Variation in Glycosidase Activity in Soluble Fractions in ICR/f Rat Lenses with the Progression of Cataract Formation

Akira KAMEI

Department of Biochemistry, Faculty of Pharmaceutical Sciences, Meijo University, 150 Yagotoyama, Tenpaku-ku, Nagoya 468–8503, Japan. Received August 14, 1998; accepted November 6, 1998

The current study reports active glycosidases in the lens of ICR/f rats, which generate a hereditary cataract approximately 90 d after birth, and the variation in enzyme activity with cataract progression. Seven active glycosidases, β -D-galactosidase, α -D-glucosidase, β -D-glucosidase, β -D-glucuronidase, β -D-galactosaminidase, β -D-glucosaminidase and α -D-mannosidase, were detected in ICR/f rat lenses. Of these, β -D-glucuronidase and β -D-galactosidase showed a tendency to increase in activity with the cataract progression. Furthermore, β -D-glucosidase and α -D-mannosidase showed a transitory increase in activity at the time of cataract formation. This result suggests that several glycosidases in the lens may be involved in the hereditary cataract formation. The optimal pH and temperature of the seven active glycosidases in rat lenses were also measured in this study.

Key words rat lens; glycosidase; hereditary cataract; Wistar rat; ICR/f rat

In the ophthalmology there is a worry that an increase in aged population will be accompanied by an increase in the number of senile cataract patients, because senile cataract is the major and most serious cause of human blindness. It is well known that senile cataract formation results ultimately from the aggregation and subsequent insolubilization of soluble lens proteins. Recently, a number of studies have been carried out in an attempt to discover the cause of lens protein aggregation and insolubilization. Several events, oxidation,¹⁾ glycation²⁾, post-translational modifications³⁾ and/or influx of various metal ions into the lens⁴⁾ have been suspected as causing lens protein aggregation. Recent studies suggest that glycation of lens proteins may play a major role in inducing such events. Thus, many studies have been reported concerning glycation, non-enzymatic glycation of lens proteins,^{2d,g,3c)} characterization of glycosidases of mammalian lenses, and the change in glycosidase activity with age.⁵⁾ Dreyfus *et al.*^{5c)} were the first to report the presence of glycosidases in mammalian lenses, with several glycosidases in human lenses exhibiting decreased activity in senile cataractous lenses. It was reported in a previous paper that the enzyme activity and K_m values of several glycosidases changed markedly with the progression of senile cataract.^{5a)} Therefore, some investigators suggested that glycosidases in the lens may play an important role of cataract formation. However, to date, no one knows the precise physiological role of glycosidases in mammalian lenses, or their role in cataract formation. To clarify these questions, we have attempted to investigate the properties of glycosidases in cataractous lenses of various mammals.

The current paper describes the active glycosidases in ICR/f rat lenses and the change in their activity with the progression of the cataract. The ICR/f rat strain was developed by Ihara, in which a spontaneous cataract occurs without exception at 3–4 months after birth and matures completely at 4–6 months of age, confirming that this strain develops a maturity-onset cataract.⁶⁾

MATERIALS AND METHODS

Animals and Lenses Male ICR/f rats were kindly donated by Dr. Ihara and then bred in our laboratory. Male Jcl

Wistar rats were purchased from Clea Japan Inc. and used as normal controls. The lens of each rat was used immediately after the animal was killed using chloroform and the lens was excised from the eyeball.

Preparation of Crude Enzyme Solution After one rat lens was homogenized in 0.5 ml 20 mM phosphate buffer (pH 7.0), the homogenate was centrifuged at $25000\times g$ for 30 min at 4 °C. The supernatant was used as a crude enzyme solution.

