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Research Article

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Preferential Binding of β^S Globin Chains Associated with Stroma in Sickle Cell Disorders

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ABSTRACT Sickle cell anemia (SS) is associated with abnormalities of the red cell membrane and decreased red cell deformability. The present study assesses globin chain binding to stroma in SS, sickle cell trait (AS), and nonsickling (AA) cells. The results indicate that there is preferential binding of newly synthesized β^s globin to red cell stroma in SS cells and preferential binding of β^s to stroma compared to β^A in AS cells. These studies show that β^s globin binding to stroma accompanies the membrane abnormalities in SS and AS patients.

INTRODUCTION

Sickle cell anemia (SS disease)[1] is associated with abnormalities of the red cell membrane and decreased red cell deformability (1–4). An increase in membrane-bound hemoglobin is associated with SS disease cells (1, 2). The role of the membrane-associated hemoglobin in SS disease in shortening the life-span of the red blood cells has not been characterized. In patients with sickle cell trait (AS disease), the red blood cell deformability may also be decreased (4) and red cell survival shortened in experimental models (5). The present study was undertaken to assess the role of individual globin chains in the pathogenesis of abnormalities of the membrane of SS and AS cells. The results indicate that newly synthesized β^s chains in reticulocytes are preferentially bound to the stroma in patients with both SS and AS disease.

METHODS

Heparinized blood was collected from patients with SS, AS, sickle cell-thalassemia (S-thalassemia) and nonsickling (AA) hemolytic disorders associated with AA hemoglobin. The plasma was removed at 2,000 rpm for 10 min, and the cells were washed twice with normal saline. 1 vol of packed cells, usually 3–6 ml, was incubated with 1 vol of Krebs Ringer bicarbonate solution, containing all amino acids except leucine, to which 1/10th vol of [^{3}H]leucine (New England Nuclear, Boston, Mass.), had been added, and the pH was adjusted to 7.6 (6). The cells were incubated for 1 h at 37° in room air. After incubation, the cells were washed three times with 10–20 vol of cold normal saline. The cells were then lysed with 20 vol of a solution containing 1 mM EDTA, pH 7.2. Stroma was recovered by centrifuging the hemolysate at 15,000 rpm for 20 min at 4°C. The supernate was decanted and saved. The stroma was washed 5–10 times with 20–30 vol of a solution containing 1 mM EDTA, pH 7.2, until the washes were visually clear. The stroma still contained some hemoglobin identified by a slight pink color after washing. Centrifugation was at 15,000 rpm for 15 min. Supernates and stromas were frozen at −20°C until use.

Analysis of globin chain content. Aliquots of ^{3}H-labeled stroma or supernatant fractions were added to ^{14}C-labeled hemolysates, obtained either from AA or SS patients, and to a varying amount of unlabeled hemolysate. The recovery of [^{3}H]leucine in α and β globin chains was corrected by using the relative recovery of [^{14}C]leucine α vs. β globin (see Table I). In the studies of AS patients, [^{14}C]hemolysates from SS and AA patients were mixed and used as labeled carrier. The ^{3}H incorporations in $(\beta^A + \beta^S)/\alpha$ were corrected by using the relative recovery of ^{14}C incorporations in $(\beta^A + \beta^S)/\alpha$. The relative recovery of [^{14}C]β^A and -β^S did not vary significantly. Globin chain chromatography was performed by using a modification of the method originally described by Clegg, Naughton, and Weatherall (7, 8). Aliquots of fractions collected from carboxymethyl cellulose (CM-cellulose) chromatography were counted in

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[1] *Abbreviations used in this paper:* AA, nonsickling; AS disease, sickle cell trait; CM-cellulose, carboxymethyl cellulose; SS disease, sickle cell anemia; S-thalassemia, sickle cell thalassemia.

