HEMOGLOBIN G TAIWAN-AMI: $\alpha_2\beta_2^{25\ Gly \rightarrow Arg}$ [1]

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Hemoglobin G Taiwan-Ami is a slow variant of human adult hemoglobin which was first found in 1964 in members of the Ami tribe of Taiwan aborigines (Blackwell et al, 1965). In a population survey of 1571 apparently normal Ami subjects, 9 or 0.57 per cent, had the slow hemoglobin in addition to Hemoglobin A. No additional cases were found among approximately 3,000 subjects from other aboriginal tribes in Taiwan; therefore, it was concluded that the hemoglobin variant is more characteristically associated with the Ami tribe.

Structure studies have now shown that the molecular anomaly in Hemoglobin G Taiwan-Ami is in the B-helix of the beta-chain; specifically, a glycyl residue normally found at position beta-25 is replaced by a residue of arginine. The additional arginine residue accounts for the altered electrophoretic mobility of the hemoglobin molecule. The structure of the variant can be expressed as $\alpha_2\beta_2^{25\ Gly \rightarrow Arg}$.

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MATERIALS AND METHODS

The standard procedures for protein structure studies developed by numerous investigators were employed for the present work in the same general manner described previously (Blackwell and Liu, 1966). The Hemoglobin G was separated from Hemoglobin A by starch block electrophoresis of the mixed hemolysate (Masri et al, 1958). After digestion of the purified hemoglobin, using crystalline trypsin (Worthington 2x, salt free), the peptides were examined by mapping procedures (Ingram, 1958; Baglioni, 1961) on Whatman 3MM paper using pyridine-acetate buffer at pH 6.4 (Baglioni, 1962) for electrophoresis, 40v/cm for 1.5 hours, followed by overnight descending chromatography with pyridine-butanol-acetic acid-water, 10:15:3:12 by volume (Hill et al, 1962).

As described below the tryptic digest of the G hemoglobin included two new arginine-containing anomalous peptides, one neutral and one acidic. Both peptides were separated from the other peptides by high voltage paper electrophoresis for further analysis. Parallel electrophoretic runs of the tryptic digests of both Hemoglobin A and Hemoglobin G were made at pH 5.4 with pyridine-acetic acid buffer (Gehring-Müller et al, 1966) at 55v/cm for two hours. Comparison of the ninhydrin-positive and arginine-positive (Jepson and Smith, 1953) acidic zones showed that the new acidic peptide was separated from the normal acidic peptides. The abnormal neutral peptide migrated with the other neutral peptides at pH 5.4 but was clearly separated from them when the neutral band was eluted and re-subjected to electrophoresis with a formic acid-acetic acid buffer (Rothman and Higa, 1962), pH 1.9 at 4500v (57v/cm) for 1.5 hours.

For amino acid composition analyses, the respective peptide samples were eluted from the electrophoresis paper with 5.7 N hydrochloric acid and hydrolysed in evacuated, sealed tubes for 18 hours or longer at 110°C. After repeated vacuum drying at room temperature the hydrolysates were analyzed by ion-exchange chromatography using Technicon Autoanalyzer equipment as outlined by the Technicon technical bulletin. External and internal standards of norleucine were employed for calibration.

The amino acid sequences in some of the peptides were determined by modified Edman degradation procedures (Doolittle, 1965; Light and Greenberg, 1965); approximately 1 micromole of each peptide was used. The PTH-amino acid derivatives released at each cycle of the degradation were identified with descending paper chromatography using systems described by Sjöquist (1953), Edman and Sjöquist (1956), and Schroeder et al (1963). The PTH derivatives were located on the paper by treatment

with iodine and azide solutions.

For C-terminal amino acid analyses the peptides, after electrophoretic separation, were eluted from the paper with 0.1 M ammonium bicarbonate and the resulting peptide solution used directly by mixing with carboxypeptidase A and B enzymes in a final enzyme/peptide weight proportion of 1 to 20. Carboxypeptidase A-DFP (Worthington 3x, COA-DFP 6139, 38 units/mg protein) and Carboxypeptidase B-DFP (Worthington COB-DFP 29, 90 units/mg protein) were added in equal amounts to the substrate solution at zero time and the mixture maintained at 37°C with aliquots taken for amino acid analysis at 0, 1, 2, 18, and 21 hours.

