THE SYNTHESIS OF HUMAN HAEMOGLOBIN A_2 DURING ERYTHROID MATURATION

by

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SUMMARY

Synthesis of the δ-chains of human haemoglobin A_2 and the δβ-chains of haemoglobin Lepore has virtually ceased by the reticulocyte stage, in contrast to the β-chain of haemoglobin A. In bone marrow δ-chain synthesis is active particularly in younger precursors. These results suggest that there is a temporal dissociation between the synthesis of β and δ-chains, δ-chains only being synthesised briefly during erythroid development while β-chains are produced throughout erythroid maturation.

INTRODUCTION

The existence of two haemoglobins in such widely differing amounts in the same cell as human Hb A and Hb A_2 [Ref 1] offers a model system for studying some aspects of the control of protein synthesis in mammalian cells. There is good genetic evidence that synthesis of the α-chains of Hbs A and A_2 is controlled by the same locus (or loci) and that therefore they are derived from a common pool.[2] Thus the low levels of Hb A_2 must result from either a reduced rate of production or increased rate of destruction of the δ-chains.

Rieder and Weatherall (1965)[Ref 3] examined the incorporation of ^{59}Fe and ^{14}C leucine into Hbs A and A_2 in peripheral reticulocytes and bone marrow. These studies ruled out the premature destruction of haemoglobin A_2 in the circulation. However, there was a discrepancy between the relative specific activities of Hbs A and A_2 in

the marrow as compared with the reticulocytes, suggesting that Hb A and A_2 synthesis might not be synchronous during erythroid maturation. The findings were inconclusive, however, since the labelling in individual globin chains was not examined; indeed it has been shown subsequently that there is a marked discrepancy in the labelling of the α and δ-chains of Hb A_2 after *in vitro* incubation of reticulocytes

In the present experiments the *in vitro* synthesis of the globin chains of Hbs A and A_2 has been studied in both bone marrow and peripheral reticulocytes. Similar experiments have also been performed on the reticulocytes of a person heterozygous for haemoglobin Lepore, which has a composite δβ-chain produced by unequal genetic crossing over.[5] The results provide unequivocal evidence of asynchronous synthesis of β and δ-chains during erythroid maturation.

METHODS

Blood or marrow samples were obtained from patients with a variety of conditions in which haemoglobin synthesis was normal. The cells were washed and incubated with ^{14}C leucine at a concentration of 1-10 μCi/ml as previously described.[6] A preliminary separation of haemoglobins A and A_2 was carried out on DEAE Sephadex A50[ref 7] and further purification effected by starch block or cellulose acetate electrophoresis. The purity of the haemoglobin fractions was checked by starch gel electrophoresis.[6] The constituent globin chains of the purified Hbs were separated on CM-cellulose columns in 8M urea/mercaptoethanol buffer[8] and the total radioactivity incorporated into each chain and its specific activity was then determined.

RESULTS AND DISCUSSION

^{14}C leucine incorporation into Hbs A and A_2 in reticulocytes

Preliminary experiments indicated that there was a linear incorporation of isotope into haemoglobin A in reticulocytes for periods of up to 4 hours. All subsequent incubations were therefore

carried out within this time period. In a series of 9 experiments the ratio of the specific activities of Hbs A and A_2 after incubation periods ranging from 1-3 hours varied from 3.0 to 8.0. The synthesis of Hb A and its constituent α and β-chains was linear up to 4 hours, whereas haemoglobin A_2 synthesis showed a linear rise up to about 1 hour and then no further incorporation (Figure 1). Almost all the radioactivity in the Hb A_2 was in the α-chains while the δ-chains

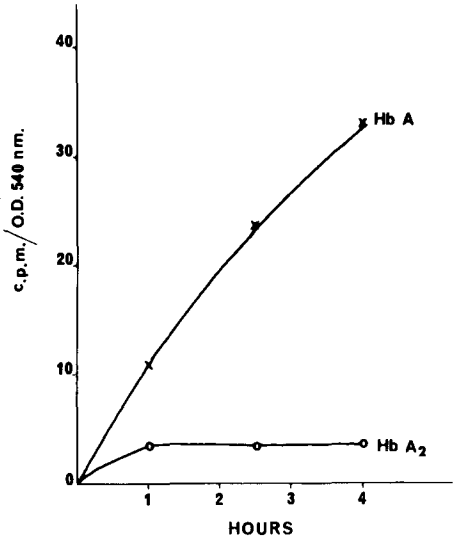

Figure 1

Incorporation of ^{14}C leucine into haemoglobins A and A_2 during *in vitro* incubation of reticulocyte-rich peripheral blood.

were virtually unlabelled (Figure 2). These results suggest that there is little or no synthesis of δ-chain at the reticulocyte stage, and that the radioactivity incorporated into Hb A_2 results from the exchange of α-chains between those of previously synthesised Hb A_2, and Hb A or the α-chain pool which is known to exist in human reticulocytes.[6] Experiments in which labelled Hb A was mixed with unlabelled Hb A_2 indicated that little exchange is likely to occur during the purification procedure after lysis of the cells.

