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Functional topography of band 3: Specific structural alteration linked to functional aberrations in human erythrocytes

(anion transport/ankyrin binding/electron paramagnetic resonance spectroscopy)

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ABSTRACT **Band 3 is the major anion transport polypeptide of erythrocytes. It appears to be the binding site of several glycolytic enzymes. Structurally, band 3 is the major protein spanning the erythrocyte membrane and connects the plasma membrane to band 2.1, which binds to the cytoskeleton. In the present study, we report an alteration of band 3 molecule that is associated with the following changes: erythrocyte shape change from discoid to "thorny cells" (acanthocytes), restriction of rotational diffusion of band 3 in the membrane, increase in anion transport, and decrease in the number of high-affinity ankyrin-binding sites. Changes in erythrocyte IgG binding, glyceraldehyde-3-phosphate dehydrogenase, fluorescence polarization (indicative of membrane fluidity), and other membrane proteins as determined by polyacrylamide gel electrophoresis were not detected. Cells containing the altered band 3 polypeptide were obtained from individuals with abnormal erythrocyte morphology. Two-dimensional peptide maps revealed differences in the M_r 17,000 anion transport segment of band 3 consistent with additions of tyrosines or tyrosine-containing peptides. The data suggest that (*i*) this alteration of band 3 does not result in accelerated aging as does cleavage and (*ii*) structural changes in the anion transport region result in alterations in anion transport.**

Band 3 is the major anion transport polypeptide of erythrocytes (1, 2) and, in addition, appears to be the binding site for the glycolytic enzymes aldolase (3) phosphofructokinase (4), glyceraldehyde-3-phosphate dehydrogenase (5), and hemoglobin (6). Water transport across the membrane has been ascribed to band 3 (7).

Senescent cell antigen, an aging protein that terminates the life-span of cells (8–13), appears to be derived from band 3 (11, 12). Appearance of senescent cell antigen initiates IgG binding and cellular removal (8–10).

Structurally, the M_r ≈95,000 band 3 molecule crosses the membrane between 3 and 12 times (14–16). A cytoplasmic segment containing the amino terminus binds to band 2.1 (ankyrin), which attaches to the internal filamentous cytoskeleton (17). Band 3 has been found in all cells examined (18–22).

To date, functional changes linked to a structural defect within the band 3 molecule have not been reported. In the present studies, we report an alteration in the band 3 molecule that increases its apparent relative mobility by 2000–4000 and is associated with erythrocyte acanthocytosis, restriction of rotational diffusion of band 3 in the membrane, an increase in anion transport to a level 2–3 times that of control cells, and a decrease in the number of high-affinity ankyrin-binding sites. Two-dimensional peptide maps are consistent with additions of chloramine-T/^{125}I-labeled peptides in the anion transport region. Thus, we have a structural alteration clearly linked to functional changes in band 3.

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MATERIALS AND METHODS

Cell Separation. Cells were separated into populations of different ages on Percoll gradients as described (10).

IgG Binding Assay. The amount of IgG on cells was quantitated by using ^{125}I-labeled protein A according to the method of Yam *et al.* (23).

NaDodSO$_4$/Polyacrylamide Gel Electrophoresis. Proteins were analyzed on 6–25% linear NaDodSO$_4$/polyacrylamide gradient gels using the discontinuous buffer system of Laemmli (24). Samples were run both with and without dithiothreitol in the sample buffer. Band 3 relative mobilities were calculated from its leading edge.

Immunostaining of the Membrane Proteins. Immunoautoradiography was performed by the immunoblotting technique of Towbin *et al.* (25) with the modifications described (11, 26, 27).

Glyceraldehyde-3-Phosphate Dehydrogenase Assay. Glyceraldehyde-3-phosphate dehydrogenase was measured by the method of Schrier (28) with the modifications described (13).

Carbohydrate Enzymatic Treatments. Washed erythrocytes were treated with 0.3 or 9 units of endoglycosidase F (New England Nuclear) or endo-β-galactosidase (Boehringer Mannheim) in 10 vol of buffer for 2 hr at 37°C. The buffer used for control (untreated) cells and endoglycosidase F treatment was 10 mM NaPO$_4$/150 mM NaCl/50 mM EDTA/0.5 mM diisopropylfluorophosphate, pH 6.5; for endo-β-galactosidase the buffer was phosphate-buffered saline with 0.5 mM diisopropylfluorophosphate (pH 7.4).

