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Complete Amino Acid Sequence of the γ Chain from the Major Fetal Hemoglobin of the Pig-Tailed Macaque, *Macaca nemestrina*†

Peter E. Nute* and Walter C. Mahoney

ABSTRACT: The complete primary structure of the γ chain of the major fetal hemoglobin from the pig-tailed macaque, *Macaca nemestrina*, was obtained by the automated sequencing of fragments produced by three nonenzymatic cleavage reactions. About two-thirds of the sequence was established from the amino terminus of the intact chain and two of the three fragments produced by cleavage at methionyl residues by cyanogen bromide. Acid cleavage at the single aspartyl-prolyl linkage and cleavage at tryptophanyl residues in intact chains yielded the two fragments necessary to complete the sequence. This γ chain, the first from a nonhuman primate to be sequenced, differs from the human $^{G}\gamma$ and $^{A}\gamma$ chains at but 4 and 5 positions, respectively. All substitutions are conservative and unlikely to produce alterations in the oxygen-binding properties of the tetrameric fetal hemoglobin. Consideration of the data presented herein, together with published observations made on portions of other primate γ chains, provides some insight into the evolutionary history of the multiple γ-globin chains observed in several anthropoid primates.

All of 109 fetal and neonatal pig-tailed macaques (*Macaca nemestrina*) examined by us have possessed two structurally distinct γ chains in their fetal hemoglobins (Nute & Stamatoyannopoulos, 1971a,b; P. E. Nute, unpublished data). Further heterogeneity of their fetal hemoglobins, traceable to α-chain variation, has also been noted, and the presence of either two or four fetal hemoglobins per individual is ascribed to the combination of products of genes at duplicate γ-chain loci with one or both of the structurally distinct α chains commonly found in members of this species (Nute & Pataryas, 1974; Nute, 1974; W. C. Mahoney & P. E. Nute, unpublished data). The two γ chains, designated γ_{slow} and γ_{fast} according to the relative electrophoretic mobilities at basic pH of the hemoglobins in which they appear, are present in a $2\gamma_{slow}:1\gamma_{fast}$ ratio in circulating erythrocytes during gestation and throughout the period of replacement of fetal by adult hemoglobin (Nute & Stamatoyannopoulos, 1971b).

The existence of at least two (and perhaps as many as four) γ-chain loci (reviewed by Wood et al., 1977; Schroeder & Huisman, 1979) may be characteristic of many of the higher primates. Both $^{A}\gamma$ (with alanine in position 136) and $^{G}\gamma$ (with glycine in position 136) chains have been found in all of several hundred normal humans from widely separated geographical locations, indicating that there are at least two γ-chain loci in *Homo sapiens* (Schroeder et al., 1968, 1972). Nonallelic γ-chain genes may also exist in gorillas (*Gorilla gorilla*), orangutans (*Pongo pygmaeus*), rhesus monkeys (*Macaca*

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mulatta) (Huisman et al., 1973), baboons (*Papio cynocephalus*) (DeSimone et al., 1979; P. E. Nute & W. C. Mahoney, unpublished data), and chimpanzees (*Pan troglydytes*) (De Jong, 1971). However, excepting the γ chains of chimpanzees, the residues of which were placed by homology with human γ-chain sequences after compositional analyses of tryptic and chymotryptic peptides (De Jong, 1971), and scattered peptides from the γ chains of a few New and Old World monkeys, gorillas, and orangutans (Huisman et al., 1973; Sullivan et al., 1976), the structures of γ chains from nonhuman primates are quite unknown. This is surprising, given the rapidly rising interest in the evolutionary relationships among primate γ and β chains, in the evolutionary origins of the multiple γ-chain loci that appear so widespread among the Anthropoidea and in the mechanisms underlying the perinatal shift from γ- to β-chain production in man. It is with these interests in mind that we present the complete amino acid sequence of the γ_{slow} chain of *M. nemestrina* and further characterize this species as one that might serve as an appropriate model in whose context analysis of the ontogenetic shifts in patterns of hemoglobin synthesis could be profitably pursued.

