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Assessment of structure, stability and aggregation of soluble lens proteins and alpha-crystallin upon non-enzymatic glycation: The pathomechanisms underlying cataract development in diabetic patients

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ABSTRACT

Total soluble lens proteins (TSPs) and α-crystallin (α-Cry) were individually subjected to the long-term glycation in the presence of D-glucose. The glycated and non-glycated protein counterparts were incubated under different stress conditions and compared according to their structure, stability and aggregation propensity by various spectroscopic techniques and gel mobility shift analyses. Extensive glycation of the lens proteins was accompanied with structural alteration, reduction in their surface hydrophobicity and increment of their surface tension. Our results suggest that glycation causes lens crystallins to partially resist against structural alteration and aggregation/fibrillation under both thermal and thermochemical systems. The conformational stability of lens crystallins was increased upon glycation, showing the reason behind resistance of glycated proteins against stress-induced structural alteration and aggregation. Due to the resistance of glycated lens crystallins against aggregation, the role of this modification in development of senile cataract can be explained with the associated damaging consequences highlighted in this article.

1. Introduction

Eye lens is composed of highly stable and long-lived proteins which perform both structural and refractive functions. These proteins (α, β and γ-crystallins) are present at extremely high concentration (>300 mg/mL), accounting for more than ninety percent of the total lens soluble proteins (TSPs) [1]. The short-range spatial order and close packing of these proteins play a vital role in flawless transparency and refractive properties of the mammalian eye lens [2]. These weak forces which usually do not produce any stable complex are thought to be compromised in lens due to accumulation of various types of modification, occurring during normal aging and cataractogenesis [3]. Due to their remarkable longevity, lens crystallins undergo a number of age-related modifications including non-enzymatic glycation which finally attenuate their ability in the maintenance of lens transparency [4]. Ocular lens does not require insulin for glucose uptake [5], therefore under chronic hyperglycemia, D-glucose and glycolytic intermediates such as glucose 6-phosphate, fructose 6-phosphate and glyceraldehyde 3-phosphate are highly available for irreversible attachment onto amino groups of crystallin proteins, forming stable and structurally divergent adducts which collectively named as advanced glycation end products (AGEs) [6]. Extracellular AGE proteins can potentially bind to the cell surface AGE receptors (RAGE) which up-regulate in the retinas of diabetic patients, activating diverse signal transduction cascades, resulting in generation of the reactive oxygen species (ROS) [7]. Additionally, D-glucose and the above mentioned glycolytic intermediates can enter into other ROS generating pathogenic pathways as indicated in Scheme 1.

As a consequence of organelle degradation process during differentiation, the cytoplasmic organelles are absent from the innermost cells of the adult vertebrate lens [8]. Therefore, the damaging effects of hyperglycemia on lens crystallins might be even intensified in

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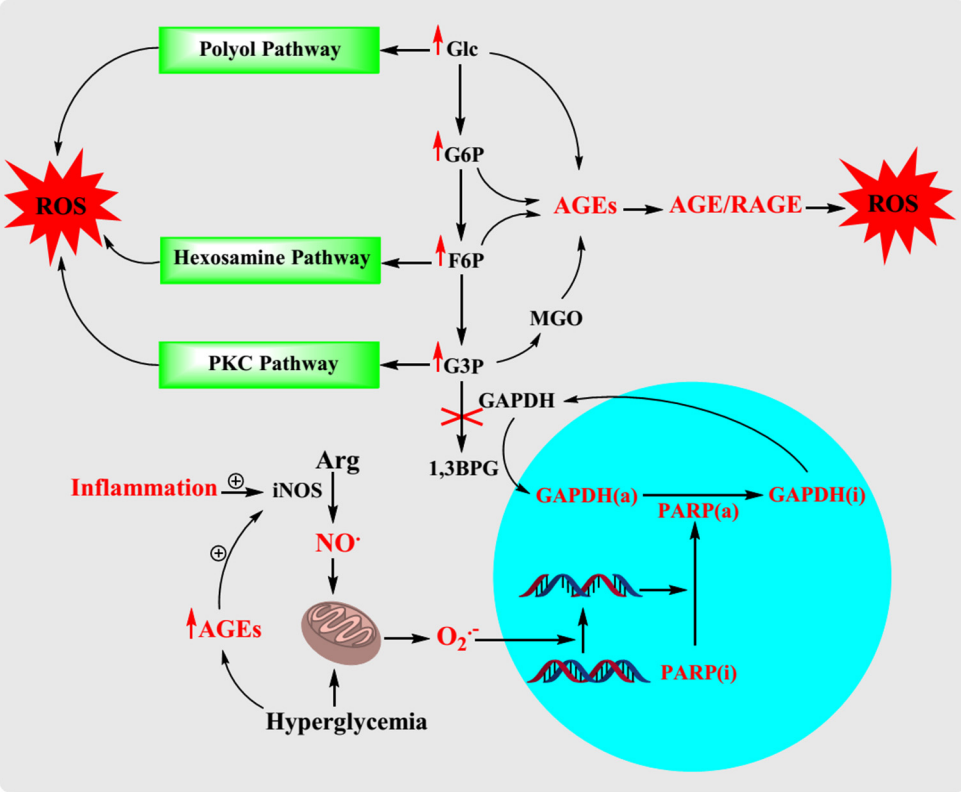

