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ScienceDirect
Experimental Eye Research 84 (2007) 914–921
EXPERIMENTAL EYE RESEARCH
www.elsevier.com/locate/yexer

Methylglyoxal inhibits glycation-mediated loss in chaperone function and synthesis of pentosidine in α-crystallin

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Received 30 June 2006; accepted in revised form 18 January 2007
Available online 2 February 2007

Abstract

α-Crystallin is a major protein in the eye lens and it functions as a molecular chaperone by preventing aggregation of mildly denatured proteins. Glycation, the reaction of sugars and ascorbate with proteins, causes covalent cross-linking and reduces the chaperone function of α-crystallin. We demonstrated that methylglyoxal (MGO), a metabolic α-dicarbonyl compound, modifies arginine residues in α-crystallin and enhances its chaperone function. We wanted to determine whether modification by MGO could protect α-crystallin from glycation-mediated cross-linking and loss of chaperone function. Our results show that MGO-modification of isolated bovine lens α-crystallin inhibits formation of pentosidine, a glycation-derived protein crosslink. Proteins in organ cultured rat lenses were similarly protected from pentosidine formation. Glycation by sugars and ascorbate resulted in almost complete loss of chaperone function of α-crystallin. Surprisingly, addition of MGO during or before glycation not only inhibited the loss of chaperone function, but it actually enhanced the chaperone function of α-crystallin. Together, these data suggest that in the aging lens, MGO inhibits glycation-mediated pentosidine synthesis and the loss of chaperone function of α-crystallin.
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Keywords: argpyrimidine; methylglyoxal; chaperone; human lens; pentosidine

1. Introduction

Because they have such a low turnover rate, proteins in the lens tend to accumulate post-synthetic modifications during aging. Among the modifications detected, many result from glycation and kynurenination (Cheng et al., 2006; Nagaraj et al., 1991; Staniszewska and Nagaraj, 2005; Tessier et al., 1999; Vazquez et al., 2002). Glycation of proteins begins with the condensation of sugars with an amino group on proteins. The initial condensation product, known as the Amadori product, undergoes a series of reactions to produce a variety of stable end products on proteins that are collectively known as advanced glycation end products, or AGEs (Thorpe and Baynes, 2003).

Some AGEs, such as pentosidine and MOLD (methylglyoxal-lysine dimer) are protein cross-linking adducts, but others, such as, pyrraline and CML (N^{ϵ}-carboxy-methyl-lysine) form adducts without cross-linking (Thorpe and Baynes, 2003). Several AGEs have been detected in the human lens, including the fluorescent products, pentosidine (Nagaraj et al., 1991) and argpyrimidine (Padayatti et al., 2001), and non-fluorescent products such as, CML (Dunn et al., 1989), MOLD (Chellan and Nagaraj, 1999), hydroimidazolones (Ahmed et al., 2003) and pyrraline (Nagaraj and Sady, 1996). Several AGEs accumulate progressively in the aging lens and accrue at a higher rate in senile cataracts (Ahmed et al., 2003; Chellan and Nagaraj, 1999; Nagaraj et al., 1991; Nagaraj and Sady, 1996). The close relationship between AGEs, lens pigmentation and protein cross-linking suggested that AGEs contribute to both yellow pigmentation and covalent cross-linking in the aging lens.

Methylglyoxal (MGO) is a metabolic α-dicarbonyl compound produced mainly from the β-elimination of the

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0014-4835/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2007.01.013

phosphate group of glycolytic intermediates, such as glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (Thornalley, 1996). MGO is found in human lenses at concentrations approximately 20 times higher than in plasma (Haik et al., 1994). The reported concentrations are 1.78 ±0.8 nmol/g in human lenses and 2.10 nmol/g in rat lenses (Haik et al., 1994; Phillips et al., 1993). These concentrations could be underestimates, as MGO is a highly reactive molecule and may not remain in a steady-state concentration in the lens. If it is not metabolized by the glyoxalase system (Thornalley, 1998), or by other enzymes, such as aldose reductase (Vander Jagt and Hunsaker, 2003), it reacts rapidly with arginine, lysine and cysteine residues on proteins (Lo et al., 1994). Low concentrations of MGO (<1 mM) produce stable adducts with arginine residues. Several arginine modifications of MGO have been identified in the lens, including argpyrimidine (Padayatti et al., 2001) and hydroimidazolones (Ahmed et al., 2003). In addition, adducts such as MOLD (Chellan and Nagaraj, 1999) and MODIC (Biemel et al., 2002) that cross-link lysine-lysine and lysine-arginine residues were detected in the human lens.

