Peptidases play an important role in cataractogenesis: An immunohistochemical study on lenses derived from Shumiya cataract rats

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Summary

The role of proteolytic enzymes in Shumiya cataract rats in alterations to lens proteins during cataract formation was studied immunohistochemically using antibodies against exopeptidases, such as lysosomal dipeptidyl peptidase II (DPP II), cytosolic dipeptidyl peptidase III, and soluble and membrane-bound alanyl aminopeptidases, and against cytosolic endopeptidases such as α- and m-calpains, and 20S proteasome. αB-crystallin was detected as a proteolytic marker in the lenses. A constant immunoreactivity against all the antibodies employed was observed in the lens epithelium independent of the strain and age of the rats. A weak immunoreactivity against exo- and endopeptidases and an intense reactivity against αB-crystallin were observed in the lens fibres of control rats at all ages. The immunoreactivity of these peptidases in lens fibres increased with age in cataract rats, but that of αB-crystallin decreased. No reactivity against exo- and endopeptidases was seen in the perinuclear region of lenses of control rats at all ages or in Shumiya cataract rats at 8 and 10 weeks of age, but an intense reactivity against these peptidases was observed in the lens perinuclear region of lenses in cataract rats at 12 and 14 weeks of age. αB-crystallin immunoreactivity was observed with ordered striations in the lens perinuclear region of all control rats whereas the striations in this area of cataract rat lens were disorganized. Membrane-bound alanyl aminopeptidase was detected feebly in the lens epithelium and fibres of both types of rat at all weeks of age. These findings indicate that exo- and endopeptidases, except for membrane-bound alanyl aminopeptidase, are expressed intensively and are age-dependent. Conversely, the amount of αB-crystallin decreased with age in lens fibres of cataract rats. Calpains (α- and m-), 20S proteasome, dipeptidyl peptidases II and III and soluble alanyl aminopeptidase are thought to induce lens opacification kinetically during cataract formation in Shumiya cataract rats through the intracellular turnover of lens proteins.

Introduction

Cataracts cause severe visual impairment in a large number of people, especially after middle age (Mizuno et al. 1992). It is widely accepted that lens proteins are damaged by infrared and ultraviolet rays, various oxidative species and inherited factors. The diminished ability to degrade the damaged proteins may cause precipitation and subsequent cataract formation (Taylor & Davies 1987). The degradation of α-, β-, and/or γ-cristallins, which are the main water-soluble proteins in the eye lens, has been demonstrated in severe cataracts (Barber 1973) and selenite nuclear cataract (Shearer et al. 1997), in congenital cataracts of Nakano mice (Piattigorsky et al. 1978) and Shumiya cataract rats (SCRs) (Inomata et al. 1997). It has been proposed that various endogenous peptidases in the lens such as calpains (Inomata et al. 1997) and proteasome (Andersson et al. 1998) may be involved in the proteolytic modification of lens proteins during cataract formation.

In our previous studies, we purified some exopeptidases such as dipeptidyl peptidase II (DPP II), dipeptidyl peptidase III (DPP III), alanyl aminopeptidase N (AAP-N) and puromycin-sensitive alanyl aminopeptidase (AAP-S) (Huang et al. 1996, Ohkubo et al. 1999, Huang et al. 1997, Yamamoto et al. 1998) and endopeptidases including 20S proteasome (Ohkubo et al. 1991) and μ- and m-calpains (Ishiguro et al. 1987, Onizuka et al. 1995, Saito et al. 1999). The physico-chemical properties of the peptidases were characterized, and we have prepared polyclonal and monoclonal antibodies against these enzymes. However, the localization of the peptidases in the lens during normal ageing and cataractous process has not yet been elucidated.

Here, we examined the distribution and reactivity of four exopeptidases and two endopeptidases in normal and
cataract lenses immunohistochemically in order to investigate whether the peptidases are involved in the induction of lens opacification during cataract formation.

Materials and methods

Animals

Breeding stock SCR, which have a hereditary cataract and in which lens opacity appears spontaneously in the nuclear and perinuclear portions at 11–12 weeks of age, were used (Shumiya 1995), Wistar rats were used as normal controls. SCRs and Wistar rats were kept in an air-conditioned, clean room and handled in compliance with the guiding principles in care and use of animals in our university. The ages of the rats examined were 8-, 10-, 12- and 14-weeks old and 10 each of SCRs and Wistar rats were used at each age for immunohistochemical experiments. The rats were perfused through the left cardiac ventricle with 10 mM phosphate-buffered saline (PBS) followed by 10% formalin under deep anaesthesia induced by intraperitoneal administration of pentobarbital (40 mg/kg). The eyes were removed and postfixed with 10% formalin for 20 h, immersed in water for 24 h, dehydrated in ethanol, substituted with benzene, and embedded in paraffin wax.

