Hemoglobin-Spectrin Complexes: Interference With Spectrin Tetramer Assembly as a Mechanism for Compartmentalization of Band 1 and Band 2 Complexes


The irreducible complexation of hemoglobin with spectrin is a natural phenomenon of red blood cell aging, positively correlating with increasing cell density and decreasing cell deformability. The current study begins to address the role of these complexes in the disruption of membrane skeletal physiology and structure. The effect of bound hemoglobin on spectrin dimer self-association was investigated in vitro. The extent of conversion of isolated spectrin dimers to tetramers was evaluated as a function of peroxide-induced globin complexation before the conversion incubations. The incremental accumulation of tetramer was observed to decrease with increasing peroxide concentration used in the globin complexation step. The role of oxidized heme in this process was made apparent by the inability of carboxyhemoglobin to inhibit tetramer accumulation. A Western blot analysis of naturally formed globin-spectrin conjugates demonstrated irreducible complexes of globin with both bands 1 and 2. The complexes are tentatively designated "h1" and "h2". This analysis also demonstrated that h1 is completely extractable from cell ghosts, whereas h2 is only 50% extractable. These findings are incorporated into a hypothesis linking globin-spectrin complexation and the consequent inhibition of spectrin dimer self-association to the clustered band 3 senescence antigen (Low et al., Science 227:531, 1985). © 1995 by The American Society of Hematology.

THE DECLINE OF red blood cell (RBC) deformability with cell age is a function of changes in cell shape, cytoplasmic viscosity, and membrane deformability. Membrane deformability, in turn, is crucially dependent on the ability of the cytoskeletal spectrin network to undergo unfolding and refolding. When circulation stresses result in forces beyond the limit of reversible deformability, then cytoskeletal protein junctional complexes are temporarily broken. Among the weakest of these junctions are the head-to-head associations of the spectrin dimers.

We have previously shown that irreducible complexation of hemoglobin (Hb) with spectrin is a natural phenomenon of RBC aging, positively correlating with increasing cell density and decreasing cell deformability.2,4 Experimentally, in vitro exposure to peroxide results in signs of accelerated cell senescence, including generation of Hb-spectrin complexes, decreased cell deformability, and cell surface alterations that lead to enhancement of phagocytosis by monocytes.5-8 Significantly, prior treatment of human RBCs with carbon monoxide completely inhibits the cellular alterations induced by peroxide, a finding that is consistent with the established role of Hb oxidation as the initial step in the production of oxygen free radicals in RBCs.9,10 The present study takes a more direct approach to the question of what effect bound Hb has on spectrin dimer self-association. In other words, does the interposition of Hb into a spectrin dimer-dimer junction under conditions of membrane shear stress constitute the wedge that initiates diminishing membrane deformability? Ektacytometric measurements of elliptocytic RBC membranes have shown that the increased spectrin dimer:tetramer ratio in these membranes was associated with a decrease in deformability to one tenth the normal value.10 The stability of these membranes was reduced as well, but only to 0.26 to 0.28 the normal level. Thus, uncoupled spectrin dimers appear to affect membrane deformability more adversely than they affect membrane stability. Moreover, the potential exists that globin-bound uncoupled dimers may facilitate an increased lateral mobility of attached band 3 molecules and, thus, their clustering into the senescence antigens described by Low et al.11 Spectrin extracted from membranes at 37°C is almost entirely in the form of αβ heterodimers that easily undergo reversible self-association in dilute solution at 30°C to form α2β2 tetramers.12,13 At 4°C, there is little or no interconversion. Our experimental design is to test whether peroxide-induced binding of Hb to spectrin dimers results in a diminished capability of spectrin self-association into tetramers, beyond that due to peroxidation of spectrin alone. We also show by Western blotting the existence of two types of natural globin-spectrin complexes within the membrane skeleton and show a partitioning of these two types within the skeleton on the basis of their differential extractability.

