Volume 170, number 2 FEBS 1450 May 1984

Degradation of actin and vimentin by calpain II, a Ca^{2+}-dependent cysteine proteinase, in bovine lens

Haruko Yoshida, Takashi Murachi* and Isamu Tsukahara

*Departments of Ophthalmology and *Clinical Science, Faculty of Medicine, Kyoto University, Kyoto 606, Japan*

Received 17 March 1984; revised version received 30 March 1984

Calpain II, a high Ca^{2+}-requiring form of Ca^{2+}-dependent cysteine proteinase (EC 3.4.22.17), isolated from bovine lens was found to cleave actin and vimentin, two major cytoskeletal elements of the lens. Polyacrylamide gel electrophoresis revealed that actin (M_r 43000) was broken down through intermediary products of approximate M_r 42000 and 40000, while vimentin (M_r 57000) was rapidly cleaved into several fragments ranging from M_r 44000 to 20000. The cleavage was dependent on Ca^{2+} and could be blocked by calpastatin, a calpain-specific inhibitor. These findings suggest that calpain might play a role in age-related degradation of the lens cytoskeleton.

Ca^{2+}-dependent cysteine proteinase *Calpain* *Actin* *Vimentin* *Proteolysis* *Bovine lens*

1. INTRODUCTION

Recently much attention has been paid to the function of cytoskeletal proteins. In the lens, the cytoskeletal filaments not only constitute the intracellular matrix but also support the elasticity and deformability of the lens for its accommodation [1–3]. The biochemical and morphological studies have revealed that the major components of cytoskeleton of the lens fiber cells are actin and vimentin [4–6]. It is known that newly produced lens fiber cells contain large amounts of 57 kDa and 43 kDa polypeptides, corresponding to vimentin and actin, respectively, but as lens fiber cells become older and displaced towards the center of the lens to form its nucleus, these two polypeptides as well as a 2000 kDa polypeptide diminish and disappear [7,8]. We previously reported limited proteolysis of bovine lens α-crystallin by calpain II, a high Ca^{2+}-requiring form of Ca^{2+}-dependent cysteine proteinase (EC 3.4.22.17; [9]) isolated from the same tissue, suggesting that the age-related degradation of α-crystallin could be an enzymatic process [10]. As a continuation along this line, we here wish to report briefly that actin and vimentin of bovine lens are also rapidly proteolyzed by lens calpain II. Ca^{2+}-induced degradation of vimentin in the lens was reported in [11], but the responsible enzyme was not isolated.

* To whom correspondence should be addressed

2. MATERIALS AND METHODS

2.1. *Purification of calpain II from bovine lens*

Calpain II was purified from fresh bovine lens, to homogeneity on polyacrylamide gel electrophoresis (PAGE), as in [10].

2.2. *Preparation of urea-soluble proteins, actin and vimentin from bovine lens*

The urea-soluble proteins were prepared by extracting the water-insoluble fraction from 50 g bovine lens with 30 ml of 50 mM Tris–HCl buffer (pH 8.6) containing 6 M urea and 1 mM EDTA at 4°C for 2 h and ultracentrifuged at 100000 × g for 90 min. The supernatant was dialyzed against water [6]. Actin and vimentin were purified from fresh bovine lens as in [2] and [12], respectively, both to apparent homogeneity on SDS–PAGE. The identity of actin was confirmed by the immunoelectrophoretic blotting method [13,14], us-

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00 © 1984 Federation of European Biochemical Societies

