UNBALANCED GLOBIN CHAIN SYNTHESIS IN RETICULOCYTES OF SICKLE CELL TRAIT INDIVIDUALS WITH LOW CONCENTRATIONS OF HEMOGLOBIN S*

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SUMMARY. Reticulocytes were isolated from the blood of eight nonanemic individuals with sickle cell trait; five donors had relatively low hemoglobin S concentrations (26 to 32%) and three had high hemoglobin S concentrations (40 to 42%). The cells were incubated with [^{3}H]leucine in a medium supporting protein synthesis. Total α chain synthesis was equal to total β chain synthesis in cells from the high hemoglobin S donors. In cells from low hemoglobin S donors the rate of α chain synthesis was about 65 to 80% that of total β chain synthesis. These data suggest that there was a deficit in α chain synthesis in the cells of sickle cell trait individuals with low hemoglobin S concentrations. The results are consistent with the presence of an α-thalassemia or α-thalassemia-like gene in the low hemoglobin S people.

Individuals with sickle cell trait have both hemoglobin A (HbA) and hemoglobin S (HbS) in their peripheral blood. In most of these people the concentration of HbS is 35 to 45% of the total blood hemoglobin, the remainder being largely HbA (1-4). In a few individuals the blood concentration of HbS is as low as 25 to 30%. This laboratory has been investigating the molecular basis for the lower concentration of the variant hemoglobin.

Several years ago Neel et al. (5) reported that the concentration of HbS in sickle cell trait people appeared to be under genetic control, because many, though not all, of the relatives of an individual with a given amount of HbS had similar amounts of the variant hemoglobin. These same investigators suggested that "modifying" genes, in addition to the sickle cell gene, might be responsible for the wide variation in HbS concentration among the sickle cell trait population. Subsequently, several investigators (6-9) reported, on the basis of genetic and

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hematological data, that sickle cell trait individuals with low concentrations of HbS, i.e. 25 to 30%, might carry a gene for α-thalassemia, an inherited disorder characterized primarily by a decrease in the production of hemoglobin α chains (10,11); however, hemoglobin synthesis studies were not done in these investigations.

The objective of the present work was to determine (a) if there was a relative deficit in α chain synthesis in erythroid cells obtained from sickle cell trait individuals with either low or high (about 40%) concentrations of HbS, and (b) the role such a deficit would have in the molecular basis for the disparity in the concentrations of the two hemoglobins.

METHODS

Venous blood (100 to 125 ml) was withdrawn from eight healthy, nonanemic adult volunteers into heparinized syringes and chilled in ice at 4°. Samples of the blood were used to make a diagnosis of sickle cell trait on the basis of (a) the pattern observed after hemoglobin electrophoresis and (b) a positive turbidity test result (Sickledex, Ortho Diagnostics). Blood hemoglobin and serum iron concentrations were within normal limits. The ratios of concentrations of HbA to HbS in the blood were determined from the absorbance of the hemoglobins, stained after electrophoresis on cellulose acetate strips, as described previously (12).

A fraction of cells with 15 to 20% reticulocytes was isolated from the blood by centrifugation on gradients of Ficoll and Renografin, as described elsewhere (13). The reticulocyte-rich cells were incubated with [^{3}H]leucine in a reaction mixture supporting protein synthesis for various times from 1.25 to 60 min (14). The postribosomal supernatants were isolated from the ^{3}H-labeled cells, and, after denaturation of the supernatants with urea and mercaptoethanol, the α, β^A, and β^S globin chains were separated by electrophoresis on cellulose acetate strips (14). The specific radioactivity (dpm per mg protein) of each globin chain was determined from the stained electrophoresis strips (14), and the ratio of total α to total β chain radioactivity of each supernatant was calculated from these specific radioactivities, as described in the legend to Table I.