MATERIALS AND METHODS

Preparation of RBC Ghosts and Spectrin Extracts

Venous human blood was drawn on heparin and immediately processed. RBCs were washed three times in 5 mmol/L sodium phosphate buffer (pH 7.4)/150 mmol/L NaCl. Membrane ghosts were prepared by hypotonic lysis in 5 mmol/L sodium phosphate (pH 8.0)/1 mmol/L EDTA in the presence of the protease inhibitor phenylmethylsulfonyl fluoride (0.1 mmol/L) at 10 vol lysate per 1 vol packed cells. Alternatively, ghosts were prepared from packed...
cells by freeze/thaw lysis (dry ice/methanol bath). Residual hemoglobin in the ghosts was removed by incubating for 10 minutes at 0°C with lysis buffer in the presence of 0.5 mmol/L diisopropyl fluorophosphate (DFP), followed by a final wash in 0.1 mmol/L sodium phosphate (pH 8.0)/0.1 mmol/L EDTA/0.1 mmol/L diithioerythritol (DTE).

For the preparation of spectrin tetramer extracts, ghosts were incubated for 16 hours at 0°C with 10 mmol/L sodium phosphate (pH 8.0) using 1 vol of extraction buffer per 1 vol packed cells. Extracted ghosts were then pelleted by centrifugation at 243,000g for 1 hour at 4°C, and the spectrin extract in the supernatant was purified by sucrose density centrifugation (below).

Spectrin extracts were also prepared from RBCs that had been treated with hydrogen peroxide, as previously outlined. Briefly, washed RBCs were adjusted to a hematocrit of 20% in glucose-enriched phosphate-buffered saline with 1.0 mmol/L NaCl. Hydrogen peroxide was added to the cell suspension to final concentrations of 0, 349, or 849 μmol/L. The cell suspension with peroxide was then incubated for 15 minutes in a shaking water bath. Ghosts and spectrin extracts were then prepared as described above.

**Spectrin Purification**

Spectrin step gradients were used for isolation of spectrin tetramers from ghost extracts (above) and for isolation of peroxide-generated Hb-spectrin complexes (below) from free Hb. Step gradients were prepared in 12.7 × 50.8-mm ultracentrifuge tubes by sequential overlay of 0.9 mL each of 20% (wt/vol) sucrose with 17%, 13%, 9%, and 5% sucrose in 10 mmol/L TRIS-HCl (pH 7.4). Spectrin extracts (above), up to 0.5 mL, were overlaid onto the step gradients and centrifuged at 82,500g, 4°C, for 16 to 18 hours. Fractions of 0.2 to 0.3 mL were sequentially removed from the top of the gradient.

Protein concentration of each fraction was determined from absorbance at 280 nm, using E_{1%}^{1cm} (absorbance of a 1% [wt/vol] solution in a cell having an absorption path of 1 cm) human erythrocyte spectrin = 8.8.

**Preparation of Hb for Complexation With Spectrin**

Venous human blood was drawn on heparin, and RBCs were pelleted and washed three times in 10 mmol/L sodium phosphate (pH 7.4)/150 mmol/L NaCl. Washed RBCs were vortexed with an equal volume of distilled water and subjected to three freeze/thaw cycles (dry ice/methanol bath). Cell membranes were pelleted at 48,000g, 4°C, for 30 minutes, and the hemolyte supernatant was recovered by aspiration. HbA was isolated from the hemolyte by anion-exchange chromatography using DE52 cellulose (Whatman, Inc, Clifton, NJ) and batch-eluting with 5 mmol/L sodium phosphate (pH 7.4). Protein concentration was determined photometrically (S PLUS; Coulter, Miami, FL).

**Preparation of Globin-Complexed Soluble Spectrin**

HbA was complexed in vitro with soluble spectrin by the heme-catalyzed Fenton reaction. Reactions of HbA (0.39 mg/mL) were prepared (2.4 μmol/L, 1.25 mg/mL) were initiated with H_{2}O_{2} (147 or 489 μmol/L) in 10 mmol/L sodium phosphate (pH 7.4)/150 mmol/L NaCl and incubating for 15 minutes at 37°C. Control incubations of spectrin included: (1) no HbA, no H_{2}O_{2}, (2) HbA, no H_{2}O_{2}; (3) no HbA, 147 μmol/L H_{2}O_{2}; (4) no HbA, 489 μmol/L H_{2}O_{2}; and (5) HbA preincubated with CO for 10 minutes at 20°C, 489 μmol/L H_{2}O_{2}. The HbA-spectrin reactions were then adjusted to 20 mmol/L DTE/0.5 mmol/L DFP/0.1 mmol/L EDTA, and incubated a further 15 minutes at 37°C, followed by dialysis against 10 mmol/L sodium phosphate (pH 8.0) at 4°C. The spectrin preparations were then isolated from free HbA by sucrose step gradients as described above.