Antibodies

Antibodies were prepared against the following enzymes: purified lysosomal DPP II from porcine seminal plasma (Huang et al. 1996), DPP III from rat liver cytosol (Ohkubo et al. 1999), AAP-S from rat liver cytosol (Yamamoto et al. 1998), membrane-bound AAP-N from human seminal plasma (Huang et al. 1997), and 20S proteasome from human red cells (Ohkubo et al. 1991). Two specific antibodies with no cross-reactivity against human µ- and m-calpains were also prepared. We raised an antibody against a peptide (N-acetyl-SEETPVYCTGVSAQVQKRARELG) of µ-calpain (Onizuka et al. 1995) and a monoclonal antibody against a peptide (MAGIAKLADREAAGELGSHERAIKYLNQD) originating from human m-calpain (Saito et al. 1999), respectively. A polyclonal antibody against the native form of αB-crystallin was purchased from Cosmo Bio (Tokyo, Japan).

Immunohistochemistry

Paraffin sections (4–µm thick) were cut using a microtome, and mounted on slides. After deparaffinization and rehydration, the sections were immersed in methanol containing 3% hydrogen peroxidase for 10 min to quench endogenous peroxidase activity and then treated in PBS containing 10% normal sheep serum for the polyclonal antibody and in PBS containing 10% rabbit serum for the monoclonal antibody. The sections were incubated with a solution of the primary antibody diluted in PBS (dilutions were 1 : 1,000–1 : 10,000 for polyclonal antibodies and 1 : 10,000 for monoclonal antibody) overnight at 4 °C. Control sections were incubated with PBS containing 10% sheep serum without primary antibody and with PBS containing the mixture of the antibody and the corresponding enzyme. The sections were incubated in a solution of biotin-conjugated IgG for 30 min and were incubated with streptavidin–biotin preformed complex for 30 min. The reaction was visualized by incubation in PBS containing 0.03% 3,3′-diaminobenzidine and 0.3% hydrogen peroxidase (Histofine SAB-Po kit, Nichirei, Tokyo, Japan). The slides were washed three times with PBS for 10 min at each interval of process and counterstained with haematoxylin. The reactivity of the peptidases in the lenses was determined by light microscopical observation based on the reactivity in 20 randomly selected sections.

Results

Localization of exopeptidases

Among the four exopeptidases tested, cytosolic DPP III (Figure 1A–H) and AAP-S (Figure 2C and D) and lysosomal DPP II (Figure 2A and B) were detected with weak reactivity in the lens epithelium from both types of rat at all ages and the density of their reactivity was constant. DPP III, DPP II and AAP-S were also observed with weak and constant reactivity in lens fibres of control rats (Figure 1A, C, E and G; Figure 2A and C), but the reactivity in the lens fibres from SCRs was stronger than that of control rats at all ages (Figure 1B, D, F and H; Figure 2B and D). The reactivity of DPP III in lens fibres of SCRs increased from moderate to intensive at 12 and 14 weeks of age, and the reactive area was extended to include the perinuclear region where the opacity of lens had first developed (Figure 1F and H). Although no reactivity of DPP III, DPP II and AAP-S was observed in the lens perinuclear region from control rats at all weeks and from SCRs at 8 and 10 weeks of age, moderate or intensive reactivity of the enzymes appeared in this region in SCRs at 12 and 14 weeks of age (Figure 1F and H; Figure 2B and D). Membrane-bound AAP-N was detected weakly in lens epithelium, feebly in lens fibres, and was not detectable in the lens perinuclear region in both control rats and SCRs at all weeks of age (Figure 2E and F). The four enzymes could not be detected in the lens nuclear region of both types of rat at all weeks of age (data not shown).

Localization of endopeptidases

Before detecting µ- and m-calpains in lenses, we prepared a mono-specific antibody against a peptide originating from human µ-calpain and also raised a monoclonal antibody against a peptide originating from human m-calpain as described in the Materials and methods section.

µ-Calpain was detected with moderate reactivity in the lens epithelium from both types of rats at all weeks of age (Figure 4A and B), and 20S proteasome was also detected...