Experimental Procedures

Erythrocytes from 31 *Macaca nemestrina*, ranging in age from 60 days' gestation to 1 week postpartum, were washed three times in saline and lysed by mixing with 2 volumes of cold, deionized water. After centrifuging to remove cell debris, the clear supernates were subjected to starch-gel electrophoresis in Tris–EDTA–borate buffer, pH 8.6 (Smithies, 1965). Visual inspection of the gels indicated that all samples contained HbF_{slow} and HbF_{fast} in, approximately, 2:1 proportions.

Ion-Exchange Chromatography. Hemoglobin F_{slow} was isolated from dialyzed hemolysates on columns of DEAE-Sephadex (A-50) as described by Nute & Stamatoyannopoulos (1971b). After preparation of globin by precipitation in cold acid–acetone (Rossi-Fanelli et al., 1958), γ_{slow} chains were isolated on columns of carboxymethylcellulose (Whatman CM-23, Advanced Fibrous) using 8 M urea buffers (Clegg et al., 1968; Nute & Pataryas, 1974). Chains were desalted on 2.5 × 45 cm columns of Sephadex G-25 (fine), equilibrated and developed with 9% formic acid, and recovered by lyophilization.

Chemical and Enzymatic Cleavages and Isolation of Products. Following S-pyridylethylation (Hermodson et al., 1973), γ_{slow} chains were cleaved at methionyl residues by cyanogen bromide. A 100-fold molar excess of cyanogen bromide over methionine was dissolved in a solution containing 10 mg of protein per mL of 70% formic acid. The reaction was allowed to proceed in the dark for 18 h at room temperature, after which the reaction mixture was diluted with 20 volumes of deionized water and lyophilized. The dried fragments were dissolved in 5 mL of 30% formic acid and separated by passage through a 2 × 195 cm column of Sephadex G-50 (superfine) equilibrated and developed with 9% formic acid.

Cleavage at the single aspartylprolyl linkage was accomplished essentially as described by Jauregui-Adell & Marti (1975). *S*-Pyridylethyl γ_{slow} chains (51 mg) were dissolved in 2 mL of 70% formic acid, 7 M in guanidine hydrochloride, and the solution was incubated at 40 °C for 24 h. The reaction was stopped by the addition of 2 mL of cold, deionized water, and the solution was immediately applied to a 2 × 95 cm column of Sephadex G-75 (superfine) equilibrated and subsequently developed with 9% formic acid. The carboxyl-terminal fragment (residues 100 through 146) was, after recovery by lyophilization, further purified by passage through a 2 × 195 cm column of Sephadex G-50 (superfine), using 9% formic acid as the eluant.

Fragmentation of the protein at tryptophanyl residues was carried out by a modification of the method of Omenn et al. (1970). The S-pyridylethylated chain (30 mg) was suspended in 2.4 mL of glacial acetic acid. After thorough wetting of the protein, 19.6 mg of BNPS-skatole[1] was added, with stirring, and completely dissolved. The protein was dissolved by the addition of 0.6 mL of distilled water, and the reaction vessel was sealed and covered with foil. The reaction proceeded for 10 h, with stirring, at room temperature, after which an equal volume of distilled water was added. Excess reagent was removed by extracting three times with glass-distilled 1-chlorobutane. The aqueous layer was placed on a 2.5 × 95 cm column of Sephadex G-50 (superfine) from which the fragments were eluted with 9% formic acid.

Various tryptic peptides were isolated on peptide maps (Clegg et al., 1966) following cleavage of unmodified γ_{slow} chains by trypsin-Tos-PheCH$_2$Cl (Worthington). Tryptophan-containing peptides were identified by application of the Ehrlich reagent (Easley, 1965) and the corresponding peptides from additional maps stained with 0.02% ninhydrin in acetone were eluted from the paper (Sanger & Tuppy, 1951) and subjected to amino acid analysis.

Amino Acid Analysis. S-Pyridylethylated chains and peptides were hydrolyzed in glass-distilled, 6 N HCl in vacuo at 110 °C for 24 h (peptides) or for 24, 48, and 72 h (whole chains). All analyses were performed on a Durrum (D-500) amino acid analyzer according to the manufacturer's instructions. *S*-Pyridylethylcysteine elutes as a discrete peak between ammonia and arginine and has a color value equal to 102% that of leucine (Friedman et al., 1970).

