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Band 3 protein degradation by calpain is enhanced in erythrocytes of old people

Neta SCHWARZ-BEN MEIR, Tova GLASER and Nechama S. KOSOWER*
Department of Human Genetics, Sackler School of Medicine, Tel-Aviv University, Ramat Aviv, Tel Aviv 69978, Israel

Band 3 protein is a major erythrocyte transmembrane glycoprotein. We compared the degradation of band 3 protein by calpain I (a cytoplasmic, micromolar-Ca^{2+}-requiring thiol proteinase) in the cells from old individuals (> 70 years old) to that in the cells from young ones (20–30 years old). In the young, little degradation of band 3 protein occurred in calpain-treated erythrocyte ghosts. In the old, significant band 3 protein degradation was found in erythrocyte ghosts treated similarly. The difference between young and old in the susceptibility of band 3 protein to calpain was retained in membrane vesicles (membranes stripped of peripheral proteins by NaOH) and in chymotrypsin-generated 60 kDa fragment (CH-60). The isolated *N*-terminal cytoplasmic 43 kDa fragment was degraded by calpain to a similar extent in old and in young. The separated 17 kDa membrane domain of the CH-60 and the trypsin-generated *C*-terminal 55 kDa membrane-spanning fragment were not degraded by calpain I in the young, nor in the old. Thus the *N*-terminal cytoplasmic domain is the domain degraded by calpain I. Enhanced sensitivity in the old is observed in intact band 3 protein and in CH-60, the isolated cytoplasmic domain being equally susceptible in young and old. The observed age-related enhanced sensitivity to calpain is consistent with the presence of modifications in band 3 protein and alterations in the association with the calpain–calpastatin system. Band 3 protein has several important functions, with modifications in the protein having implications for altered cell behaviour in the old individual.

INTRODUCTION

Band 3 protein is a major transmembrane glycoprotein in the erythrocyte and normally exists in the membrane as a dimer or tetramer, the molecular mass for the monomer being about 100 kDa [1–4]. The amino acid sequences of several band 3 and band 3-related proteins have been elucidated, but the structures are not yet known [5–8]. Band 3 protein is divided into two rather different domains of similar size. The *C*-terminal domain is hydrophobic and certainly includes multiple membrane crossings. The *N*-terminal domain is water-soluble and located within the cell [3]. Band 3 protein has several important functions. The *C*-terminal domain contains the anion-transport functional region that mediates chloride–bicarbonate exchange across the erythrocyte membrane [1–4]. It also contains one site for the attachment of an oligosaccharide chain that carries blood-group antigenic determinants [1–3]. The *N*-terminal cytoplasmic domain contains binding sites for cytoskeletal and cytoplasmic proteins [3].

Band 3 protein may be involved in the generation of the senescence signals that lead to the recognition and removal of old erythrocytes from the circulation [1–3,9,10]. It has been proposed that degradation of band 3 protein plays a role in the generation of a senescence signal [9]. Some degradation of band 3 protein seems to occur *in vivo* during erythrocyte aging in the circulation [11,12], but the relationships of the degradation to erythrocyte removal and the proteinase(s) responsible are not known. A shortened erythrocyte survival has been found in old individuals [13,14]. The factors involved have not been clarified.

The cytoplasmic Ca^{2+}-activated thiol endopeptidase calpain is known to be responsible for limited protein degradation of some membrane and cytoskeletal proteins and plays a role in membrane-associated events [15–20]. Calpastatin, a highly specific endogenous calpain inhibitor, is as widely distributed as the proteinase. The concentrations of the different types of calpain and calpastatin vary from tissue to tissue and from organism to organism. Suggestions that the calpain–calpastatin system plays a role in aging processes are up to now unsupported by data.

We have studied the susceptibility of erythrocyte membrane proteins to the micromolar-Ca^{2+}-requiring proteinase calpain I. We show here that the degradation of band 3 protein by calpain I is enhanced in erythrocytes of old individuals. The results indicate age-related changes in band 3 protein or in its interaction with membrane and/or cytoplasmic components. Counterparts of band 3 protein have been found in other cells [1–4,21–23], making studies of the behaviour of band 3 protein in erythrocytes of aged individuals potentially important for understanding the behaviour of certain cell membrane proteins in aging.

MATERIALS AND METHODS

Erythrocyte ghosts

Heparinized blood was obtained from healthy humans 20–30 years old ('young') and 70–90 years old ('old'). Blood was centrifuged, plasma and buffy coat were removed and the erythrocytes washed three times with 150 mM-NaCl. Haemoglobin-free ghosts were prepared by haemolysing erythrocytes in 5 mM-phosphate buffer, pH 8.0 (5P8), washing in 5P8 and followed by washing with 10 mM-NaCl.