Anion Transport Measurements. The "self-exchange flux" of sulfate was determined at Donnan equilibrium, essentially by following the methods of Lepke and Passow (29) and Schnell *et al.* (30). Erythrocytes were assayed either the same day or 1 day after the blood was collected. Density separation was performed as described above, and the cells were washed four times in 40 vol of saline.

Ankyrin-Binding Studies. Ankyrin was labeled with ^{125}I using Bolton–Hunter reagent (31) (1800 Ci/mmol; 1 Ci = 37 GBq; Amersham) as described by Bennett and Stenbuck (32). Binding of radiolabeled ankyrin (2–20 μg/ml) to inside-out vesicles (40–60 μg/ml) depleted of spectrin, actin, ankyrin, and band 4.1 was investigated essentially by following the procedure of Hargreaves *et al.* (33) as described by Goodman *et al.* (34). Incubation was for 120 min at 4°C and data were corrected for nonspecific binding (34) and plotted by using the Scatchard equation (35). Protein was determined according to Lowry *et al.* (36).

Abbreviation: ST-EPR, saturation transfer electron paramagnetic resonance.

Enzymatic Treatment of Erythrocytes. Fragments are referred to by the nomenclature of Steck *et al.* (37, 38). Washed erythrocytes were treated with proteases as described (12) to generate defined fragments of band 3. Chymotrypsin digests band 3 yielding fragments of M_r 55,000 and 38,000 (37, 38), designated CH-55 and CH-38, respectively (37). The M_r 41,000 (TR-41) cytoplasmic segment of band 3 was produced by mild trypsin digestion of spectrin-depleted NaOH-stripped inverted vesicles (39). The M_r 17,000 (CH-17) intramembranous fragment of band 3 was generated by chymotrypsin treatment at both sides of the membrane (38–40).

Two-Dimensional Peptide Mapping. Two-dimensional peptide maps were obtained by the method of Elder *et al.* (41). The same number of cpm (2.5×10^5) were loaded per chromatography plate.

Electron Paramagnetic Resonance (EPR) Spectroscopy Studies. Unseparated and middle-aged erythrocytes were shipped on ice to Vanderbilt University. Shipping time was <24 hr. Intact erythrocytes shipped as described above were pelleted by low-speed centrifugation and the supernatant medium was aspirated from the cells. The loosely packed cells were washed three times in 50 vol of 14 mM sodium phosphate buffer containing 0.15 M NaCl (pH 7.4). After the final wash, the cells were resuspended to 50% hematocrit in 106 mM sodium phosphate buffer (pH 7.4) and were labeled with 50 μM bis(sulfosuccinimidyl)-4-deoxypimelate as described by Beth *et al.* (42). Cells labeled in 106 mM sodium phosphate (pH 7.4) were washed free of unbound spin label by first suspending them in 40 vol of 5 mM sodium phosphate containing 0.154 M sodium chloride and 1% bovine serum albumin for 30 min at room temperature followed by centrifugation (10 min at 10,000 rpm in a Sorvall SS-34 rotor) and removal of the supernatant fluid. The packed cells were then washed three times in 40 vol of 5 mM sodium phosphate containing 0.15 M NaCl (pH 7.4) by centrifugation and resuspension. After the final wash, the loosely packed intact cells were taken for saturation transfer-EPR (ST-EPR) measurements. For studies of membrane fluidity, a 5-nitroxylstearate spin label was used.

ST-EPR measurements were made with a Varian E-112 spectrometer operating at X-band microwave frequency. Samples were introduced into the E-238 cavity in a high volume aqueous flat cell (WG-813, Wilmad). Sample temperature was regulated at 22°C with an E-257 variable temperature accessory by passing precooled air into the cavity through the front optical port. Sample temperature was continuously monitored with a Baily BAT12 digital thermometer by placing a temperature probe into the flat cell in contact with the erythrocyte suspension. The out-of-phase position was determined by the self-null method (43). ST-EPR signals were accumulated digitally using an on-line PDP 11 microcomputer to drive the spectrometer's magnetic-field sweep. All signals were recorded using a microwave observer power of 0.2 Gauss (calibrated; ref. 44) and a 50 kHz Zeeman field modulation of 5-Gauss amplitude (100 kHz detection). Effective rotational correlation times of labeled band 3 were estimated from the experimental ST-EPR spectra by comparison of the motionally sensitive ratio parameter L″/L with plots of the same parameter versus rotational correlation time from a model system study of spin-labeled hemoglobin in various glycerol/buffer solutions (43). The spectral contribution from spin-labeled lipids has been digitally subtracted from each display so that rotational diffusion of spin-labeled band 3 is the principal determinant of the spectral shape as described by Beth *et al.* (42).