Designation of Peptides. Peptides 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 complete γ_{slow} chain. Cyanogen bromide fragments are thus designated CB-1 (residues 1 through 55), CB-2 (56 through 133), and CB-3 (134 through 146). Cleavage at the single Asp–Pro bond produced fragments AP-1 (1 through 99) and AP-2 (100 through 146), while cleavage at tryptophanyl residues was expected to yield fragments Trp-1 (1 through 15), Trp-2 (16 through 37), Trp-3 (38 through 130) and Trp-4 (131 through 146). Tryptic peptides were not used in the proof of sequence, but four served as aids in locating tryptophanyl residues and in further documenting the areas of overlap between some fragments. These peptides are designated T-1+2 (1 through 17), T-4 (31 through 40), T-15 (121 through 132), and T-16 (133 through 144).

Sequence Analysis. Whole *S*-pyridylethyl γ_{slow} chains and selected fragments (3–6 mg) were degraded in a Beckman Model 890 C sequencer according to the method of Edman & Begg (1967) as modified by Hermodson et al. (1972), using the "peptide program" of Hermodson et al. (1977). The products generated by the sequencer were kept at 4 °C in 1-chlorobutane containing 0.3% ethanethiol prior to conversion to phenylthiohydantoin derivatives. After drying under a stream of purified nitrogen, conversion to Pth-amino acids was

[1] Abbreviations used: BNPS-skatole, the compound formed by the reaction of *N*-bromosuccinimide with 2-(2-nitrophenylsulfenyl)-3-methylindole, the most likely product being 2-(2-nitrophenylsulfenyl)-3-methyl-3-bromoindolenine; Tos-PheCH$_2$Cl, L-(tosylamido-2-phenyl)ethyl chloromethyl ketone; Pth, phenylthiohydantoin; PITC, phenyl isothiocyanate; 2,3-DPG, 2,3-diphosphoglyceric acid.

accomplished according to Hermodson et al. (1972). The ethyl acetate soluble Pth-amino acids were converted to their trimethylsilyl derivatives by adding 25 μL of a 50% (v/v) solution of *N,O*-bis(trimethylsilyl)acetamide in ethyl acetate and heating to 60 °C for 2–3 min.

Silylated products were identified using a Hewlett-Packard 5700A gas–liquid chromatograph equipped with a flame ionization detector, an automatic sampler (7671A), and a reporting integrator (3380S). The instrument was programmed for a linear temperature gradient ranging from 200 to 290 °C. A 4 °C/min increase in temperature after a 2-min hold at the starting temperature, and a similar hold at the final temperature, permitted detection of all ethyl acetate soluble Pth-amino acids. From the yields of those silylated Pth-amino acids that can be quantitated by gas–liquid chromatography (e.g., alanine, glycine, valine, and leucine), it was calculated that at least 60% of the amount of each derivative expected (based on the amount and size of the peptide degraded) was recovered after each cycle. Stepwise yields for the degradations ranged from 94 to 96% and, with one exception (see Results), only one sequence was observed.

Two of the three Pth-amino acids that remain in the aqueous phase after cyclization, Pth-arginine and Pth-pyridylethylcysteine, were identified by spot tests on paper or by thin-layer chromatography as described by Hermodson et al. (1972). The third, Pth-histidine, was identified using the Pauly test. After drying the aqueous phase under a stream of purified nitrogen, the Pth derivative was dissolved in 25 μL of methanol. Five microliters of the methanolic solution was spotted on a strip of Whatman No. 3 chromatography paper. The Pauly reagent was prepared by mixing 1 mL each of a solution of 1% (w/v) sulfanilic acid in 10% HCl and 5% (w/v) aqueous $NaNO_2$. The diazotization reaction was allowed to proceed for 2 min, after which 2 mL of saturated Na_2CO_3 was added to neutralize excess acid. The paper strip was dipped in the reagent and allowed to dry. A stable, dark red color developed within 1 min in the presence of as little as 5 nmol of Pth-histidine. Because diazonium ions are rather unstable, the staining reagent was prepared just before use. This test is less sensitive to temperature and chemical changes, is easier to use, and produces more intense color than does the diazotized *p*-anisidine test described by Sanger & Tuppy (1951).