Scheme 1. Activation of multiple pathogenic ROS-generating pathways under chronic hyperglycemia. Under chronic hyperglycemia and inflammation the mitochondrial overproduction of superoxide radical causes the nuclear DNA strand break which subsequently results in activation of PARP-1 enzyme. This enzyme modifies the glycolytic enzyme GAPDH which possesses a reversible nucleo-cytoplasmic translocation. Accordingly, accumulation of the upstream glycolytic metabolites results in activation of multiple pathogenic and ROS-generating pathways including polyol pathway, protein kinase C pathway, hexosamine pathway and AGE pathway.

the outer layers of lens with the young fiber cells keeping yet their cellular organelles. During chronic hyperglycemia, AGE formation increases beyond its normal levels and these pro-inflammatory mediators activate inducible nitric oxide synthase (iNOS), leading to overproduction of nitric oxide which can be also achieved during inflammatory condition (Scheme 1) [9].

Nitric oxide inhibits mitochondrial electron transport chain and subsequently increases the production of superoxide radical which causes strand breaks in nuclear DNA, subsequently activating Poly (ADP-ribose) polymerase 1 (PARP1) [10]. This enzyme which has been indicated to play a role in several nuclear processes can also modify the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) which demonstrates a reversible nucleo-cytoplasmic translocation, thus reducing its activity. As a result of GAPDH inhibition, the accumulation of upstream glycolytic metabolites activates multiple pathogenic and ROS-generating pathways including polyol pathway, protein kinase C pathway, hexosamine pathway and AGE pathway (Scheme 1) [11]. Additionally, hyperglycemia in diabetic patients pushes more electron donors (NADH and FADH2) into the electron transport chain which subsequently increases the voltage gradient ($\Delta\mu H+$) across the mitochondrial inner membrane beyond a critical threshold [12]. At this point, the electron transfer inside complex III is partially inhibited, resulting in a backup of electrons to coenzyme Q and their donation to molecular oxygen, thereby generating increased amount of superoxide (Scheme 1). Overall, the current believes on etiology of cataracts in diabetic patients are related to the progressive glycation of lens crystallins and to the harmful effects of oxidative stress associated with hyperglycemia in eye lens [13]. In this study, TSPs and α-Cry were subjected to long-term glycation and subsequently the structural properties and aggregation/fibrillation propensity of the modified and non-modified proteins were assessed under various conditions, using different techniques.

2. Materials and methods

2.1. Materials

Sephacryl S-300 HR, 1-anilino-8-naphthalene sulfonate (ANS), thioflavin-T (ThT), ortho-phthaldialdehyde (OPA), Congo red (CR), fluorescamine and other chemicals were purchased from Sigma. D-Glucose and guanidine hydrochloride (GuHCl) were supplied by Merck. All solutions were prepared with double-distilled water and kept at 4 °C before use.

2.2. Methods

2.2.1. Preparation of bovine soluble lens proteins

The total soluble lens proteins (TSPs) were prepared from the bovine eye lens according to our previous publications [14]. In brief, bovine lenses which obtained from a local slaughterhouse were dissected from the eyeballs. A 10% homogenate of the lenses was prepared in 25 mM Tris (pH 8.0), containing 0.1 M NaCl, 0.5 mM EDTA, 0.01% NaN_3, and 10 mM β-mercaptoethanol (β-ME). Then, the homogenate was centrifuged at 14,000 rpm, for 30 min at 4 °C and the supernatant collected as TSPs.

2.2.2. Purification of bovine α-Cry

Purification of lens α-Cry was performed according to our previous publications [15]. In brief, the prepared TSPs were applied on a Sephacryl S-300 HR (100 cm × 1.5 cm) gel filtration column which pre-equilibrated with the same buffer. The crystallin proteins were collected in different test tubes at a flow rate of 0.25 mL/min with