RESULTS

The only abnormality consistently observed in affected family members was acanthocytosis. Acanthocytes (Greek: akantha, thorn; akis, spine) are irregularly spiculated erythrocytes with projects of various lengths and positions. They are also called "spiny" or "spur" cells. The percentage acanthocytes (dry preparation) was as follows: propositus, 21%; sibling 2, 25%; sibling 1, 0.6%. The clinical details are described elsewhere.

To control for the effect of acanthocytosis alone on the biochemical and cellular parameters tested, we included erythrocytes from an individual with abetalipoproteinemia in our study because these individuals have >85% acanthocytes. The individual tested had 85–87% acanthocytes.

Band 3 from Affected Family Members Has an Apparent M_r of 97,000–99,000 Instead of 95,000. $NaDodSO_4$/polyacrylamide gel electrophoresis revealed that band 3 of erythrocytes from affected family members migrated at an apparent M_r of 97,000–99,000 (Fig. 1). Affected family members were propositus (M_r, ≈99,000) and sibling 2 (M_r, ≈97,000). Sibling 1 was normal (M_r, ≈95,000). This represents an apparent increase in M_r of 2000–4000 greater than that of normal band 3 (control M_r, ≈95,000). No increase in band 3 breakdown products was observed in immunoblotting studies (Fig. 1). No differences were detected in the amount of band 3 present in erythrocytes of affected family members by densitometry. Glycophorin A was present as determined by silver stains of polyacrylamide gels and the presence of MN and Ss blood group antigens. Changes in membrane proteins other than band 3 were not detected. Therefore, we refer to this alteration as "slow-migrating" band 3.

Contribution of Carbohydrate to the Apparent Increased Molecular Weight of Band 3. To determine whether the apparent increased molecular weight of band 3 was due to changes in glycosylation, erythrocytes were treated with endoglycosidase F or endo-β-galactosidase followed by $NaDodSO_4$/polyacrylamide gel electrophoresis. The mobil-

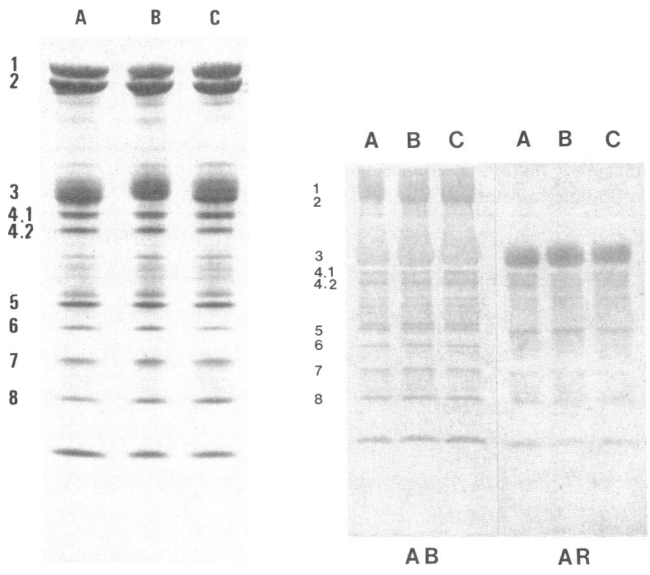

FIG. 1. Polyacrylamide gel electrophoresis and immunoblotting studies of erythrocyte membranes with normal and slow-migrating band 3. (*Left*) Coomassie blue stain of polyacrylamide gel. Lanes: A, control membranes; B, propositus membranes; C, abetalipoproteinemia membranes. (*Right*) Lanes: A, membrane standard consisting of membranes prepared from erythrocytes of normal individuals maintained as donors by our laboratory and prepared immediately; B, control membranes drawn and prepared with those of the experimental subjects; C, propositus membranes. AB, amido black protein stain; AR, autoradiograph stained with antibodies to band 3 followed by ^{125}I-labeled protein A. Control membranes were obtained from erythrocytes of a normal individual whose blood was drawn at the same time as that of the propositus and handled the same way.

ity of band 3 from control cells was increased, as would be expected following removal of glycosyl groups. Slow-migrating band 3 still migrated at a higher molecular weight (propositus: M_r, 98,500 ± 500 before; M_r, 95,700 ± 700 after 9 units of endoglycosidase F) than the control (M_r, 95,000 ± 300 before; M_r 91,500 ± 200 after 9 units of endoglycosidase F) following enzymatic treatment, indicating that glycosylation was not responsible for the apparent increased molecular weight of band 3.