RESULTS AND DISCUSSION

The pattern of peptide spots found after tryptic digestion of the Hemoglobin G Taiwan-Ami appeared normal except for the spot corresponding to the βT3 peptide which had migrated less than normal toward the anode. The slow migration of the new acidic peptide was consistent with the slower anodal mobility of the Hemoglobin G molecule in starch gel.

A second new peptide was revealed after arginine staining of the peptide map of Hemoglobin G Taiwan-Ami. In place of the normal arginine-positive βT3 peptide, two new arginine-positive spots appeared; one was the new acidic peptide revealed by ninhydrin staining mentioned above and the other was a neutral peptide in the area occupied by βT13 and therefore not visible as a separate distinct peptide by ninhydrin staining.

These results indicated that the structural anomaly of Hemoglobin G Taiwan-Ami was located in the βT3 section of the beta-chain which extends from the valine residue at position beta-18 to the arginine residue at beta-30. Since both the new acidic peptide and the new neutral peptide contained arginine, the structural difference in G Taiwan-Ami apparently involved the replacement by arginine of one of the normally-occurring amino acid residues. The additional arginine peptide bond in the variant consequently would be hydrolysed by the tryptic digestion to release two arginine peptides in place of the single arginine-containing βT3 peptide normally found.

The hydrolysates of both new abnormal peptides and the hydrolysate of normal βT3 peptide were analyzed for amino acids by ion-exchange chromatography and compared with a mixture of amino acids made to simulate the composition of peptide βT3. Results of the analyses are summarized in Table 1.

Table 1. Amino Acid Composition Of The Abnormal Peptides From Hemoglobin G Taiwan-Ami Compared With Their Normal Counterpart, Peptide βT3

Amino Acid Residue	Expected βT3 Molar Ratio	Observed						Combined Molar Ratios of Both Peptides
		Peptide βT3		Acidic Peptide		Neutral Peptide		
		Micro-moles	Molar Ratio	Micro-moles	Molar Ratio	Micro-moles	Molar Ratio	
Arg	1	0.151	1.0	0.187	1.0	0.129	1.0	2
Asp	2	0.352	2.2	0.356	1.9	-	0	2
Glu	2	0.321	2.0	0.189	1.0	0.145	1.1	2
Gly	3	0.452	2.8	0.204	1.1	0.134	1.0	2
Ala	1	0.171	1.1	-	0	0.139	1.0	1
Val	3	0.485	3.0	0.542	2.9	-	0	3
Leu	1	0.164	1.0	-	0	0.137	1.0	1

The expected molar ratios for the normal βT3 peptide were obtained. The combined molar ratios of the two abnormal peptides also agreed with the values for βT3 except for the replacement of one glycine by one arginine residue. The neutral peptide, containing one residue each of glutamyl, alanyl, leucyl, glycyl, and arginyl, corresponds to the normal positions from beta-26 to beta-30. The neutrality of that peptide resulted from the presence of one acidic and one basic amino acid residue in addition to the three neutral residues. The occurrence of the neutral pentapeptide containing amino acid residues beginning with position beta-26 suggested that the adjacent position, beta-25, was the site of the additional arginine residue which would provide the additional arginyl bond for hydrolysis during tryptic digestion. Furthermore, the component amino acids in the abnormal acidic peptide matched those normally present in the beta-chain between the valine residue at position 18 and the glycine residue at position 24. The additional arginine residue replaces the glycine residue normally found at position beta-25. If it is assumed that only one position is changed, i.e.,arginine replacing glycine at beta-25, all other positions between beta-18 and beta-30 are established. The relationships of the two abnormal peptides are shown in Figure 1.