Band 3 protein and fragments

Erythrocyte membranes were stripped of peripheral proteins by established procedures [24]; 10 vol. of 0.1 M-NaOH were added to 1 vol. of ghost suspension at 4 °C for a few seconds,

Abbreviations used: PMSF, phenylmethane sulphonyl fluoride; Tos-Phe-CH_2Cl, *N*-tosyl-L-phenylalaninechloromethane ('TPCK'); SBTI, soybean trypsin inhibitor; PBS, phosphate-buffered saline; DTT, dithiothreitol.

* To whom correspondence should be sent.

followed by centrifugation at 40000 *g* and washing with 10 mM-NaCl. This procedure yielded vesicles containing only traces of spectrin and other cytoskeletal proteins.

Band 3 protein fragments were generated by established procedures [25,26]. Intact erythrocytes, suspended in 135 mM-NaCl/10 mM-sodium phosphate buffer, pH 7.4 (phosphate-buffered saline, PBS), were treated with chymotrypsin (Sigma, 1 mg/ml of 50% cell suspension) for 30 min at 37 °C. Cells were then washed once in PBS containing 1.0% of BSA and twice with buffer alone. Chymotrypsin-treated cells were then haemolysed and membranes prepared as described above to yield chymotrypsin band 3 protein fragments of 60 kDa (CH-60) and 35 kDa (CH-35).

Erythrocyte ghosts, suspended in PBS buffer, were treated with trypsin (Sigma; 0.05 mg/ml of 20% ghost suspension) for 30 min at 25 °C [16]. The reaction was stopped by the addition of 0.2 mg of SBTI/ml, washing in 10 mM-NaCl and stripping of peripheral proteins, to generate the transmembrane band 3 protein *C*-terminal fragment of 55 kDa (TR-55) [26]. The transmembrane band 3 protein 17 kDa fragment was generated by digestion of intact cells with chymotrypsin, followed by trypsin digestion of the cell membranes and stripping the preparations of peripheral proteins.

The *N*-terminal cytoplasmic fragment was prepared by established procedures [27] with slight modifications. Chymotrypsin was added to pH 11 stripped membrane vesicles (2 μg of chymotrypsin/ml of vesicle suspension) at 0 °C for 20 min. The reaction was stopped by the addition of 0.1 mM-phenylmethanesulphonyl fluoride (PMSF) and 0.1 mM-*N*-tosyl-L-phenylalanylchloromethane ('TPCK'). The supernatant containing the *N*-terminal cytoplasmic 43 kDa fragment was obtained by centrifugation of the treated vesicle suspensions at 40000 *g* for 20 min.

Calpain and calpastatin

Human erythrocytes (obtained from young individuals) were haemolysed in 10 vol. of 10 mM-Tris/HCl (pH 7.4)/1.0 mM-EGTA/1.0 mM-EDTA/0.5 mM-dithiothreitol (DDT). Membrane-free haemolysate (obtained by centrifugation of the lysed cells at 40000 *g* for 20 min) was used for the isolation of calpain and calpastatin by published procedures [15], with some modifications, as previously described [28]. Calpain activity was measured with casein as substrate, and analysis of acid-soluble products was carried out by established procedures [15,28]. A unit of calpain is defined as the proteolytic activity giving an A_{750} of 1.0 in the colour reaction for the acid-soluble products [15]. A unit of calpain activity is usually obtained from about 1–2 ml of erythrocytes. A unit of calpastatin activity is defined as the amount inhibiting 1 unit of calpain.

Treatment of erythrocyte ghosts, stripped membranes and band 3 protein fragments with calpain

White erythrocyte ghosts were suspended in 10 mM-Tris/HCl buffer, pH 7.4, containing 0.1 mM-PMSF. The ghost suspensions were mixed with calpain solution (0.5–1.0 unit/ml of 10% ghost suspension) and kept at 4 °C for 15 min. A solution of NaCl, Ca^{2+} and DTT was then added (final concentrations of 135 mM-NaCl, 1.0 mM-Ca^{2+} and 0.5 mM-DTT), and suspensions were incubated at 30 °C for 30–60 min. In some experiments calpastatin was added together with calpain (2.0 units of calpastatin/unit of calpain). At the end of the incubation period, Laemmli buffer [29] was added to stop the reaction and solubilize the membranes.

Stripped membranes and band 3 protein fragments were suspended in 10 mM-Tris/HCl buffer, pH 7.4, containing 0.1 mM-PMSF, 135 mM-NaCl, 1.0 mM-Ca^{2+} and 0.5 mM-DTT and incubated with or without calpain (0.2–0.5 unit of calpain/ml of stripped membranes containing 0.5 mg of protein; 0.1–1.0 unit of calpain/ml of band 3 protein fragments containing 0.2–0.5 mg of protein). Samples were incubated at 30 °C for 30–60 min and then solubilized as described above.