IgG Binding to Erythrocytes. IgG binding to unseparated young and middle-aged cells from individuals with high molecular weight band 3 was the same as that of both the normal control and sibling (Table 1). Thus, premature aging of erythrocytes as determined by IgG binding did not occur. Old erythrocytes showed increased IgG binding as in normally observed (8–10).

Anion Transport Studies. Anion transport studies revealed that middle-aged erythrocytes from the propositus and sibling 2 had abnormally increased anion transport (increased V_{max}; Table 2).

Ankyrin Binding Studies. Ankyrin binding studies revealed that the number of high-affinity ankyrin-binding sites was significantly reduced for the propositus and for sibling 2 (Table 3). The dissociation constant (K_d) was reduced for the propositus. It is tempting to speculate that the observed acanthocytosis results from changes in the interaction of band 3 with ankyrin. As we envision it, loss of anchorage of band 3 to the erythrocyte cytoskeleton would permit evagination of the membrane.

ST-EPR Studies. The observed changes in anion transport function suggest that an alteration in band 3 may be in the anion transport region. As another approach to analyzing activity in the anion transport region, we used ST-EPR and bis(sulfosuccinimidyl)-4-deoxypimelate, a spin-label that binds to the anion transport region of band 3. ST-EPR data from normal control erythrocytes have been compared with those from the propositus and sibling 2 exhibiting abnormal erythrocyte function. Representative data, as shown in Fig. 2, have been analyzed by standard approaches (43) to yield effective rotational correlation times for spin-labeled band 3 protein in intact erythrocytes as described in detail by Beth *et al.* (42). These data and those from the normal sibling 1 are tabulated in Tables 4 and 5. The significant finding has been the positive correlation between the morphologic finding of acanthocytosis with the biological findings of increased molecular weight of band 3, a decrease in the high-affinity ankyrin-binding sites on band 3, and the increased rotational correlation time for band 3 protein from the ST-EPR measurements (Table 5). Increased rotational correlation time indicates slowing or restriction of band 3 motion or freedom in the membrane. The ST-EPR data suggest that the alteration in band 3 is in the anion transport region.

Peptide Mapping Analysis of Band 3 and Defined Proteolytic Products of Band 3. Both anion transport studies and ST-EPR data suggest that the anion transport region is

Table 1. IgG binding to erythrocytes from individuals with normal or high molecular weight band 3 as determined by a ^{125}I-labeled protein A binding assay.

Exp.	Individual	Cell age	Molecules of IgG per cell
1	Control	Unsep	6 ± 1
	Propositus	Unsep	7 ± 1
	Control	MA	7 ± 1
	Propositus	MA	4 ± 1
	Control	Old	98 ± 7
	Propositus	Old	70 ± 6
2	Control	Unsep	7 ± 1
	Propositus	Unsep	7 ± 1
	Control	YG	7 ± 1
	Propositus	YG	8 ± 1
	Control	MA	7 ± 1
	Propositus	MA	7 ± 1
3	Control	MA	7 ± 1
	Sibling 1	MA	9 ± 1
	Sibling 2	MA	6 ± 1
	Aβ-lipoprot	MA	5 ± 1
	Control	Old	70 ± 5
	Sibling 1	Old	252 ± 16
	Sibling 2	Old	120 ± 4

Only results of three of the four experiments performed are presented. The results of the other experiment were the same. Data are presented as the mean ± 1 SD. Exp., experiment; Aβ-lipoprot, abetalipoproteinemia; Unsep, unseparated; YG, young; MA, middle-aged; Old, second band of 4 bands of old cells.

Table 2. Anion (sulfate) transport by erythrocytes from individuals with normal or high molecular weight band 3

