# RAPID TURNOVER OF NEWLY-SYNTHESIZED $\beta^S$ CHAINS IN RETICULOCYTES FROM INDIVIDUALS WITH SICKLE CELL TRAIT*

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SUMMARY. Reticulocytes were isolated from the blood of four non-anemic individuals with sickle cell trait who had low concentrations of hemoglobin S (25 to 30%). The cells were incubated with [$^3$H]leucine in a medium supporting protein synthesis for various times from 1.25 to 60 min. The ratio of specific radioactivity of the $\beta^S$ globin chain to that of the $\beta^A$ globin chain ($\beta^S/\beta^A$) of the total supernatant isolated from the labeled cells decreased toward unity from initial values of greater than 1.3 with increasing time of incubation. These results suggest that the disparity in peripheral blood concentrations of hemoglobins S and A in these individuals may be explained, at least in part, by a more rapid turnover of some of the newly-synthesized $\beta^S$ chains compared to the $\beta^A$ chains.

The peripheral blood concentration of hemoglobin S (HbS) is lower than that of hemoglobin A (HbA) in individuals with sickle cell trait (heterozygotes for the sickle cell gene). Most of these individuals have a blood hemoglobin composition of 35 to 45% HbS with the remainder largely HbA; a few individuals have only 25 to 30% HbS (1-3). The molecular basis for the lower blood concentration of the variant hemoglobin remains unknown. Presumably the HbS tetramer is itself a stable molecule in the blood cells of these individuals. Several investigators suggested that there is a relative decrease in synthesis of HbS which is proportional to the lower blood concentration. Thus, when erythroid cells of these heterozygotes were exposed to radioactive precursor molecules, either *in vivo* or *in vitro*, the resulting specific radioactivity of HbS was equal to that of HbA (4-7). Results of the current investigation

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suggest that in individuals with low concentrations of HbS (25 to 30%), some of the newly-synthesized $\beta^S$ chains are rapidly turned over or removed from the cellular soluble phase compared to the $\beta^A$ chains.

## METHODS

Blood was obtained from four healthy adult volunteers. A diagnosis of sickle cell trait was made on the basis of (a) the pattern observed after hemoglobin electrophoresis and (b) a positive turbidity test (Sickledex, Ortho Diagnostics) result. Serum iron concentrations were within normal limits. The ratios of concentrations of HbA to HbS in the blood (Table I) were determined from the absorbance of the hemoglobins, stained after electrophoresis

TABLE I

Globin Chain Specific Radioactivities

| Donor* (sex) | Ratio HbA/HbS | Incubation Type | Incubation Time | Specific Radioactivity $\beta^S$ | Specific Radioactivity $\beta^A$ | Ratio $\beta^S/\beta^A$ |
|---|---|---|---|---|---|---|
| | | | min | dpm/mg x $10^{-5}$ | | |
| E.P. | 71/29 | Pulse+ | 1.25 | 0.40 | 0.30 | 1.33 |
| (F) | | Chase | 2.50 | 0.63 | 0.56 | 1.13 |
| | | Chase | 5.00 | 0.57 | 0.61 | 0.93 |
| | | Chase | 10.00 | 0.53 | 0.59 | 0.90 |
| | | Pulse | 40.00 | 13.34 | 14.58 | 0.91 |
| J.P. | 71/29 | Pulse | 2.50 | 0.62 | 0.49 | 1.27 |
| (M) | | Chase | 10.00 | 1.13 | 1.22 | 0.93 |
| | | Pulse | 5.00 | 1.36 | 1.15 | 1.18 |
| | | Pulse | 10.00 | 3.60 | 3.32 | 1.08 |
| | | Pulse | 20.00 | 6.99 | 6.77 | 1.03 |
| | | Pulse | 40.00 | 11.00 | 11.73 | 0.94 |
| R.P. | 74/26 | Pulse | 2.50 | 1.59 | 1.23 | 1.29 |
| (F) | | Pulse | 5.00 | 2.97 | 2.49 | 1.19 |
| | | Pulse | 20.00 | 11.23 | 10.43 | 1.08 |
| | | Pulse | 60.00 | 17.79 | 18.48 | 0.96 |
| B.L. | 72/28 | Pulse | 2.50 | 2.30 | 1.81 | 1.27 |
| (F) | | Pulse | 5.00 | 5.26 | 4.39 | 1.20 |
| | | Pulse | 60.00 | 49.54 | 46.50 | 1.07 |

*J.P. and R.P. are son and daughter, respectively, of E.P.; B.L. is unrelated to the other three donors.

