cycle of the sequencer appears in Table II. The complete sequence of the γ chain of *M. mulatta* is presented in Figure 7.

Discussion

In comparing the few cercopithecoid γ chains of known sequence, one is struck by the very slight extent of structural dissimilarity among them (Table III). None differs from any other at more than two positions (75 and 117), and the $^{1}\gamma$

Table II: Yields and Methods of Identification of Products Generated by Automated Degradation of Selected γ-Chain Fragments

cycle	residue	yield (nmol)[a]	identified by[b]	cycle	residue	yield (nmol)[a]	identified by[b]
			Fragment CB-1 (140 nmol degraded)				
1	Gly	66.9	A	23	Ala	56.7	A
2	His	ND[c]	B	24	Gly	27.9	A
3	Phe	100.7	A	25	Gly	31.9	A
4	Thr	ND	A	26	Glu	35.3	A
5	Glu	100.9	A	27	Thr	ND	A
6	Glu	110.0	A	28	Leu	27.7	A
7	Asp	103.3	A	29	Gly	22.7	A
8	Lys	34.8	A	30	Arg	ND	C
9	Ala	93.9	A	31	Leu	28.1	A
10	Thr	ND	A	32	Leu	32.9	A
11	Ile	127.6	A	33	Val	31.9	A
12	Thr	ND	A	34	Val	37.6	A
13	Ser	ND	A	35	Tyr	20.0	A
14	Leu	58.0	A	36	Pro	12.0	A
15	Trp	49.5	A	37	Trp	37.6	A
16	Gly	36.3	A	38	Thr	ND	A
17	Lys	50.9	A	39	ND	ND	
18	Val	52.3	A	40	Arg	ND	C
19	Asn	35.9	A	41	Phe	24.3	A
20	Val	63.8	A	42	Phe	34.2	A
21	Glu	45.0	A	43	Asp	17.0	A
22	Asp	59.2	A				
			Fragment CB-2 (93 nmol degraded)				
1	Gly	52.1	A	25	Asp	52.0	A
2	Asn	64.3	A	26	Leu	24.8	A
3	Pro	46.1	A	27	Lys	11.4	A
4	Lys	36.1	A	28	Gly	12.0	A
5	Val	66.0	A	29	Thr	ND	A
6	Lys	40.1	A	30	Phe	26.8	A
7	Ala	72.8	A	31	Ala	13.3	A
8	His	ND	B	32	Gln	20.2	A
9	Gly	37.6	A	33	Leu	17.9	A
10	Lys	22.0	A	34	Ser	ND	A
11	Lys	28.6	A	35	Glu	15.3	A
12	Val	54.3	A	36	Leu	14.4	A
13	Leu	44.6	A	37	His	ND	B
14	Thr	ND	A	38	Cys	ND	D
15	Ser	ND	A	39	Asp	15.6	A
16	Leu	35.6	A	40	Lys	4.5	A
17	Gly	25.4	A	41	Leu	11.0	A
18	Asp	50.7	A	42	His	ND	B
19	Ala	43.3	A	43	Val	12.2	A
20	Ile	34.7	A	44	Asp	12.5	A
21	Lys	15.1	A	45	Pro	4.4	A
22	Asn	9.0	A	46	Glu	9.6	A
23	Leu	30.1	A	47	Asn	2.5	A
24	Asp	42.1	A	48	Phe	9.6	A
			Fragment CB-3 (100 nmol degraded)				
1	Val	89.2	A	8	Leu	56.7	A
2	Ala	94.1	A	9	Ser	ND	A
3	Gly	53.1	A	10	Ser	ND	A
4	Val	85.9	A	11	Arg	ND	C
5	Ala	86.0	A	12	Tyr	48.2	A
6	Ser	ND	A	13	His	30.7	E
7	Ala	79.0	A				
			Fragment Trp-3 (75 nmol degraded)				
1	Thr	ND	A	12	Ser	ND	A
2	Gln	45.6	A	13	Ser	ND	A
3	Arg	ND	C	14	Ala	33.1	A
4	Phe	57.2	A	15	Ser	ND	A
5	Phe	61.5	A	16	Ala	32.9	A
6	Asp	42.5	A	17	Ile	20.0	A
7	Ser	ND	A	18	Met	13.9	A
8	Phe	52.2	A	19	Gly	9.3	A
9	Gly	23.5	A	20	Asn	8.4	A
10	Asn	16.2	A	21	Pro	4.4	A
11	Leu	22.8	A	22	Lys	6.7	A
			Fragment AP-2 (120 nmol degraded)				
1	Pro	106.8	A	20	Gly	15.3	A
2	Glu	108.5	A	21	Lys	18.5	A
3	Asn	84.6	A	22	Glu	15.8	A
4	Phe	100.1	A	23	Phe	30.3	A
5	Arg	ND	C	24	Thr	ND	A

