Point Mutation in the β-Spectrin Gene Associated with αI/74 Hereditary Elliptocytosis

Implications for the Mechanism of Spectrin Dimer Self-Association

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Abstract

αI/74 hereditary elliptocytosis (HE) is a subgroup of HE in which patients exhibit an impaired self-association of spectrin dimers and an abnormal proteolytic cleavage of the αI domain of spectrin. We studied a family in which the proband presented with a severe neonatal hemolytic anemia with poikilocytosis. Biochemical analysis of erythrocytes from the proband and his family members allowed us to ascertain a diagnosis of homozygosity for αI/74 HE in the proband and heterozygosity in his parents and several of their offspring. Results of polymorphism linkage analysis suggested that the defect in this family was located in β rather than α spectrin. We analyzed the 3′ end of the β-spectrin gene of the proband and detected a mutation that changes a codon for alanine to one for proline. Allele-specific oligomer hybridization on slot blots of DNA from other family members confirmed the presence of the mutation only in members heterozygous for the disorder. This is the first example of a point mutation in the β-spectrin chain that is associated with defective spectrin dimer self-association and an abnormal proteolytic cleavage of the α chain. Based on this finding, we propose a model for the mechanism of interaction between the α- and β-spectrin chains. (*J. Clin. Invest.* 1990. 86:909–916.) Key words: hereditary pyropoikilocytosis • polymerase chain reaction • restriction fragment length polymorphism • genomic cloning • genetic linkage analysis

Introduction

Hereditary elliptocytosis (HE)[1] is a heterogeneous group of disorders characterized by the presence of elliptically shaped erythrocytes in the blood (1). In most patients with HE, there is a variable number of elliptocytic cells without evidence of hemolysis. However, severe hemolytic anemia may be observed in some patients in the first year of life (HE with transient poikilocytosis in infancy) that evolves into typical mild HE. In a subset of patients, the disease persists with a chronic compensated hemolytic anemia of variable severity. Sometimes the severity of the hemolysis requires repeated blood transfusions and splenectomy to ameliorate the condition. This form of the disorder, characterized by prominent poikilocytosis and increased membrane fragility to heat and mechanical stress, is consistent with a diagnosis of either homozygous HE or hereditary pyropoikilocytosis (HPP) (2).

Over the last few years, defects in several of the erythrocyte membrane skeleton proteins have been shown to be the cause of HE and HPP (3, 4). The erythrocyte membrane skeleton is a meshwork of proteins on the undersurface of the red cell membrane, which is responsible for the maintenance of the shape and flexibility of the red cell (5). The erythrocyte membrane skeleton proteins include spectrin, protein 4.1, ankyrin, and actin. Spectrin (Sp), the major membrane skeleton protein, is a heterodimer composed of an α and a β subunit. Each α and β subunit can be resolved into several peptide domains by limited tryptic cleavage, named αI to αV and βI to βIV (6). The αI 80-kD domain corresponds to the α chain NH_2-terminal region and the βI domain to the β chain COOH-terminal region. Spectrin dimers associate head to head to form tetramers that represent the basic unit of the membrane skeleton. The αI and βI domains participate in the spectrin dimer self-association. The exact mechanism of interaction between the subunits is, however, unknown.

Recently, subgroups of HE and HPP have been shown to be associated with a defective ability of the spectrin dimers to self-associate into tetramers (7–9). In many cases, structural alterations have been detected in the αI domain of the α-spectrin chain after partial tryptic digestion (10–25). These alterations are characterized by tryptic peptide patterns with partial or complete loss of the normal 80-kD fragment and appearance of abnormal or enhanced smaller peptide fragments. To date, seven distinct disorders have been described and each mutant has been classified according to the molecular weight of the abnormal or enhanced peptide fragment: Sp αI/78 kD (10, 11), Sp αI/74 kD (12–15), Sp αI/65–68 kD (16–18), Sp αI/61 kD (19), Sp αI/46–50a kD (20–23), Sp αI/50b kD (24), and Sp αI/43 kD (25). Among these, the subgroup of HE associated with an abnormal 74-kD proteolytic fragment (HE αI/74) is of particular interest since the impairment of spectrin dimer self-association in this subgroup is among the most severe in all HE subgroups examined (26). Delineating the mo-

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1. *Abbreviations used in this paper:* ASO, allele-specific oligonucleotide; HE, hereditary elliptocytosis; HPP, hereditary pyropoikilocytosis; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; Sp, spectrin.