Assay of Glycosidase Activity The glycosidase activity was measured using 4-methylumbelliferone (MU)-sugar derivatives (Wako Pure Chemical Industries Ltd., Japan) as substrates according to the method of Dreyfus *et al.*^{5c)} Briefly, 150 μ l substrate solution of 4-MU-sugar derivative and 150 μ l buffer solution of optimal pH were mixed, and the mixture was pre-incubated for 5 min at the respective optimal temperature. Subsequently, 45 μ l enzyme solution was added to the mixture and incubated for 30 min at the respective optimal temperature. The reaction was terminated by adding 1.0 ml 0.2 M glycine-NaOH buffer, pH 10.7, and the fluorescence of liberated 4-MU at $Em=450$ nm ($Ex=365$ nm) was measured with a Shimadzu RF-5000 Spectrofluorophotometer. One unit (U) of enzyme activity was defined as the amount hydrolyzing 1.0 μ mol substrate/min under optimal conditions.

The optimal pH of the enzyme was measured in 0.2 M sodium phosphate–0.1 M citric acid buffer over a range of pH values from 3.0 to 8.5 and in 0.1 M potassium phosphate–0.05 M sodium borate buffer, pH 9.0, following a 30 min-incubation at 37 °C. The optimal temperature was measured in 0.2 M sodium phosphate–0.1 M citric acid buffer at the optimal pH and for a 30 min incubation period at various temperatures.

Protein Assay Protein was measured by the method of Bradford⁷⁾ using bovine serum albumin as a standard.

RESULTS

Detection of Active Glycosidases in ICR/f Rat Lenses Active glycosidases in an ICR/f rat lens of a 60-d old animal were examined initially by using 15 different 4-MU-sugar derivatives as substrates. These were: 4-MU- α -D-arabinopy-

* To whom correspondence should be addressed.

Fig. 1. Variation in Specific Activity of Glycosidases in ICR/f Rat Lenses with the Progression of the Cataract

The detailed measurement of the specific activity is described in "Materials and Methods." □, specific activity of ICR/f rat with the progression of the cataract; ●—, specific activity of Wistar rat with age was used as a normal control. These activities were measured at the optimal pH. The experimental data represent the mean $\pm$ S.D. of four separate experiments.

ranoside, 4-MU- β -D-xyloside, 4-MU- α -D-glucopyranoside (Glc), 4-MU- β -D-Glc, 4-MU- α -D-galactoside (Gal), 4-MU- β -D-Gal, 4-MU- α -D-mannopyranoside (Man), 4-MU- β -D-Man, 4-MU- β -D-glucuronide (GlcUA), 4-MU- β -D-glucosaminide (GlcNAc), 4-MU- β -D-galactosaminide (GalNAc), 4-MU- β -L-fucopyranoside (Fuc), 4-MU- α -D-Fuc, 4-MU- β -lactoside (Lac), and 4-MU- α -D-cellobioside. As a result, seven types of active glycosidases were detected: β -D-galactosidase, α -D-glucosidase, β -D-glucosidase, β -D-glucuronidase, β -D-galactosaminidase, β -D-glucosaminidase and α -D-mannosidase.

Optimal pH and Temperature First, the optimal pH was measured at 37°C. The optimal pHs of β -D-galactosidase, α -D-glucosidase, β -D-glucosidase, β -D-glucuronidase, β -D-galactosaminidase, β -D-glucosaminidase, and α -D-mannosidase were pH 4.5, 7.0, 5.0, 5.0, 6.5, 4.5 and 6.5, respectively. Furthermore, α -D-glucosidase, β -D-glucosidase, β -D-galactosaminidase and α -D-mannosidase had a secondary optimal pH with lower activity. However, in the current study we did not investigate this phenomenon further.

Then, the optimal temperature was determined under the condition of the respective optimal pH. As a result, the optimal temperatures of β -D-glucosaminidase, β -D-galactosaminidase and β -D-glucuronidase was found to be 45°C, 55°C and 60°C, respectively. The temperature for the other four enzymes was approximately 40°C.

Glycosidase Activities in ICR/f Rat Lenses with the Progression of Cataract The ICR/f rat develops a cataract at approximately 90 d after birth and this then progresses to a mature cataract.⁶⁾ Thus, glycosidase activities were measured at 60 d (before cataract formation), 90 d (cataract just

formed), 120 d (progressive cataract) and 160 d (mature cataract) under respective optimal conditions. In this experiment, Wistar rats were used as normal controls.