Table II (*Continued*)

cycle	residue	yield (nmol)[a]	identified by[b]	cycle	residue	yield (nmol)[a]	identified by[b]
			Fragment AP-2 (120 nmol degraded)				
6	Leu	74.5	A	25	Pro	9.0	A
7	Leu	101.7	A	26	Glu	10.9	A
8	Gly	41.0	A	27	Val	11.3	A
9	Asn	59.2	A	28	Gln	9.8	A
10	Val	95.1	A	29	Ala	9.3	A
11	Leu	63.8	A	30	Ser	ND	A
12	Val	89.9	A	31	Trp	4.3	A
13	Thr	ND	A	32	Gln	5.4	A
14	Val	96.1	A	33	Lys	8.5	A
15	Leu	46.8	A	34	Met	7.1	A
16	Ala	65.9	A	35	Val	6.6	A
17	Ile	46.0	A	36	Ala	6.6	A
18	His	ND	B	37	Gly	2.9	A
19	Phe	38.3	A	38	Val	5.7	A

[a] Only 25% of each product generated by the sequencer was analyzed by LC; yields listed above are normalized to 100% injection. [b] A, LC; B, spot test using Pauly reagent; C, spot test using phenanthrenequinone reaction; D, thin-layer chromatography; E, amino acid analysis of material extracted from the cup of the sequencer. [c] ND, not determined.

Table III: Structural Comparisons of Primate γ Chains[a]

species	residue no. 75	77	104	117	135	136	ref
H. sapiens	Ile	His	Lys	His	Thr	Gly/Ala	Schroeder et al. (1963); Schroeder & Huisman (1979)
P. troglodytes[b]	Ile	His	Lys	His	Thr	Gly/Ala	De Jong (1971)
M. mulatta	Ile	Asn	Arg	His	Ala	Gly	this report
M. nemestrina γ_{slow}	Ile	Asn	Arg	Arg	Ala	Gly	Nute & Mahoney (1979b)
P. cynocephalus	Ile/Val	Asn	Arg	His	Ala	Gly	Nute & Mahoney (1979a)