**Analysis of Soluble Spectrin Self-Association**

**Dimer-tetramer interconversion.** After the isolation of globin-spectrin complexes from free HbA, the spectrin dimers were allowed to reversibly convert to tetramers. These reactions were performed at 1.25 mg/mL in 5 mmol/L sodium phosphate (pH 7.4)/150 mmol/L NaCl/1 mmol/L DFP/0.1 mmol/L DTE and were allowed to reach equilibrium by incubation for 4 hours at 30°C. The uncomplicated spectrin controls were incubated similarly.

**Agarose-acrylamide gel electrophoresis and calculation of dimer-tetramer conversion.** The spectrin dimer-tetramer mixtures were evaluated both before and after the 4-hour interconversion incubations, using electrophoresis under nondenaturing conditions on 0.3% agarose. 2.5% acrylamide composite cylindrical gels run at 4°C.

**Calculation of Extractable Spectrin**

ghosts and spectrin extracts from peroxide-treated cells (described above) were electrophoresed on 4% cylindrical gels by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), using the Fairbanks buffer system, then stained and scanned as described for spectrin dimer-tetramer analysis. The unextracted ghosts used for SDS-PAGE analysis were suspended in the same volume as that used for the spectrin extractions. From the scan printouts, the peak areas corresponding to the dimer and tetramer bands were carefully cut out and weighed to the nearest 0.05 mg. The extent of conversion from dimer to tetramer forms during the 4-hour incubation was expressed as the percentage of spectrin in the tetrameric form: ie, tetramer weight/dimer weight + dimer weight) at 4 hours less that at 0 hours. The percent conversion at each time point for each trial mixture was averaged from two runs.

**Quantitation of Globin-Spectrin Complex Concentration**

Western blots of cell ghosts, extracted spectrin, and residual membranes. SDS-PAGE of cell ghosts, extracted spectrin, and residual membranes was performed with the Laemmli buffer system, a 6% separating gel in the mini format (55 × 85 × 0.75 mm; Bio-Rad Laboratories, Hercules, CA), and a five-slot, 4% stacking gel. One lane was reserved for high-molecular-weight markers (45 to 200 kD; Bio-Rad Laboratories) to calculate relative molecular mass (M) values of reactive sample bands. Samples of known protein content (bicinchoninic acid method; Pierce, Rockford, IL) were heated (100°C, 5 minutes) in sample buffer containing dithioerythritol and electrophoresed (200 V) so that the pyronin Y marker dye traversed 5.0 cm. The electrophoresed samples were transferred (150 mA) to a nitrocellulose membrane (0.10 μm pore; Micron Separations Inc, Honeyoye Falls, NY). After transfer, lane positions were determined, and sample lanes were split vertically. For each sample lane, one of the half-lanes was stained for total protein using colloidal gold (Bio-Rad Laboratories). The other half-lane was probed with the antigen monoclonal antibody-hb-1-294, 15 μg IgG per milliliter, for 1 hour at 25°C. The buffer TTBS (0.2% Tween 20 in TBS: 20 mmol/L Tris-HCl, pH 7.5/0.500 mmol/L NaCl) was used to block the membrane before antibody incubation, to carry both first and enzyme-conjugated secondary antibodies, and to wash the membrane after each antibody incubation. Development of the blot was then performed with romochloriodophenyl/nitroblue tetrazolium (BCIP/ NBT; Moss Inc, Pasadena, MD) for 15 minutes at 25°C.
Densitometry. Densitometric analysis of the developed blots was performed at 540 nm reflectance, scanning each half-lane (7 to 8 mm in width) three times at 2-mm intervals with a 0.05 × 2.0-mm beam and using a CS-9000 scanner and the CSTURBO 1D Analysis Program (Shimadzu Corp, Kyoto, Japan). Antibody-reactive bands identified by molecular weight shifts as globin-spectrin complexes were densitometrically integrated by the analysis program, converting average band colorimetric intensities into area units. Quantities of each globin-spectrin complex were expressed both as raw area units and as specific activities relative to total sample protein. The gold-stained components in the residual membrane sample were identified by molecular weight shifts as globin-spectrin complexes from ascites fluid essentially followed that previously described for the human HbA and immune splenic B lymphocytes derived from an RBF/Dn mouse. The reactivity of hh-1-294 versus isolated globin chains was assessed by dot blot. The alpha and beta globins were isolated from Hb in the oxidized state, reflects an intermolecular crosslinking of spectrin rather than tetramer formation. Supporting this view, carboxyhemoglobin/peroxide-treated spectrin showed no increased tetramer concentration at 0 hours (Fig 1G v E or A). Moreover, Western blot analysis of spectrin + Hb ± peroxide, incubated as described for globin complexation (Materials and Methods) and then brought to hypotonic, 0°C conditions, showed an irreducible high-molecular-weight band that reacted with monoclonal antibodies specific for Hb, spectrin α, and spectrin β (C.R.K., unpublished data, December 1994). Although we have not yet definitively established the relative molecular mass of this band, we tentatively conclude that this represents globin-complexed, cross-linked spectrin heterodimers.

