Biochimie 132 (2017) 19–27

Contents lists available at ScienceDirect

Biochimie

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

Review

Paraoxonase and atherosclerosis-related cardiovascular diseases

Dimitry A. Chistiakov [a], Alexandra A. Melnichenko [b, c], Alexander N. Orekhov [b, c, d, e], Yuri V. Bobryshev [b, f, g, *]

[a] Department of Molecular Genetic Diagnostics and Cell Biology, Division of Laboratory Medicine, Institute of Pediatrics, Research Center for Children's Health, 119991, Moscow, Russia
[b] Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, 125315, Russia
[c] Institute for Atherosclerosis Research, Skolkovo Innovative Center, Moscow, 121609, Russia
[d] Department of Biophysics, Biological Faculty, Moscow State University, Moscow, 119991, Russia
[e] National Research Center for Preventive Medicine, Moscow, 101000, Russia
[f] Faculty of Medicine, School of Medical Sciences, University of New South Wales, Sydney, NSW, 2052, Australia
[g] School of Medicine, University of Western Sydney, Campbelltown, NSW, 2560, Australia

ARTICLE INFO

Article history:
Received 25 July 2016
Accepted 18 October 2016
Available online 19 October 2016

Keywords:
Paraoxonase
PON1
PON2
PON3
Atherosclerosis
Atheroprotective properties

ABSTRACT

In humans, three paraoxonase (PON1, PON2, and PON3) genes are clustered on chromosome 7 at a locus that spans a distance around 170 kb. These genes are highly homologous to each other and have a similar protein structural organization. PON2 is the intracellular enzyme, which is expressed in many tissues and organs, while two other members of PON gene family are produced by liver and associate with high density lipoprotein (HDL). The lactonase activity is the ancestral. Besides lactones and organic phosphates, PONs can hydrolyze and therefore detoxify oxidized low density lipoprotein and homocysteine thiolactone, *i.e.* two cytotoxic compounds with a strong proatherogenic action. Indeed, PONs possess numerous atheroprotective properties, which include antioxidant activity, anti-inflammatory action, preserving HDL function, stimulation of cholesterol efflux, anti-apoptosis, anti-thrombosis, and anti-adhesion. PON genetic polymorphisms contribute to susceptibility/protection from atherosclerosis-related diseases. The bright antiatherogenic activity of the PON cluster makes it a promising target for the development of new therapeutic strategies.

Contents

* Corresponding author. Faculty of Medicine, University of New South Wales, Sydney, NSW, 2052, Australia.
E-mail address: y.bobryshev@unsw.edu.au (Y.V. Bobryshev).

http://dx.doi.org/10.1016/j.biochi.2016.10.010
0300-9084/

1. Introduction

In humans, paraoxonase (PON) was first considered as an enzyme that hydrolyses and thereby neutralizes organoposphate xenobiotics such as paraoxon (insecticide), sarin, soman, VX (all three are nerve gases), chlorpyrifos oxon, diazoxon, and other related toxic compounds [1,2]. PON1 can perform a reverse reaction (i.e. lactonization) of γ- and δ-hydroxy-carboxylic acids [3]. This enzyme can also metabolize some drugs that have lactone and cyclic carbonates [4]. More importantly, PON is able to detoxify oxidized low density lipoprotein (oxLDL) and degrade homocysteine thiolactone, an intermediate that can induce protein N-homocysteinylation. It was well established that PON serve as a cardioprotective agent in atherosclerosis and related vascular diseases [5].

In fact, there are three human PON genes (PON1, PON2, and PON3), which are clustered on chromosome 7q23.1-q22.3 [6]. All human PON genes have 9 exons and share around 70% of homology at the nucleotide sequence and 60% of homology at the amino acid (a.a.) sequence. In mammals, the comparative similarity of each of those three genes is higher and reaches 81–90% homology at the nucleotide sequence and 79%–90% of homology at the a.a. sequence [7].

The evolution of the gene cluster occurs through the duplication of a common ancestor. Is it likely that the enzyme lactonase from *Fusarium oxysporium* (a fungus) may serve as the best candidate to be an ancestor due to significant homology with human PON [8]. Genes encoding lactone cycle-breaking enzymes were also identified in many other organisms belonging to bacteria, plants, worms, insects, fishes, amphibians, reptilians, and birds thereby suggesting a substantial PON sequence conservation among different species [9]. Among PON genes, PON2 seems to be the oldest on the basis on structural homology and evolutionary distance. PON2 appears to give rise to both PON1 and PON2 as a result of a second duplication event [9]. The ability of hydrolyze lactons is the common evolutionary conserved feature of PON-like enzymes since lactones can be frequently meet in plants (especially in fruits) where they contribute to flavors [10]. Structure-reactivity studies of PON1 suggest that its native activity is lactonase indicating that PON1 in fact is lactonase [11].

