·Basic Research·

Carbamylation-induced inactivation of glyceraldehydes 3-phosphate dehydrogenase and thioltransferase in bovine lens

Jie Zhang, Hong Yan

Foundation items: International Development Research Award (No.070667/Z/03); Excellent Scientific Talents of Military Medicine Grant (No.04J011)

Department of Ophthalmology, Tangdu Hospital, the Fourth Military Medical University, Xi'an 710038, Shaanxi Province, China
Correspondence to: Hong Yan. Department of Ophthalmo-logy, Tangdu Hospital, the Fourth Military Medical Univer-sity, Xi'an 710038, Shaanxi Province, China.yhongb@fmmu.edu.cn

Received:2008-12-05 Accepted:2009-01-04

Abstract

• AIM: To investigate whether potassium cyanate can inactivate glyceraldehydes 3-phosphate dehydrogenase (GAPDH) and thioltransferase (TTase) in bovine lens.

• METHODS: Fresh intact bovine lenses were incubated with 100mmol/L potassium cyanate (KCNO) for 7 and 12 days respectively. Then all lens were incubated in 50mmol/L DMEM solution. The proteins in the water-soluble fractions from the normal control and the cyanate-modified lens were extracted. The activity of GAPDH and TTase in the water-soluble fraction after incubation at 37℃ was measured by spectrophotometer.

• RESULTS: GAPDH activity was significantly lower in the cyanate-modified lens proteins than that of the normal control($P<0.01$), and considerably diminished in protein incubated with 100mmol/L potassium cyanate for 12 days. There were statistically significant differences in the activity of TTase between the normal control lenses and the carbamylated lenses incubated for 7 days($P<0.05$) and 12 days($P<0.01$). However, there was no statistical difference between the samples incubated with 100mmol/L KCNO for 7 and 12 days ($P=0.19296$).

• CONCLUSION: This study provides evidence to show carbamylation is able to inactivate GAPDH and TTase in bovine lenses. This may have implications for the susceptibility of lenticular GAPDH and TTase to carbamylation, and also for the research on pathogenesis of cataract.

• KEYWORDS: carbamylation; cataract; potassium cyanate; glyceraldehydes 3-phosphate dehydrogenase;thioltransferase

Zhang J, Yan H. Carbamylation-induced inactivation of glyceraldehydes 3-phosphate dehydrogenase and thioltransferase in bovine lens. *Int J Ophthalmol* 2009;2(1):41-44

INTRODUCTION

Post-translational modifications, known to occur during aging, such as glycation, carbamylation, oxidation, deamidation, phosphorylation and truncation, can cause conformational changes and thus cataract [1]. Carbamylation, an important post-translational modification of proteins occurring *in vivo*, may contribute to cataract formation in populations with high levels of blood urea. Urea in blood and other body fluids, including aqueous humour is always in equilibrium with isocyanic acid, the reactive species of carbamylation. Cyanate reacts with proteins at physiological pH by reversible binding to thiol groups, or by covalent binding to amino groups [2]. Binding of cyanate to lysyl residues, by eliminating positive charges, would alter protein-protein or protein-water interactions, and destabilize the protein. Such changes of protein surface charge produced by carbamylation lead to opacification of the lens [3]. Our earlier studies showed that the primary targeted lens proteins of carbamylation were αA-, βB2- and γS-crystallin. It indicates that these lens proteins are prone to be exposed to isocyanate (data were not published). The importance of carbamylation of lens crystallins, its effect on protein conformation and aggregation, and the potential to induce cataract have been discussed extensively [4-8], but little is known about the inactivation of enzymes induced by carbamylation.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a

key glycolytic enzyme, oxidizes and subsequently phosphorylates glyceraldehydes 3-phosphate in the glycolytic pathway. Its activity decreased in clear human lenses with aging and in cataract lenses [9,10]. *In vitro* incubations of GAPDH with sugar, cyanate and prednisolone-21-hemisuccinate, will lead to significant loss of enzyme activity[11].

Thioltransferase (TTase), which is a member of the oxido-reductase family, regulates thiol/disulfide homeostasis[12]. Its active site sequence (Cys-Pro-Tyr-Cys) is responsible for this activity [13]. TTase has been postulated to repair oxidant-sensitive sulfhydryls by facilitating thiol-disulfide interchange between glutathione (GSH) and protein thiol mixed disulfides [12,14,15]. It is likely that this mechanism keeps the lens crystallins and membrane proteins in the reduced form by preventing their cross-linking or inactivation, and initial event in cataractogenesis.