SDS/PAGE and immunostaining methods

Proteins were analysed by SDS/PAGE (using 10% acrylamide in the gels) as described by Laemmli [29]. Immunoblotting was carried out by standard procedures [30]. Polyclonal antibody (rabbit) to human band 3 protein was a gift from Dr. David Gershon, the Technion, Haifa, Israel. Polyclonal antibody (rabbit) to the cytoplasmic *N*-terminal 43 kDa fragment was a gift from Dr. Philip S. Low, Purdue University, West Lafayette, IN, U.S.A. Alkaline phosphatase-conjugated anti-rabbit IgG antibody was used as a secondary antibody, using 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt and *p*-NitroBlue Tetrazolium chloride Bio-Rad reagents [31] as substrates.

RESULTS

Degradation of band 3 protein in erythrocyte membranes

Erythrocyte ghosts, prepared from cells of young and of old individuals, were incubated in the presence or absence of calpain. Membranes were then solubilized and analysed by SDS/PAGE and by immunoblotting. Coomassie Blue staining is shown in Fig. 1(*a*) (lanes a–d) and immunoblotting with polyclonal antibodies to band 3 protein (AbI) is shown in Fig. 1(*b*) (lanes e–h). Membranes from young and old individuals incubated without calpain appeared similar in most cases or showed very slight differences (noted in the lower-molecular-mass proteins) when viewed in Coomassie Blue-stained gels (Fig. 1, lanes a and c). In the presence of calpain, band 3 protein in erythrocyte membranes of old individuals was degraded to an appreciable extent (Fig. 1*b*), whereas little degradation of band 3 protein from

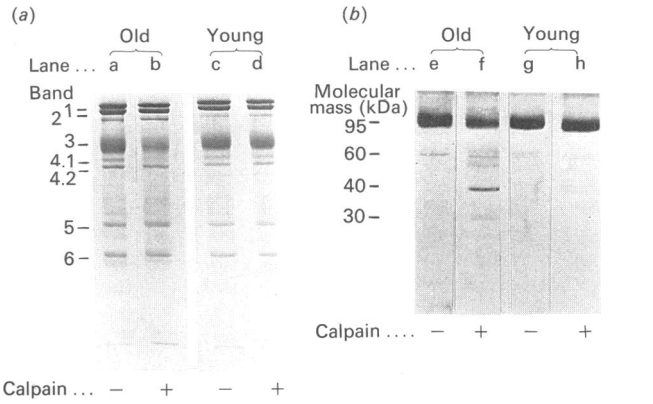

Fig. 1. Profiles of proteins from control and from calpain-treated erythrocyte membranes: SDS/PAGE and immunoblots obtained with antibody to band 3 protein (AbI)

Erythrocyte-ghost suspensions were incubated at 30 °C for 60 min with or without calpain (0.5 unit of calpain/ml of 10% erythrocyte ghost suspension) and processed as described in the Materials and methods section. (*a*) (Lanes a–d), Coomassie Blue-stained gel; (*b*) (lanes e–h), immunoblots with AbI. Lanes a, b, e and f, membranes from an old individual; lanes c, d, g and h, membranes from a young individual; lanes a, c, e and g, control membranes incubated without calpain; lanes b, d, f and h, membranes incubated with calpain. Erythrocyte samples from seven out of 11 old individuals showed calpain-induced proteolysis similar to that shown here, whereas samples from two out of nine young donors showed slight proteolysis under the same experimental conditions.

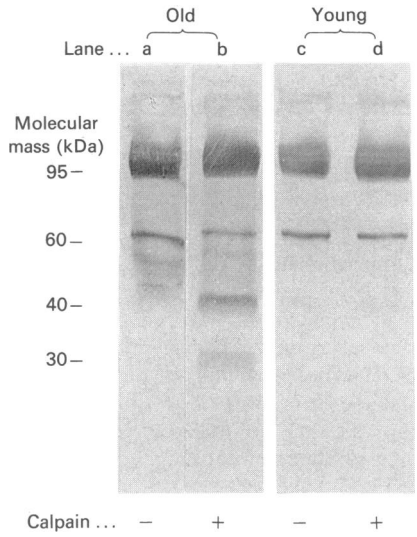

Fig. 2. Immunoblots, obtained with antibody to 43 kDa (AbII), of proteins from control and from calpain-treated erythrocyte membranes

Ghost suspensions were incubated at 30 °C for 40 min with or without calpain (0.5 unit of calpain/ml of 10% erythrocyte ghost suspension) and processed as described in the Materials and methods section. Lanes a and b, membranes from an old individual; lanes c and d, membranes from a young individual; lanes a and c, control membranes; lanes b and d, calpain-treated membranes. Calpain-induced degradation patterns similar to that shown here for the old donor were obtained in five out of eight old individuals. No such degradation was observed in any of the six young donors studied under the same experimental conditions.