Glycosidase activities in the lens of Wistar rats fell with age as shown in Fig. 1, except for β -D-galactosidase. Only the activity of β -D-galactosidase remained unchanged by age. However, ICR/f rat lenses exhibited three types of variation in glycosidase activity with the progression of the cataract. One was a reduction in glycosidase activity, as shown with α -D-glucosidase, β -D-galactosaminidase and β -D-glucosaminidase, which was observed in the control Wistar rat lens. Another was an increase in glycosidase activity with the progression of the cataract, as in the case of β -D-galactosidase and β -D-glucuronidase. Their activity increased several fold compared with the Wistar rat. The third type was an abrupt and transitory increase in glycosidase activity at approximately 90 d (cataract formation), as in the case of β -D-glucosidase and α -D-mannosidase. In particular, β -D-glucosidase activity increased approximately 4-fold compared with the Wistar rat. Hence, it was presumed that at least four glycosidases, β -D-galactosidase, β -D-glucosidase, β -D-glucuronidase and α -D-mannosidase, may play an important role in cataract formation in the rat.

Among the four glycosidases with a secondary optimal pH, only β -D-mannosidase showed a markedly change at 90 d similar to the change in the activity measured at the main optimal pH. Thus, at 90 d the enzyme activity increased suddenly and then rapidly decreased.

DISCUSSION

The current paper demonstrates the existence of seven active glycosidases in ICR/f rat lenses, and variations in their enzyme activity with age and the progression of the cataract. Seven active glycosidases, β -D-galactosidase, α -D-glucosidase, β -D-glucosidase, β -D-glucuronidase, β -D-galactosaminidase, β -D-glucosaminidase and α -D-mannosidase were detected. Among them, four glycosidases, β -D-galactosidase, β -D-glucosidase, β -D-glucuronidase, and α -D-mannosidase, exhibited changes in their enzyme activity with age and with the progression of the cataract compared with Wistar rat used as normal controls. Among them, β -D-galactosidase and β -D-glucuronidase showed a tendency to increased activity after cataract formation. On the other hand, β -D-glucosidase and α -D-mannosidase showed a transitory increase in enzyme activity at 90 d, which is the period of cataract formation.

Concerning glycosidases in the rat lens, Carlin *et al.*^{5e)} reported that in the Albino rat lens these were six active glycosidases, β -D-galactosidase, β -D-glucosidase, β -D-galactosaminidase, β -D-glucosaminidase, α -D-mannosidase and β -D-fucosidase. However, we were unable to find fucosidase activity in the lenses of Wistar an ICR/f rats. Instead, we found two other glycosidases, α -D-glucosidase and β -D-glucuronidase, in addition to the five glycosides described above. This discrepancy may be attributable to the difference in rat species.

The variation in glycosidase activity with the progression of the cataract was examined using the ICR/f rat. Several enzymes showed a tendency to increased activity at the period of cataract formation or with the progression of the cataract. We believe that this is the first report of this phenomenon and we believe that at least four glycosidases, α -D-galactosidase, β -D-glucosidase, β -D-glucuronidase and α -D-mannosidase, may play a role in cataract formation in the rat. In previous papers, we have reported that glycation of lens protein may be a major cause of human senile cataract^{5a,b)} and that the monosaccharides responsible for glycation of lens proteins may be supplied by glycogen,⁸⁾ polysaccharides, proteoglycans and/ or glycolipids following enzymatic hydrolysis by glycosidases such as α -D-glucosidase and β -D-glucosidase. However, it is difficult to understand whether several glycosidases are involved in cataract formation in the ICR/f rat, because the cataract of ICR/f rats is hereditary. Furthermore, it is also incomprehensible that α -D-mannosidase activity increased during the period of cataract formation, because no-

body knows the substrate for α -D-mannosidase in the mammalian lens at the present time.

The current study revealed that the activity of some glycosidases in the lens of hereditary cataracts changed with the progression of the disease. Species differences and the role of active glycosidases in the mammalian lens should be investigated further to clarify the precise mechanism of cataract formation.

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