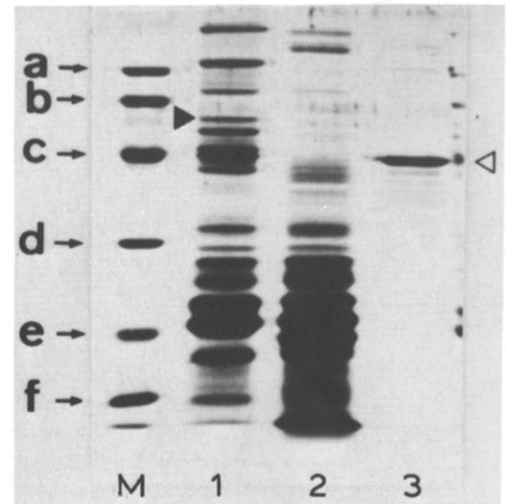

Fig.1. The effect of lens calpain on the urea-soluble fraction of bovine lens. Urea-soluble extracts (50 μg) were incubated with 0.06 μg calpain II without Ca^{2+} (lane 1) and with 1 mM Ca^{2+} (lane 2). Lane 3 shows lens actin used as a marker. Lane M shows the marker proteins which include: a, phosphorylase (M_r 94000); b, bovine serum albumin (M_r 67000); c, ovalbumin (M_r 43000; d, carbonic anhydrase (M_r 30000); e, soybean trypsin inhibitor (M_r 20100); f, α-lactalbumin (M_r 14400). The pattern of degradation was analyzed by SDS-polyacrylamide gel (12%) electrophoresis. The solid arrowhead denotes the position of vimentin, and the open arrowhead denotes the position of actin.

ing rabbit anti-chicken actin antibody, which was a generous gift from Dr Ichiro Yahara, Tokyo Metropolitan Institute of Medical Science, Tokyo.

2.3. *Proteolysis of actin and vimentin by calpain II*

Proteolysis of urea-soluble proteins, actin and vimentin, was carried out at 30°C in an incubation mixture which contained various amounts of calpain II, 50 mM imidazole–HCl buffer (pH 7.5), 5 mM cysteine and 1 mM $CaCl_2$. The reaction was stopped by transferring a 25-μl aliquot of the reaction mixture into 25 μl of 50 mM Tris–HCl buffer (pH 6.8) containing 2% SDS, 10% glycerol, and 5 mM 2-mercaptoethanol, followed by heating at 100°C for 5 min. Then the pattern of proteolysis was analyzed by SDS-polyacrylamide gel (12%)

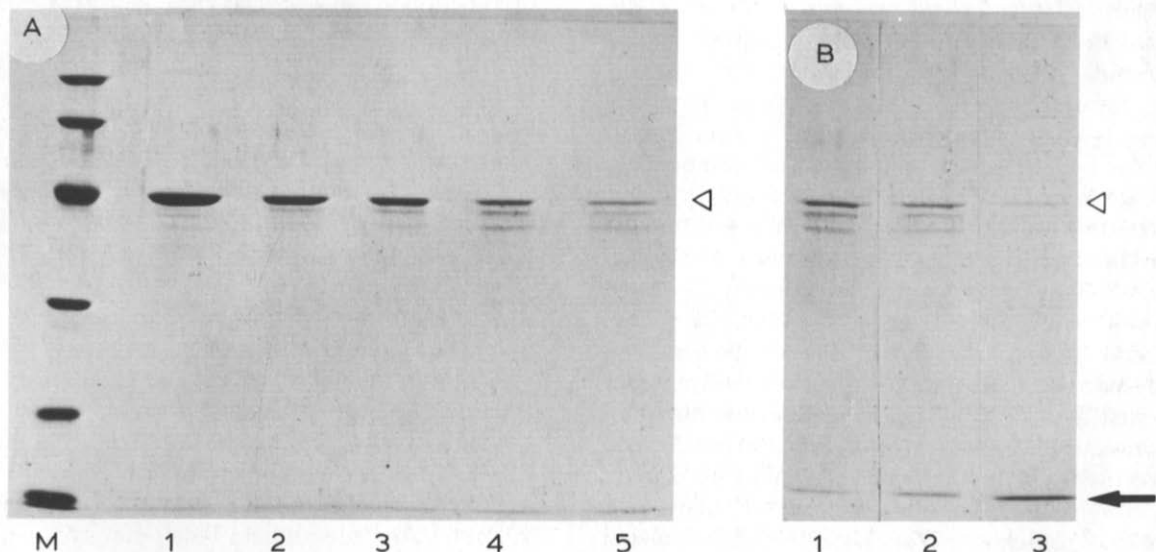

Fig.2. Degradation of lens actin by calpain II purified from bovine lens. Lens actin (20 μg) was incubated with calpain II in the standard incubation mixture, and the mode of degradation was analyzed by SDS-polyacrylamide gel (12%) electrophoresis. (A) Time course with 0.06 μg of calpain II. (B) Dose dependence for 20 min incubation. The incubation periods in A were: lane 1, 0 min; lane 2, 15 s; lane 3, 1 min; lane 4, 5 min; lane 5, 20 min. Lane M, marker proteins (see fig.1). The amounts of calpain II in B: lane 1, 0.015 μg; lane 2, 0.06 μg; lane 3, 3 μg. The open arrowhead denotes the position of actin (M_r 43000). The arrow shows the front of the gel.

