

Diminished Synthesis of an Alpha Chain Mutant, Hemoglobin I ($\alpha^{16 \text{ lys} \rightarrow \text{glu}}$)

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ABSTRACT In patients heterozygous for abnormal hemoglobins there is usually less than 50% of the mutant hemoglobin present in peripheral blood. The synthetic rates of α -chain mutants compared to α^A have not been reported to date. In this study the production of α^A - and α^I -chains has been measured in peripheral blood and bone marrow of two patients with approximately 30% hemoglobin I, an α -chain abnormality ($\alpha^{16 \text{ lys} \rightarrow \text{glu}}$). The results suggest that the decreased amount of α^I compared to α^A is due solely to diminished biosynthesis of the α^I -chains. The relative rates of synthesis of α^I - and α^A -chains are similar in both nucleated red cells and reticulocytes indicating that no change occurs during erythroid cell maturation which preferentially affects either α^I or α^A production.

INTRODUCTION

More than 30 mutants of human hemoglobin have been isolated in which the amino acid substitution is in the α -chain (1). In every case, the amount of abnormal hemoglobin present in peripheral blood has been considerably less than that of normal hemoglobin (HbA) (1, 2). Premature and selective destruction of cells containing the abnormal hemoglobin, resulting in the preferential survival of cells with predominantly HbA could account for the low concentration of the abnormal hemoglobin in peripheral blood. Alternatively a diminished rate of synthesis of the abnormal α -chain might account for this observation. Indeed, decreased production has been documented for several β -chain mutants (3-5). Duplicated α -chain genes (2), decreased mRNA for abnormal chains, or decreased translation of the mRNA for globin mutants (3, 6) have been suggested as possible mechanisms for diminished α -chain production. Synthetic rates for α -chain mutations have not been reported to date. This paper presents experiments per-

formed on blood from a family with hemoglobin I, HbI ($\alpha^{16 \text{ lys} \rightarrow \text{glu}}$), in an attempt to elucidate mechanisms which account for the lesser amounts of abnormal chain in the peripheral blood. The results indicate that α^I is synthesized at a diminished rate compared to α^A in both reticulocytes and nucleated red cells of patients heterozygous for HbI. In addition, there is no change in the relative rates of α^I and α^A during erythroid cell development. The rate of synthesis of α^I is proportional to its concentration in peripheral blood indicating that a diminished synthetic rate for α^I can adequately explain the decreased amount of HbI present in peripheral blood.

METHODS

Clinical and hematologic findings. The peripheral blood and bone marrow of the propositus, a 23 yr old Negro female heterozygote and the peripheral blood of her newborn son who was also heterozygous for the mutant hemoglobin were studied. Both mother and child had normal hemoglobin levels and red cell morphology. The newborn had a 10% reticulocyte count which returned to normal after 1-2 wk. The father of the child had a normal hemoglobin electrophoretic pattern and was hematologically normal. No other relatives in the family were available for study. These findings in patients with HbI are consistent with those described by others (7-13).

Hemoglobin studies. Both cellulose acetate and starch-gel electrophoresis were performed at pH 8.6. The hemoglobins were quantitated by elution from starch gel and measurement of cyanmethemoglobin at OD 415 (14) and by densitometric measurement from cellulose-acetate electrophoresis using a Beckman Spinco densitometer Model R-110 (15). Fetal hemoglobin concentration was estimated by alkali denaturation (16). Hybridization studies using hemoglobins isolated from starch-gel electrophoresis were performed as described previously (17).

Measurement of globin chain synthesis. Specimens from peripheral blood or bone marrow were incubated with ^{14}C - or ^3H -labeled valine in modified Krebs-Ringer bicarbonate buffer as previously described (18). Incubations were performed at either 30°C or 37°C for periods up to 120 min. After incubation the cells were lysed, and the stroma removed by centrifugation at 50,000 g in a 50.1 rotor for 2 hr in a Spinco Model L ultracentrifuge. Globin was prepared from the hemolysate as previously described (18). The

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globin chains were separated by column chromatography using carboxymethyl-cellulose (CMC) in 8 M urea at pH 6.8 (19). The globin chain content of the eluate was measured by its optical density at 280 m μ (OD 280) in a Gilford spectrophotometer (18). The tubes containing the specific globin chain peaks were pooled, aminoethylated, and freed from excess reagents on Sephadex G-25 in 0.5% formic acid (19). The peaks were then lyophilized, dissolved, in water, and an OD 280 obtained. Radioactivity was measured on 0.5–1.0 ml of this sample by adding 12 ml of Bray's solution and counting in a Packard Tri-Carb liquid scintillation counter. Adequate cpm were accumulated in order to reduce the counting error to less than 1%. The results are expressed as specific activity (SA) in cpm/OD 280 for each globin chain.

