Colloids and Surfaces B: Biointerfaces 125 (2015) 170–180

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

The impact of calcium ion on structure and aggregation propensity of peroxynitrite-modified lens crystallins: New insights into the pathogenesis of cataract disorders

Maryam Ghahramani [a], Reza Yousefi [a,*], Kazem Khoshaman [a], Mohammad-Mehdi Alavianmehr [b]

[a] Protein Chemistry Laboratory (PCL), Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran
[b] Department of Chemistry, Shiraz University of Technology, Shiraz, Iran

ARTICLE INFO

Article history:
Received 26 August 2014
Received in revised form
30 September 2014
Accepted 3 November 2014
Available online 24 November 2014

Keywords:
Peroxynitrite (PON)
Calcium
Lens crystallins
Aggregation
Cataract

ABSTRACT

As a highly potent reactive oxygen and nitrogen species, peroxynitrite (PON) has been indicated in the pathogenesis of various ocular disorders. The PON induces mobilization of intra cellular calcium which plays an important function in structure and activity of lens proteins. Moreover, the amount of calcium increases to the pathogenic level in the cataractous lenses. The aim of this study was to assess the impact of calcium ion on structure and aggregation of PON-modified lens crystallins, using spectroscopic techniques and gel mobility shift assay. The PON modification of lens proteins was confirmed with detection of the significantly increased quantity of carbonyl group, dityrosine, nitrotyrosine and nitrotryptophan. Moreover, the modified proteins indicated high levels of solvent exposed hydrophobic surfaces and markedly elevated proteolytic instability which can be explained with their structural alteration upon this type of modification. The results of UV–vis absorption studies suggest that PON-modified lens crystallins are highly sensitive to aggregation in the presence of both physiological and pathological ranges of calcium ion. Also, the results of thioflavin T fluorescence study indicated absence of any ordered aggregate entity in the calcium-induced aggregate samples. The results of gel mobility shift assay demonstrated the importance of calcium ion in the induction of disulfide and dityrosine covalent cross-linking and formation of the oligomeric structure with relatively larger sizes in the PON-modified crystallins compared to the non-modified protein counterparts. Overall, this study may suggest that a simultaneous raise of calcium ion and PON in the eye ball is an important risk factor for development of cataract diseases.

1. Introduction

As a highly potent reactive oxygen and nitrogen species, peroxynitrite (PON) is formed in a reaction between two free radicals, superoxide anion ($O_2^{\bullet -}$) and nitric oxide ($NO^{\bullet}$) [1–3]. While superoxide is a byproduct of normal metabolism, nitric oxide is produced by the activity of two constitutive nitric oxide synthases (nNOS and eNOS) and one inducible enzyme counterpart (iNOS) which can be stimulated with certain immunologic or inflammatory stimuli [4–7]. The rate of PON generation is significantly enhanced under pathological conditions such as inflammation and diabetes, where stimulated cells release $NO^{\bullet}$ and $O_2^{\bullet -}$ at the elevated rates which favor formation of this oxidative agent [8–11]. PON which influences the inflammatory responses at multiple levels has been indicated to contribute in the pathogenesis of diabetic complications [12,13]. The three NOS isoforms have been identified in different parts of the eye [14,15]. Moreover, the induction of iNOS and overproduction of PON have been indicated in ocular inflammatory pathologies and in the retinas of human subject with diabetic retinopathy [14,16–18]. The enhanced formation of PON has been indicated in ocular fluid of various optical disorders such as uveitis, corneal damages, glaucoma, retinopathy, and senile cataract [19]. The pathway of PON pathobiology can be explained by the ability of this potent oxidizing agent to modify variety of biomolecules such as proteins, lipids, sugars, DNA and small antioxidant molecules, e.g., glutathione [2,20–24]. Moreover, PON demonstrates a very high affinity for Tyr, Trp and Cys residues in proteins [25–27]. Accordingly, various biomarkers of PON-induced protein modifications have been introduced as nitrotyrosine, dityrosine, nitrotryptophan, protein carbonyls, cysteine oxidation, protein

* Corresponding author. Tel.: +98 7116137665; fax: +98 7112280916.
E-mail address: ryousefi@shirazu.ac.ir (R. Yousefi).