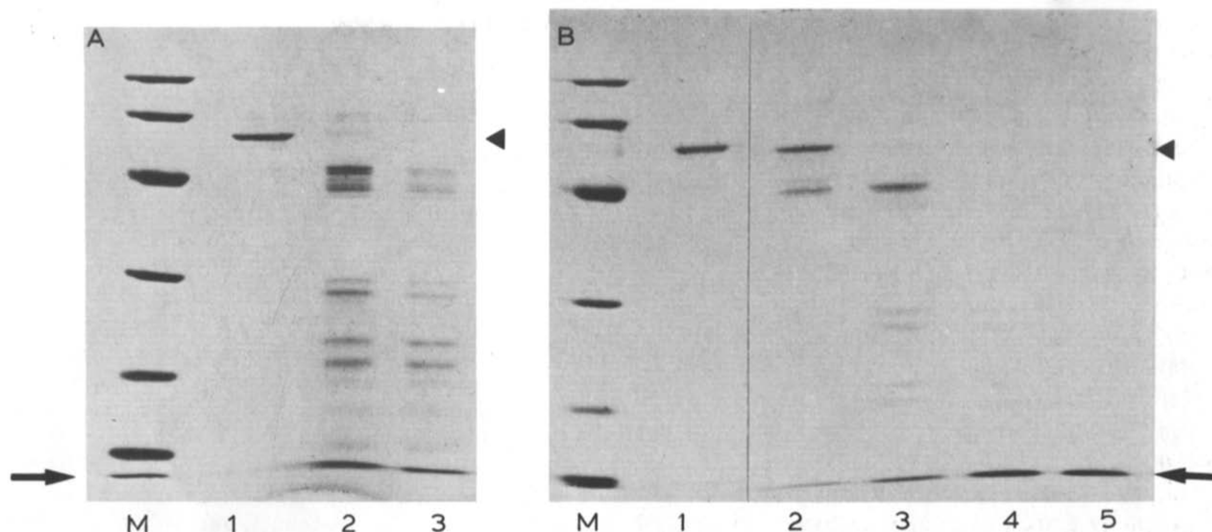

Fig.3. Degradation of lens vimentin by calpain II from bovine lens. Lens vimentin (20 μg) was incubated with calpain II in the standard incubation mixture, and the mode of degradation was analyzed by SDS-polyacrylamide gel (12%) electrophoresis. (A) Time course with 0.06 μg calpain II. (B) Dose response for 20 min incubation. The incubation periods in A were: lane 1, 0 min; lane 2, 1 min; lane 3, 20 min. The amounts of calpain II in B were: lane 1, 0 μg; lane 2, 0.015 μg; lane 3, 0.06 μg; lane 4, 1.5 μg; lane 5, 3 μg. Lane M, marker proteins (see fig.1). The solid arrowhead denotes the position of vimentin (M_r 57000). The arrow shows the front of the gel.

electrophoresis, performed as in [15]. Protein markers were the products of Pharmacia (Uppsala). Protein was determined as in [16] with bovine serum albumin as standard.

3. RESULTS

Fig.1 shows the effect of lens calpain II on the urea-soluble extracts of the lens. Several protein bands disappeared almost completely which included those corresponding to actin (43 kDa) and vimentin (57 kDa). No cleavage took place in the absence of Ca^{2+}. The actions of calpain II on the isolated lens actin and lens vimentin are shown in fig.2,3. Both actin and vimentin were broken down in time- and dose-dependent manners. The progress of the degradation for completion seemed to be more rapid with vimentin than with actin, but it was always accompanied by gradual accumulation of small peptide products on the gel front. Actin gave intermediary products of approximate M_r 42000 and 40000, while vimentin was degraded through the formation of several fragments of 44–20 kDa.

The proteolysis of actin and vimentin also occurred, with the resultant fragments similar to those above, in the presence of 10 mM Sr^{2+} and 10 mM Ba^{2+} added instead of 1 mM Ca^{2+}, whereas 1 mM Sr^{2+}, 1 mM Ba^{2+} and each 10 mM Mg^{2+}, Mn^{2+} or Zn^{2+} had no effect. It was also found that Ca^{2+}-dependent proteolysis of actin and vimentin was completely inhibited by 2 mM EGTA, 1 mM monoiodoacetic acid, E-64 (20 μg/ml), leupeptin (20 μg/ml) and calpastatin (300 units/ml), the endogenous inhibitor of calpain isolated also from bovine lens [10]. These data are all compatible with the proposition that the observed proteolysis is nothing but the result of the action of calpain II.