young individuals occurred under these conditions (Fig. 1, d). Immunoblotting with AbI showed, in addition to band 3 protein, one or several minor fractions in the region of 60 kDa in the control membranes, this being noted somewhat more in the membranes from old individuals (Fig. 1, e) than in those from young ones (Fig. 1, g). In calpain-treated membranes from old individuals, several additional, lower-molecular-mass fractions were observed in the regions of 40 kDa and 30 kDa, with diminution of the 95 kDa fraction (Fig. 1, f). Little or no change was noted in calpain-treated membranes from young individuals

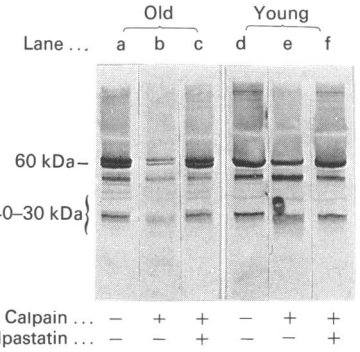

Fig. 4. Immunoblots of control and of calpain-treated band 3 protein chymotrypsin-generated fragments

Immunoblotting was carried out with AbII. Suspensions of chymotrypsin-generated fragments were incubated at 30 °C for 60 min with or without calpain (0.2 unit of calpain/ml of suspension containing 0.5 mg of protein), with or without calpastatin (0.4 unit/ml of fragment suspension). Lanes a, b and c, CH-60 from an old individual; lanes d, e and f, CH-60 from a young individual; lanes a and d, control; lanes b and e, calpain-treated; lanes c and f, calpastatin- and calpain-treated. Six out of nine old individuals showed significant proteolysis of CH-60 fragment, similar to the one shown here. Six out of nine young donors showed slight proteolysis, similar to the one shown here, with three showing very little proteolysis.

(Fig. 1, h). Degradation of band 4.1 also occurred in membranes from old individuals (Fig. 1, b). Little or no degradation of band 4.1 was observed in the membranes of erythrocytes from young individuals (Fig. 1, d).

Immunoblotting with polyclonal antibodies to the *N*-terminal cytoplasmic domain (AbII) also showed a fraction of 60 kDa in untreated membranes of both young and old individuals (Fig. 2, a and c). In addition, immunoblotting with AbII showed multiple weak bands between 95 and 60 kDa and 60 and 40 kDa in membranes from some old individuals (in three out of eight cell-ghost preparations), appearing mostly as diffuse staining in these regions (Fig. 2, a). In calpain-treated membranes from old individuals, protein bands of 40 kDa and 30 kDa were observed (Fig. 2, b), with little of such bands noted in immunoblots of calpain-treated membranes from young individuals (Fig. 2, d).

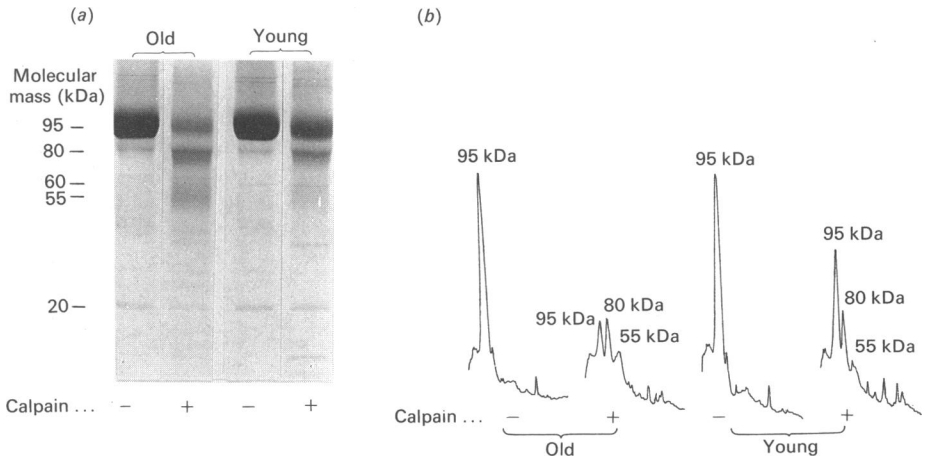

Fig. 3. SDS/PAGE profiles (*a*) and densitometric scans (*b*) of proteins from membranes stripped of peripheral proteins

Suspensions of stripped membranes were incubated at 30 °C for 40 min with or without calpain (0.4 unit of calpain/ml of stripped membrane suspensions containing 0.5 mg of protein). Stripped membranes from seven out of ten old individuals showed calpain-induced proteolysis similar to, or greater than, that shown here. Samples from three out of eight young donors showed proteolysis similar to that shown here, with the rest showing little or no proteolysis.