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lecular defect in this disorder will help elucidate the mechanism of spectrin dimer self-association.

We studied a family in which the proband presented with a severe neonatal hemolytic anemia with poikilocytosis, requiring regular transfusion (14). A splenectomy recently was performed on the proband, eliminating the need for transfusion, and making possible analysis of his blood cells in the absence of transfused cells. Biochemical studies of erythrocytes from the proband and his relatives allowed us to ascertain a diagnosis of homozygous αI/74 HE in the proband and heterozygous αI/74 HE in his parents and several siblings. We report here the identification of a β-spectrin mutation associated with the disorder in this family.

Methods

Thermal sensitivity and morphology of erythrocytes. The thermal sensitivity of erythrocytes was studied as previously described (2). Normal and heated cells were examined by light phase-contrast microscopy after fixation in 1% glutaraldehyde (vol/vol) in 5 mmol phosphate buffer, 150 mmol NaCl, pH 7.4.

Erythrocyte deformability and membrane stability. These studies were performed using an ektacytometer. Whole-cell deformability was followed as a function of the osmolality of the suspending medium as previously described (27). The membrane resistance to shear-induced fragmentation was measured as described (28).

Preparation and analysis of red cell membranes. Red cell membranes were prepared as described (11). Membrane proteins were analyzed by electrophoresis in SDS-PAGE either with a 5–15% polyacrylamide gradient as described by Laemmli (29) or using a 3.5–15% polyacrylamide gradient as described by Fairbanks et al. (30). To estimate Sp/band-3 ratios, SDS polyacrylamide slab gels (30) were scanned after Coomassie blue staining using a DU8 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) at 550 nm.

Study of Sp dimer-tetramer equilibrium. Sp was extracted as described (31), by incubating white ghosts overnight at 4°C in low ionic strength buffer. The content of spectrin dimers and tetramers was determined by nondenaturing gel electrophoresis as described (14).

Limited tryptic digestion of Sp. Limited tryptic digests of spectrin extracts were prepared as described (11). Sp peptides were separated by SDS-PAGE in a 7–15% polyacrylamide gradient and by two-dimensional electrophoresis as described by O'Farrell (32) and modified by Speicher et al. (6). One-dimensional SDS-PAGE of Sp tryptic digests were scanned at 550 nm after Coomassie blue staining.

Partial amino acid sequencing of SpαI/74-kD peptide. After separation by SDS-polyacrylamide gel electrophoresis, tryptic peptides were transferred onto polyvinylidene difluoride membranes (Immobilon; Millipore Corp., Bedford, MA). After staining with Coomassie blue, the band corresponding to the 74-kD peptide was cut out, destained in 50%/7% methanol/acetic acid and stored at −20°C before microsequencing analysis performed with a gas-phase sequencer (model 740A; Applied Biosystems, Foster City, CA) (33).

Cloning and characterization of the 3′ end of the human β-spectrin gene. A human genomic DNA library in bacteriophage lambda (34) was screened using a 0.64-kb Xho I/Eco RI DNA fragment corresponding to the 3′ end of the human β spectrin cDNA (35).

Polymorphism linkage analysis. The α spectrin αII domain peptide polymorphism was determined as described (36, 22). Briefly, partial tryptic digestion of spectrin generates either a 35-kD αII peptide or a 37-kD peptide on one-dimensional electrophoresis and polymorphism type 1, 2, or 3 on two-dimensional electrophoresis as described (36). α-spectrin restriction fragment length polymorphisms (RFLPs) were detected by digesting genomic DNA with Xba I, Pvu II, or Msp I enzymes and hybridizing the Southern blots with a 13-kb α-spectrin probe to determine haplotypes (37, 38). The β-spectrin RFLP was detected by digesting with Hind III enzyme a 800-bp polymerase chain reaction (PCR) product amplified from genomic DNA using flanking primers (see Results). In the presence of the polymorphic restriction site, two fragments, 350 and 450 bp in length, will be obtained.

PCR primers. The sequences of the forward and reverse PCR primers used for amplification of the 3′ end of the β-spectrin gene are 5′-GTGGCTGAGGCGTGGCTGATTGC-3′ (primer A) and 5′-CACCTGGGCTGAGCTAGTAG-3′ (primer C), respectively. The primers flank a 1.2-kb segment of the gene that contains three exons and two introns (see Results).

