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**Research Article**

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# Preferential Binding of $\beta^S$ Globin Chains Associated with Stroma in Sickle Cell Disorders

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A B S T R A C T   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 $\beta^S$ globin to red cell stroma in SS cells and preferential binding of $\beta^S$ to stroma compared to $\beta^A$ in AS cells. These studies show that $\beta^S$ 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 $\beta^S$ chains in reticulocytes are preferentially

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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.

bound to the stroma in patients with both SS and AS disease.

## METHODS

## Table I
### Globin Synthesis in Patients with SS Disease and AA Disorders

| Patient | Diagnosis | Reticulocytes | Supernate ³H cpm βᴬ | α | ³H βᴬ/α | ¹⁴C βᴬ/α | Corrected βᴬ/α | Stroma ³H cpm βᴬ | α | ³H βᴬ/α | ¹⁴C βᴬ/α | Corrected βᴬ/α |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | | | | | | | 1.1 | | | | | 1.8 |
| ±SD | | | | | | | ±0.2 | | | | | ±0.9 |

| Patient | Diagnosis | Reticulocytes | Supernate ³H cpm β^s | α | ³H β^s/α | ¹⁴C β^s/α | Corrected β^s/α | Stroma ³H cpm β^s | α | ³H β^s/α | ¹⁴C β^s/α | Corrected β^s/α |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | | | | | | | 0.96 | | | | | 2.4 |
| ±SD | | | | | | | ±0.2 | | | | | ±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 βᴬ | β^s | α | Corrected (βᴬ + β^s)/α* | βᴬ† 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).

‡ $\beta^A$ or $\beta^s$ as % of total cpm in stroma/$\beta^A$ or $\beta^s$ as % of total cpm in supernate.

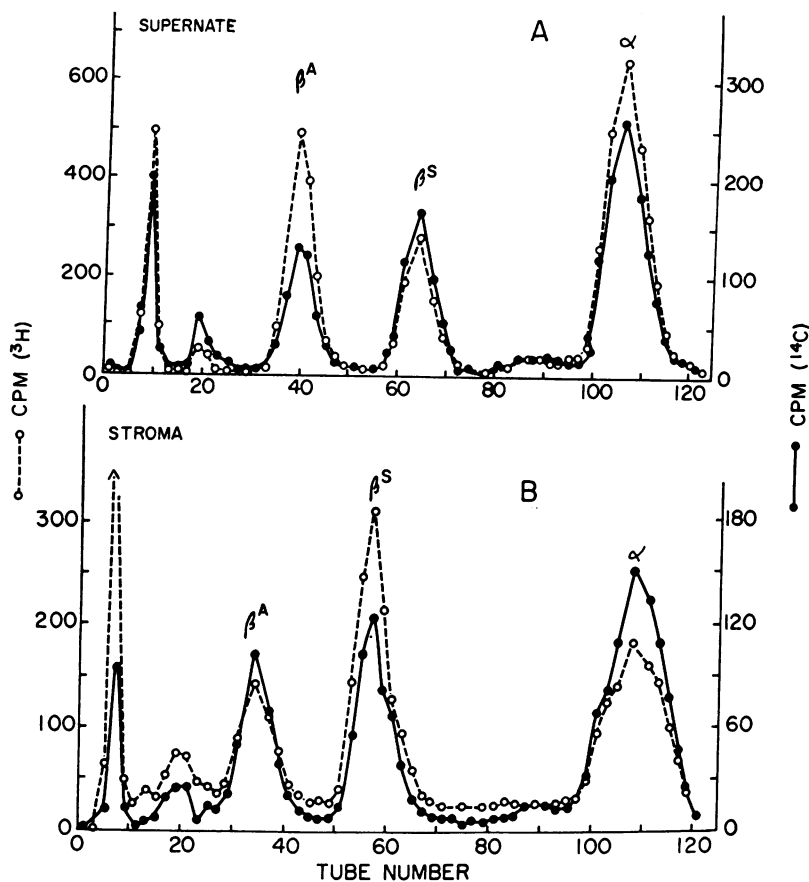806    A. Bank, G. Mears, R. Weiss, J. V. O'Donnell, and C. Natta