Exp.	Individual	Cell age	K_m, mM	V_{max}, mol × 10^{-8} per 10^8 cells per min
1	Control	Unsep	0.7 ± 0.2	12.1 ± 2.0
	Propositus	Unsep	0.7 ± 0.2	30.4 ± 2.5*
	Control	MA	0.6 ± 0.1	11.1 ± 0.6
	Propositus	MA	0.5 ± 0.1	22.3 ± 1.0*
	Control	Old	1.0 ± 0.1	6.5 ± 0.3†
	Propositus	Old	1.2 ± 0.1	8.2 ± 0.4†‡
2	Control	Unsep	0.8 ± 0.1	14.2 ± 1.4
	Propositus	Unsep	0.8 ± 0.1	34.5 ± 1.1*
	Control	MA	0.8 ± 0.1	12.1 ± 2.0
	Propositus	MA	0.7 ± 0.1	32.3 ± 1.5*
3	Control	Unsep	0.9 ± 0.1	13.8 ± 1.1
	Sibling 1	Unsep	0.9 ± 0.2	12.4 ± 1.4
	Sibling 2	Unsep	0.9 ± 0.1	23.5 ± 2.8*
	Aβ-lipoprot	Unsep	1.5 ± 0.2‡	6.9 ± 1.2‡
	Control	MA	0.6 ± 0.1	12.1 ± 1.3
	Sibling 1	MA	0.6 ± 0.1	10.8 ± 0.9
	Sibling 2	MA	0.7 ± 0.1	18.8 ± 1.1*
	Aβ-lipoprot	MA	1.4 ± 0.1‡	5.8 ± 0.8‡
4	Control	Unsep	1.1 ± 0.1	12.8 ± 1.4
	Propositus	Unsep	0.9 ± 0.2	24.8 ± 2.0*
	Control	MA	0.9 ± 0.1	12.0 ± 1.0
	Propositus	MA	0.6 ± 0.2	24.6 ± 1.3*

Data are presented as the mean ± 1 SD. Exp., experiment; Aβ-lipoprot, abetalipoproteinemia; Unsep, unseparated; MA, middle-aged; Old, second band.
*$P \leq 0.001$ compared to control.
†$P \leq 0.001$ compared to middle-aged.
‡$P \leq 0.01$ compared to control.

Table 3. Ankyrin binding by middle-aged erythrocyte ghosts from individuals with normal or high molecular weight band 3

Exp.	Individual	High-affinity binding sites, no.*	K_d, nM
1	Control	45 ± 3	28 ± 2
	Propositus	29 ± 2†	15 ± 1†
2	Control	57 ± 7	34 ± 5
	Propositus	27 ± 5†	24 ± 4
3	Control	42 ± 6	30 ± 5
	Sibling 1	60 ± 9	43 ± 8
	Sibling 2	22 ± 2†	26 ± 3
	Aβ-lipoprot	100 ± 12†	33 ± 5

Results are presented as the mean ± 1 SD based on three experiments and a total of 20–24 determinations. Exp., experiment; Aβ-lipoprot, abetalipoproteinemia.
*μg of ankyrin per mg of membrane protein after removal of ankyrin, spectrin, actin, and band 4.1.
†$P \leq 0.01$.

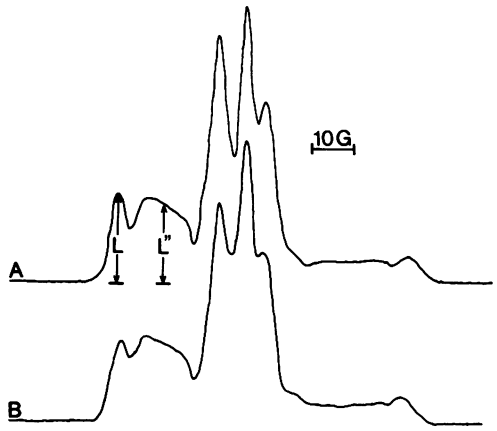

FIG. 2. ST-EPR spectra from spin-labeled intact erythrocytes with normal or high molecular weight band 3. The data shown are representative spectra demonstrating a normal tracing (*A*) or slowing of rotational correlation times (*B*; sibling 2).

altered in individuals with slow-migrating band 3. As a more definitive approach to localizing and defining alterations in band 3, two-dimensional peptide maps of band 3, altered band 3, and their proteolytic products were compared (Fig. 3). Changes were observed in a M_r ≈17,000 transmembrane anion transport segment of band 3. A prominent peptide appears in tryptic maps of altered band 3 (Fig. 3*D*, center arrow). We suspect that this represents increased amounts of a peptide observed in normal band 3 (Fig. 3*C*, center arrow). However, it could also be a new peptide or the same peptide with increased amounts of tyrosine, since the chloramine-T method of ^{125}I labeling that was used labels tyrosine. Other changes include the appearance of a peptide to the lower right of the prominent peptide (indicated by arrow in Fig. 3*D*) and a peptide on the lower left of the prominent peptide (left arrow, Fig. 3*D*). Similar changes were observed in a M_r ≈55,000 chymotrypsin fragment of band 3, which contains the M_r ≈17,000 intramembranous fragment (Fig. 3 *E* and *F*). Changes in a M_r ≈38,000 reciprocal fragment to the M_r ≈55,000 fragment (Fig. 3 *A* and *B*) and M_r ≈40,000 cytoplasmic segment (not shown) were not observed. These results indicate that alterations in slow-migrating band 3 occur in the anion transport region.

