Translation of Human Globin mRNA: Globin Synthesis in Cells Containing Hb Leiden

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Most structurally abnormal hemoglobins are present in smaller amounts than HbA in the erythrocytes of heterozygous subjects. In the presence of a hemoglobinopathy, α and β globin synthesis remains balanced with equal production of the two types of chains. In reticulocytes of subjects with Hb Leiden (β6 or 7 glu → 0) there is greater production of α than β globin in vitro (β/α = 0.67), and slightly more βA is synthesized than βLeiden (βA/βLeiden = 1.28). Differences in specific mRNA content, rates of initiation of chain synthesis, or rates of chain elongation could be responsible for such differential polypeptide synthesis. In the present study, the ribosomal assembly of βA, βLeiden, and α globin chains was examined in peripheral blood. The translation times of the three chains did not differ significantly (average times: βA = 65.4 sec, βLeiden = 70.8 sec, α = 53.5 sec). These results indicated that an altered rate of translation was not the source of the anomalous globin synthesis observed in vitro in cells containing Hb Leiden. The experiments suggested that the observed imbalance in α/β production was due to either differential rates of initiation of globin chain synthesis or quantitative differences in the amounts of the specific mRNAs present in the cells.

A BNORMAL human hemoglobins occur in widely varying amounts in the blood of heterozygous subjects. With few exceptions the mutant hemoglobins constitute less of the total circulating hemoglobin than HbA. Despite the variability in the circulating levels of the different structurally altered hemoglobins, there is generally balanced total production of α and β chains. This finding is in contrast to the unequal globin production characteristic of the thalassemia syndromes. Neither the mechanisms nor the specific stages of protein synthesis responsible for the different proportions of most of the structurally different hemoglobins or for the coordination of α and β production are well understood.

Recently, an unusual imbalance in globin synthesis with marked predominance of α globin production was demonstrated in vitro in reticulocytes containing unstable Hb Leiden β6 or 7 glu → 0. In the present investigation of the translation stage of globin chain production, the assembly times of βA, βLeiden and α chains were measured. No differences in the rates of translation were found to explain either the greater synthesis of βA than βLeiden or the predominance of α over β globin production.
MATERIALS AND METHODS

Hemoglobin synthesis by reticulocytes in vitro. Erythrocytes from a subject with Hb Leiden were washed with a solution containing 0.13 M NaCl, 0.005 M KCl, and 0.0074 M MgCl₂ and pre-incubated for 10 min at 37°C in the medium of Lingrel and Borsook without valine as previously described. Then 200 µCi of ³H-valine was added, and after exactly 3, 6, and 9.5 min of incubation portions of the cells were removed and immediately hemolyzed. Hemoglobin uniformly labeled with ¹⁴C was obtained by incubating peripheral blood from the same subject for 6 hr with ¹⁴C-valine.

Measurement of globin chain assembly times. The method was based on the procedure of Dintzis as previously detailed. Membrane-free hemolysates prepared from the 3-, 6-, and 9.5-min incubations with ³H-valine were mixed with an amount of ¹⁴C-valine hemolysate calculated to give identical ³H-hemoglobin: ¹⁴C-hemoglobin concentration ratios for all three time samples. Heme was removed from the combined hemoglobin samples by acid-acetone treatment, and the constituent globin chains were separated on carboxymethyl cellulose columns in 8 M urea using a modification of the method of Clegg, Naughton, and Weatherall. The separated chains were freed of urea by dialysis, aminoethylated, digested with trypsin, and peptide maps were prepared. The valine-containing peptides were identified by staining with ninhydrin, cut from the paper, eluted into counting vials, and the ³H- and ¹⁴C-radioactivity was measured in a liquid scintillation counter as previously described.