+See Fig. 1 for $\beta^S/\beta^A$ ratios for several additional incubation times of E.P.'s cells.

on cellulose acetate strips, as described previously (8).

For synthesis studies, venous blood (100 ml) was withdrawn in heparinized syringes and chilled in ice at 4°. A fraction of cells, rich in reticulocytes (15 to 25%), was obtained by centrifugation of the blood on gradients of Ficoll and Renografin, as described elsewhere (9). Packed reticulocytes (0.05 ml) were added to 0.15 ml of a 1 1/3-fold concentration of an incubation medium prepared without plasma as described by Boyer et al. (10) except for the omission of L-leucine in the amino acid supply. After incubation of this reaction mixture for 5 min at 37°, 0.8 mCi (0.05 ml) of L-[4,5-$^{3}$H]leucine (60,000 mCi per mmole) was added. Incubation of various assays at 37° was continued for specific times as indicated in the table and figure legend. The post-ribosomal supernatants were isolated from the labeled cells (8).

The specific radioactivities of the total $\beta^{S}$ and $\beta^{A}$ globin chains in the isolated cell supernatants were determined. Mercaptoethanol (10 $\mu$l) was added to 20 $\mu$l samples of the supernatants. After the mixture was incubated for 1 hr at 4°, 15 $\mu$l of 8 M urea were added, and the incubation was continued for an additional 15 min. About 8 $\mu$l of this mixture were applied in duplicate to cellulose acetate strips (Sepraphore III, Gelman Instrument Co.). To separate the globin chains, electrophoresis was done for 3 hr at 400 volts and 5° in a buffer (pH 8.8) of the following composition: 0.075 M Tris, 0.63 mM EDTA, 0.08 M boric acid, 0.05 M mercaptoethanol, and 6 M urea. After electrophoresis, the strips were stained with naphthol blue black and dried. The central regions of each globin chain band were excised and pooled in a glass vial, and 10 ml of a scintillation fluor (8) were added. The radioactivity was determined in a liquid scintillation counter, and the amount of protein (mg) in each solution was determined from the absorbance of the stain at 640 nm using an appropriate working curve. The globin chain specific radioactivity (dpm per mg) was calculated from these data.

## RESULTS

Reticulocytes were isolated from the blood of healthy, non-anemic individ-

uals with sickle cell trait who had low concentrations of HbS. The cells were incubated with [$^3$H]leucine in a reaction mixture which supported protein synthesis for various times from 1.25 to 60 min. The rate of incorporation of [$^3$H]leucine into soluble phase protein was nearly constant throughout the incubation period; most of the protein radioactivity comigrated with authentic Hbs A and S or their precursor chains during appropriate electrophoretic analyses (Shaeffer et al., unpublished data). Figure 1 (inset) shows the pattern of

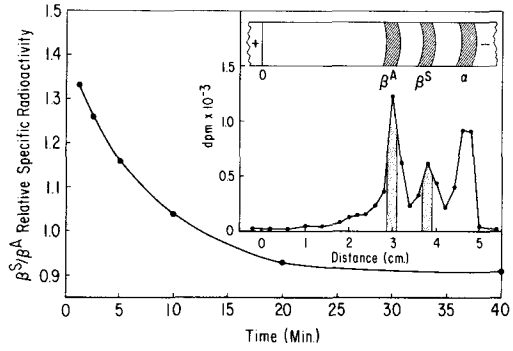


Fig. 1 Ratio of the specific radioactivities of the $\beta^S$ and $\beta^A$ globin chains. Replicate batches of E.P.'s cells were incubated for various times with [$^3$H]-leucine, and the globin chains of the labeled cell supernatants were separated by electrophoresis (see "Methods"). The inset shows the pattern of protein radioactivity obtained from cells incubated for 2.5 min. The globin chain specific radioactivities were determined from excised central regions (shaded areas) of the β chain bands.

globin chain radioactivity after electrophoresis, in the presence of urea and mercaptoethanol, of the supernatant isolated from E.P.'s cells incubated with [$^3$H]leucine for 2.5 min. Similar patterns were obtained after electrophoresis of supernatants isolated from cells incubated for other times, although the distribution of radioactivity among the globin chains varied with time of incubation.