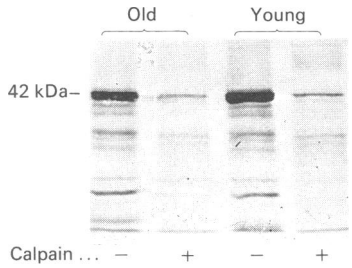

Fig. 5. Immunoblots of control and of calpain-treated isolated *N*-terminal soluble cytoplasmic 43 kDa domain of band 3 protein

Aliquots were incubated at 30 °C for 30 min with or without calpain (0.1 of calpain/ml of suspension containing 0.25 mg of protein). Immunoblotting was carried out with AbII. Results similar to those shown here were obtained in four old and four young individuals.

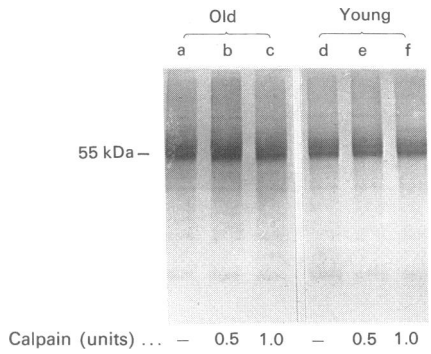

Fig. 6. SDS/PAGE profiles of control and of calpain-treated trypsin-generated 55 kDa membrane fragment (TR-55)

Suspensions of TR-55 were incubated at 30 °C for 60 min with or without calpain (0.5 or 1.0 unit of calpain/ml of suspension containing 0.5 mg of protein). Lanes a, b and c, TR-55 from an old individual; lanes d, e and f, TR-55 from a young individual; lanes a and c, control; lanes b, d, e and f, calpain-treated. Results similar to those shown here were obtained in four old and four young individuals.

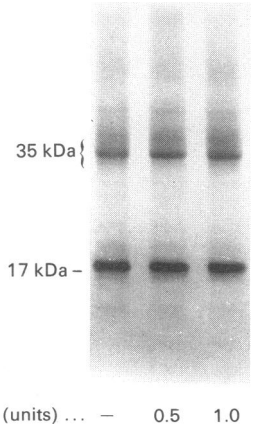

Fig. 7. SDS/PAGE profiles of control and of calpain-treated 17 kDa membrane domain from an old individual

Suspensions were incubated at 30 °C for 60 min with or without calpain (0.5 or 1.0 unit of calpain/ml of fragment suspension containing 0.5 mg of protein). Lane a, control; lanes b and c, calpain-treated. Results similar to those shown here were obtained in four old and four young individuals.

That AbII seems to 'recognize' the 60 kDa fraction and minor fractions better than AbI is possibly due to the different preparations used for obtaining the antibodies (intact band 3 used for AbI and the isolated *N*-terminal cytoplasmic domain used for AbII).

Degradation of band 3 protein in membranes stripped of peripheral proteins

In order to find out whether the difference in the degradation of band 3 protein between old and young individuals was dependent on, or independent of, intact membrane structure, erythrocyte ghosts were stripped of peripheral proteins by a short treatment with NaOH as described in the Materials and methods section. The stripped membrane preparations were incubated without or with calpain, solubilized, and analysed by SDS/PAGE. Coomassie Blue staining (Fig. 3*a*) and densitometry of the stained gel (Fig 3*b*) showed some degradation of band 3 protein (95 kDa) in stripped membrane preparations from young individuals. Band 3 protein of old individuals was degraded by calpain more than that of young individuals. Thus band 3 protein in stripped membranes of young individuals seems to be more susceptible to calpain than the protein in the intact membrane, but the difference between young and old in the susceptibility of band 3 protein to calpain is clearly observed in membrane vesicles after the removal of most of the peripheral proteins.

Degradation of band 3 protein fragments

Erythrocytes were treated with chymotrypsin (to yield the CH-60 and CH-35 fragments [26]), after which the membranes were isolated and stripped of peripheral proteins. Aliquots were incubated with or without calpain, each matched by a duplicate including calpastatin. Samples were then solubilized, electrophoresed and immunoblotted. Control preparations from old and young showed several fractions in addition to the CH-60 fragment, as shown by the appearance of bands of lower molecular mass in immunoblots, using AbII (Fig. 4, a and d). In some old individuals (four out of 11 from whom CH-60 fragment was obtained), the CH-60 fragment appeared as two discrete bands (Fig. 4, a). Only one CH-60 band was found in preparations from nine young individuals studied under these conditions (Fig. 4, d). Incubation with calpain caused a significant degradation of CH-60 and of the lower-molecular-mass fractions in preparations from old individuals, with both CH-60 bands degraded to a similar extent (Fig. 4, b). A slight degradation of CH-60 was seen in preparations from young individuals (Fig. 4, e). Calpastatin inhibited the calpain-induced degradation (Fig. 4, c and f). Thus the CH-60 fragments were degraded by calpain, with the difference between young and old still apparent.