2. PON1 structure and intracellular/tissue distribution

PON1 includes 354 a.a. and has a molecular mass of 43 kDa [12]. The N-terminus of the enzyme retains a highly hydrophobic leader sequence that is responsible for binding high density lipoprotein (HDL) [13]. PON1 contains three cysteines (Cys42, Cys284, and Cys353) of whom two (Cys42 and Cys353) generate a disulfide bond. The replacement of either Cys42 or Cys353 by alanine leads to loss of the catalytic activity and substantial reduction of enzyme secretion [14]. Change of Cys284 to alanine or serine results in reduction but remaining a residual paraoxonase and arylesterase activities [15]. In addition, Cys284 was shown to protect LDL from Cu^{2+}-dependent oxidation [16], an evidence that stimulated Aviram et al. [17] to discuss about a possibility of existence of two (i.e. hydrolytic and antioxidant) catalytic centers in the PON1 molecule.

In PON1, there are two Ca^{2+}-binding sites, one of which has a high affinity and is needed for enzyme stabilization while another is necessary for hydrolytic activity. Removal of Ca^{2+} leads to irreversible loss of the enzymatic activity and destabilization. Ca^{2+} replacement with other divalent cations such as Mg^{2+} or Zn^{2+} maintains enzyme stability but not activity [18]. Histidine residues (His115, His134, His155, His243) and Trp281 were found to be involved in the esterase enzymatic activity of PON1 [14].

To evaluate a three-dimensional (3D) structure, a rabbit PON1 that is highly identical to human PON1 was used [19]. Overall, the enzyme has a propeller-like 3D structure with six vanes (each containing four β-sheets) and central tunnel. The disulfide bond Cys42-Cys353 covalently links the N- and C-termini, a feature preserved in the PON family. A distance of 7.4 Å separates two Ca^{2+} ions, one of which is located in the central region whereas another is present on top of the molecule [19]. The top Ca^{2+} ion is suggested to play a catalytic role through interaction with side chain oxygens of Asn224, Asn270, Asn168, Asp269, and Glu53 [20]. The centrally located Ca^{2+}can potentially contribute to PON1 stability [20]. PON1 is a glycoprotein, with Asn253 and Asn324 as predicted glycosylation sites [21]. The abovementioned a.a. residues are highly conserved indicating that all members of the PON family keep the common catalytic site structure that diverge to three independent genes during evolution. Compared with human PON2, human PON1 and PON3 have hydrophobic N-terminal regions (at a.a. 7–18 and 19–28 respectively) that contribute in HDL binding with the involvement of hydrophobic a.a. of the second helix [19].

Liver is the major site of expression of human PON1 and PON3. Low PON3 expression was also detected in human kidney [22]. In contrast, PON2 is widely expressed in multiple tissues [23]. Liver releases PON1 and PON3 into the circulation where the enzymes bind to HDL particles [23]. PON2 is located intracellularly, mainly close to the nucleus, where the enzyme aggregates to the endoplasmic reticulum and nuclear membrane [24]. The abundance of PON2 in the endoplasmic reticulum can be explained by enrichment of the endoplasmic reticulum with antioxidants that along with local proteins stabilize the enzyme [25]. PON1 and PON3 can be found in atherosclerotic lesions since both enzymes are associated with HDL. In plaques, PON2 continues to be expressed in endothelial cells (ECs), vascular smooth muscle cells (VSMCs), and macrophages (i.e. in main lesion cell components) and has anti-atherogenic effects on these cells [10].

3. Physiological substrates of paraoxonases

As mentioned above, the lactonase activity is the primary PON activity. Oxidized polyunsaturated fatty acids (PUFAs) form lactone-like structures that can serve as PON substrates [11,26]. In oxLDL, PON1 was shown to hydrolyze 19% of lipid peroxides and up to 90% cholesteryl linoleate hydroperoxides [27] suggesting a key atheroprotective role of this enzyme by clearance of LDL from lipid peroxides. Aviram et al. [28] also showed the ability of PON1 to hydrolyze an ester link between cholesterol and linoleate hydroperoxide or peroxide, a highly atherogenic and prooxidant intermediate.

Another protective function of PON1 is to neutralize homocysteine thiolactone to prevent protein modification [29]. The thiolactone can form amide links with protein lysine that can alter protein activity and functionality. Indeed, homocysteine thiolactone can contribute to atherogenesis by inducing atherogenic damage of ECs and VSMCs either by toxicity itself or by homocysteinylation of cell proteins. PON1 hydrolyzes the thiolactone to homocysteine thus detoxifying this highly reactive compound [30]. PON1 and PON2 were shown to reduce atherosclerosis-related oxidative stress induced by hydrogen peroxide (H_2O_2) [23]. PON2 is also able to decrease intracellular reactive oxygen species (ROS) production in endoplasmic reticulum stress [24].

Thus, advanced capacity to hydrolyze various oxidized intermediates and homocysteine thiolactone makes PONs to be an important protector against oxidative stress and inflammatory disorders such as atherosclerosis.