[a] Only those positions at which structural differences occur are presented. The listing of two amino acids under a single residue number is indicative of structural heterogeneity of the γ chains from members of the same species. [b] Sequences of the $^{A}\gamma$ and $^{G}\gamma$ chains of *P. troglodytes* were inferred from the amino acid compositions of small peptides.

```
                5                  10                  15
 1  Gly His Phe Thr Glu Glu Asp Lys Ala Thr Ile Thr Ser Leu Trp
16  Gly Lys Val Asn Val Glu Asp Ala Gly Gly Glu Thr Leu Gly Arg
31  Leu Leu Val Val Tyr Pro Trp Thr Gln Arg Phe Phe Asp Ser Phe
46  Gly Asn Leu Ser Ser Ala Ser Ala Ile Met Gly Asn Pro Lys Val
61  Lys Ala His Gly Lys Lys Val Leu Thr Ser Leu Gly Asp Ala Ile
76  Lys Asn Leu Asp Asp Leu Lys Gly Thr Phe Ala Gln Leu Ser Glu
91  Leu His Cys Asp Lys Leu His Val Asp Pro Glu Asn Phe Arg Leu
106 Leu Gly Asn Val Leu Val Thr Val Leu Ala Ile His Phe Gly Lys
121 Glu Phe Thr Pro Glu Val Gln Ala Ser Trp Gln Lys Met Val Ala
136 Gly Val Ala Ser Ala Leu Ser Ser Arg Tyr His
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FIGURE 7: The complete amino acid sequence of the γ chain from *M. mulatta*.

(Ile-75) chain from the baboon, *P. cynocephalus*, is identical in sequence with the γ chain from *M. mulatta*. In addition, comparison of these cercopithecoid γ chains with the $^{A}\gamma$ and $^{G}\gamma$ chains from man and chimpanzees (*P. troglodytes*) yields only three to five differences in sequence (Table III).

The conservative nature of γ-chain evolution among higher primates is underscored upon comparison of the sequences of β chains from the same species. Although β chains from various species of macaques differ by no more than one substitution, that of *P. cynocephalus* differs from its counterparts among macaques at three or four sites and from the human β chain at eight positions (Nute & Mahoney, 1980). On the basis of these comparisons alone, it is clear that considerably less evolutionary change has occurred among the γ chains of hominoids and cercopithecoids than has occurred among their β chains during the 20 million or more years since Old World monkeys, apes, and man last shared a common ancestry (Szalay & Delson, 1979). Unfortunately, the factors responsible for this discrepancy in rates of evolutionary change remain obscure. That selective constraints have set limits on the kinds of substitutions permitted does, however, seem likely. None of the substitutions by which hominoid and cercopithecoid γ chains differ are known to affect residues that participate in the binding of 2,3-DPG or the formation of contacts between like or unlike chains in the heterotetramers. Hydrophobic amino acids (Val or Ile) occupy position 75 in the various primate γ chains, in keeping with retention of the hydrophobic character of the interior of the molecule. At position 77, one finds either an asparaginyl or a histidinyl residue; both are polar, and the substitution of one by the other at this site would seem to be of little functional import. Either lysine or arginine at position 104 should be effective in maintaining the hydrophilic nature and stability of the central cavity of the heterotetramer, and the presence of one or the other of these residues at the corresponding position in the β chains of different higher primates (Nute & Mahoney, 1980) supports this assertion. In position 117, which is occupied by an externally oriented residue, primate γ chains bear either histidine or arginine; these two residues should be equally effective in maintaining the surface charge and solubility of the molecule. Finally, the exchanges involving threonine and alanine in position 135 and glycine and alanine in position 136 involve only minor alterations in polarity and size of the side groups bordering on the central cavity of an $\alpha_2\gamma_2$ tetramer; no functional or configurational changes can, at this time, be attributed to either of these substitutions.

In the light of evidence that nonallelic γ-chain genes exist in man (Schroeder et al., 1968, 1972), the great apes *P. troglodytes*, *G. gorilla*, and *P. pygmaeus* (De Jong, 1971; Huisman et al., 1973; Schroeder et al., 1978), and the cercopithecoid monkeys *P. cynocephalus* and *M. nemestrina*

(Nute & Mahoney, 1979a,b), it is tempting to assume that nonallelic γ-chain genes also exist in *M. mulatta*. The possibility that rhesus monkeys carry nonallelic γ genes is not denied by our failure to detect any sign of structural ambiguity in the course of determining the γ-chain sequence. In fact, given the slight extent of structural dissimilarity among the γ chains of higher primates, one might reasonably expect to find single species in which the products of genes at nonallelic γ-chain loci are identical in sequence, as is true of the products of duplicate α-chain genes in *Homo sapiens* [reviewed by Forget (1979)]. Moreover, although we have assumed that the minor component of fetal hemoglobin, which was isolated from the lysed red cells of *M. mulatta* by ion-exchange chromatography, contains γ chains that are identical in sequence with those of the major component, this assumption rests solely upon circumstantial evidence (see Results). While rhesus macaques may bear nonallelic γ-chain genes that encode chains of identical sequences, confirmation of their presence will, most likely, depend upon characterization of the non-α-gene complex in this species.

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