In order to characterize further the calpain-sensitive domains, the *N*-terminal 43 kDa fragment and the C-terminal 55 kDa fragments [26,27] were treated with calpain. The 43 kDa fragment was degraded by calpain, with a similar degree of degradation observed in preparations from young and old individuals (Fig. 5).

The *C*-terminal 55 kDa membrane-spanning domain was not degraded by calpain in either young or old individuals under the experimental conditions used (Fig. 6). The 17 kDa membrane domain, obtained by chymotrypsin treatment of intact cells, followed by trypsin treatment of the erythrocyte membranes [26], was not susceptible to calpain (Fig. 7).

Thus the degradation of band 3 protein by calpain involves mainly the cytoplasmic *N*-terminal 43 kDa domain, with enhanced sensitivity to degradation observed in old people in the intact band 3 protein and in the CH-60 fragment. The isolated

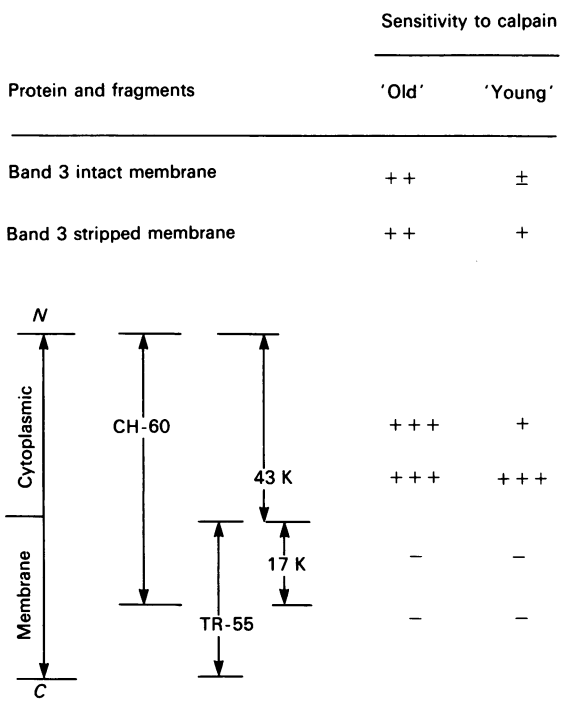

Fig. 8. Sensitivity of band 3 protein and band 3 protein fragments from old and young individuals to calpain

N, band 3 protein *N*-terminus; *C*, band 3 protein C-terminus; CH-60, the chymotrypsin-generated *N*-terminal fragment; 43 K, the *N*-terminal 43 kDa cytoplasmic domain; 17 K, the transmembrane 17 kDa domain of CH-60; TR-55, the trypsin-generated *C*-terminal transmembrane fragment.

43 kDa domain is equally sensitive in the 'young' and 'old'. The results are summarized in Fig. 8.

DISCUSSION

The enhanced sensitivity of band 3 protein in old individuals ('old' band 3 protein) to degradation by calpain appears to be quantitatively, rather than qualitatively, different from that in young individuals ('young' band 3 protein). The differential sensitivity to calpain, observed in intact membranes, was apparent also in the disrupted membrane preparations containing CH-60, whereas the isolated *N*-terminal cytoplasmic domain derived from 'old' and from 'young' band 3 protein was equally susceptible to calpain. These results point to the CH-60 fragment itself and/or association with other membrane components (i.e., phospholipids, proteins) as being altered in old individuals. The hinge region between the cytoplasmic and transmembrane domain of band 3 protein, being intact in the CH-60, may contain a site which is degraded more easily in cells of old people. When CH-60 is present in the 'native' conformation, alterations in other parts of band 3 protein or in other membrane components could also influence the sensitivity of the protein to calpain. An altered sensitivity of globin to degradation by calpain has been shown to be associated with mutations either near or far from the cleaved sites [32].

The enhanced sensitivity of band 3 protein from old individuals to calpain could be due to post-translational modifications of band 3 protein or of other membrane components. Several age-related post-translational modifications in proteins from senescent tissues have been described, including changes due to oxidation, deamidation, non-enzymic glycosylation, and alterations in conformation without evidence for covalent modification [33,34]. Band 3 protein is known to undergo some modifications, such as phosphorylation of tyrosine-8 and methylation of aspartic acid residues at the hinge region between the cytoplasmic and the transmembrane domains of the protein [35]. Age-associated alterations in the band 3 protein primary amino acid sequence are not excluded, however. No information is available at present on any such change in 'old' band 3 protein.