4. DISCUSSION

The present experiment clearly demonstrates proteolysis of two important cytoskeletal proteins, actin and vimentin, in the lens fiber cells by calpain II, which is known to be also present in the cytosol of the same cells [10]. During the aging process of the lens, the decrease or disappearance of lens cytoskeletal proteins has been reported [7,8]. The degradation of these cytoskeletal proteins may induce the disappearance of protein architecture of

the cell or produce the disintegration of the cell membrane [17], resulting in several irreversible changes which include cataract formation. The present results, although obtained from in vitro studies, suggest the involvement of calpain in these degradative processes. It is particularly interesting to note that the same enzyme, lens calpain II, was also shown to proteolyze easily both A and B chains of α-crystallin which is the major component of water-soluble proteins in the lens [10]. Thus, the possibility is that once Ca^{2+} is made available to activate calpain in a lens fiber cell, it triggers the degradation of not only major water-soluble proteins but also major water-insoluble proteins.

Authors in [11] reported that lens vimentin was degraded by a Ca^{2+}-activated protease, but they did not isolate the enzyme. They found that the enzymatic action was blocked by iodoacetic acid and had no effect on actin [11]. Authors in [19] isolated a vimentin-specific Ca^{2+}-protease from Ehrlich ascites tumor cells and found that this enzyme did not proteolyze actin. Therefore, it remains unsolved whether the vimentin-degrading Ca^{2+}-proteases described by those authors are really different enzymes from calpain II, or whether the authors failed to demonstrate the susceptibility of actin to their enzymes which could be identical with calpain II.

ACKNOWLEDGEMENTS

We thank Mrs M. Hatanaka for useful discussions and Miss Y. Sugihara for secretarial assistance. This research was supported in part by a grant-in-aid for Scientific Research, Ministry of Education, Science and Culture of Japan.

REFERENCES

[1] Rafferty, N.S. and Goossens, W. (1978) Exp. Eye Res. 26, 177–190.
[2] Kibbelaar, M.A., Selten-Versteegen, A.M.E., Dunia, I. and Benedetti, E.L. (1979) Eur. J. Biochem. 95, 543–549.
[3] Kibbelaar, M.A., Ramaekers, F.C.S., Ringens, P.J., Selten-Versteegen, A.M.E., Poels, L.G., Jap, P.H.K., Van Rossum, A.L., Feltkamp, T.E.W. and Bloemendal, H. (1980) Nature 285, 506–508.
[4] Ramaekers, F.C.S., Polls, L.G., Jap, P.H.K. and Bloemendal, H. (1982) Exp. Eye Res. 35, 363–369.
[5] Ramaekers, F.C.S., Osborn, M., Schmid, E., Weber, K., Bloemendal, H. and Franke, W.W. (1980) Exp. Cell Res. 127, 309–327.
[6] Kibbelaar, M.A. and Bloemendal, H. (1979) Exp. Eye Res. 29, 679–688.
[7] Bradley, S.N.R., Alcala, J. and Maisel, H. (1980) Exp. Eye Res. 30, 109–113.
[8] Ringens, P.J., Hoenders, H.J. and Bloemendal, H. (1982) Exp. Eye Res. 34, 201–207.
[9] Murachi, T. (1983) Trends Biochem. Sci. 8, 167–169.
[10] Yoshida, H., Murachi, T. and Tsukahara, I. (1984) Biochim. Biophys. Acta, in press.
[11] Roy, D., Chiesa, R. and Spector, A. (1983) Biochem. Biophys. Res. Commun. 116, 204–209.
[12] Geisler, N. and Weber, K. (1981) FEBS Lett. 125, 253–256.
[13] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[14] Hawkes, R., Niday, E. and Gordon, J. (1982) Anal. Biochem. 119, 142–147.
[15] Laemmli, U.K. (1970) Nature 227, 680–685.
[16] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[17] Garner, M.H., Roy, D., Rosenfeld, L., Garner, W.H. and Spector, A. (1981) Proc. Natl. Acad. Sci. USA 78, 1892–1895.
[18] Nelson, W.J. and Traub, P. (1982) J. Biol. Chem. 257, 5544–5553.