The first 12 residues of fragment CB-3 were identified as described above. After cleavage of the phenylthiocarbamoyl derivative of the penultimate residue, the twelfth cycle was stopped before the carboxyl-terminal residue could be coupled with PITC. The carboxyl-terminal residue was identified by amino acid analysis (without prior hydrolysis) of the material remaining in the cup.

Results

The amino acid sequence of the γ_{slow} chain of *Macaca nemestrina* is presented in Figure 1. The range of overlap between partial sequences is slight (1–3 residues). However, the amino acid sequences of the human chains (Schroeder et al., 1963; Schroeder & Huisman, 1979), with which the γ_{slow} chain is homologous, support completely the positioning of the γ_{slow} fragments as indicated in Figure 1. In addition, the close correspondence of amino acid compositions of the whole chain and fragments therefrom with the compositions determined from the sequence indicates that the sequence, as presented, is complete.

Isolation and Identification of Fragments. Elution profiles produced by gel filtration of CB and AP fragments from intact, *S*-pyridylethyl γ_{slow} chains appear in Figures 2 and 3, respectively, while that produced by gel filtration of the products

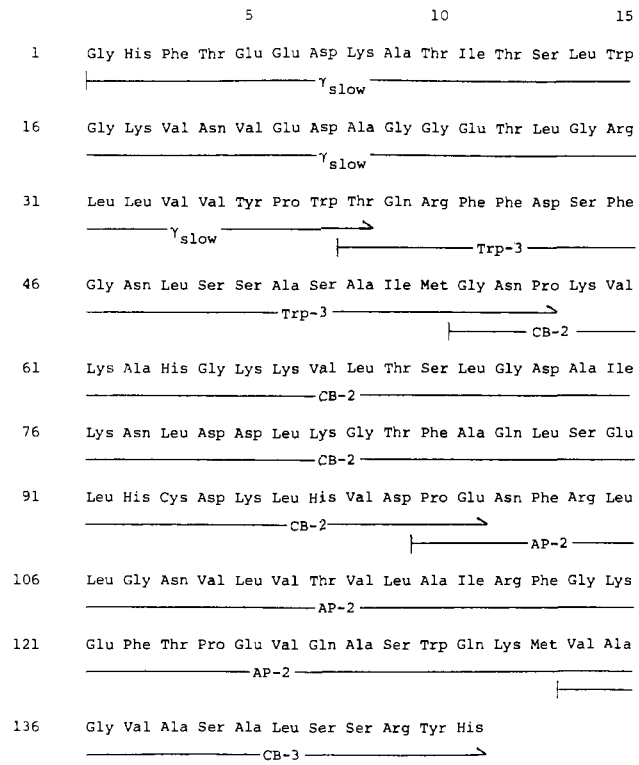

FIGURE 1: The amino acid sequence of the γ_{slow} chain from *Macaca nemestrina*. The lines with designations of fragments denote the analyses used to determine the sequence.

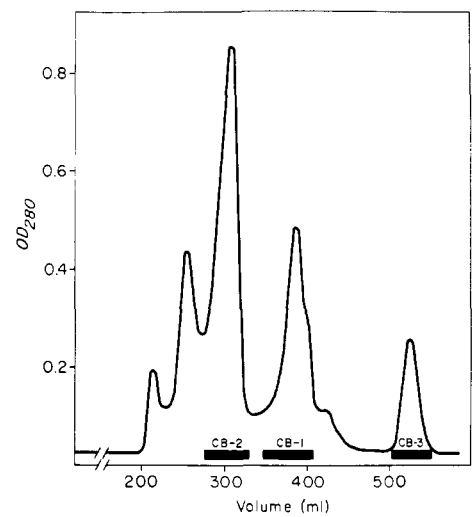

FIGURE 2: Gel filtration of fragments produced by cleavage of the *S*-pyridylethyl γ_{slow} chain at methionyl residues. Fragment CB-1 was identified by amino acid analysis, but was not further analyzed.

of cleavage at tryptophanyl residues appears in Figure 4. Amino acid compositions of the whole *S*-pyridylethyl γ_{slow} chain, the fragments required for completion of the sequence (CB-2, CB-3, AP-2, and Trp-3), and four tryptic peptides (T-1+2, T-4, T-15, and T-16) appear in Table I.