http://dx.doi.org/10.1016/j.colsurfb.2014.11.002
0927-7765/

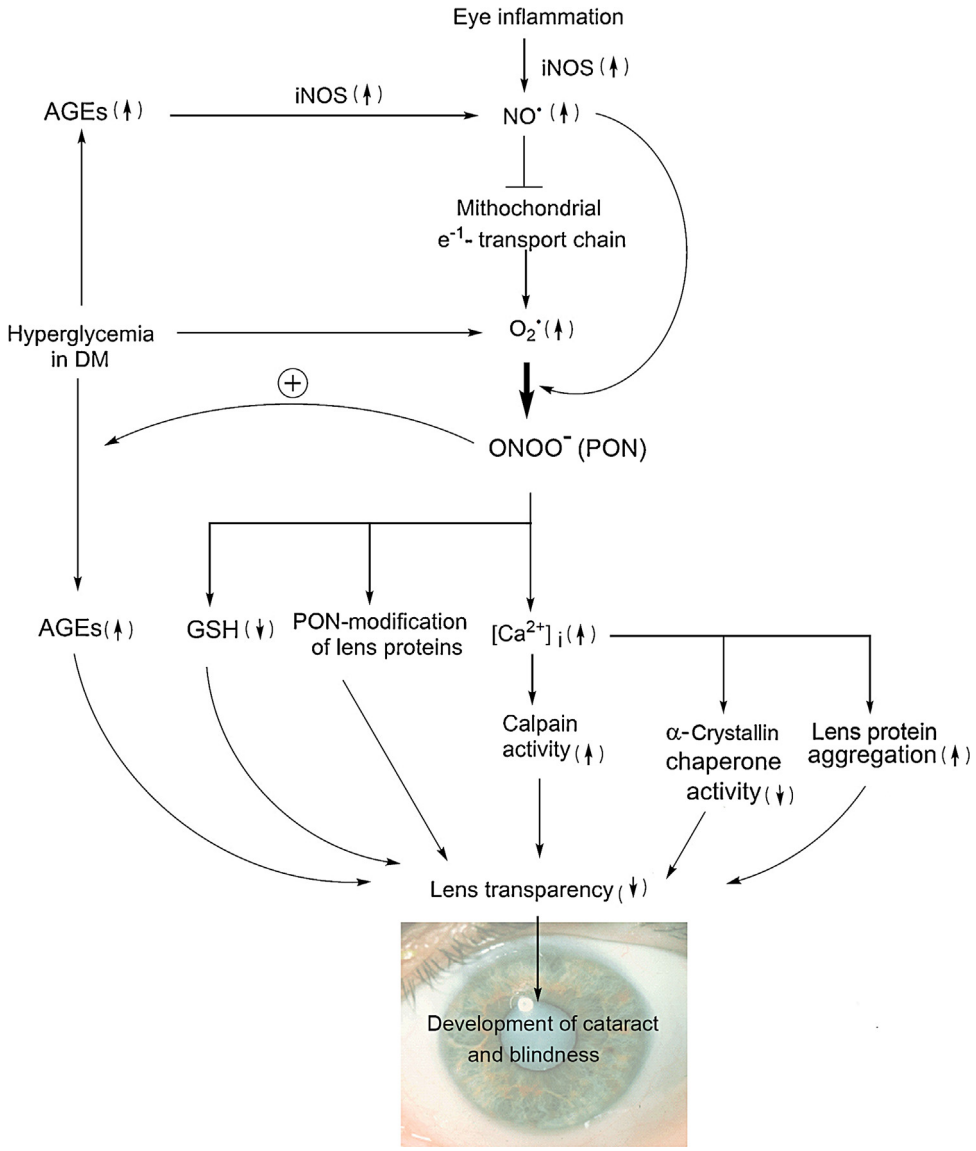

Fig. 1. The possible routes for contribution of PON and calcium in development of cataract disorder and blindness. Chronic hyperglycemia in diabetes mellitus (DM) and inflammation are indicated as two important pathological states which could increase the level of PON to the significant extent in eye ball. PON may contribute in lens opacification either directly via modification of lens crystallins or indirectly by elevation of calcium level. Moreover, PON decreases the level of reduced glutathione (GSH) which is the principal lenticular antioxidant, existing in an unusually high concentration in the lens where it functions to maintain the tissue's transparency. Also, these two cataract causing agents (PON and calcium) may contribute in the pathomechanism of cataract diseases either in additive- or in sequential manner.

fragmentation and disruption of metal sulfur clusters [20,25–28]. Overall, more than 60 human disorders have been indicated to be associated with the PON-mediated protein modifications [29].

The eye lens is composed of highly stable and long-lived proteins, α-, β- and γ-crystallins (Cry) which are accounted for almost ninety percent of the total lens proteins (TSPs), and these proteins perform both structural and refractive functions [30]. Due to their limited turnover during the life span, the accumulation of various modifications on these proteins have important effect on their structure and short-range ordered packing which are all necessary for the maintenance of lens transparency [30–33]. Due to the mass production of this oxidative agent in diabetes and during inflammation, TSPs are among those proteins which are easily subjected to the PON modification. Also, PON has been indicated to induce mobilization of intra cellular calcium (calcium efflux from mammalian mitochondria) which plays an important role in both structure and activity of lens proteins [34]. As reported previously, in the cataractous lenses, the concentration of this metal ion increases to a significant level [35]. Due to the involvement of both calcium and PON in pathogenesis of cataract disorders, the current study was done with the aim of investigating the impact of calcium ion on structure and aggregation propensity of PON-modified TSPs. As indicated in this study, calcium ion was shown to induce the structural alteration and subsequently oligomerization/aggregation of PON-modified TSPs to significantly higher levels, compared to their unmodified protein counterparts. The obtained results may indicate that a simultaneous increase in both calcium and PON as occurring in the cataract disorder may exacerbate the pathological conditions by enhancing the rate of disease development.

2. Materials and methods

2.1. Materials

Thioflavin T (ThT), 2,4-dinitrophenyl hydrazine (DNPH), 1-anilino-8-naphthalene sulfonate (ANS) and other chemicals were purchased from Sigma Chemical Company.