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 $\beta^A$ compared to $\alpha$ chains bound to stroma. In two patients (patients 4 and 6, Table I), there is a modest increase in the relative amount of $\beta^A$ compared to $\alpha$ globin ($\beta^A/\alpha$) bound to stroma as compared to that present in the supernate. In patient 3, Table I, there is a marked increase in the $\beta^A/\alpha$ bound to stroma compared to that in supernate. There is no statistically significant difference in the $\beta^A/\alpha$ ratios in supernate and stroma of the AA patients as a group ($P > 0.20$). The percent of $\beta^A$ counts bound to stroma compared to the total $\beta^A$ 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 $\beta^s/\alpha$ 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 $\beta^s/\alpha$ globin in stroma com-

pared to supernate is significant at the 0.1% level ($P < 0.001$). Between 9.4 and 15% of the total $\beta^s$ present in the cells is bound to stroma. The $^3$H-labeled stroma of AA patients gave no significant radioactivity in the $\beta^s$ region; similarly, $^3$H-labeled stroma of SS patients gave no significant radioactivity in the $\beta^A$ 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 $\beta$ compared to $\alpha$ 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 $\beta$ globin associated with stroma compared to $\alpha$ globin (Table II). The increase in total $\beta$ counts bound to stroma in the reticulocytes of these patients is preferentially an increase in $\beta^s$ counts associated with stroma (Table II). The $\beta^A$ cpm in

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supernate and stroma expressed as percent of total globin cpm is similar, while the percent of total globin cpm in stroma which are $\beta^s$ counts is increased between 1.4- and 2.4-fold (Table II). The relative increase in $\beta^s$ over $\beta^A$ 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 $\beta$ counts per $\alpha$ 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 $\beta^s$ globin, while in the other, both $\beta^A$ and $\beta^s$ counts bind to stroma. In the AS and S-thalassemia patients, the percent of $\beta^s$ 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 $\beta^A$ binding to AA stroma, there is no statistically significant preferential $\beta^A$ binding. By contrast, there is a significant increase in the amount of $\beta^s$ globin relative to $\alpha$ 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 $\beta^A$ and $\beta^s$ chains are synthesized in the same cells. In the four AS patients studied, the preferential binding of $\beta$ globin chains to stroma is due primarily to increased binding of $\beta^s$ chains.

It is unclear from these studies whether $\beta^s$ globin chains are preferentially bound to stroma in vivo in SS or AS cells or whether the preferential $\beta^s$ binding occurs only in vitro during incubation, lysis, and subsequent stromal isolation. Only reticulocytes are synthesizing globin as measured in these experiments; thus, if $\beta^s$ 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 $\beta^s$ over $\alpha$ chains to stroma. This result may be due to limited availability of free $\beta^s$ 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 $\alpha$ chains present in different cells was not accurately measured and might affect the relative binding of $\alpha$ and $\beta$ globin to stroma. Despite these limitations, the experiments presented here show that under the conditions used, there is a preferential binding of $\beta^s$ globin to stroma in human red cells. In addition, there is preferential binding of $\beta^s$ globin to AS and SS stroma compared to $\beta^A$ globin binding to AA red cell stroma. Schneider, Takeda, Gustavson, and Alperin (2) have reached similar conclusions by using different methods. The stromal-bound $\beta^s$ 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 $\beta^s$ chains to stroma occurs during early erythroid cell development, then $\beta^s$ globin binding to stroma in bone marrow cells may lead to preferential destruction of the cells containing stromal-bound $\beta^s$ chains and favor the survival of AS cells that contain decreased amounts of $\beta^s$ 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 $\beta^s$ 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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