RESULTS

Effect of Globin Complexation on Soluble Spectrin Self-Association

The potential of bound hemoglobin to obstruct spectrin dimer self-association was tested in a cell-free system, measuring shifts in dimer:tetramer ratios at equilibrium as a function of peroxide concentration used in the globin complexation step. The results of the soluble spectrin dimer-tetramer equilibration trials are shown in Fig 1. Each stained gel pair depicts dimer and tetramer concentrations both at the start of and after the 4-hour equilibration. The incremental accumulation of tetramer is seen to be much less in the case of spectrin complexed with globin at 489 μmol/L H₂O₂ (Fig 1F, 4 hours v 0 hours) than occurs with spectrin treated with the same concentration of peroxide alone (Fig 1E) or with the untreated spectrin (Fig 1A). Spectrin complexed with globin at 147 μmol/L H₂O₂ shows a less inhibited accumulation of tetramer (Fig 1D v C or A). It is noted that the concentration of soluble tetramer at the start of the incubation appears to be higher for the globin-complexed spectrins than for the corresponding peroxide-treated or untreated spectrins (Fig 1F, 0 hours v E or A, 0 hours; Fig 1D, 0 hours v C or A, 0 hours). Even the treatment by Hb without peroxide appears to induce a small increase in tetramer concentration at 0 hours (Fig 1B). As the reaction conditions before the start of the incubation (hypotonic environment, 0°C) do not favor tetramer formation, we suggest that the apparent increase in tetramer concentration at 0 hours, particularly with Hb in the oxidized state, reflects an intermolecular crosslinking of spectrin rather than tetramer formation. Supporting this view, carboxyhemoglobin/peroxide-treated spectrin showed no increased tetramer concentration at 0 hours (Fig 1G v E or A). Moreover, Western blot analysis of spectrin + Hb ± peroxide, incubated as described for globin complexation (Materials and Methods) and then brought to hypotonic, 0°C conditions, showed an irreducible high-molecular-weight band that reacted with monoclonal antibodies specific for Hb, spectrin α, and spectrin β (C.R.K., unpublished data, December 1994). Although we have not yet definitively established the relative molecular mass of this band, we tentatively conclude that this represents globin-complexed, cross-linked spectrin heterodimers.

To compensate for elevated pre-equilibration tetramer backgrounds, cited above, in assessing the self-association trials, the parameter used to compare the variously treated spectrins was the incremental increase in tetramer concentration expressed as a percentage difference: i.e., Tᵢ(Tᵢ + Dᵢ) - T₀(T₀ + D₀), where T and D refer to the tetramer and dimer integrated concentrations, respectively, at 4 or 0 hours of incubation. The incremental percent tetramer increases for control spectrin, globin-spectrin complexes at 147 and 489 μmol/L H₂O₂, and carboxyhemoglobin/peroxide-treated spectrin are presented in Table 1, which summarizes the equilibration trials shown in Fig 1A, D, F, and G. It is noted that the incremental increase in tetramer accumulation decreases with increasing concentration of H₂O₂ used to create the oxyhemoglobin-complexed spectrin.

Spectrin Extractability

To determine the effect on self-associational properties of spectrin extracted from peroxide-treated RBCs, similar di-
Table 1. Incremental Increase in Spectrin Tetramer Concentration as a Function of Peroxide Treatment In Vitro

<table>
<thead>
<tr>
<th>H₂O₂ (μmol/L)</th>
<th>% Tetramer Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>40 ± 4</td>
</tr>
<tr>
<td>147</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>489</td>
<td>9 ± 4</td>
</tr>
<tr>
<td>CO + 489</td>
<td>39 ± 5</td>
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</table>

Incremental (percentage) increases in tetramer concentration over a 4-hour incubation were quantified from equilibration trials of globin-spectrin complexes produced at 0, 147, and 489 pmol/L H₂O₂ and of carboxyhemoglobin/peroxide-treated spectrin, as shown for one trial in Fig 1, A, D, F, and G. Values are average of two trials.