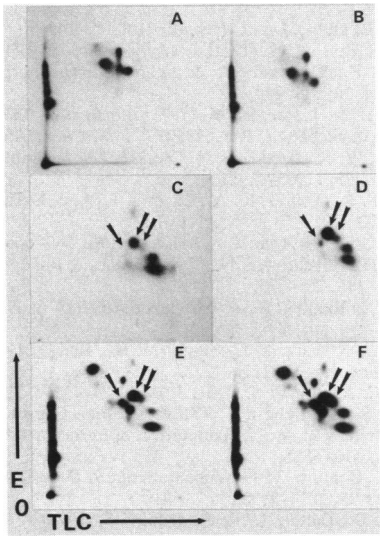

FIG. 3. Two-dimensional peptide maps of proteolytic fragments of normal band 3 (left) and slow-migrating band 3 (Right). (*A* and *B*) CH-38; (*C* and *D*) CH-17; (*E* and *F*) CH-55. O, origin; E, electrophoresis; TLC, thin-layer chromatography.

Table 4. Electron paramagnetic spectroscopy studies of the anion transport region of band 3 in intact middle-aged erythrocytes

Sample	L"/L	Rotational correlation times, sec
Control	0.80	200
Propositus	0.91	350
Control	0.79	200
Sibling 1	0.82	200
Sibling 2	0.92	350

The parameter L"/L was measured from the indicated experimental ST-EPR spectrum as defined in Fig. 1. Effective rotational correlation times were estimated from the parameter L"/L by comparison with the model system plots of Thomas *et al.* (43) to the nearest 50 μsec.

DISCUSSION

Results of the studies presented here indicate that a specific structural alteration of band 3—namely, an addition in the anion transport region—is associated with acanthocytosis and changes in erythrocyte function as determined by anion (sulfate) transport and ankyrin-binding assays. Increased binding of IgG to unseparated or middle-aged cells is not observed.

Anion transport by slow-migrating band 3 cells is characterized by an increased V_{max}, suggesting that it is more efficient. Differences in glyceraldehyde-3-phosphate dehydrogenase activity, cation transport (both ouabain-sensitive and ouabain-resistant inside-out efflux of ^{22}Na), and fluorescence polarization were not observed (results not shown). The latter studies suggest that membrane fluidity was normal.

Band 2.1 (ankyrin or syndein) links spectrin, bands 1 and 2, to band 3, which anchors the membrane skeleton to the membrane. Ankyrin-binding studies revealed a decrease in the number of high-affinity binding sites on membranes from the propositus and sibling 2 ($P \leq 0.01$). In addition, the propositus may have a decreased dissociation constant. It has been suggested that the differences between high- and low-affinity ankyrin-binding sites may be due to differences in band 3 conformation. Perhaps the alteration in slow-migrating band 3 results in a conformation change that eliminates or renders some ankyrin-binding sites inaccessible.

Changes in the parameters studied were not due to acanthocytosis alone because cells from an individual with 85–87% acanthocytes exhibited changes that were the opposite of those observed with slow-migrating band 3. Band 3 in this individual's erythrocytes migrated normally in polyacrylamide gels.

The rotational correlation times measured from spin-labeled band 3 in intact erythrocytes provide an indication of the degree of association between band 3 monomers and the interaction of the membrane oligomer with other membrane proteins. Previous work using ST-EPR (42) has supported

Table 5. Summary of changes associated with high molecular weight band 3

Individual	BD3 M_r	Acan	Anion trans, (V_{max})	Ankyrin-binding sites	Rotat. corr. time
Propositus	↑	↑	↑	↓	↑
Sibling 1	nl	nl	nl	nl	nl
Sibling 2	↑	↑	↑	↓	↑
Aβ-lipoprot	nl	↑↑↑	↓	↑	

BD3 M_r, band 3 molecular weight; Acan, acanthocytes; trans, transport; Rotat. corr., rotational correlation; Aβ-lipoprot, abetalipoproteinemia. ↑, Increase; ↓, decrease; nl, normal.

the optical work of Nigg and Cherry (45), which suggests that the rotational diffusion of band 3 in the erythrocyte membrane is highly restricted due to interactions of its N-terminal cytoplasmic domain with membrane skeletal proteins. The observation of increased rotational correlation times for spin-labeled band 3 in erythrocytes of the propositus and sibling 2 indicates that the rotational freedom, averaged over all of the copies of band 3 present, has decreased due to an increase in hydrodynamic size (i.e., protein–protein interaction). This could have arisen from self association, from increased association with other membrane proteins, or from a significant change in effective viscosity of the bilayer. We suspect that it is due to increased self association since membrane fluidity is normal and the number of high-affinity ankyrin binding sites is decreased, not increased, in affected individuals. However, we do not have sufficient spectroscopic and biochemical data at the present time to conclusively discriminate between these alternatives.