It has been suggested that, in the presence of micromolar concentrations of Ca^{2+}, cytoplasmic inactive calpain associates with the cell membrane. The binding results in calpain activation [15–18,36–39]. Interaction with phospholipids lowers the requirement of calpain for Ca^{2+} [17]. An altered phosphorylation state of membrane components (due, e.g., to changes in metabolic activity of the erythrocytes in old individuals) may change calpain activity and/or change the association of calpastatin with the phospholipids and with calpain [40,41]. We have found an enhanced calpain binding and activation in erythrocyte membranes from old individuals, along with a diminution in the binding of calpastatin to these membranes (N. Schwarz-Ben Meir, T. Glaser & N. S. Kosower, unpublished work). The altered association of the calpain–calpastatin system with the erythrocyte membranes from old individuals is consistent with the enhanced band 3 protein degradation in these membranes shown here, but the relationship between the two remains to be studied.

The erythrocytes of old people have a shortened life span in the circulation as compared with that of cells from young individuals [13,14]. It has been suggested that the erythrocyte in the old individual is released from the bone marrow into the circulation with a diminished capacity to protect the cell against damage [13]. Such a cell would undergo membrane alterations qualitatively similar to those occurring in the cells of the young individual, but at an accelerated rate, resulting in an early removal from the circulation [13]. The enhanced band 3 protein degradation in old individuals shown here is consistent with these assumptions, being quantitatively different from, but qualitatively similar to, that in young individuals.

The exact nature of the membrane alterations leading to the removal of the erythrocyte from the circulation is not known [14]. It has been suggested that a fraction of band 3 protein may, via limited oxidation and/or proteolysis, undergo conformational modifications and serve as a source for senescence signal(s), marking the erythrocyte for removal from the circulation [9,10]. An enhanced band 3 protein degradation may play a role in the events leading to the shortened life of the erythrocyte in old individuals.

In some old individuals we observed two kinds of band 3 protein, differing slightly in molecular mass. This was not discerned when comparing the appearance in SDS/PAGE of the 95 kDa wide bands of 'old' and 'young', but the two bands were easily observed by SDS/PAGE analysis of CH-60 fragments, which lack the carbohydrate chain and appear as narrow bands. The appearance on SDS/PAGE of two bands of CH-60 fragments seen in some old people may reflect post-translational modifications or be due to a limited degradation *in vivo* of some of the cytoplasmic *N*-terminal molecules, resulting in a slightly shortened molecule. Differences among individuals in the rate and magnitude of the appearance of any alteration may account for the fact that only some of the old individuals show changes obvious on gels.

The degradation of the *N*-terminal cytoplasmic domain would affect its associations with cytoskeletal and cytoplasmic components [3,42,43]. As a result, cell structural stability and glycolytic-pathway activity may be altered [42–44]. We propose

that a calpain-induced limited degradation of a fraction of the *N*-terminal cytoplasmic domain of band 3 protein leads to perturbation of the outer membrane surface via conformational changes communicated from the inside and via metabolic changes, with the end result of the elimination of the cell. Several variant forms of band 3 protein have been described [11,45]. Of interest is the case of haemolytic anaemia associated with increase in the amount of band 3 protein degradation products and erythrocyte IgG binding [45]. The defect responsible for the enhanced susceptibility to degradation in that case is not known.

The enhanced susceptibility of band 3 protein from old people to calpain-induced degradation demonstrated here presents a well-defined age-associated altered behaviour of a specific important membrane protein. Counterparts of band 3 protein are found in other cells [1–4,21–23]. The erythrocyte membrane thus provides a useful model for the study of age-associated alterations in membrane structure in general and band 3-like proteins in particular.