In this communication, this is the first evidence to show carbamylation is able to inactivate GAPDH and TTase in bovine lenses. It may have implications for the susceptibility of lenticular GAPDH and TTase to carbamylation. The relationship between carbamylation and cataract pathogenesis is explored.

MATERIALS AND METHODS

Materials Six 2-year-old bovine lenses were obtained from a local abattoir (Oxford, UK). Potassium cyanate and all other chemicals and enzymes were obtained from Sigma-Aldrich (Poole, Dorset, UK), and were of the highest purity available.

Methods

Incubation of bovine lens with potassium cyanate The intact bovine lenses were dissected, and then four clear bovine lenses were incubated with 100mmol/L potassium cyanate (KCNO) for 7 and 12 days respectively in a 37℃ shaking waterbath [4,16]. All lens incubations were carried out in 50mmol/L DMEM with 15mmol/L Hepes, D-199 and added antibiotics (penicillin, streptomycin and amphotericin B, Sigma). At the end of the incubation, each intact bovine lens was decapsulated and gently homogenized in a hand-held homogenizer over ice with 7 times the volume of double purified water (containing 13mmol/L EDTA) of its wet weight, to extract the water-soluble fraction of the lens proteins. And then it was centrifuged at 22 440g for 40 minutes at 4℃. The supernatant containing the water-soluble proteins was dialyzed against distilled water at 4℃ for 24 hours and freeze-dried, then stored at -20℃ before enzyme determination and used for all the experiments described.

GAPDH activity assay The activity of glycerldehyde 3-phosphate dehydrogenase (GAPDH) was determined spectrophotometrically by measuring the decrease in absorbance at 340nm against water within 1 minute at 30℃. The reaction mixture (3.0mL) consisted of 2.258mL of 0.1mol/L triethanolamine buffer with pH adjusted to 7.6, using 1mol/L NaOH, 200μL of 50g/L solution of glycerate-3-p, 100μL of 20g/L ATP, 100μL of 10g/L EDTA solution, 50μL of 0.1mol/L magnesium sulphate, 50μL of 10g/L NADH, 12μL of 3-phosphoglyceric phosphokinase suspension containing 7 units and finally 230μL of the incubation sample (containing 3mg lens protein). All of these reagents were pipetted into a 3mL quartz cuvette in a Hitachi U-2001 spectrophotometer with 1cm light path, and then the absorbance values were recorded. The enzyme activity was calculated from the linear decrease in absorbance between time 0 and 1 minute. By definition, one international unit GAPDH reduces 1.0μmol of 3-phosphoglycerate to D-glyceraldehyde 3-phosphate in a coupled system with 3-phosphoglyceric phosphokinase at pH 7.6 in 1 minute at 30℃.

TTase activity assay TTase was assayed following the method of Mieyal *et al* [17]. Reaction mixtures without the cell homogenate were used as blanks in enzyme assay. The reaction was carried out in the presence of NADPH, GSH and glutathione reductase (GR) with the synthetic disulfide, hydroxyl ethyl disulfide (HEDS), as substrate. The decreased A340nm for NADPH was monitored for enzyme activity. The enzyme activity was measured in an order by which one sample from each group (controls, incubated for 7 days, and incubated for 12 days) was measured in succession, to ensure that all the samples were treated similarly. The change in absorbance was corrected for any change in parallel assays without supernatant.

Statistical Analysis All experiments were repeated at least three separate times and differences were assessed by student's paired *t* -test where relevant. All comparisons were made with control solution unless stated otherwise.

RESULTS

Activity of GAPDH from Normal Control Lenses and Carbamylated Lenses The activity of GAPDH in clear

lenses was tested and compared with samples containing clear lenses incubated with 100mmol/L KCNO for 7 and 12 days respectively. Potassium cyanate at concentration of 100mmol/L inactivated GAPDH in a time-dependent manner (Figure 1). GAPDH activity in control group was very high, 341.6 ±24.7U/μg lens protein in samples containing clear lens supernatant incubated without KCNO. All the carbamylated groups offered a statistically significant decrease in activity. The significant decrease in activity (52.4 ± 4.4U/μg lens protein) was achieved in the group incubated with potassium cyanate for 7 days (P<0.01), whereas the greatest decrease (8.5±1.5U/μg lens protein) was achieved in the group incubated for 12 days (P<0.01) compared with the normal control group. All of the results were highly statistically significant.