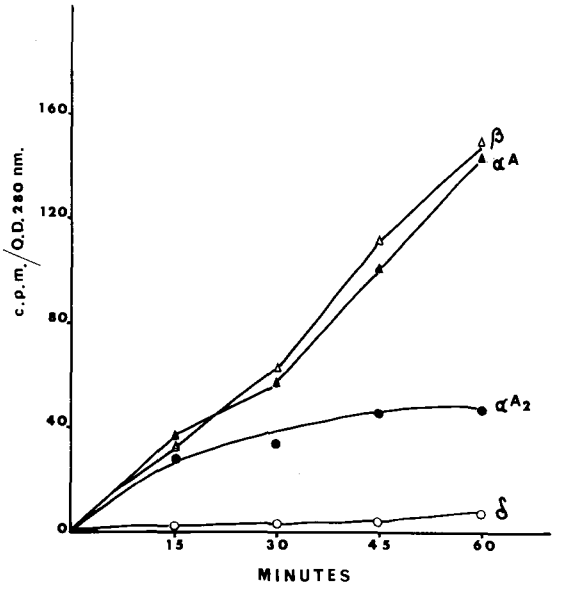

Figure 2

Incorporation of ^{14}C leucine into the individual chains of haemoglobins A and A_2 during reticulocyte incubations.

In a further experiment reticulocyte rich peripheral blood was separated into 'young' and 'old' populations by differential centrifugation.[6] After incubating the fractions with ^{14}C leucine the ratio of the specific activities of Hb A/Hb A_2 was 2.9 and 3.9 in 'young' and 'old' populations respectively.

The incorporation of ^{14}C leucine in Hb Lepore

Reticulocytes from an individual heterozygous for Hb Lepore Washington were incubated for 2 hours and samples removed at regular intervals. Hbs A, Lepore and A_2 were isolated and their specific activities determined. The relative incorporation into Hbs A and A_2 was similar to that seen in Figure 1 while that of Hb Lepore was intermediate between the two. When the constituent chains of Hb Lepore were analysed it was found that, as in Hb A_2, nearly all the counts were in the α-chain, presumably due to exchange with the α-chain pool, while the δβ-chains contained little incorporated

radioactivity, again suggesting that they are not being actively synthesised in reticulocytes.

All these experiments indicate that there is virtually no δ-chain synthesis in reticulocytes, except possibly in very young cells. For this reason haemoglobin A_2 synthesis was examined in bone marrow samples.

Incorporation of ^{14}C leucine into Hbs A and A_2 in marrow incubations

Bone marrow cells were incubated with ^{14}C leucine under identical conditions to those used for the reticulocyte experiments. After the cells had been washed, equal amounts of normal adult red cells were added to each sample as a source of carrier Hb. There was linear incorporation of ^{14}C leucine into both haemoglobins A and A_2 and into the individual α, β and δ-chains over a period of at least 90 minutes (Figure 3)

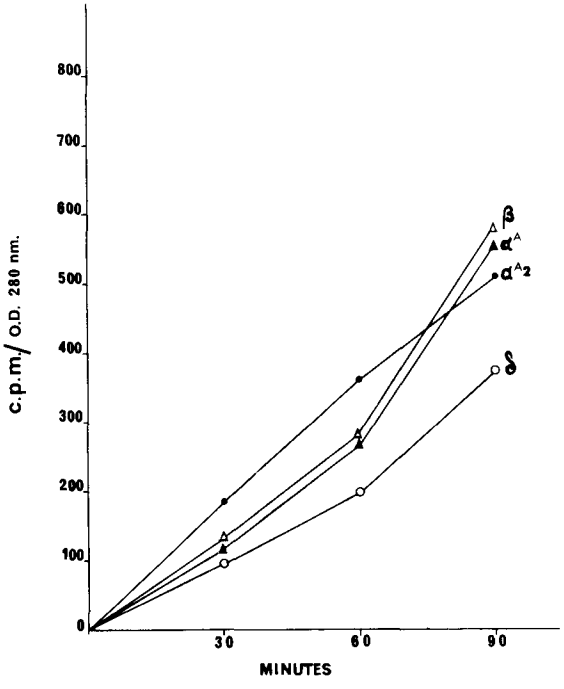

Figure 3

Incorporation of ^{14}C leucine into the chains of haemoglobins A and A_2 during in vitro incubation of marrow cells.

These results provide clear evidence that δ or δβ-chain production only occurs during the earlier stages of erythroid maturation and is almost complete at the late normoblast stage. The lack of δ-chain production is unlikely to be an *in vitro* artefact since linear δ-chain synthesis occurs in bone marrow samples incubated under identical conditions, and the previous investigation[3] indicates that the δ-chains of haemoglobin A_2 are stable and are not rapidly turned over in erythroid precursors.

The rapid decline in δ-chain synthesis during erythroid maturation may result from a very limited period of activity of the δ-chain locus in young red cell precursors or from a reduced rate of translation, or decreased stability, of mRNA for the δ-chain. The latter might follow from a reduced rate of initiation for δ-chain mRNA giving rise to small polysomes which are relatively more susceptible to ribonuclease.

We have not been able to confirm previous reports[9,10] which suggested that the translation time for δ-chain mRNA is increased compared with that for β-chain mRNA and find that, in bone marrow where Hb A and A_2 synthesis is linear, the assembly times of the β and δ-chains are not significantly different (AVR *et al*, MS in preparation). However, by examining the relative rates of synthesis of β and δ-chains in erythroid precursors separated by age on albumin density gradients,[11] we have obtained preliminary evidence that δ-chain synthesis is relatively more active in very young precursors. These findings suggest a true temporal dissociation between δ and β-chain production during erythroid maturation.

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