α-Crystallin is a major lens protein that accounts for nearly 50% of the total protein in the lens. It is composed of two subunits αA- and αB-crystallin, both with the same molecular weight of ∼20 kDa. Both subunits function as molecular chaperones (Horwitz, 2003) and prevent aggregation of denatured proteins. Several studies confirm that glycation makes α-crystallin a weaker chaperone (Cherian and Abraham, 1995; Van Boekel et al., 1996). Surprisingly, reaction with MGO improves the chaperone function of α-crystallin (Nagaraj et al., 2003). We found that the enhanced chaperone function is due to chemical modification of selected arginine residues on αA-crystallin (Nagaraj et al., 2003). In addition, site directed mutagenesis of R21 and R103 to A21 and A103 showed that the positive charge on R21 and R103 underlies the improved chaperone function (Biswas et al., 2006).

Since MGO has a high propensity to react with arginine residues, and pentosidine synthesis requires arginine residues on proteins (Biemel et al., 2001), we reasoned that prior reaction with MGO could inhibit pentosidine synthesis from sugars and ascorbate (Grandhee and Monnier, 1991). We also considered that arginine modification by MGO might enhance chaperone activity of α-crystallin by preventing glycation of the protein. Accordingly, we investigated these possibilities in experiments with purified bovine lens α-crystallin and organ cultured rat lenses.

2. Materials and methods

2.1. Reagents

Lysozyme, D-ribose, heptafluorobutyric acid, 40% methylglyoxal, L-ascorbic acid, TC-199 medium and citrate synthase were from Sigma Chemical Co. (St. Louis, MO, USA). D,L-glyceraldehyde was from Aldrich Chemical Co., Milwaukee, WI, USA.

2.2. Preparation of bovine α-crystallin

α-Crystallin was isolated from calf lenses. Lenses were decapsulated and homogenized with a motorized homogenizer in buffer (4.0 ml/lens) containing 50 mM sodium phosphate (pH 7.4). The homogenate was centrifuged at 30,000 × *g*, and the supernatant was subjected to column chromatography as described by Sharma et al. (1997). The purity of the preparation was established by SDS–PAGE.

2.3. Protein glycation in the presence of MGO

(A) *Pre-incubation with MGO*: α-crystallin (5.0 mg/ml) in 0.1 M sodium phosphate buffer (pH 7.4) was filtered through a 0.22 μm filter, then incubated with 0.1–5 mM MGO for 24 h followed by incubation for 72 h with or without 10 mM ribose or 10 mM ascorbate at 37 °C.
(B) *Co-incubation with MGO*: α-crystallin (5 mg/ml) in 0.1 M sodium phosphate buffer (pH 7.4) was incubated with 0.1–5 mM MGO and 10 mM ribose together for 96 h at 37 °C.

Proteins without either MGO or ribose or ascorbate were included as controls in all experiments.

2.4. HPLC assay for pentosidine and argpyrimidine

Protein samples were incubated as described above and then mixed with an equal volume of 10% trichloroacetic acid and left on ice for 20 min. The precipitated protein was collected by centrifugation at 2000 × *g* for 10 min and hydrolyzed with 6 N HCl for 16 h at 110 °C. The acid was evaporated in a Speed Vac system, and the pellet was suspended in 250 μl of water and filtered through 0.45 μm centrifugal filters. The amino acid content of each hydrolysate was estimated using ninhydrin as described by Nagaraj and Monnier (1992). Aliquots of all samples were separated by HPLC to measure argpyrimidine and pentosidine. The HPLC conditions were essentially as described by Wilker et al. (2001), but with minor modifications in solvent B and the linear gradient program. Solvent A was water with 0.01 M heptafluorobutyric acid (HFBA), and solvent B was 70% acetonitrile in water with 0.01 M HFBA. The solvent program was as follows: 0–39 min, 16%B; 40–50 min, 20%B; 50–60 min, 22%B; 60–62 min, 28%B; 62–71 min, 100%B; 71–80 min, 16%B. The column eluate was monitored with an on-line fluorescence detector set at excitation/emission wavelengths of 335/385 nm. Under the conditions used, argpyrimidine and pentosidine had a R_T of ∼17 min and ∼25 min. Argpyrimidine and pentosidine in protein samples were quantified by comparison with the peak areas of known amounts of synthetic standards.

2.5. Rat lens organ culture

The procedure for rat lens organ culture was described by Shamsi et al. (2000). Eight hours after dissection and

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