DNA amplification and sequencing. Genomic DNA of the proband was obtained by standard procedures and PCR amplified using a DNA thermal cycler (Perkin Elmer Cetus Corp., Norwalk, CT) (39, 40). The PCR product was agarose-gel purified and subcloned into a pGEM7 plasmid (Promega Biotec, Madison, WI). Nucleotide sequencing was performed on the double-stranded plasmid using the Sequenase enzyme kit (United States Biochemical Corp., Cleveland, OH). The sequencing primers used were the T7 and SP6 primers of the vector and an internal primer that anneals to an intronic sequence next to the second exon of the amplified segment. The sequence of the internal primer is 5′-CTTGGCAGAGCATTCAGCTCC-3′ (primer B).

Slot-blot hybridization. Genomic DNA of every member of the kindred was PCR amplified by the same procedure as above. Two-tenths of the PCR product of each sample were transferred to a nylon membrane (Nytran; Schleicher and Schuell, Keene, NH) using a slot blot apparatus and hybridized as described with allele-specific oligonucleotide probes containing either the normal or mutant sequence (41). The final washing step was at 65°C for 10 min. The sequences of the normal and mutant probes are 5′-TCTCAAAAGCCTCATGCCTC-3′ and 5′-TCTCAAAAGGCTCATGCCTC-3′, respectively.

Case report

The clinical course and hematologic features of the proband (III-3 in Fig. 4), a male from Mali, born in 1981, whose parents are first cousins, have been previously reported (14, 42). Briefly, at the time of the first studies, he had severe hemolytic anemia with prominent poikilocytosis, requiring frequent blood transfusions. The patient underwent a partial splenectomy in 1987, but complete splenectomy was necessary one year later. Since then, the proband has not received any blood transfusions but hemolysis persists (Hb level: 8–9.8 g/dl; MCV: 69–72 fl; reticulocytes: 22% or 800,000/mm^3).

Results

The proband. In the absence of blood transfusion four months after the complete splenectomy, morphological and biochemical studies of the proband's erythrocytes were available. Blood smear showed prominent fragmentation with numerous spherocytes and microspherocytes, microcytes (MCV: 71 fl), few triangulocytes and discocytes (< 5%). Remarkably, no elliptocytes were observed.

The proband's erythrocytes were extremely sensitive to heat treatment: 40% of the cells fragmented after a 5-min incubation at 45°C (n = 2). Similar features could be observed after incubation for 40 min at 37°C, suggesting spontaneous fragmentation at body temperature. Erythrocytes from control individuals did not fragment below 49°C. Mechanical resistance of resealed ghosts was greatly decreased, estimated at 5% (n = 2) of the normal control.

Erythrocyte deformability of the proband, studied using osmotic gradient ektacytometry showed an ektacytometric profile similar to that observed in severe hereditary spherocytosis.

Analysis of erythrocyte membrane proteins on SDS-PAGE indicated significant decrease in the amount of spectrin as estimated by a Sp/band 3 ratio of 0.76 (n = 2) (normal value: 1.04±0.13, n = 21).

