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The structural damages of lens crystallins induced by peroxynitrite and methylglyoxal, two causative players in diabetic complications and preventive role of lens antioxidant components

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ABSTRACT

Peroxynitrite (PON) and methylglyoxal (MGO), two diabetes-associated compounds, are believed to be important causative players in development of diabetic cataracts. In the current study, different spectroscopic methods, gel electrophoresis, lens culture and microscopic assessments were applied to examine the impact of individual, subsequent or simultaneous modification of lens crystallins with MGO and PON on their structure, oligomerization and aggregation. The protein modifications were confirmed with detection of the significantly increased quantity of carbonyl groups and decreased levels of sulfhydryl, tyrosine and tryptophan. Also, lens proteins modification with these chemical agents was accompanied with important structural alteration, oligomerization, disulfide/chromophore mediated protein crosslinking and important proteolytic instability. All these structural damages were more pronounced when the lens proteins were modified in the presence of both mentioned chemical agents, either in sequential or simultaneous manner. Ascorbic acid and glutathione, as the main components of lens antioxidant defense mechanism, were also capable to markedly prevent the damaging effects of PON and MGO on lens crystallins, as indicated by gel electrophoresis. The results of this study may highlight the importance of lens antioxidant defense system in protection of crystallins against the structural insults induced by PON and MGO during chronic hyperglycemia in the diabetic patients.

1. Introduction

As the highly abundant proteins in ocular lens, α-, β-, and γ-crystallins, are important structural and functional proteins playing an essential role in the transparency and refractive power of lenticular tissue [1]. Disruption of the intra- or inter-protein interactions in the vastly ordered and interactive assemblies of these proteins can alter their delicate structures, leading to loss of accommodation and opacification of eye lenses [2]. Due to their limited turnover over decades, these proteins are highly vulnerable to the environmental insults which are the major causative players in the etiology of cataract disorders [3]. In addition, cataract is a frequent complication in diabetic patients and the most common cause of blindness worldwide [4]. Recent studies have shown that lens crystallins play an instrumental role in diabetes and its complications. Diabetic cataracts seem to be a part of multifactorial mechanism; therefore, several reactive metabolites may act either in an additive or synergistic manner, during chronic hyperglycemia, to modify the crystallin proteins, leading to lens opacification [4,5]. Both carbonyl and oxidative stresses are major contributing factors in the pathogenesis of diabetic complications [6,7]. Methylglyoxal (MGO), the highly reactive α-dicarbonyl metabolite, has been suggested to significantly increase in diabetic tissues during hyperglycaemia and in ketoacidosis [8,9]. Glyceraldehyde 3-phosphate, Maillard reaction of sugars, glucose autoxidation, lipid peroxidation, threonine degradation, metabolism of ketone bodies and ascorbate oxidation products are important potential sources of MGO generation in the

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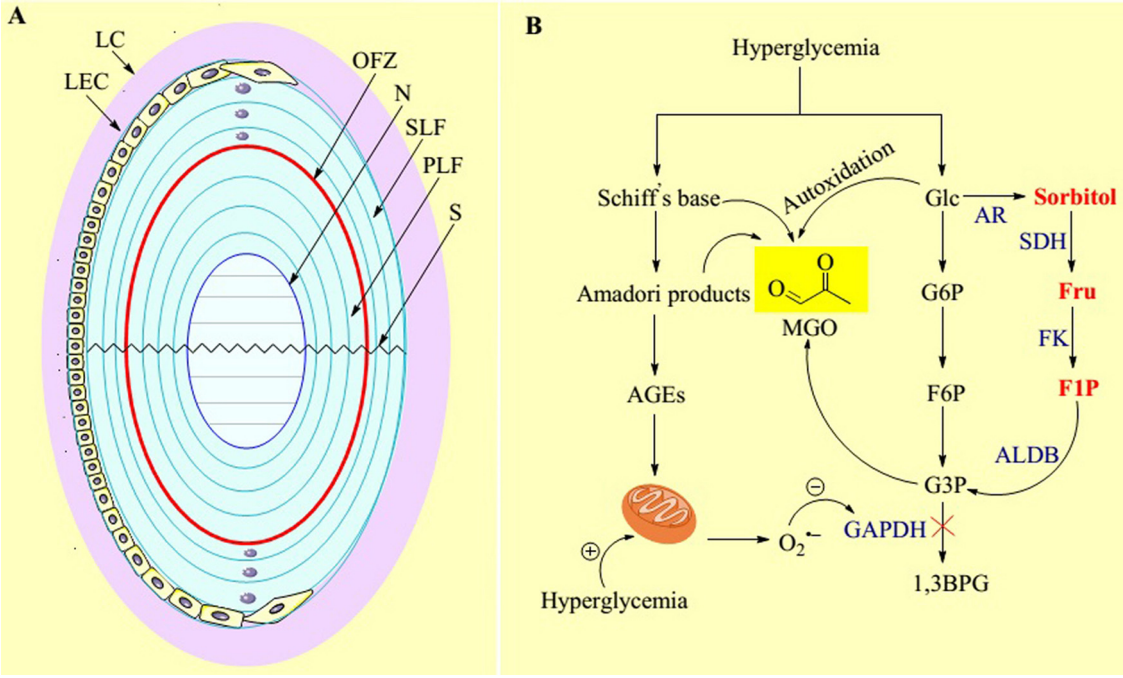