TABLE I

Globin Synthesis in Patients with SS Disease and AA Disorders

Patient	Diagnosis	Reticulocytes	Supernate ³H cpm β^A	Supernate ³H cpm α	Supernate ³H $\frac{\beta^A}{\alpha}$	Supernate ¹⁴C $\frac{\beta^A}{\alpha}$	Supernate Corrected $\frac{\beta^A}{\alpha}$	Stroma ³H cpm β^A	Stroma ³H cpm α	Stroma ³H $\frac{\beta^A}{\alpha}$	Stroma ¹⁴C $\frac{\beta^A}{\alpha}$	Stroma Corrected $\frac{\beta^A}{\alpha}$
1	Iron deficiency	10	55,368	54,529	1.0	0.8	1.2	26,524	27,037	1.0	0.8	1.2
2	Iron deficiency	12	385,483	324,954	1.2	0.9	1.2	48,832	33,832	1.4	1.1	1.2
3	Pernicious anemia	11	7,923	15,761	0.5	0.7	0.8	11,062	3,780	2.9	0.8	3.6
4	Myelofibrosis	10	7,740	9,680	0.8	0.7	1.1	16,758	11,932	1.3	0.7	1.9
5	Auto-immune hemolytic anemia	—	3,911	6,343	0.6	0.6	1.0	7,986	12,522	0.6	0.6	1.0
6	Iron deficiency	9	24,643	31,482	0.8	0.6	1.4	12,800	10,160	1.2	0.7	2.0
Mean ±SD							1.1 ±0.2					1.8 ±0.9
			³H cpm β^S	³H cpm α	³H $\frac{\beta^S}{\alpha}$	¹⁴C $\frac{\beta^S}{\alpha}$	Corrected $\frac{\beta^S}{\alpha}$	³H cpm β^S	³H cpm α	³H $\frac{\beta^S}{\alpha}$	¹⁴C $\frac{\beta^S}{\alpha}$	Corrected $\frac{\beta^S}{\alpha}$
7	Sickle cell anemia	15	103,428	129,784	0.8	0.8	1.0	45,478	36,883	1.2	0.8	1.7
8	Sickle cell anemia	15	40,760	54,358	0.8	0.8	1.0	45,011	30,531	1.4	0.8	2.0
9	Sickle cell anemia	20	49,055	60,078	0.8	0.8	1.0	19,886	12,019	1.7	0.8	2.0
10	Sickle cell anemia	18	40,237	42,865	0.9	0.9	1.0	70,805	32,063	2.3	0.9	2.6
11	Sickle cell anemia	21	32,813	42,733	0.8	1.0	0.8	47,210	13,125	3.6	1.0	3.6
Mean ±SD							0.96 ±0.2					2.4 ±0.7

a Packard tricarb liquid scintillation counter on double label settings (Packard Instrument Co., Inc., Downers Grove, Ill.) which gave an efficiency for ³H of 10% and for ¹⁴C of 30%. The counting error was less than 1%.

RESULTS

Comparison of globin bound to SS and AA cells stroma. Supernate and stroma-bound globin chains

TABLE II

Globin Synthesis in AS and S-Thalassemia Patients

		³H cpm β^A	³H cpm β^S	³H cpm α	Corrected $(\beta^A + \beta^S)/\alpha$*	β^A↑ str/sup‡	β^S↑ str/sup‡
AS Patient							
1	Supernate	12,978	10,185	23,009	1.0	—	—
	Stroma	3,746	5,087	4,336	2.0	1.0	1.8
2	Supernate	28,274	22,272	39,053	1.2	—	—
	Stroma	10,617	12,203	13,081	1.8	0.9	1.4
3	Supernate	7,099	4,476	15,569	0.8	—	—
	Stroma	3,603	6,299	5,985	1.7	0.9	2.4
4	Supernate	15,464	12,235	34,445	0.8	—	—
	Stroma	8,668	17,526	17,340	1.5	0.8	2.1
Mean±SD						0.9±0.07	1.9±0.38
S-Thalassemia patient							
5	Supernate	4,329	12,658	32,646	0.5	—	—
	Stroma	1,156	3,569	3,014	1.7	1.7	1.8
6	Supernate	5,958	10,405	19,767	0.8	—	—
	Stroma	7,541	19,175	11,589	2.3	1.2	1.7

* ³H incorporation in $(\beta^A + \beta^S)/\alpha$ corrected for relative recovery of ¹⁴C incorporations in $(\beta^A + \beta^S)/\alpha$ carrier globin (mixed SS and AA hemolysates).