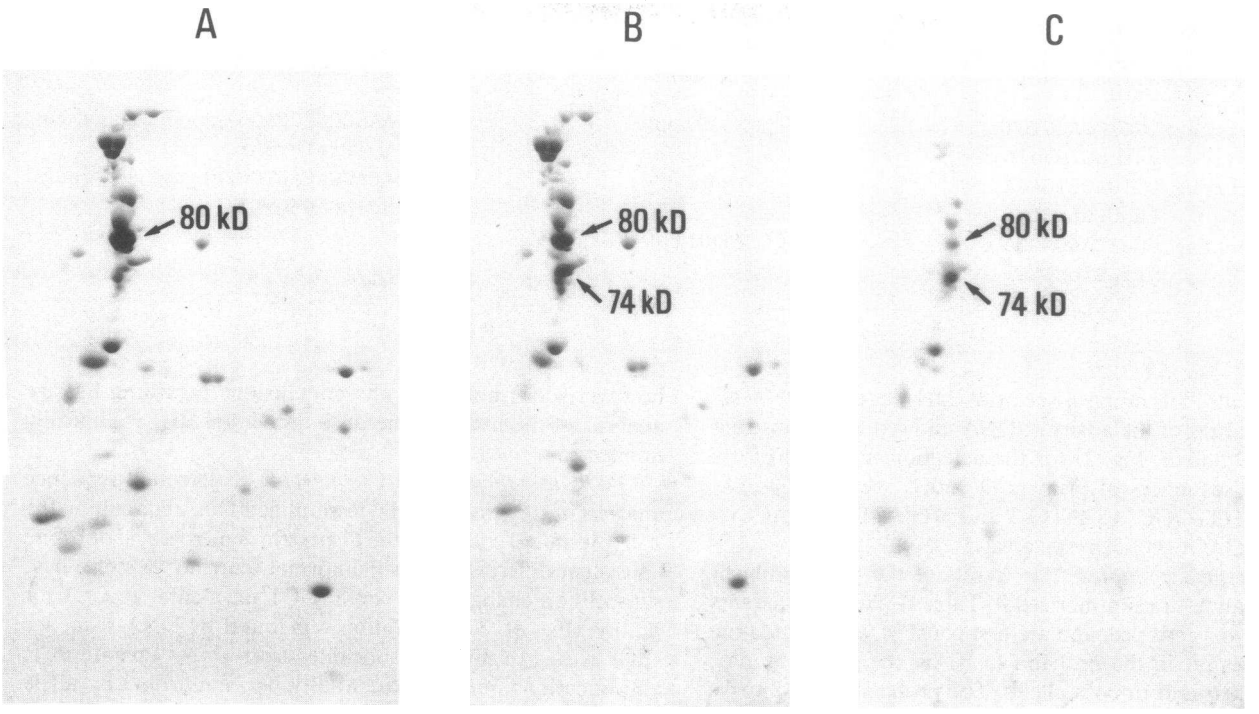

Figure 1. Two-dimensional separation (isoelectric focusing/SDS-PAGE) of tryptic digests of spectrin. (*A*) normal control; (*B*) proband's mother (II-1) and (*C*) proband (III-3). The 80- and 74-kD peptides are indicated by arrows.

The proband's spectrin had impaired ability to undergo self-association as manifested by the high percentage of dimers in the 4°C extract: 82% vs. a normal value of 3.6%±1.6%. The tryptic digestion pattern of the proband's spectrin, separated in one- and two-dimensional gels (Fig. 1), revealed a marked decrease in the 80-kD peptide and a high amount of 74-kD peptide: the 74 kD/74 kD + 80 kD peptide ratio estimated in the one-dimensional gel was 87% (n = 3).

Partial amino acid sequencing of the 74-kD peptide from the proband's spectrin, revealed that the NH_2 terminus of the 74-kD peptide is a leucine, the tryptic cleavage having occurred at lysine 42. The amino acid sequence was normal up to leucine 59.

Other family members. Several other members of the kindred were diagnosed to be heterozygous for αI/74 HE. There was no obvious elliptocytosis exhibited on blood smears but ektacytometric studies of their erythrocytes showed trapezoidal curves characteristic of heterozygous HE (14, 42). Membrane mechanical stability studied in two HE patients (III-5, III-6) was markedly decreased with a fragmentation half-time value of 14 and 23% of the control value, respectively. In contrast to the increased thermal sensitivity of the proband's erythrocytes, erythrocytes from the heterozygous family members began to fragment only after 40 min of incubation at 47°C. Membrane spectrin content, estimated by Sp/band 3 ratio was normal in all heterozygous members. There was an excess of spectrin dimers in 4°C spectrin extracts (between 20 and 32%) and an increased amount of 74-kD peptide (between 43 and 49%) associated with a decrease of the 80-kD peptide after partial tryptic digestion. In unaffected family members, all biochemical results were within normal ranges.

Cloning and characterization of the 3′ end of the normal human β-spectrin gene. A number of recombinants were isolated that contained an 8.0-kb Eco RI fragment that hybridized to the screening probe. The region of hybridizing DNA in this fragment was localized to the 3′-terminal 2.5 kb of the fragment (Fig. 2). DNA sequence analysis of this region revealed that the mRNA encoding sequences were distributed among three exons (labeled X, Y, and Z in Fig. 2). The nucleotide sequences of the exon/intron boundaries are listed in Table I.