Scheme 1. The lens anatomy and pathways of lenticular MGO generation during chronic hyperglycemia.
(A) The lens capsule (LC) is transparent and the basement membrane forms its outermost layer. The lens epithelial cells (LECs) are responsible for growth and development of the transparent ocular lens. The lens fiber cells (LFCs) form the bulk of interior part of the lens. The organelle free zone (OFZ) is interior part of lens containing the enucleated fiber cells. The secondary lens fiber cells (SLF) are highly polarized organelle-containing cells surrounding OFZ. The primary lens fiber cells (PLF) are organelle-free fiber cells in OFZ. Lens nucleus (N) is the central part of lens filled with the most aged fiber cells. Sutures (S) are specialized junctions joining the LFCs at their apical and basal ends. (B) Hyperglycemia induces different pathological pathways which finally culminated in MGO over production. Also, MGO is derived from Amadori product in the pathway of AGEs formation. Autoxidation of glucose is another important source of MGO. Both hyperglycemia and AGEs stimulate superoxide anion overproduction by mitochondria which subsequently results in inhibition of glycolytic enzyme, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Inhibition of GAPDH results in accumulation of glycolytic intermediates particularly glyceraldehyde 3-phosphate (G3P) which is an important precursor of MGO generation. This MGO precursor can be also generated from the aldose reductase (AR) pathway which indicates hyperactivity in the lenticular tissues. In this pathway, SDH, FK and ALDB stand for sorbitol dehydrogenase, fructokinase and aldolase B.

lenticular tissues. In addition, hyperglycemia in diabetic patients can elevate the rate of MGO generation in eye lenses (Scheme 1).

Among different glycation agents, MGO is believed to possess a very high glycation potential with a concomitant generation of reactive oxygen species (ROS) during its synthesis and degradation [10]. As suggested before, the reaction of MGO with amino acid resulted in ROS generation [11]. Although oxidation is not always required, many types of advanced glycation end products (AGEs) are generated by a combination of glycation and oxidation [12]. Therefore, formation of so-called glycoxidation products can be triggered by oxidative stress. This dicarbonyl compound has 20,000 times more glycating potential than glucose and its concentration increases many folds in lens, kidneys and blood of diabetes patients [13,14]. MGO reacts rapidly with amino, guanidino and thiol functional groups in proteins, leading to browning, formation of fluorescent products, cross-linking and denaturation of proteins [15]. Moreover, the specific binding of MGO modified proteins resulted in immunological complications in diabetes patients [16,17]. The elevated plasma level of MGO in diabetic patients has been also suggested to inactivate antioxidant enzymes and thereby accumulate an oxidative stress [18,19]. As indicated in Scheme 1, during hyperglycemia due to contribution of mitochondria in oxidative stress, the damaging effect of MGO might be even intensified in the outer layers of lens, keeping yet their cellular organelles. According to an *in vitro* study, MGO can also induce the oxidative damages of DNA [20].

While MGO has been indicated to accelerate the browning and cross-linking of proteins, leading to the development of long-term diabetic complications, its detoxification occurs with the activity of glutathione-dependent glyoxalase and NADPH-dependent aldose reductase which respectively convert this reactive metabolite to D-lactate and 1,2-propanediol [21–25] (Scheme 2).

Various sources of MGO generation and its detoxification/damaging pathways are summarized in Scheme 2. It seems that detoxification of this reactive metabolite is linked to the oxidization of a highly important molecule (NADPH) constituting an important part of the antioxidant defense mechanism in the eye lens. Furthermore, it has been reported that with age the activity of MGO detoxification system in the human lens significantly declines [26]. Additional to the carbonyl stress, accumulating evidences support the hypothesis that diabetes is associated with increased nitrosative stress and peroxynitrite (PON) formation in numerous tissues [27]. Hyperglycemia favors the increased generation of nitric oxide through expression of inducible nitric oxide synthase (iNOS) and induces generation of superoxide anion (Scheme 3A) [28].

As shown in Scheme 3, nitric oxide combines with superoxide anion to yield the strong oxidant agent PON, which attacks proteins, leading to oxidation of tryptophan, cysteine, and tyrosine residues [28]. As reported earlier, the formation of PON increases in the diabetes-associated pathological processes and this oxidative/nitrative agent induces the formation of AGEs by the cleavage of Amadori product and generation of glucosone and glyoxal from glucose [29]. Simultaneous increase in both MGO and PON as seen during hyperglycemia (Scheme 3B) [8,9,30] and in the cataract disorder [31–33] may exacerbate the pathological conditions by enhancing the rate of disease development. Therefore, the main objective in the current study was to examine structure and aggregation propensity of the lens crystallins in the presence of both MGO and PON and to evaluate the possible role of eye lens antiox-