‡ β^A or β^S as % of total cpm in stroma/β^A or β^S as % of total cpm in supernate.

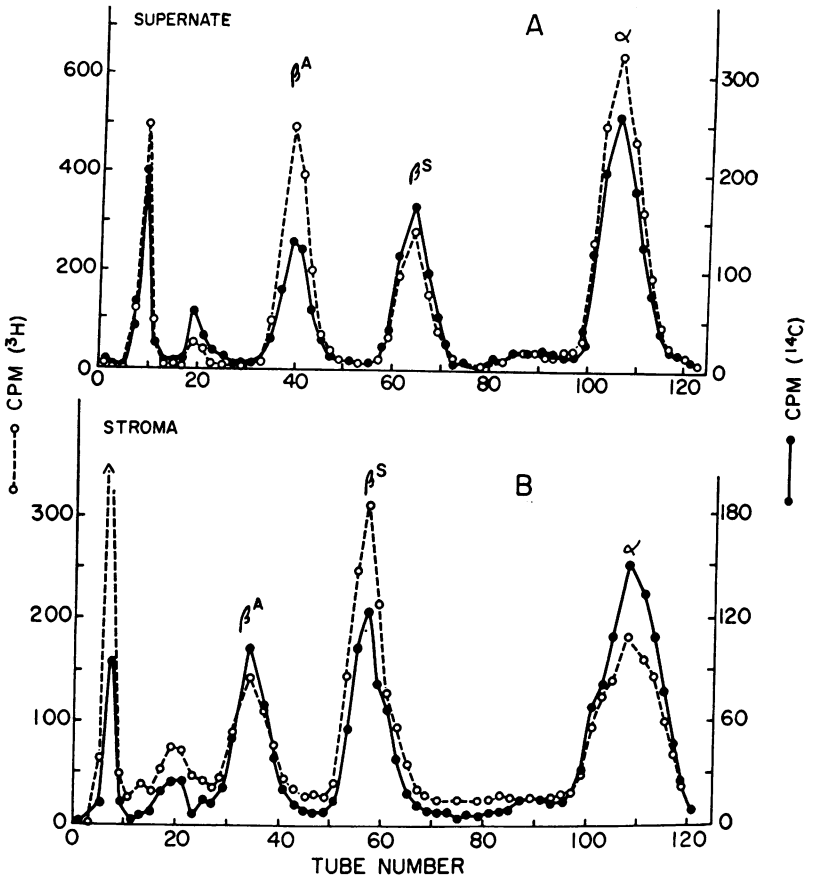

FIGURE 1 Globin chromatography of supernate and stroma obtained from AS patient 1 (Table II). (A) Supernate; (B) Stroma. (●----●) ^{3}H-labeled globin from patient. (●——●) [^{14}C]globin carrier from SS and AA intact cells.

were examined in six patients with AA reticulocytoses (Table I). In three of the six patients (patients 1, 2, and 5, Table I), there is no increase in the relative amount of β^A compared to α chains bound to stroma. In two patients (patients 4 and 6, Table I), there is a modest increase in the relative amount of β^A compared to α globin (β^A/α) bound to stroma as compared to that present in the supernate. In patient 3, Table I, there is a marked increase in the β^A/α bound to stroma compared to that in supernate. There is no statistically significant difference in the β^A/α ratios in supernate and stroma of the AA patients as a group ($P > 0.20$). The percent of β^A counts bound to stroma compared to the total β^A present varies from 1.3–8% and was greatest in patients 3 and 4, respectively. Similar studies were performed on five patients with SS disease (Table I). The average increase in β^s/α bound to stroma as compared to supernate is 2.5-fold, significantly higher than that in the AA patients (Table I). The range is between 1.7- and 4.5-fold. The increase in the β^s/a globin in stroma compared to supernate is significant at the 0.1% level ($P < 0.001$). Between 9.4 and 15% of the total β^s present in the cells is bound to stroma. The ^{3}H-labeled stroma of AA patients gave no significant radioactivity in the β^s region; similarly, ^{3}H-labeled stroma of SS patients gave no significant radioactivity in the β^A region from CM-cellulose chromatography.