Approximately 7.5 kb 3′ to the Xho I site, the cloned genomic DNA contains a polymorphic Hind III site that can be

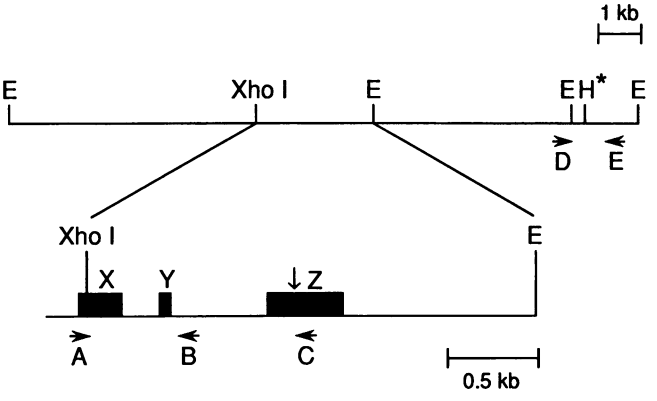

Figure 2. Exon structure of the β-spectrin gene. The 3′ end of the β-spectrin mRNA encoding sequence is distributed among 3 exons, X, Y, and Z, and terminates in the middle of the last exon (*vertical arrow*). A partial restriction map is shown (E, Eco RI; H, Hind III) and the polymorphic Hind III site indicated (H*). The positions of the oligomer primers used for PCR amplification of genomic DNA for RFLP analysis and nucleotide sequencing are indicated by horizontal arrows.

Table I. Exon/intron Boundaries at the 3' End of the β Spectrin Gene

3' Acceptor sequence (intron/exon)	Exon	5' Donor sequence (exon/intron)
gttccccctggcag/TGCTGGAGGTGT. . . euLeuGluValC	X (197 bp)	. . .AAGCCCACCACG/gtgagcagggaaca LysProThrThr
ttctcctcctctag/CTTGAGCTGAAA. . . LeuGluLeuLys	Y (50 bp)	. . .AGAGGAGACTGG/gtgagtggtggccg aGluGluThrGl
tgtgtcttccttag/GCCTCAAGAGGA. . . yProGlnGluGl	Z (401 bp)	—

detected with 3'-terminal β-spectrin cDNA probes. DNA sequence analysis of the adjacent DNA allowed us to construct primers (*D* and *E*, Fig. 2) for the detection of the RFLP by PCR. The sequences of primers D and E are 5'-GCGGAATTCAGTTGTGGCACGATG-3' and 5'-GCGTCTAGACCATAAATGGAGGTG-3', respectively.

Polymorphism studies. The results of the polymorphism linkage analysis are summarized in Table II. Since the parents of the homozygous proband are first cousins, we expected the αI/74 mutation in the proband to be on the same chromosomal background in both alleles. The proband was homozygous for the 37-kD allele of the αII peptide polymorphism, consistent with possible linkage of the mutation to this allele. However, one of his siblings, (III-4), heterozygous for the disorder, was also homozygous in the same allele. This disparity thus made it unlikely that the mutation was linked to α-spectrin. The finding that the proband was heterozygous for the α-spectrin RFLPs supported the same conclusion (Table II). In contrast, the data on the β-spectrin RFLP was consistent with the possibility that the αI/74 mutation was linked to allele 2 of the β-spectrin polymorphism and suggested that the mutation might lie in the β-spectrin gene. The fact that individual III-1 has β-spectrin alleles 1/1 even though his father is homozygous for allele 2 raises the possibility of nonpaternity. This finding, however, does not affect the conclusions regarding linkage analysis of the mutation because individual III-1 is clinically unaffected.

PCR amplification and sequencing. Nucleotide sequence analysis of the proband's PCR amplified DNA containing the 118 3' terminal codons of the β-spectrin gene revealed only one consistent difference from the normal sequence: a single base substitution changing the codon GCT for alanine to CCT for proline (Fig. 3). This mutation was found by DNA sequence analysis in five different subclones from three separate PCR amplifications and its authenticity was confirmed by allele specific oligonucleotide hybridization (see below). The affected amino acid residue, No. 2053 of the β-spectrin chain (43), is located in the last typical repeat (No. 17) of the β-spectrin chain at position 74 of the repeat, near the middle of helix 2 in the triple helical model of Speicher and Marchesi (35, 44).