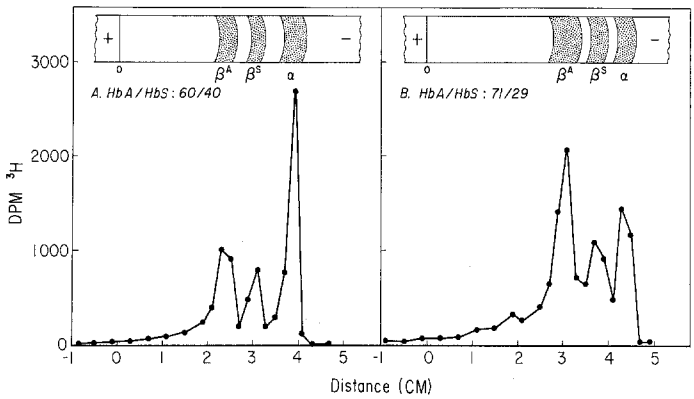

Fig. 1. Patterns of protein radioactivity obtained after separation of the globin chains of ^{3}H-labeled cell supernatants by electrophoresis on cellulose acetate strips. Reticulocyte-enriched cells were incubated with [^{3}H]leucine for 5 min as described in "Methods" and ref. (14), and 20 µl samples of the isolated supernatants were denatured with 10 µl of mercaptoethanol and 15 µl of 8 M urea. After electrophoresis of this mixture the strips were stained, dried, and cut into 2 mm sections for radioactivity assay. The results were obtained from cells of (A) an individual (I.B.) with a high HbS concentration, and (B) a donor (E.P.) with a low HbS concentration. The positions of the stained globin bands are shown at the top of each panel.

RESULTS

Reticulocyte-rich fractions of cells isolated from the blood of five donors with low HbS concentrations (26 to 32%) and three donors with high HbS concentrations (40 to 42%) were incubated with [^{3}H]leucine. Fig. 1 shows the patterns of protein radioactivity obtained after denaturation and electrophoresis, in the presence of urea and mercaptoethanol, of representative supernatants from the cells of individuals with (A) high and (B) low concentrations of HbS. Most of the protein radioactivity migrated with authentic globin chains, which were identified in separate experiments by coelectrophoresis of the ^{3}H-labeled supernatants with purified, uniformly ^{14}C-labeled Hbs A or S obtained from cells incubated with [^{14}C]leucine (data not shown). The fraction of globin radioactivity which migrated with the α chain compared to that of the two β chains was substantially higher for individuals with high HbS concentrations (Fig. 1A) than for low HbS donors (Fig. 1B).

The ratio of total α chain to total β chain radioactivity of each supernatant was calculated from the globin chain specific radioactivities determined,

TABLE I

Globin Chain Specific Radioactivities and Total α/β Synthesis Ratio

Donor* (A/S Ratio)	Incubation time	Specific Radioactivity β^S	β^A	α	Synthesis Ratio+ αdpm/βdpm
	min	dpm/mg x 10^{-5}			
J.S.	5.0	-	1.54	1.54	1.00
(A/A Control)	20.0	-	5.88	6.03	1.02
I.B.	5.0	0.87	0.91	0.90	1.01
(60/40)	20.0	3.97	3.95	4.00	1.01
M.C.	1.25	0.22	0.25	0.23	0.97
(59/41)	20.0	8.76	9.13	8.64	0.96
E.S.	20.0	11.12	10.62	11.60	1.07
(58/42)	60.0	22.66	21.28	21.57	0.99
A.C.	1.25	1.63	1.14	0.99	0.76
(68/32)	5.0	7.67	6.05	4.99	0.76
B.L.	5.0	5.57	4.72	3.99	0.80
(72/28)	60.0	70.82	67.35	52.09	0.76
E.P.	5.0	1.97	1.70	1.16	0.65
(71/29)	10.0	4.67	4.49	3.03	0.67
J.P.	2.5	0.62	0.49	0.41	0.78
(71/29)	40.0	11.00	11.73	8.57	0.74
R.P	5.0	6.05	4.50	3.47	0.71
(74/26)	20.0	19.18	17.39	12.78	0.72

Replicate batches of each donor'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 Fig. 1). The specific radioactivities were determined from excised central regions of each globin chain band, as described previously (14).