Determination of globin chain structure. The phenyl isothiocyanate degradation method was employed to determine the sequence of amino acids at the NH₂-terminal of the purified globin chains (20). Stepwise degradations were carried out by the automated sequencer procedure in a Beckman protein/peptide Sequencer¹ using a program essentially the same as that of Edman and Begg (21). Approximately 0.25 μ mole of globin chain was used for the analysis. Edman and Begg using humpback whale apomyoglobin have demonstrated that this method is capable of accurately determining up to 60 residues by NH₂-terminal amino acid sequence analysis (21). Identification of the 3-phenyl-2-thiohydantoin (PTH) derivatives of the amino acids was generally performed by thin-layer chromatography (21). PTH-arginine and PTH-histidine were identified by specific color reactions (22).

RESULTS

Studies of hemoglobins. Starch-gel electrophoresis revealed a rapidly moving hemoglobin band in the peripheral blood of the mother and the newborn. This fast moving component represented 25% of the total hemoglobin in the mother and 34% in the newborn. The electrophoretic pattern of the newborn revealed that both the normal and rapidly migrating hemoglobin fractions were slightly closer to the origin than comparable components in the propositus. The newborn's hemolysate contained 78% alkali-resistant hemoglobin. This is consistent with the presence of the γ -chain in both the hemoglobins of the newborn's hemolysate. Densitometric tracings of the cellulose-acetate strips of these specimens confirmed these observations (Fig. 1).

Hybridization studies. The rapidly moving abnormal hemoglobin was isolated from starch-gel electrophoresis and hybridized with HbC as previously described (17). HbC, a slow moving hemoglobin with two charge differences per β -chain ($\beta^{\text{Asu}} \rightarrow \beta^{\text{Lys}}$) was obtained from a patient homozygous for this β -chain abnormality. Cellulose acetate and starch-gel electrophoresis of the hybridized hemoglobin mixture revealed a new component migrating in the region of HbA. This experiment indicated that the abnormal hemoglobin component was an

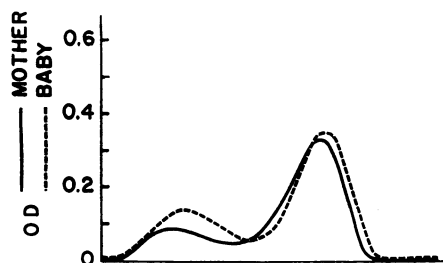


FIGURE 1 Densitometric tracings of amounts of hemoglobins in HbI heterozygote. The solid line (●—●) represents the optical density at 415 m μ of the propositus; the dotted line (○---○) is the optical density at 415 m μ in the hemolysate of the newborn. The origin is at the right.

α -chain abnormality with two charge differences from normal α -chains.

Identification of the abnormal globin chain (α^1) peak. When globin was prepared from the total stroma-free hemolysate of the mother and newborn, a new peak of OD 280 was eluted from CMC-chromatography between normal β^A and α^A -chains (Fig. 2). Normal α -chains and the abnormal globin chain were each subjected to 40 cycles of the phenyl isothiocyanate degradation in the Sequencer in separate experiments. Essentially only one amino acid was present at each step of the degradation, demonstrating the purity of the preparation and the efficiency of the method. The amino acid sequence of the 40 residues at the NH₂-terminal end was found to be the same in the abnormal chain as in α^A , with the exception of the replacement of the lysine at residue 16 by glutamic acid. This substitution is identical with that previously described for α^1 (12).

Synthesis of α^1 - and α^A -chains. The SA of α^1 in the peripheral blood of the propositus was 69 cpm/OD 280 while that of α^A was 71 cpm/OD 280 (Table I, Fig. 2); the α^1/α^A ratio of SA was 1.0. The SA of α^1 in the bone marrow of the propositus was 557 cpm/OD 280, and 510 cpm/OD 280 for α^A with an α^1/α^A ratio of 1.1 (Table I). There was no significant difference between the ratios obtained after a 7 min incubation at 30°C, and the incubations of 60 and 120 min at 30°C or 37°C. In the peripheral blood of the newborn, the α^1/α^A ratio of SA was also 1.0 (Table I).