Anion transport studies confirm our previous findings (13) indicating that normal old cells have an increased K_m and decreased V_{max}. Old cells with high molecular weight band 3 show the same changes (i.e., increased K_m and decreased V_{max} relative to middle-aged cells). Although cells with slow-migrating band 3 show normal aging changes, the young and middle-aged cells do not age prematurely. The anion transport changes observed in these cells are different than those of normal aging in that the K_m of young and middle-aged cells is not changed, while V_{max} increased rather than decreased. Premature binding of IgG to cells with slow-migrating band 3 is not observed. Thus, this addition to the band 3 molecule does not result in cellular aging changes (12, 13).

We initially studied anion transport as a function of cellular age because impairment of anion transport was anticipated (13) based on our data indicating that senescent cell antigen is generated by cleavage of band 3 in the anion transport region (12). However, it is unlikely that the decreased anion transport (increase in K_m, decrease in V_{max}) can be explained on the basis of band 3 degradation alone. Band 3 is present in $\approx 1.2 \times 10^6$ copies per cell; whereas the amount of senescent cell IgG bound is only ≈ 100 molecules per cell. Decreased anion transport function probably precedes degradation and, presumably, follows subtle structural changes in band 3 that have yet to be detected. Other changes in addition to degradation of band 3 may be required to initiate senescent cell IgG binding. If this were the case, the number of IgG molecules bound would be far less than the number of band 3 molecules degraded. Another explanation is that band 3 molecules are altered so that they no longer function together as transport units. The appealing aspect of this approach is that band 3 is visualized as a membrane functional unit of interacting molecules rather than as individual proteins floating in a lipid environment. Any change that would impair the ability of molecules to interact would impair their function. Thus, the integrative function of molecules may be important for molecular aging.

Note Added in Proof. Glucose transport was decreased in erythrocytes from the proposito and sibling 2 ($P \leq 0.01$ compared to control). Glucose transport of erythrocytes from sibling 1 was normal. Cells from both parents show changes in all of the parameters described in our paper and in glucose transport, although to a lesser degree than their children. Our studies suggest that this band 3 mutation is an autosomal recessive.

The proposito was brought to our attention by Dr. David Nanus, a resident in the Department of Medicine of the Bronx Municipal Hospital Center. We are grateful to Albert H. Beth, Thomas E. Conturo, and J. V. Staros at Vanderbilt University for performing ST-EPR studies. We are grateful to Dr. Richard A. Cooper (Medical College of Wisconsin) for performing the lipid assays and the fluorescence polarization studies. The patient with abetalipoproteinemia was referred to us by Dr. I. H. Scheinberg. Ankyrin was a generous gift from Dr. S. R. Goodman. Preliminary studies by Dr. Olga Blumenfeld and Dr. Jiri Palek showed that band 3 of the proposito migrated more slowly than normal band 3 by NaDodSO$_4$/polyacrylamide gel electrophoresis. We appreciate the excellent technical assistance of D. Danzeiser and J. Earle-Hughes. This work was supported by the Veterans Administration Research Service and the Research Corporation. The work at Vanderbilt was supported by Grants HL34737 and AM31880. Albert H. Beth is the recipient of a Searle Scholar Award/Chicago Community Trust.