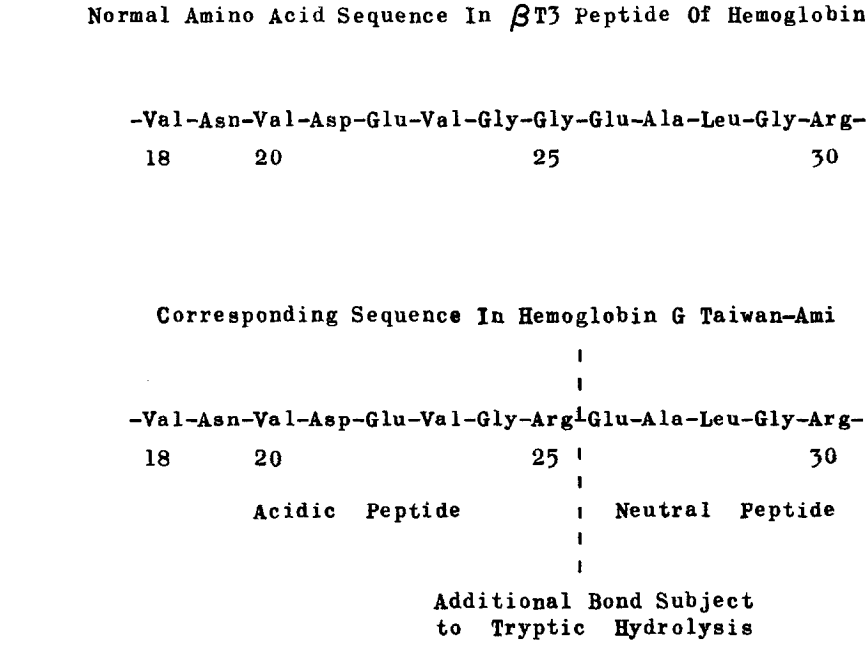

Figure 1. Comparison of the Relevant Amino Acid Sequences in the Beta-Chain of Hemoglobin A and Hemoglobin G Taiwan-Ami.

The above results were verified by those obtained in the step-wise degradation studies which were made on the acidic peptide; for purposes of comparison the same degradation procedure was run simultaneously on the βT3 peptide. Ten sequences were determined in the βT3 peptide:

Val-Asn-Val-Asp-Glu-Val-Gly-Gly-Glu-Ala- .

Those results agreed with the established sequence of the first 10 residues in βT3 as illustrated in Figure 1. In the abnormal acidic peptide from Hemoglobin G Taiwan-Ami the results were the same for the first seven residues:

Val-Asn-Val-Asp-Glu-Val-Gly-

The eighth degradation step produced greatly diminished amounts of PTH-glycine and no further derivatives were found after a ninth cycle was attempted.

As a further verification of the amino acid sequences in the abnormal peptides the acidic peptide and normal βT3 peptide were compared for their C-terminal amino acids. In both peptides the relative rates of release of amino acids from the C-terminal ends indicated that arginine was first, followed by glycine. In the βT3

peptide the third amino acid released was leucine whereas valine was third in the abnormal peptide.

These combined results are considered sufficient to establish that the structural anomaly in Hemoglobin G Taiwan-Ami involves the replacement of glycine by arginine at position beta-25.

The substitution of arginine for glycine is one of several possible results of glycine mutation involving single codon changes (Nirenberg et al, 1965); two previous examples of such a change are Hemoglobin Russ (Reynolds and Huisman, 1966) in which arginine replaces glycine at position alpha-51 and Hemoglobin Dβ-Bushman (Wade et al, 1967) in which the same replacement occurs at position beta-16. Arginine also occurs in place of glutamine at position alpha-54 in Hemoglobin Shimonoseki (Hanada and Rucknagel, 1963) and in place of histidine at position beta-63 in Hemoglobin Zurich (Muller and Kingma, 1961).

Although detailed clinical and hematological studies have not yet been made on the subjects with the Hemoglobin G Taiwan-Ami and their kindred, preliminary blood studies on some of the subjects indicate normal values for their hemoglobin concentrations, hematocrits, and red blood cell counts. In addition, the subjects appear to have no specific complaints suggesting anemia.

No hemoglobin variants except G Taiwan-Ami have been found thus far in the aboriginal people of Taiwan; furthermore, that hemoglobin variant has been found only in the Ami tribesman. Studies among the kindreds of the original 9 Ami subjects have disclosed approximately 40 additional individuals with the same hemoglobin variant. A current survey is being made in a further attempt to detect the possible occurrence of hemoglobin variants among the people of the other aboriginal tribes. It also is of interest to determine whether the same hemoglobin occurs in other Proto-Malayan people of Southeast Asia.

SUMMARY

Hemoglobin G Taiwan-Ami is an adult hemoglobin variant seen among members of the Ami aboriginal tribe in Taiwan. The structural anomaly responsible for its slow electrophoretic mobility involves the replacement of a glycine moiety by one of arginine in the B-helix of the beta-chain at position 25.

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