DISCUSSION

Previous structural studies of HbI have been performed by tryptic digest analysis of unseparated globin chains. Early studies reported an aspartic acid substitution for lysine at position 16 (9, 10), while later analysis demonstrated the presence of a glutamic acid residue in this position (12). The experiments reported here are the first in which the α^1 -chain has been separated from β -chains prior to analysis of its sequence; in addition,

¹ Beckman Instruments, Inc., Spinco Division, Palo Alto, Calif.

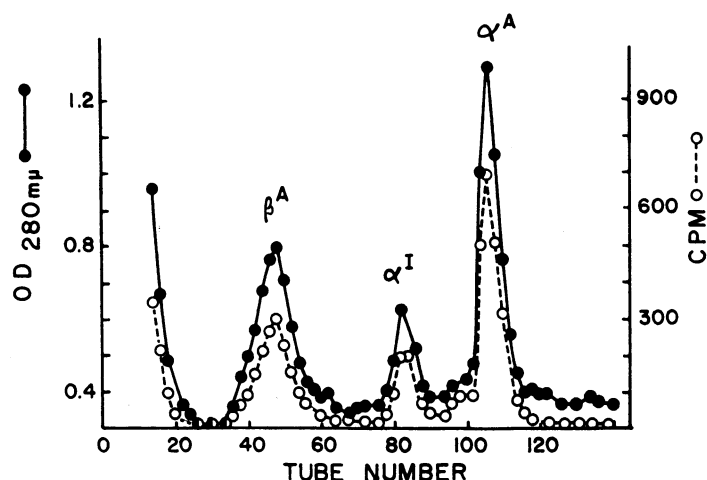


FIGURE 2 CMC-chromatography of the globin prepared from the total stroma-free hemolysate of the bone marrow of the propositus with HbI. The solid line (●—●) represents OD 280 of globin; the dotted line (○---○) represents the radioactivity.

this is the first report of a human globin chain in which the amino acid substitution was determined directly by NH₂-terminal sequence analysis on the intact chain.

All of the hemoglobins described to date with single amino acid substitutions in the α -chain are present in heterozygotes in much lower amounts than HbA (1, 2). The present study attempts to define the mechanisms responsible for the decreased per cent of abnormal hemoglobin in an α -chain heterozygote. Since there is three times as much α^A as α^I in peripheral blood the ratio of the SA of α^I/α^A should be 3.0 if α^I and α^A are synthesized at the same rate. The finding of SA ratio (α^I/α^A) of 1.0 in both bone marrow and peripheral blood of the propositus and newborn suggests that diminished synthesis of α^I chains alone may account for the lower amount of HbI in peripheral blood. In addition, the similar α^I/α^A ratios in bone marrow and peripheral blood indicate that changes in the capacity for hemoglobin synthesis during

erythroid cell maturation do not preferentially affect either α^I or α^A production. The absence of anemia or hemolysis in the propositus in addition to the constant α^I/α^A ratios with development make it unlikely that selective destruction of α^I -containing cells leads to diminished amounts of α^I in the peripheral blood. There is a possibility that rapid destruction of newly synthesized α^I -chains could explain the relatively lower than expected α^I labeling. The lack of a change in the α^I/α^A SA ratios in short-term incubations compared to that of long-term incubations of bone marrow (Table I) do not support this interpretation.

The mechanisms which account for diminished α^I -chain synthesis remain obscure. A nucleotide substitution in the structural gene for α^I -chains could result in decreased production of mRNA molecules for α^I . Alternatively, multiple α -chain genes could result in more structural genes for α^A -chains than for α^I -chains (2). The presence of multiple α -chain genes has been documented in several species (23-25). An abnormal rate of translation of the mRNA for α^I chains is another explanation for decreased synthesis for HbI. A delay in the release of completed α^I chains due to abnormal binding of these chains to the ribosomes might also prolong the time necessary for α^I synthesis. Further experiments are in progress to distinguish between these possibilities.

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TABLE I
Relative Rates of Synthesis of α^I and α^A

| Source | Incubation time | Temperature | α^I * | α^A * | α^I/α^A * |
|---------|-----------------|-------------|--------------|--------------|-----------------------|
| | min | °C | | | |
| Mother | P. B.† | 120 | 37 | 69 | 71 |
| | B. M.‡ | 7 | 30 | 268 | 258 |
| | B. M. | 120 | 37 | 557 | 510 |
| | B. M. | 60 | 30 | 1465 | 1370 |
| Newborn | P. B. | 120 | 37 | 307 | 318 |

* Specific activity (cpm/OD 280).

† Peripheral blood.

‡ Bone marrow.

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