Slot blot analysis. The presence of this nucleotide substitution in the family was confirmed and shown to be associated with the disorder in each member by allele-specific oligomer (ASO) hybridization (Fig. 4). PCR-amplified genomic DNAs of family members were hybridized to the ASO probes and the results showed that all individuals clinically heterozygous for the disorder (II-1, II-2, III-2, III-4, III-5, III-6) have both the normal and mutant allele. The clinically homozygous proband (III-3) has only the mutant allele. All clinically normal family members (II-3, III-1, III-7, III-8, III-9) have only the normal allele.

Table II. Polymorphism Linkage Analysis

Individual	αII domain polymorphism 1-D	αII domain polymorphism 2-D	α-spectrin RFLP†	β-spectrin RFLP
II-1	35/37 kD	1/3	−+−/−+−	1/2
II-2	35/37 kD	ND*	+−+/+−+	2/2
II-3	37 kD	2/2	ND	1/2
III-1	37 kD	3	ND	1/1
III-2	35/37 kD	ND	ND	2/2
III-3	37 kD	3	−+−/+−+	2/2
III-4	37 kD	ND	ND	1/2
III-5	35/37 kD	1/3	ND	2/2
III-6	37 kD	2/3	ND	1/2
III-7	37 kD	2/3	ND	1/2
III-8	35/37 kD	1/2	ND	1/2
III-9	35/37 kD	1/2	ND	2/2

* Not determined.

† The haplotype denotes the presence (+) or absence (−) of polymorphic Xba I, Pvu II, and Msp I sites in each allele, as described (38).

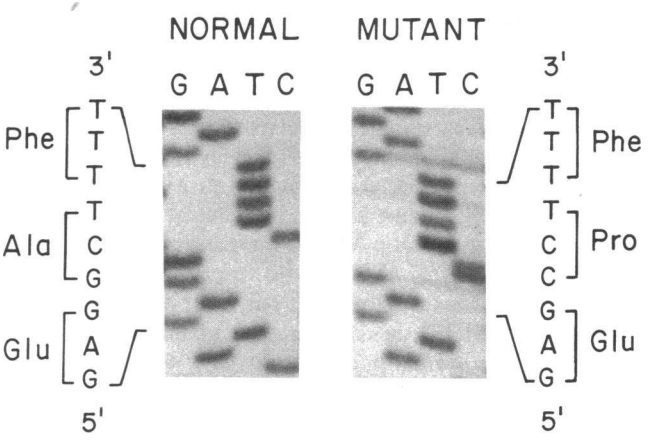

Figure 3. Nucleotide sequence of the αI/74-kD HE mutation. Genomic DNA of the 3' end of the β-spectrin gene from the proband was amplified by PCR, subcloned, and sequenced. A point mutation was detected that changes a codon for alanine, GCT, to one for proline, CCT.

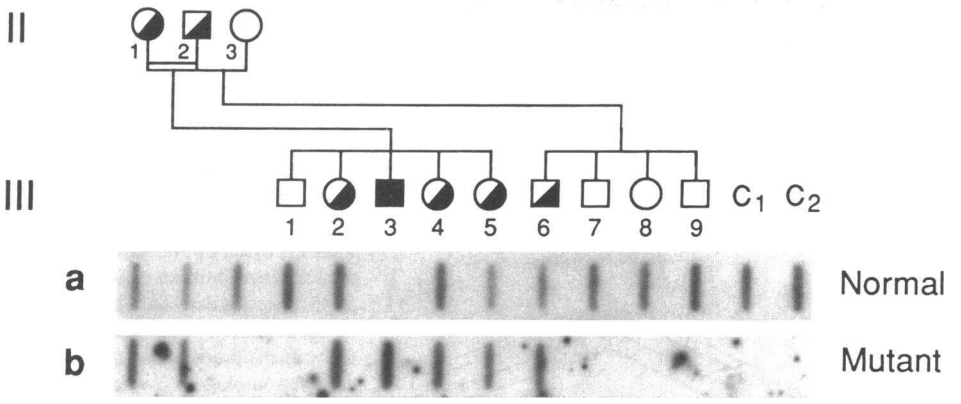

Figure 4. Hybridization of amplified genomic DNA to allele-specific oligomer probes. Genomic DNA from family members was amplified by PCR, slot blotted onto nylon membranes and hybridized with allele-specific oligomer probes containing either the normal or mutant sequence. *a*, normal probe. *b*, mutant probe. C_1 and C_2 are two unrelated normal controls.