Sequence Analysis. The strategy employed in obtaining the primary structure of the γ_{slow} chain is diagrammed in Figure 5. The residues occupying positions 1 through 38 were identified by stepwise degradation of the intact chain. Subsequent analyses of fragments CB-2 and CB-3 established the sequences of residues 56 through 101 and 134 through 145, respectively. The penultimate residue of CB-3 was identified as tyrosine by gas–liquid chromatography. The material removed from the cup after extraction of the anilinothiozo-

Table I: Amino Acid Compositions of the γ_{slow} Chain and Selected Fragments[a]

amino acid	whole chain[b]	CB-2 56-133[i] 81[j]	CB-3 134-146[i] 93[j]	AP-2 100-146[i] 26[j]	Trp-3 38-130[i] 42[j]	amino acid	whole chain[b]	CB-2 56-133[i] 81[j]	CB-3 134-146[i] 93[j]	AP-2 100-146[i] 26[j]	Trp-3 38-130[i] 42[j]
Asp	14.1 (14)	9.0 (9)		2.5 (2)	11.0 (11)	Ile	3.9[d] (4)	2.0 (2)		1.0 (1)	2.9 (3)
Thr	9.0[c] (9)	3.9 (4)		2.2 (2)	4.7 (5)	Leu	16.9[d] (17)	11.1 (11)	1.1 (1)	5.2 (5)	11.9 (12)
Ser	10.6[c] (11)	3.1 (3)	2.6 (3)	3.6 (4)	6.4 (7)	Tyr	1.9 (2)		0.9 (1)	0.8 (1)	
Glu	12.4 (12)	7.3 (7)		5.0 (5)	6.9 (7)	Phe	7.8 (8)	4.1 (4)		3.4 (3)	7.2 (7)
Pro	3.8 (4)	3.3 (3)		1.9 (2)	3.0 (3)	Lys	10.7 (11)	8.6 (9)		2.0 (2)	7.8 (8)
Gly	12.8 (13)	5.8 (6)	1.0 (1)	3.2 (3)	7.1 (7)	His	4.8 (5)	2.7 (3)	0.9 (1)	1.0 (1)	2.9 (3)
Ala	11.7 (12)	5.4 (5)	3.0 (3)	4.9 (5)	7.1 (7)	Arg	4.6 (5)	1.9 (2)	0.9 (1)	2.8 (3)	2.9 (3)
Val	12.7[d] (13)	7.4 (7)	2.1 (2)	5.8 (6)	7.0 (7)	Cys[g]	0.7 (1)	0.8 (1)			1.1 (1)
Met	1.7 (2)	present[f]		0.4 (1)	0.8 (1)	Trp[h]	ND (3)	ND (1)	ND (1)	ND (1)	de-stroyed (1)

amino acid	T-1+2 1-17[i] 10[j]	T-4 31-40[i] 11[j]	T-15 121-132[i] 12[j]	T-16 133-144[i] 12[j]	amino acid	T-1+2 1-17[i] 10[j]	T-4 31-40[i] 11[j]	T-15 121-132[i] 12[j]	T-16 133-144[i] 12[j]
Asp	1.3 (1)				Ile	0.7 (1)			
Thr	2.7 (3)	1.1 (1)	1.1 (1)		Leu	0.9 (1)	1.9 (2)		1.1 (1)
Ser	1.0 (1)		1.2 (1)	2.9 (3)	Tyr		0.9 (1)		
Glu	2.1 (2)	1.1 (1)	4.2 (4)		Phe	1.0 (1)		1.0 (1)	
Pro		1.0 (1)	0.9 (1)		Lys	1.8 (2)		1.0 (1)	
Gly	2.1 (2)			1.2 (1)	His	0.8 (1)			
Ala	0.9 (1)		1.0 (1)	3.2 (3)	Arg		0.8 (1)		0.9 (1)
Val		1.3[e] (2)	1.0 (1)	2.0 (2)	Cys[g]				
Met				0.7 (1)	Trp[h]	+ (1)	+ (1)	+ (1)	