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REFERENCES

1. Passow, H. (1986) Rev. Physiol. Biochem. Pharmacol. **103**, 62–203
2. Cabantchik, Z. I. (1983) in Structure and Function of Membrane Proteins (Quagliariello, E. & Palmieri, F., eds.), pp. 271–281, Elsevier, Amsterdam
3. Low, P. S. (1986) Biochim. Biophys. Acta **864**, 145–167
4. Jennings, M. L. (1989) Annu. Rev. Biophys. Biophys. Chem. **18**, 397–430
5. Tanner, M. J. A., Martin, P. G. & High, S. (1988) Biochem. J. **256**, 703–712
6. Kopito, R. R. & Lodish, H. F. (1985) Nature (London) **316**, 234–238
7. Cox, J. V. & Lazarides, E. (1988) Mol. Cell. Biol. **8**, 1327–1335
8. Lux, S. E., John, K. M., Kopito, R. R. & Lodish, H. F. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 9089–9093
9. Bosman, G. J. C. G. M. & Kay, M. M. B. (1988) Blood Cells **14**, 19–35
10. Lutz, H. U., Fasler, S. Stammler, P., Bussolino, F. & Arese, P. (1988) **14**, 175–195
11. Morrison, M., Grant, W., Smith, H. T., Mueller, T. J. & Hsu, L. (1985) Biochemistry **24**, 6311–6315
12. Czerwinski, M., Wasniowska, K., Steuden, I., Duk, M., Wiedlocha, A. & Lisowska, E. (1988) Eur. J. Biochem. **174**, 647–654
13. Gershon, H. & Gershon, D. (1988) Blood Cells **714**, 93–98
14. Danon, D. & Marikovsky, Y. (1988) Blood Cells **14**, 7–15
15. Murachi, T. (1984) Biochem. Soc. Symp. **49**, 149–167
16. Suzuki, K., Imajoh, S., Emori, Y., Kawasaki, H., Minami, Y. & Ohno, S. (1987) FEBS Lett. **220**, 271–277
17. Melloni, E., Pontremoli, S. (1989) Trends Neurosci. **12**, 438–444
18. Mellgren, R. L. (1987) FASEB J. **1**, 110–115
19. Glaser, T. & Kosower, N. S. (1986) Eur. J. Biochem. **159**, 387–392
20. Kosower, N. S. & Glaser, T. (1990) in Intracellular Ca^{2+}-Dependent Proteolysis (Mellgren, R. L. & Murachi, T., eds.), pp. 163–180, CRC Press, Boca Raton, FL
21. Passow, H., Raida, M., Wendel, J., Legrum, B., Bartel, D., Lepke, S. & Furuto-Kato, S. (1989) Biochem. Soc. Trans. **17**, 812–815
22. Rothstein, A. (1989) in Membrane Transport (Tosteson, D. C., ed.), pp. 203–235, Waverly Press, Baltimore
23. Kopito, R. R., Lee, B. S., Simmons, D. M., Linsey, A. E., Morgans, C. W. & Schneider, K. (1989) Cell (Cambridge, Mass.) **59**, 927–937
24. Steck, T. L., Ramos, B. & Strapazon, E. (1976) Biochemistry **15**, 1154–1161
25. Jennings, J. L. & Passow, H. (1979) Biochim. Biophys. Acta **554**, 498–519
26. Cousin, J. L. & Motais, R. (1982) Biochim. Biophys. Acta **687**, 147–155
27. Korsgren, C. & Cohen, C. M. (1988) J. Biol. Chem. **263**, 10212–10218
28. Glaser, T. & Kosower, N. S. (1986) FEBS Lett. **206**, 115–120
29. Laemmli, U. K. (1970) Nature (London) **227**, 680–685
30. Towbin, H., Staehelin, T. & Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4350–4354.
31. Harlow, E. & Lane, D. (1988) Antibodies, a Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
32. Melloni, E., Salamino, F., Sparatore, B., Michetti, M. & Pontremoli, S. (1984) Biochim. Biophys. Acta **788**, 11–16
33. Dice, J. F. & Goff, S. A. (1987) in Modern Biological Theories of Aging (Warner, H. R., Butler, R. N., Sprott, R. C. & Schmeider, E. L., eds.), pp. 155–168, Raven Press, New York
34. Harding, J. J., Beswick, H. T., Ajiboye, R., Huby, R., Blakytny, R. & Rixon, K. C. (1989) Mech. Ageing Dev. **50**, 7–16
35. Lowenson, J. & Clarke, S. (1988) Blood Cells **14**, 103–117
36. Suzuki, K. (1987) Trends Biochem. Sci. **12**, 103–105
37. Kuboki, M., Ishii, H. & Kazama, M. (1987) Biochim. Biophys. Acta **929**, 164–172
38. Sakai, K., Hayashi, M., Kawashima, S. & Akanuma, H. (1989) Biochim. Biophys. Acta **985**, 51–54
39. Inomata, M., Hayashi, M., Nakamura, M., Saito, Y. & Kawashima, S. (1989) J. Biol. Chem. **264**, 18838–18843
40. Mellgren, R. L., Lane, R. D. & Mericle, M. T. (1989) Biochim. Biophys. Acta **999**, 71–77
41. Kapprell, H. P. & Goll, D. E. (1989) J. Biol. Chem. **264**, 17888–17896
42. Davis, L., Lus, S. E. & Bennett, V. (1989) J. Biol. Chem. **264**, 9665–9672
43. Willardson, B. M., Thevenin, B. J. M., Harrison, M. L., Kuster, W. M., Benson, M. D. & Low, P. S. (1989) J. Biol. Chem. **264**, 15893–15899
44. Boivin, P. (1988) Biochem. J. **256**, 689–695
45. Kay, M. M. B., Flowers, N., Goodman, J. & Bosman, G. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 5834–5838

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