Table 2. Spectrin Extractability From Peroxide-Treated RBCs

<table>
<thead>
<tr>
<th>H₂O₂ (μmol/L)</th>
<th>% Extracted Spectrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80 ± 13</td>
</tr>
<tr>
<td>349</td>
<td>71 ± 18</td>
</tr>
<tr>
<td>849</td>
<td>42 ± 18</td>
</tr>
</tbody>
</table>

Equal numbers of cell ghosts and equal volumes of extracted spectrin from each peroxide treatment were electrophoresed. Data are representative of total spectrin in corresponding cell ghosts and are the average of three runs.

Fig 2. Split-lane Western blot analysis of RBC membrane proteins for high-molecular-weight globin complexes. Reduced proteins from unfractionated cell ghosts (2.00 μg), residual membranes after spectrin extraction (0.60 μg), and the extracted spectrin were electrophoresed and transferred to nitrocellulose. The blotted lane positions are noted at the top. Vertical cuts through the center of each lane created four panels, two of which were probed with the antiglobin monoclonal antibody hh-1 and developed using the alkaline phosphatase:BCIP/NBT system. The other two (unlabeled) panels were stained with colloidal gold to reveal the total protein profile of each lane. Positions of the globin-spectrin complexes (h₁ and h₂), bands 1, 2, and 3, and globin are indicated on the left.
The current study recognizes both P and occur with apparent equimolar stoichiometry. This suggests globin-spectrin binding are limited to the identity of the head region. At this point, our data on the specifics of from steric or allosteric effects of globin binding at or near globin complexation in vitro (Fig 1 and Table 1) may result that the inhibition of spectrin dimer self-association after globin chains composing the h1 and h2 complexes (this study and our previous report). Previously, we had shown that a globin-specific monoclonal antibody (P-thalassemic cells (excess, unpaired α globin) being more defective than α-thalassemic cells.

**Compartmentalization of Complexes**

The h2 globin-spectrin complexes are far less extractable than the h1, remaining in the residual membranes (Fig 2 and Table 3). This suggests that h2 complexes (but not h1) are tightly associated with integral membrane proteins, eg, the anion channel band 3 protein, through atypical interactions catalyzed or mediated through the globin components. The h2 complex (human α globin-β spectrin) appears to be associated with disrupted membrane skeletal structure, which can be appreciated in β-thalassemic cells. Yuan et al have demonstrated that the erythroid precursors of severely β-thalassemic cells exhibit accumulative α globin deposition beginning as early as the proerythroblast stage. Preliminary double-immunolabeling studies have demonstrated sites of colocalization of α globin with spectrin in these precursors. At these sites, the spectrin often appeared clumped rather than exhibiting usual smooth distribution (rim fluorescence).

Although we have used peroxide to experimentally accelerate the rate of formation of globin-spectrin complexes, the complexes themselves did not appear to differ physiologically from those encountered naturally (as shown in Fig 2). Thus, peroxide-generated h1 complexes were completely extractable regardless of the peroxide concentration used (not shown). We suggest that under natural conditions of oxidative stress, the heme iron of the spectrin-bound globin catalyzes intermolecular covalent bonding through generation of hydroxyl radicals from H$_2$O$_2$—a natural by-product of aerobic metabolism. Heme iron has been shown to be capable of catalyzing cyclical generation of hydroxyl radicals via the Fenton reaction. We have demonstrated the natural occurrence of globin-spectrin irreducible covalent bonds (Fig 2), but do not rule out the possibility that other types of intermolecular crosslinkages may result from the suggested mechanism, cyclical generation of hydroxyl radicals, or from other undefined mechanisms. As pointed out, inextractable h2 accounts for about one half of total h2 naturally found in unfractioned RBCs (Table 3), but only about one third of total inextractable β spectrin; ie, h2/(h2 + band 2) (Table 4).