[a] Values in parentheses refer to compositions determined from the sequence. [b] Values are averages over duplicate 24-, 48-, and 72-h hydrolyses, except where otherwise noted. [c] Values determined by extrapolation to zero time. [d] Values are averages of duplicate 72-h hydrolyses. [e] Low yield of valine attributable to incomplete hydrolysis of the Val^{33}-Val^{34} bond. [f] Detected as homoserine and homoserine lactone. [g] Detected as *S*-pyridylethylcysteine. [h] ND, not determined; tryptophan was detected on tryptic peptide maps by application of the Ehrlich reagent. [i] Residues. [j] Yield (%).

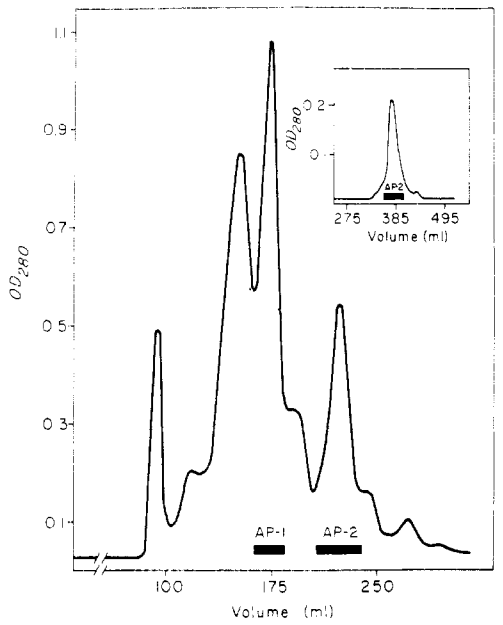

FIGURE 3: Gel filtration of fragments produced by cleavage of *S*-pyridylethyl γ_{slow} chains with 70% formic acid. Inset: further purification of the AP-2 fragment by gel filtration (see text for details). Fragment AP-1 was identified by amino acid analysis, but was not further analyzed.

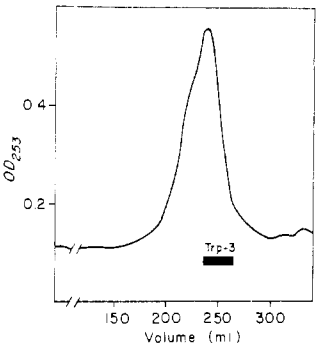

FIGURE 4: Second passage of material containing Trp-3 and contaminants through a 2.5 × 95 cm column of Sephadex G-50 (superfine) equilibrated and developed with 9% formic acid. In the original separation of Trp fragments, read at 280 nm, only one major peak, which eluted at the same effluent volume as that depicted above, was observed (see Results).

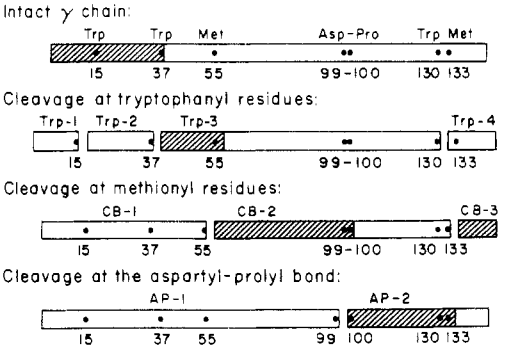

FIGURE 5: Summary of the sequencing strategy. Cross-hatching indicates those portions of the intact chain and selected fragments which were degraded. The fragments designated Trp-1, Trp-2, and Trp-4 were not recovered.

linone derivative of tyrosine yielded only tyrosine (47.2 nmol), histidine (296.2 nmol), and ammonia (2230.6 nmol) upon amino acid analysis. Repetition of this procedure, using a second preparation of the CB-3 fragment, produced comparable results (91.1 nmol of tyrosine, 1373.4 nmol of histidine). Thus, we conclude that histidine occupies the carboxyl-terminal position of the γ_{slow} chain.