*J.P. and R.P are son and daughter, respectively, of E.P.; the other donors are unrelated to each other and to the P. family.

+The ratio of total α chain to β chain radioactivity of the cell supernatant was calculated from the globin chain specific radioactivities (α, β^A, β^S) and the peripheral blood HbA/HbS concentration ratio (A/S) by using the following formula:

$$\alpha\text{dpm}/\beta\text{dpm} = [\alpha(A/S + 1)]/[\beta^A (A/S) + \beta^S]$$

which was derived with the assumptions that (a) the total cellular amount (mg) of α chains equals the combined amounts of β^A and β^S chains and (b) the cellular pools of globin and hemoglobin chains precursor to Hbs A and S are small in amount compared to those present in the tetramer hemoglobins. Each α or β chain has 18 residues of leucine, and thus the αdpm/βdpm ratio represents the ratio of subunit chain synthesis. Similar results were obtained when the chain synthesis ratios were determined directly from the total radioactivities associated with each globin chain peak in the electrophoretic patterns rather than from the specific radioactivities of the central regions; however, because of the approximately 10 to 20% overlap of radioactivity among different globin chains (see Fig. 1), the synthesis ratios determined from the specific radioactivities are more accurate.

as previously described (14), from electrophoresis strips similar to those of Fig. 1. Table I shows that, for several incubation times, total α chain synthesis was equal to total β chain synthesis in cells from individuals with high HbS con-

centrations or from a control person, homozygous for normal HbA. Conversely, in cells from donors with low HbS concentrations, the rate of α chain synthesis was about 65 to 80% that of total β chain synthesis. These data suggest that there was a deficit in α chain synthesis in the cells of individuals with low HbS concentrations. Moreover, the red cell indices and morphology of the low HbS donors showed microcytosis and hypochromia in the absence of iron deficiency and elevated HbA_2 concentrations. Collectively, these results are consistent with the presence of an α-thalassemia or α-thalassemia-like gene in these individuals (10).

DISCUSSION

The molecular basis for the lower blood concentration of HbS compared to that of HbA in individuals with sickle cell trait remains unknown. The presence of an α-thalassemia gene may be partially responsible for the lower HbS concentration in those people who have it. Thus, we reported earlier that newly synthesized β^S chains turned over or were removed from the soluble phase more rapidly than newly synthesized β^A chains in reticulocytes of individuals with low HbS concentrations (14). The following is one possible model of hemoglobin assembly, modified slightly from that proposed earlier (14), consistent with these data. Newly synthesized α, β^A, and β^S globin chains are released from their respective polyribosomes into small soluble pools. The β^A chains inherently combine more readily with α chains than do β^S chains. When α chains are made in limiting amounts because of the presence of an α-thalassemia gene, HbA is assembled in preference to HbS. The excess β^S globin chains are unstable and are removed from the soluble phase. A similar model was proposed by Weatherall (7,15) to explain the putative association of α-thalassemia with low peripheral blood concentrations of HbS and the concomitant failure to detect a β_4^S molecule (9); a tetramer of β^S chains was not observed also in the present work. Past studies suggested that similar phenomena may be occurring in the cells of individuals who have other stable β chain variants, e.g. Hbs C (7,8,16) and E (17,18), in association with α-thalassemia, although hemoglobin synthesis experiments were not done.

The major finding of the present work is a relative deficit of α chain synthesis compared to β chain synthesis in reticulocytes of sickle cell trait individuals with low HbS concentrations. Although this result is consistent with the presence of an α-thalassemia gene in these individuals, additional studies of the members of their families are in progress to provide evidence confirming the inherited nature of the association of the chain synthesis imbalance with low HbS concentrations. Other investigators have reported, in a preliminary manner (19), results of hemoglobin synthesis studies of sickle cell trait individuals similar to those documented here.

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