Activity of TTase from Normal Control Lenses and Carbamylated Lenses Potassium cyanate (100mmol/L) inactivated TTase after an incubation period of 7 days and 12 days. The activity of TTase was greater in the normal control lenses than that in the carbamylated lenses. However, there was no statistical difference between the samples incubated with 100mmo/L KCNO for 7 and 12 days (P=0.19296). After an incubation period of 7 days, the activity of TTase decreased to 32.1 ±2.1U/μg lens protein compared with 38.7±2.7 in the normal control lenses (P<0.05). The activity of TTase lowered to 30.1±0.4U/μg lens protein after an incubation of 12 days (P<0.01) compared with the normal control group (Figure 2).

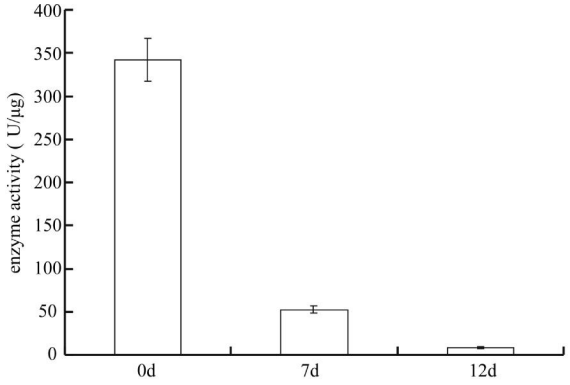

Figure 1 GAPDH activity was measured in bovine lenses in the absence of, or presence of 100mmol/L potassium cyanate for 7 days or 12 days

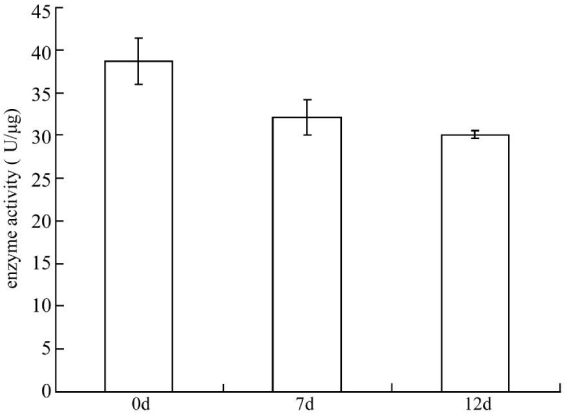

Figure 2 TTase activity was measured in bovine lenses in the absence of, or presence of 100mmol/L potassium cyanate for 7 days or 12 days

DISCUSSION

Our data showed that the activity of GAPDH in carbamylated supernatant was much lower than that in clear lens supernatant. The activity in lenses obtained by incubation with 100mmol/L potassium cyanate for 12 days was lower than that of the lenses supernatant incubated with same concentration of potassium cyanate for 7 days, which was found to be time-dependent.

The role of GAPDH in many biochemical processes has been studied extensively. GAPDH activity decreases in experimentally induced cataracts and in human diabetic cataract [3]. This is paralleled by increased glycation of the lens proteins of diabetics [18] or modification of glucocorticoids in the case of patients who had undergone glucocorticoid therapy[19]. Earlier studies reported that GAPDH activity was significantly decreased in pooled lenses from cataract groups compared to clear lenses of similar average age [20]. Carbamylation of essential lysine residues can inhibit the normal function of proteins. GAPDH contains a highly conserved and reactive lysine near its active site [21]. Reaction of this residue with a small molecule could lead directly to its inactivation.

TTase activity tested in this study was only slightly reduced when clear lenses were exposed to 100mmol/L potassium cyanate for 7 days, while its activity was significantly affected by potassium cyanate after incubation for 12 days.

The physiological function of TTase has been speculated to be a repair enzyme for oxidation damaged proteins/enzymes through its dethiolation capability. The lens depends on a balanced redox state and SH/-S-S- ratio for maintaining its transparency. TTase can specifically dethiolate protein-S-S-glutathione and restore protein free SH groups for proper enzymatic or protein functions and maintain the function

of the lens effectively [22]. This is the first evidence to show carbamylation is able to inactivate TTase in bovine lenses.

A unifying mechanism by which environmental cataracts may develop could be accelerated by post-translational modification of lenticular proteins such as carbamylation. It is possible that inactivation of these enzymes was due to cumulative post-translational modification which induces conformational changes of proteins and affects the active sites, resulting in the inactivation of enzymes.

Acknowledgements: The authors gratefully acknowledge the Wellcome Trust (International Development Research Award, 070667/Z/03) and Excellent Scientific Talents of Military Medicine Grant (04J011) for financial support.