Binding of globin chains to stroma in patients with AS disease. In four patients studied with AS disease (Table II, Fig. 1), there is also a significant increase in the relative amount of total β compared to α globin ($[\beta^A + \beta^s]/\alpha$) present in the stroma as compared to that in the supernate ($P < 0.01$). While there is relatively balanced ($[\beta^A + \beta^s]/\alpha$) synthesis in the supernate of these four patients, there is a 1.8- to 2.2-fold increase in the relative amount of total β globin associated with stroma compared to α globin (Table II). The increase in total β counts bound to stroma in the reticulocytes of these patients is preferentially an increase in β^s counts associated with stroma (Table II). The β^A cpm in

supernate and stroma expressed as percent of total globin cpm is similar, while the percent of total globin cpm in stroma which are β^S counts is increased between 1.4- and 2.4-fold (Table II). The relative increase in β^S over β^A in stroma as compared to supernate is statistically significant ($P < 0.01$). In two patients with S-thalassemia (Table II), there is also a relative increase in total β counts per α counts in stroma as compared to those in the supernate. In one of these patients, this increase appears to be due preferentially to binding of β^S globin, while in the other, both β^A and β^S counts bind to stroma. In the AS and S-thalassemia patients, the percent of β^S bound to stroma is between 5 and 15%.

DISCUSSION

In this study, the binding of newly synthesized globin chains to stroma has been studied by comparing the relative amounts of labeled globin chains present in the supernate with globin chains associated with red cell stroma. Although there is some variability in β^A binding to AA stroma, there is no statistically significant preferential β^A binding. By contrast, there is a significant increase in the amount of β^S globin relative to α globin bound to stroma in AS, SS, and S-thalassemia patients. Patients with AS were examined in order to control for differences between SS and AA cells. In AS patients, both β^A and β^S chains are synthesized in the same cells. In the four AS patients studied, the preferential binding of β globin chains to stroma is due primarily to increased binding of β^S chains.

It is unclear from these studies whether β^S globin chains are preferentially bound to stroma in vivo in SS or AS cells or whether the preferential β^S binding occurs only in vitro during incubation, lysis, and subsequent stromal isolation. Only reticulocytes are synthesizing globin as measured in these experiments; thus, if β^S binding to stroma is occurring in vivo, the observations reported here only reflect events in reticulocytes. In one experiment in which ^{3}H-labeled SS supernate was added to unlabeled AA stroma in vitro (data not shown), there was no preferential binding of β^S over α chains to stroma. This result may be due to limited availability of free β^S chains for binding to AA stroma or differences between in vitro and in vivo conditions. In addition, there is no information on the number of unlabeled globin chains or hemoglobin molecules already bound to stroma or the number of binding sites available in SS or AA cells which may affect subsequent binding of labeled globin chains.

Several other variables must be controlled before these observations are well understood in terms of their physiologic significance. No special attention was taken to the state of oxygenation; the cells were incubated in room air. The pH and ion concentrations used for cell lysis and stromal isolation may also affect the findings. In addition, the pool of α chains present in different cells was not accurately measured and might affect the relative binding of α and β globin to stroma. Despite these limitations, the experiments presented here show that under the conditions used, there is a preferential binding of β^S globin to stroma in human red cells. In addition, there is preferential binding of β^S globin to AS and SS stroma compared to β^A globin binding to AA red cell stroma. Schneider, Takeda, Gustavson, and Alperin (2) have reached similar conclusions by using different methods. The stromal-bound β^S globin chains may contribute to the decreased deformability and increased hemolysis of SS cells. AS cells have been reported to have a decreased deformability even when oxygenated (4) and shortened survival when injected into rats (5). If preferential binding of β^S chains to stroma occurs during early erythroid cell development, then β^S globin binding to stroma in bone marrow cells may lead to preferential destruction of the cells containing stromal-bound β^S chains and favor the survival of AS cells that contain decreased amounts of β^S globin. This phenomenon could explain the decreased amount of Hb S relative to Hb A produced by reticulocytes and present in mature AS red cells. In addition, genetic differences in membrane structure may affect the relative binding of β^S and Hb S to stroma and may help explain the wide variations in degree of anemia and clinical manifestations observed in different patients with SS disease.

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