RESULTS

Imbalance in α/β Globin Synthesis

When reticulocyte-rich (5%) peripheral blood containing Hb Leiden was incubated with radioactive amino acid, there was a marked imbalance in α/β globin synthesis with a predominance of α globin production. Figure 1A shows the column chromatography of globin prepared after mixing a 3-min ³H-valine incubation with a 6-hr ¹⁴C-valine incubation. The ratio of total β to total α synthesis was 0.67. This ratio was identical in the 3-min and 6-hr samples, indicating that loss of radioactive unstable β chain during the incubation was not a factor contributing to unequal radioactivity in α and β globin. There was a greater synthesis of β⁴ than βLeiden, the β⁴/βLeiden synthesis ratio was 1.28. The total β/α synthesis ratios for 6 min and 9.5 min ³H-valine pulses were 0.67 and 0.63, respectively.

Imbalance in α/β globin synthesis is not found in association with other unstable structural mutants of hemoglobin. Figure 1B shows the results of column chromatography of globin prepared after mixing 3-min ³H-hemoglobin and 6-hr ¹⁴C-hemoglobin from a subject with Hb Gun Hill. The synthesis of α and β chains was balanced: the total β/α synthesis ratio was 1.06.

Assembly of Globin Chains

The technique for measuring the elongation time of globin chains depends upon the fact that polypeptide synthesis proceeds by stepwise addition of amino acids from the NH₂-terminus to the COOH-terminus. When radioactive amino acid is added to cells actively synthesizing protein, it is first incorporated onto the growing ends of partially completed chains on the polyribosomes. Initially, radioactivity appears at the COOH-terminal end of the hemoglobin in solution. With continued incubation, gradients of radioactivity extend in from the COOH-terminus toward the NH₂-terminus, and eventually radioactivity appears at the NH₂-terminus. The time elapsed between the appearance of radio-
activity at the COOH-terminus and the labeling of the NH2-terminal residue represents the assembly time for the polypeptide.

Figure 2 shows the gradients of radioactivity in $\beta^A$, $\beta^{Leiden}$, and $\alpha$ chains after 3, 6, and 9.5 min of incubation of peripheral blood with $^3$H-valine. The slopes of the $^3$H/$^{14}$C ratios are similar for all samples. Although an absolute difference in specific activity persists between the NH2- and COOH-termini, with increasing time of incubation the chains approach uniform labeling and the percentage difference between the specific activities of the two ends of the chain decreases. Table 1 shows the relative per cent labeling by $^3$H of the NH2-terminus compared to the COOH-terminus for the three chains at the various time points.9 The results are comparable for all three polypeptides and indicate that $\beta^A$, $\beta^{Leiden}$, and $\alpha$.
Fig. 2. Gradients of radioactivity in valine residues in (A) \( \beta^A \), (B) \( \beta^{\text{leiden}} \), and (C) \( \alpha \) globin after 3, 6, and 9.5 min of incubation with \(^3\)H-valine. After mixing the tritiated samples with a hemolysate uniformly labeled with \(^{14}\)C-valine, the globin chains were separated and fingerprinted. The valine-containing peptides were eluted from the paper and counted for \(^3\)H- and \(^{14}\)C-radioactivity. The specific activity of each tryptic peptide containing valine is expressed as the \(^3\)H/\(^{14}\)C ratio and is plotted against the corresponding position of the valine residue in the chain. In peptides containing more than one valine, the average position was used. Peptides \( \alpha \) T\(\beta\)XII and \( \alpha \) T\(\beta\)XIII were not isolated and the valine residues in those peptides are not indicated. The straight lines were determined by the least-squares method.

<table>
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<tr>
<th>Globin Chain</th>
<th>Relative Per Cent (^3)H(NH\textsubscript{2}/COOH)*</th>
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<tbody>
<tr>
<td>( \beta^A )</td>
<td>52.5 74.5 88.8</td>
</tr>
<tr>
<td>( \beta^{\text{leiden}} )</td>
<td>43.5 75.6 86.2</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>54.1 82.1 88.4</td>
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*Calculated by dividing the specific activity (\(^3\)H/\(^{14}\)C) of the NH\textsubscript{2}-terminus by the specific activity of the COOH-terminus. The specific activities were derived from the ends of the regression lines in Fig. 2. The results indicate the rate at which the specific activity of the NH\textsubscript{2}-terminus of a chain approaches that of the COOH-terminus with increasing time of incubation.