The specific radioactivities (dpm per mg protein) of the $\beta^A$ and $\beta^S$ globin chains were determined from the central regions (see shaded areas, Fig. 1 inset) of the stained globin chains excised from the strips after electrophoresis

(see "Methods"). The ratio of the specific radioactivity of the $\beta^S$ chain to that of the $\beta^A$ chain decreased with time of incubation of E.P.'s cells (Fig. 1). Similar data were obtained from the supernatants of the $^3H$-labeled cells of the other three individuals (Table I). In two experiments, the cells from different individuals (E.P. and J.P.) were pulse-labeled with [$^3H$]leucine for 1.25 or 2.5 min and isolated by centrifugation; samples were incubated for additional times in a non-radioactive medium (chase). The $\beta^S/\beta^A$ specific radioactivity ratios of the supernatant globin chains decreased from values of 1.33 and 1.27 to values close to 0.9 during these chase incubations (Table I).

## DISCUSSION

During the incubations of these cells with [$^3H$]leucine an amount of protein very small compared to that already in existence was synthesized and labeled. Thus, the total cellular amount of $\beta^S$ or $\beta^A$ globin chains remained essentially unchanged. Determination of the globin chain specific radioactivities allows a comparison to be made of the amount of newly-synthesized chains to that of chains already extant. In experiments with cells from individuals heterozygous for a variant $\beta$ chain, the $\beta^{variant}/\beta^A$ ratio of specific radioactivities can be used as a measure of the relative rates of synthesis and/or degradation of abnormal and normal $\beta$ chains (11). For example, in studies on the synthesis patterns of unstable hemoglobin Köln (12) or Sabine (8) in blood reticulocytes, the $\beta^{variant}/\beta^A$ specific radioactivity ratio decreased toward unity as the time of cellular incubation with radioactive amino acids increased. These data suggested that some of the variant $\beta$ chains were degraded or removed from the soluble phase of the cell at a faster rate than the normal $\beta^A$ chains; thus, the results were consistent with the unstable nature of the variant hemoglobins.

In the current study of synthesis patterns in the cells of heterozygotes with low concentrations of HbS, the $\beta^S/\beta^A$ specific radioactivity ratios were close to unity when the cells were incubated with [$^3H$]leucine for times suf-

ficiently long, i.e. generally greater than 20 min. These results confirm those of other workers (13). However, the findings that the values of the $\beta^S/\beta^A$ specific radioactivity ratio were substantially greater than unity for short times of incubation and that these values decreased with time suggest that some of the newly-synthesized $\beta^S$ chains were removed from the soluble phase at a rapid rate compared to the $\beta^A$ chains. Moreover, the observation that the $\beta^S/\beta^A$ ratio of pulse-labeled cells decreased during chase incubations is consistent with this conclusion.

We suggest that the following is one possible model, consistent with these results, for hemoglobin synthesis and assembly in reticulocytes of individuals with sickle cell trait who have low concentrations of HbS. Newly-synthesized $\beta^S$ and $\beta^A$ globin chains are released from their respective polyribosomes into small soluble pools. All or nearly all of the $\beta^A$ chains combine with soluble $\alpha$ chains to form HbA. Free $\beta^S$ globin chains are less stable than $\beta^A$ globin chains, and some of the former are rapidly removed from the soluble phase before they have a chance to combine with $\alpha$ chains; other $\beta^S$ globin chains combine with $\alpha$ chains to form the relatively stable HbS tetramer. Perhaps $\beta^A$ chains inherently combine more readily with $\alpha$ chains than do $\beta^S$ chains (14). Experiments are currently in progress to determine if (a) the disparity in blood concentrations of HbA and HbS in individuals with low concentrations of HbS (25 to 30%) can be explained entirely by the proposed rapid turnover of some of the newly-synthesized $\beta^S$ chains, (b) individuals with higher concentrations of HbS have the same pattern of hemoglobin synthesis as those with low concentrations of HbS, and (c) the newly-synthesized $\beta^S$ chains lost from the soluble phase appear in the cell ghosts, as was reported recently for newly-synthesized $\beta^S$ chains in blood cells isolated from individuals with sickle cell anemia (15). The patterns of synthesis and degradation of other stable β-chain variants of HbA, such as Hbs C and E, may be similar to that suggested for HbS.

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