1. Cabantchik, Z. I. & Rothstein, A. (1974) *J. Membr. Biol.* **15,** 207–226.
2. Lepke, S., Fasold, H., Pring, M. & Passow, H. J. (1976) *J. Membr. Biol.* **29,** 147–177.
3. Strapazon, E. & Steck, T. L. (1977) *Biochemistry* **16,** 2966–2971.
4. Karadsheh, N. S., Uyeda, K. & Oliver, R. M. (1977) *J. Biol. Chem.* **252,** 3515–3524.
5. Kliman, H. J. & Steck, T. L. (1975) *J. Biol. Chem.* **255,** 6314–6321.
6. Salhany, J. M. & Shaklai, N. (1979) *Biochemistry* **18,** 893–899.
7. Brown, P. A., Feinstein, M. B. & Shuaki, R. I. (1975) *Nature (London)* **254,** 523–525.
8. Kay, M. M. B. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3521–3525.
9. Kay, M. M. B. (1978) *J. Supramol. Struct.* **9,** 555–567.
10. Bennett, G. D. & Kay, M. M. B. (1981) *Exp. Hematol.* **9,** 297–307.
11. Kay, M. M. B., Goodman, J., Sorensen, K., Whitfield, C., Wong, P., Zaki, L. & Rudoloff, V. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1631–1635.
12. Kay, M. M. B. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5753–5757.
13. Kay, M. M. B., Bosman, G. J. C. G. M., Shapiro, S., Bendich, A. & Bassel, P. S. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2463–2467.
14. Steck, T. L. (1978) *J. Supramol. Struct.* **8,** 311–324.
15. Ramjeesingh, M. & Rothstein, A. (1982) *Membr. Biochem.* **4,** 259–269.
16. Kopito, R. & Lodish, H. F. (1985) *Nature (London)* **316,** 234–238.
17. Bennett, V. & Stenbuck, P. J. (1979) *Nature (London)* **280,** 468–473.
18. Kay, M. M. B., Tracey, C., Goodman, J., Cone, J. C. & Bassel, P. S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6882–6886.
19. Drenckhahn, D., Zinke, K., Schauer, U., Appell, K. C. & Low, P. S. (1984) *Eur. J. Cell Biol.* **34,** 144–150.
20. Drenckhahn, D., Schülter, K., Allen, D. P. & Bennett, V. (1985) *Science* **230,** 1287–1289.
21. Cox, J. V., Moon, R. T. & Lazarides, E. (1985) *J. Cell Biol.* **100,** 1548–1555.
22. Kay, M. M. B. (1986) in *Red Cell Antigens and Antibodies*, ed. Garratty, G. (Am. Assoc. Blood Banks, Arlington, VA), pp. 35–82.
23. Yam, P., Petz, L. D. & Spath, P. (1982) *Am. J. Hematol.* **12,** 337–346.
24. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
25. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
26. Kay, M. M. B., Sorensen, K., Wong, P. & Bolton, P. (1982) *Mol. Cell. Biochem.* **49,** 65–85.
27. Kay, M. M. B. in *Methods in Hematology: Red Cell Membranes*, eds. Shohet, S. & Mohandas, N. (Churchill Livingstone, New York), in press.
28. Schrier, S. L. (1966) *Am. J. Physiol.* **210,** 139–145.
29. Lepke, S. & Passow, H. (1971) *J. Membr. Biol.* **6,** 158–182.
30. Schnell, K. F., Gerhardt, S. & Schoppe-Fredenburg, A. (1977) *J. Membr. Biol.* **30,** 319–350.
31. Bolton, A. E. & Hunter, W. M. (1973) *Biochem. J.* **133,** 529–539.
32. Bennett, V. & Stenbuck, P. J. (1980) *J. Biol. Chem.* **255,** 6424–6432.
33. Hargreaves, W. R., Giedd, K. N., Verkley, A. & Branton, D. (1980) *J. Biol. Chem.* **255,** 11965–11972.
34. Goodman, S. R., Shiffer, K. A., Casoria, L. A. & Eyster, M. E. (1982) *Blood* **60,** 772–784.
35. Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51,** 660–666.
36. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
37. Steck, T. L., Ramos, B. & Strapazon, E. (1976) *Biochemistry* **15,** 1153–1161.
38. Steck, T. L., Koziarz, J. J., Singh, M. K., Reddy, G. & Kohler, H. (1978) *Biochemistry* **17,** 1216–1222.
39. Appell, K. C. & Low, P. S. (1981) *J. Biol. Chem.* **256,** 11104–11111.
40. Markowitz, S. & Marchesi, V. T. (1981) *J. Biol. Chem.* **256,** 6463–6468.
41. Elder, J., Pickett, R. A., II, Hampton, J. & Lerner, R. A. (1977) *J. Biol. Chem.* **252,** 6510–6515.
42. Beth, A. H., Conturo, T. E., Venkataramu, S. D. & Staros, J. V. (1986) *Biochemistry* **25,** 3824–3832.
43. Thomas, D. D., Dalton, L. R. & Hyde, J. S. (1976) *J. Chem. Phys.* **65,** 3006–3024.
44. Beth, A. H., Balasubramanian, K., Robinson, B. H., Dalton, L. R., Benkataramu, S. D. & Park, J. H. (1983) *J. Phys. Chem.* **87,** 359–367.
45. Nigg, E. A. & Cherry, R. J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4702–4706.