REFERENCES

1 Harding JJ. Protein glycation and cataract: a conformational disease. In: Uversky VN, Fink AL, editors. Protein misfolding, aggregation, and conformational diseases. Part B. US: Springer; 2007:499–514

2 Martin S, Harding JJ. Site of carbamoylation of bovine gamma–II–crystallin by potassium [14C] cyanate. *Biochem J* 1989;262(3):909–915

3 Harding JJ. Cataract: biochemistry, epidemiology and pharmacology. London: Chapman & Hall; 1991:155–159

4 Beswick HT, Harding JJ. Conformational changes induced in bovine lens alpha–crystallin by carbamylation. Relevance to cataract. *Biochem J* 1984;223(1):221–227

5 Beswick HT, Harding JJ. High–molecular–weight crystallin aggregate formation resulting from non–enzymic carbamylation of lens crystallins: relevance to cataract formation. *Exp Eye Res* 1987;45(4):569–578

6 Harding JJ, Crabbe MJC. The lens: development, proteins, metabolism and cataract. In: Davson H, editor. The Eye. 3rd ed. New York: Academic Press 1984:207–492

7 Smith JB, Shun–Shin GA, Sun Y, Miesbauer LR, Yang Z, Yang Z, Zhou X, Schwedler J, Smith DL. Glutathione adducts, not carbamylated lysines, are the major modification of lens alpha–crystallins from renal failure patients. *J Protein Chem* 1995;14(3):179–188

8 Derham BK, Harding JJ. Alpha–crystallin as a molecular chaperone. *Prog Retin Eye Res* 1999;18(4):463–509

9 Friedburg D. Enzyme activity patterns in clear human lenses and in different types of human senile cataract. *Ciba Foundation Symposium* 1973;19:117–133

10 Dovrat A, Scharf J, Gershon D. Glyceraldehyde 3–phosphate dehydrogenase activity in rat and human lenses and the fate of enzyme molecules in the aging lens. *Mech Ageing Dev* 1984;28:187–191

11 Hook DW, Harding JJ. Inactivation of glyceraldehyde 3–phosphate dehydrogenase by sugars, prednisolone–21–hemisuccinate, cyanate and other small molecules. *Biochim Biophys Acta* 1997;1362:232–242

12 Mieyal JJ, Gravina SA, Mieyal PA, Srinivasan U, Starke DW. Glutathionyl specificity of thioltransferase: Mechanistic and physiological implications. In: Packer L, Cadenas E, eds. Biothiols in health and disease. New York: Marcel Dekker, Inc; 1995:305–372

13 Porras A, Valladares A, Alvarez AM, Roncero C, Benito M. Differential role of PPAR gamma in the regulation of UCP–1 and adipogenesis by TNF–alpha in brown adipocytes. *FEBS Lett* 2002;520(1–3):58–62

14 Ziegler DM. Role of reversible oxidation–reduction of enzyme thiols–disulfides in metabolic regulation. *Annu Rev Biochem* 1985;54:305–329

15 Wells WW, Yang Y, Deits TL, Gan ZR. Thioltransferases. *Adv Enzymol Relat Areas Mol Biol* 1993;66:149–201

16 Owers J, Duncan G. The viability of the bovine lens in organ culture. *Exp Eye Res* 1979;28(6):739–742

17 Mieyal JJ, Starke DW, Gravina SA, Dothey C, Chung JS. Thioltransferase in human red blood cells: purification and properties. *Biochemistry* 1991;30(25):6088–6097

18 Oimomi M, Maeda Y, Hata F, Kitamura Y, Matsumoto S, Baba S, Iga T, Yamamoto M. Glycation of cataractous lens in non–diabetic senile subjects and in diabetic patients. *Exp Eye Res* 1988;46(3):415–420

19 Manabe S, Bucala R, Cerami A. Nonenzymatic addition of glucocorticoids to lens proteins in steroid–induced cataracts. *J Clin Invest* 1984;74(5):1803–1810

20 Yan H, Hanrding JJ, Xing K, Lou MF. Revival of glutathione reductase in human cataractous and clear lens extracts by thioredoxin and thioredoxin reductase, in conjunction with alpha–crystallin or thioltransferase. *Curr Eye Res* 2007;32(5):455–463

21 Wassarman PM, Major JP. The reactivity of the sulfhydryl groups of lobster muscle glyceraldehyde 3–phosphate dehydrogenase. *Biochemistry* 1969;8(3):1076–1082

22 Lou MF. Redox regulation in the lens. *Prog Retin Eye Res* 2003;22(5):657–682