Discussion

In this report, we have presented the results of a hematological and biochemical analysis of erythrocytes from a kindred with αI/74 HE and identified a mutation in the β-spectrin chain that is very closely associated with the disorder.

The homozygous αI/74 HE proband presented with severe hemolysis, growth retardation, and frontal bossing. The severity of the hemolysis was correlated with a high percentage of dimeric (unassembled) Sp and Sp variant. Similar findings were previously described in another SpαI/74 homozygote (45). These SpαI/74 variants have completely lost their self-association function in contrast to other Sp variants, such as SpαI/65, which retains some propensity to self-associate and does not lead to severe hemolysis even in homozygotes (46).

Studies of the homozygous proband revealed a phenotype with extreme spherocytosis, poikilocytosis, markedly increased thermal sensitivity, and a decreased amount of Sp in erythrocyte membranes. This phenotype is similar to that described in patients with HPP (1, 47).

Several results suggested that the basic defect responsible for the disorder in this family resided in the β- and not the α-spectrin chain. The results of genetic linkage analysis were inconsistent with a single α chain defect but suggested a β chain defect (Table II). Sequence analysis of exons 1 and 2 of the α-spectrin genes of the patient revealed no mutation in the first 82 amino acids of the αI domain (48). The results of spectrin chain reconstitution experiments showed that the presence of β and not α chains from the patient contributed to the elevated level of αI/74 fragment following partial tryptic digestion (48a).

To identify the molecular defect of the disorder in this family, we analyzed the region of the β-spectrin gene that encodes the carboxyl end of the chain, which is the end involved in dimer self-association. The availability of the polymerase chain reaction technique made it possible to rapidly examine the whole family for the presence of a particular mutation (49, 50). By nucleotide sequencing of PCR amplified products of this region of the proband's β-spectrin gene, we identified a point mutation in the gene that changes a codon for alanine to one for proline. This mutant allele was found by allele-specific oligomer hybridization to be present in, and only in, members of the family diagnosed by biochemical analyses to be heterozygous or homozygous for the αI/74 disorder. This absolute correlation between the molecular results and the biochemical data strongly suggest that the base substitution is the cause of the αI/74 disorder in this family.

The major portion of each spectrin chain is thought to consist of a repeating triple helical structure as suggested by Speicher and Marchesi (44). The repeat motifs of both genes show a distinct heptad pattern, with periodic alternation of hydrophilic and hydrophobic residues, suggesting that the triple helices form a coiled-coil structure, which is resistant to proteolysis (51). Examination of the sequences shows that in the β-spectrin chain the last repeat structure terminates after the second helix of the Speicher and Marchesi model (35), while in α-spectrin the repeat structure begins as the third helix of the model (44).

We propose that the ends of the repeat structures of the β and α chains interact to form a single triple helical unit in a coiled-coil configuration, with the β chain contributing the first two helices and the α the third (Fig. 5). In a spectrin tetramer, this proposed interaction is between the α and β chains from opposite dimers; in a dimer, the NH_2 terminus of the α chain may fold back to form a hair-pin structure with the COOH terminus of the β chain of the same dimer.

The proline substitution described in this report occurs near the middle of a potential α-helix of the β chain repeat participating in the inter-chain interaction proposed in the model (Fig. 5). Since a proline residue has a high propensity to break an α-helical structure, such a substitution at this site could disrupt the local conformation and prevent the formation of the coiled-coil structure between the two chains. Such an occurrence would cause an impairment in spectrin dimer self-association and expose the single helical coil at the end of the α-chain to enhanced proteolysis. The cleavage site that gives rise to the 74-kD fragment, Lys 42 in our case, lies near the middle of this particular helical coil. This model would thus explain how a mutation in the β chain could give rise to the phenotype of αI/74 HE. As shown in Fig. 5, the α chain cleavage would occur in the facing α chain of the head-to-head tetramer but could also occur in the side-to-side α chain of the dimeric structure described above.