The poor extractability of the h2 complexes is postulated to result from their inclusions in localized disruptions of membrane skeletal structure. A current working hypothesis regarding the formation of the membrane signal for senescent RBC destruction holds that hemoglobin denaturation leads to hemichrome formation, the binding of hemichrome tetramers to high affinity sites on band 3 dimers, and the subsequent clustering of band 3 dimers to form the senescence antigen on the cell surface, to which IgG binds, thereby signaling RBC removal by the reticuloendothelial system. Band 3 clustering implies an atypical lateral mobility within the plane of the cell membrane, ie, one that is unrestricted by the constraints of the spectrin network. We envision that globin-spectrin complex formation and the con-

**Table 4. Inextractable Spectrin Components**

<table>
<thead>
<tr>
<th>Component</th>
<th>Area Units*</th>
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<tbody>
<tr>
<td>h1</td>
<td>0</td>
</tr>
<tr>
<td>Band 1</td>
<td>6.65</td>
</tr>
<tr>
<td>h2</td>
<td>6.29†</td>
</tr>
<tr>
<td>Band 2</td>
<td>13.31†</td>
</tr>
</tbody>
</table>

* Integrated densitometric scan of gold-stained residual ghost profile, Fig 2.
† Area units calculated from divided chromatogram peak.

Judged by their molecular weight shifts (Fig 2), the irreducible covalent complexes of globin and spectrin chains occur with apparent equimolar stoichiometry. This suggests that the inhibition of spectrin dimer self-association after globin complexation in vitro (Fig 1 and Table 1) may result from steric or allosteric effects of globin binding at or near the head region. At this point, our data on the specifics of globin-spectrin binding are limited to the identity of the globin chains composing the h1 and h2 complexes (this study and our previous report). Previously, we had shown that a β globin-specific monoclonal antibody (β10-1-18946), recognizing an epitope centered approximately at β10 Ala, detected an h1 complex found in sickle cell membranes, but not h2 complex. As the monoclonal antibody used in the current study (hb-1-294) recognizes both β and α globin, one may conclude that the h2 complex contains α globin. Thus, the human h1 complex is β globin-α spectrin, and the h2 complex is α globin-β spectrin. Preliminary data from the baboon system indicate that the globin compositions are reversed, ie, α globin-α spectrin and β globin-β spectrin (not shown).

An alternative interpretation of the inhibited dimer self-association after globin complexation may be that the junctional sites on the spectrin subunits are oxidatively damaged by hydroxyl radicals generated from H$_2$O$_2$ by the heme iron in the complexed globin, as described below. In a cellular context, this would probably require that the location of the complexed Hb be at or near the dimer-dimer junction because of the limited effective range of the free radical.

Lamchiagdha et al have reported defective spectrin dimer self-association in thalassemic RBCs. In their study, spectrin was extracted from various hemoglobinopathic subjects, and dimer-tetramer conversion measurements were performed by methods similar to those described herein. Spectrin extracted from severely thalassemic cells showed significantly defective dimer self-association properties, with β-thalassemic cells (excess, unpaired α globin) being more defective than α-thalassemic cells.

**Physiologic Effects of Complexes**

Physiologic Effects of Complexes

<ref>Table 3</ref> lists the relative proportions of the inextractable spectrin (band 1, h2, band 2) as scanned from the gold-stained residual membrane profile in Fig 2. It can be seen that h2 accounts for about one third (6.29/19.60 area units) of the total inextractable β spectrin (h2 + band 2).

The h2 globin-spectrin complexes are far less extractable than the h1, remaining in the residual membranes (Fig 2 and Table 3). This suggests that h2 complexes (but not h1) are tightly associated with integral membrane proteins, eg, the anion channel band 3 protein, through atypical interactions catalyzed or mediated through the globin components. The h2 complex (human α globin-β spectrin) appears to be associated with disrupted membrane skeletal structure, which can be appreciated in β-thalassemic cells. Yuan et al have demonstrated that the erythroid precursors of severely β-thalassemic cells exhibit accumulative α globin deposition beginning as early as the proerythroblast stage. Preliminary double-immunolabeling studies have demonstrated sites of colocalization of α globin with spectrin in these precursors. At these sites, the spectrin often appeared clumped rather than exhibiting usual smooth distribution (rim fluorescence).

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sequent inhibition of spectrin dimer self-association provides for the loosening of constraints on band 3 lateral mobility.

ACKNOWLEDGMENT

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REFERENCES