The sequence of Trp-3 overlapped that of the amino-terminal end of the intact chain by a single residue (threonine at position 38) and extended the sequence from position 38 through position 58, thus overlapping the CB-2 sequence by three residues. The overlap at Thr_{38} was further documented

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

species	residue no. 75	77	104	117	135	136	references
Homo sapiens	Ile/Thr	His	Lys	His	Thr	Gly/Ala	Schroeder et al. (1963); Schroeder & Huisman (1979)
Pan troglodytes[b]	Ile	His	Lys	His	Thr	Gly/Ala	De Jong (1971)
Gorilla gorilla	?	?	?	?	Thr	Gly/Ala	Huisman et al. (1973)
Pongo pygmaeus	Ile/Val[b]	?	?	?	Thr/Ala	Gly	Huisman et al. (1973); Schroeder & Huisman (1979)
Papio cynocephalus	Ile/Val	?	?	?	Ala	Gly	DeSimone et al. (1979); Sullivan et al. (1976); P. E. Nute & W. C. Mahoney (unpublished)
Macaca mulatta	?	?	?	?	Ala	Gly	Huisman et al. (1973)
Macaca nemestrina							
γ_{slow}	Ile	Asn	Arg	Arg	Ala	Gly	this report
γ_{fast}[b]	?	?	?	?	Ala	Gly	P. E. Nute (unpublished)

[a] Only those positions at which structural differences occur are presented. The listing of two amino acids under one residue number is indicative of structural heterogeneity of the γ chains from members of the same species. [b] These residues were placed by inference of sequences from amino acid compositions of small peptides.

by amino acid analysis of tryptic peptide T-4 (Table I). The amino acid composition of this peptide, which does not fit the sequences around Trp_{15} or Trp_{130}, indicates that the peptide is composed of residues 31 through 40.

Sequence analysis of AP-2 began at position 100, providing a two-residue overlap with CB-2, and continued through residue 135, overlapping the sequence of CB-3 by two residues and completing the sequence of the γ_{slow} chain. Designation of the overlap at positions 134 and 135 was supported by the results of amino acid analysis of tryptic peptide T-16 (Table I). The composition of T-16 corresponds to that of residues 133 through 144 in the sequence and is in accord with the sequence through this region as determined from fragments AP-2 and CB-3.

In all but one instance, a single sequence was observed. The exception involved Trp-3. Initial attempts at analyzing the material constituting the major peak produced by gel filtration of Trp fragments revealed that two sequences were present in nearly equal proportions. One sequence corresponded to that starting at Gly_{16}, the other to that beginning at Thr_{38}. Presumably, partial cleavage at Trp_{37}–Thr_{38} and Trp_{130}–Gln_{131} resulted in a mixture of fragments that were inadequately separated by the procedures employed. This mixture was again subjected to gel filtration and only the material in the trailing half of the major peak was recovered (Figure 4). Analysis of this material still produced the sequence starting at Gly_{16}, but in yield so low (~6% of that of the major fragment) that it no longer interfered with the unambiguous ascertainment of the sequence of the first 21 residues in Trp-3.

Discussion

There are but four differences in sequence between the γ_{slow} chain of *Macaca nemestrina* and the human $^{G}\gamma$ chain and five between the γ_{slow} and human $^{A}\gamma$ chains (Table II). These differences all involve essentially conservative substitutions that can be effected by alterations of single nucleotides in their respective codons. The sites at which these substitutions occur do not, in human fetal hemoglobins, contain residues that participate in interchain contacts or in the binding of 2,3-diphosphoglyceric acid (Perutz & TenEyck, 1971; Lehmann & Kynoch, 1976). Thus, in the presence of physiological levels of 2,3-diphosphoglyceric acid, the high oxygen affinity of human fetal hemoglobin, relative to that of adult hemoglobin, is likely to be paralleled by that of HbF_{slow} from *M. nemestrina*. Such a relationship between the oxygen-binding properties of adult and fetal bloods has been observed in three other species of macaque, *M. mulatta*, *M. fascicularis* (= *irus*) (Novy et al., 1969), and *M. fuscata* (Takenaka & Morimoto, 1976).