The model presented above is consistent with a number of previously reported observations. It predicts that other mutations disrupting this region of either the α or β chain involved in the terminal coiled-coil interaction may also result in the αI/74 HE phenotype. Recent molecular studies on spectrin Culoz α40 (52), spectrin Lyon α43 (52), spectrin α22 (53), and spectrin α25 (Garbarz, M., unpublished data), all causing the αI/74 HE phenotype, detected the presence of point mutations in this region of the α chain. In HE associated with shortened β chains presumably truncated at their carboxyl end, spectrins

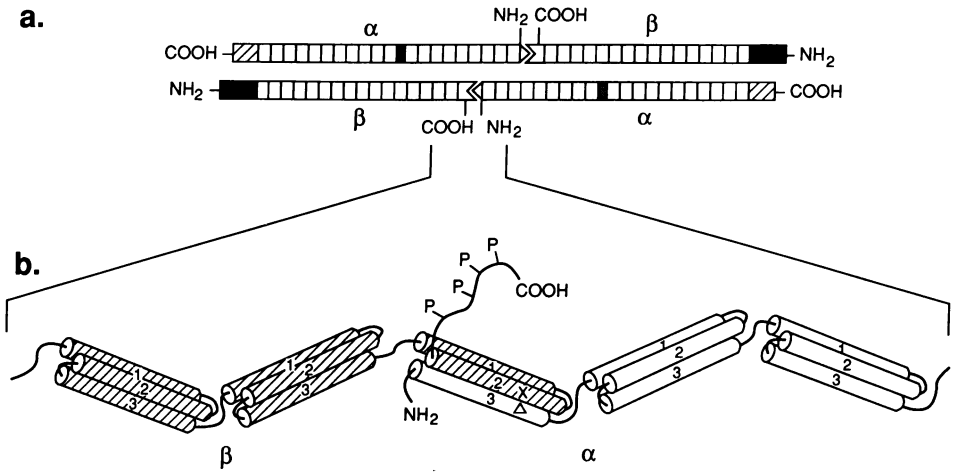

Figure 5. A model for the mechanism of interaction between the α- and β-spectrin chains. (*a*) the α- and β-spectrin chains are arranged in an anti-parallel fashion to form a dimer and two such dimers associate head-to-head to form a tetramer. The model for the various repeats and segments of the spectrin chains is based on the sequences of the full length human α-(60) and β-(43) spectrin cDNAs. The open blocks represent homologous repeats; the stippled block, the atypical nonhomologous α-spectrin repeat 10; the crosshatched and filled blocks, the segments at the NH_2 terminus and COOH terminus, respectively, of the α- and β-spectrin chains that share homology with analogous segments at the ends of the α-actinin chain (43, 60). (*b*) the ends of the α- and β-spectrin chains interact to form a single triple helical unit in a coiled-coil configuration. The αI/74 HE mutation found in the β chain (×) could disrupt this coiled-coil interaction, impairing spectrin dimer self-association and exposing the α chain to enhanced proteolysis at a normally protected site (*triangle*).

Nice and Rouen, there is also an elevated level of the αI 74-kD fragment on tryptic digestion (54, 55). The model explains the elevated amount of the 74-kD fragment seen after tryptic digestion of the isolated α chain because its β chain partner to form the coiled-coil structure is absent (15, 52). Previous observations that isolated peptides with an intact αI domain associate with the β chain in a head to tail and not side to side fashion are explained by the model (56). The inability of the 74-kD fragment from the αI domain to participate in tetramer formation also agrees with the predictions of the model (57).

Our model predicts a tight interaction between the ends of the α and β chains in the dimer form. Therefore, the association of dimers to form tetramers requires breaking of this intra-dimer interaction before tetramers can form. This process predicts a high activation energy of spectrin dimer self-association, which has been observed (58). In a related experiment, dimers with a defective αI domain were incubated with dimers with a truncated β chain. The rate of hybrid tetramer formation observed was six orders of magnitude higher than that of tetramer formation using normal dimers (59). Similar results were observed for the tetramer formation between SpαI/74(β mutation) and SpαI/74(α mutation) (Dhermy, D., unpublished data). According to our model, a high rate of association between these mutant dimers is expected because an intra-dimer interaction does not occur in these dimers while an inter-dimer interaction occurs between the normal chain of each dimer to form a hybrid tetramer.

The mutation described in this report is the first example of a point mutation in the β-spectrin chain that is associated with defective spectrin dimer self-association and an abnormal proteolytic cleavage of the α chain. Its identification should help elucidate the mechanism of association between the α- and β-spectrin chains.

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