\( \beta^{\text{leiden}} \), and \( \alpha \) globin become uniformly labeled at the same time. By 9.5 min the NH\textsubscript{2}/COOH specific activity ratio was 0.86–0.89 for all three chains. In addition, the specific activities of the NH\textsubscript{2}-terminus and the COOH-terminus at the three time points were plotted against time, the slopes of those curves were determined, and the average translation time of each of the three chains was calculated according to the following equation:

\[
\text{Translation time (sec)} = \frac{(\text{mean sp. act. C-term}) - (\text{mean sp. act. N-term})}{(\text{slope C-term (sec}^{-1}) + (\text{slope N-term (sec}^{-1}))}
\]
The average translation times thus calculated for $\beta^A$, $\beta^{Leiden}$, and $\alpha$ globin were 65.4, 70.8, and 53.5 sec, respectively.

From the results of these sets of calculations, given the precision of the method, the translation times of all three globin chains appear to be the same.

**DISCUSSION**

Polypeptide chain biosynthesis is a multistep process, depending upon a number of nuclear and cytoplasmic events. Theoretically, any of several components or reactions could be involved in differentially altering the levels of structurally different hemoglobins within the erythrocyte. The present analysis indicates that the anomalies of globin chain biosynthesis, i.e., $\alpha/\beta$ imbalance and unequal $\beta^A$ and $\beta^{Leiden}$ production, observed in reticulocytes containing Hb Leiden, cannot be ascribed to altered rates of polypeptide elongation. The translation times of $\beta^A$, $\beta^{Leiden}$, and $\alpha$ globin are all approximately 1 min. However, the translation rates in peripheral blood reticulocytes may not reflect those in the younger nucleated erythroid elements of the bone marrow where the bulk of hemoglobin synthesis takes place. In addition, the method is probably not precise enough to demonstrate slight, but perhaps important, differences in elongation rates.

In previous studies of the synthesis of human globin chains by normal and thalassemic reticulocytes, translation times similar to those found in the present experiments were observed. Experiments on cells from patients with other hemoglobinopathies have also indicated that it takes in the range of 60-100 sec to complete a globin chain even if the chain is structurally abnormal. Although there is variation in the translation times of human globin reported in these separate studies, in individual experiments the rates of translation of normal and abnormal $\beta$ chains have been equal. The present study and the previous report by White, Lang, and Lehmann also suggest that human $\alpha$ and $\beta$ chains are translated at the same rate.

Several reports indicate that rabbit globin is translated much faster in reticulocytes than is human globin. This difference may be a function of the younger reticulocyte population one can induce in experimental animals. In the rabbit system, $\alpha$ and $\beta$ globin also appear to be translated at equal rates. In both rabbit and human reticulocytes, synthesis of $\alpha$ globin takes place on smaller polysomes than $\beta$ globin. This difference in the number of ribosomes per mRNA strand is thought to indicate that the rate of initiation of translation for $\alpha$ chains is lower than for $\beta$ chains. Equal production of $\alpha$ and $\beta$ globin must then depend upon a greater amount of $\alpha$ than $\beta$ mRNA within the cells.

The present type of experiment indicates only the time required for polypeptide chain elongation and release from the polyribosome. Alterations in the initiation process are not measured. $\beta^{Leiden}$ has a deletion near the NH$_2$ terminus, and it is conceivable that there is some overall defect in the initiation of $\beta$ globin mRNA translation in these cells. Otherwise, it would appear that an alteration in the relative amounts of $\alpha$ and $\beta$ globin mRNA is the other most likely explanation for the unbalanced globin synthesis in reticulocytes containing Hb Leiden.
REFERENCES

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