A third form of human γ chain, originally described as the variant chain in HbF Sardinia (Grifoni et al., 1975), has been found at fairly high frequencies in various human populations (Ricco et al., 1976; Huisman et al., 1977). This chain, in which threonine replaces isoleucine at position 75, appears to contain alanine at position 136 and may be specified by an allele at the $^{A}\gamma$ locus (Schroeder & Huisman, 1979). The γ_{slow} chain of *M. nemestrina*, in containing isoleucine at position 75 and glycine at position 136, would appear to differ from the human $^{TA}\gamma$ chain (with threonine at position 75 and alanine at position 136) by six amino acid residues, assuming that the $^{TA}\gamma$ chain is identical with the $^{A}\gamma$ chain in the remainder of its sequence.

Although structural characterization of the γ_{fast} chain from *M. nemestrina* is far from complete, compositional analysis of the CB-3 fragment from this chain indicates that position 136 is occupied by a glycyl residue (P. E. Nute, unpublished data); although there are structurally different γ chains in all fetal and neonatal *M. nemestrina* screened to date, the difference(s) appear(s) to reside elsewhere.

In spite of the rather slight extent of divergence between the γ_{slow} chain of *M. nemestrina* and the human $^{A}\gamma$ and $^{G}\gamma$ chains, analyses of γCB-3 fragments from several nonhuman primates indicate that there is a considerable amount of heterogeneity in γ-chain structure both within and between species (Table II). Both $^{A}\gamma$ and $^{G}\gamma$ chains have been found in chimpanzees (*Pan troglodytes*), and these chains appear to be identical in primary structure with their human counterparts (De Jong, 1971). In addition, the sequences of CB-3 fragments from the γ chains of a single *Gorilla gorilla* are identical with those of the corresponding fragments from the human $^{A}\gamma$ and $^{G}\gamma$ chains (Huisman et al., 1973). As we move to our more distant relatives, signs of interspecific differences in γ-chain structure appear, although the extent of such differences is immeasurable owing to the incomplete nature of the available data. For example, Huisman et al. (1973) showed that the γ chains from each of five orangutans (*Pongo pygmaeus*) differed from those of man by the presence of both threonine and alanine in position 135 and only glycine in position 136. Position 136 is occupied by glycine alone and position 135 by alanine alone in the γ chains of the Old World monkeys *Papio cynocephalus* (Sullivan et al., 1976; DeSimone et al., 1979; P. E. Nute & W. C. Mahoney, unpublished CB-3 sequence), *Macaca mulatta* (Huisman et al., 1973), and *M. nemestrina*. Moreover, the threonyl residue in position 135 of one orangutan chain matches that found in the same position of the γ chains from humans, gorillas, and chimpanzees (Huisman et al., 1973). Thus, based on comparisons of γCB-3 fragments alone, the orangutan appears to fall between man,

gorilla, and chimpanzee, on the one hand, and baboon and macaque on the other.

The relationships noted above are in accord with the results of studies illustrating that structural heterogeneity at position 75 is not unique to human γ chains (Table II). The orangutan fetal hemoglobins examined to date (from an unspecified number of animals) each show a Val/Ile ambiguity in position 75 of their γ chains (Schroeder & Huisman, 1979); the same ambiguity was found in the γ chains of three baboons (*Papio cynocephalus*) examined by W. A. Schroeder (reported by DeSimone et al., 1979) and in the course of sequencing the γCB-2 fragments from a fourth member of this species (P. E. Nute & W. C. Mahoney, unpublished data).

Clearly, most analyses of portions of primate γ chains have been conducted on mixtures of structurally different components. Hence, it appears that at least two γ-chain loci are present in members of most, if not all, of the species examined. Clarification of the evolutionary relationships among these chains must await the determination of complete sequences of γ chains from a wide range of primates. Furthermore, the assignment of residues at positions showing structural ambiguity to specific chains will be of importance both in estimating the number of γ-chain loci and/or alleles in a given species, and in assessing the evolutionary relationships among the γ chains encoded at these loci. Given that the γ_{slow} and γ_{fast} chains of *Macaca nemestrina* are separable by conventional methods, establishment of the sequence of the former chain, in which no structural ambiguity was detected, constitutes a step toward the attainment of these goals.

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