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Oxidized-phospholipids in reconstituted high density lipoprotein particles affect structure and function of recombinant paraoxonase 1 ☆

Subhabrata Kar [1,3], Mitulkumar A. Patel [1,2], Rajan K. Tripathy, Priyanka Bajaj, Abhay H. Pande *

Department of Biotechnology, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S. Nagar (Mohali), 160062 Punjab, India

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ABSTRACT

Paraoxonase 1 (PON1) is an HDL-associated enzyme and exhibits anti-inflammatory, anti-diabetic, and anti-atherogenic properties. Association of PON1 to HDL particles increases the stability and activity of PON1 and is important for the normal functioning of the enzyme. HDL particles are made up of lipid and protein constituents and apolipoprotein A-I (apoA-I) is a principal protein constituent of HDL that facilitates various biological activities of HDL. In many disease conditions the oxidized phospholipid (Ox-PL) content of HDL is found to be increased and an inverse correlation between the activity of PON1 and oxidation of the HDL is observed. However, the molecular details of the inhibitory action of the Ox-PL-containing HDL on the function of PON1 are not clear yet. In this study we have assembled reconstituted HDL (rHDL) particles with and without Ox-PL and compared their effect on the structure and function of ^{13}C-labeled recombinant PON1 (^{13}C-rPON1) by employing attenuated total reflectance Fourier transformed infrared (ATR-FTIR) spectroscopy and enzymatic assay. Our results show that the presence of the Ox-PL in the rHDL particles alters the structure of rPON1 and decreases its lactonase activity.

1. Introduction

Human paraoxonase 1 (PON1)[1] is an ~45 kDa, Ca^{2+}-dependent enzyme that acts on a broad range of substrates [1,2]. The various activities of PON1 can be grouped under three categories, namely, lactonase, arylesterase and phosphotriesterase activities [1,2]. PON1 is a multi-tasking enzyme and possesses anti-inflammatory, anti-oxidative, anti-atherogenic, and anti-diabetic properties [3–5]. It is believed that, by virtue of its lactonase activity, PON1 enzyme plays an important role in the prevention of atherosclerotic lesion development [6]. The phosphotriesterase activity enables the enzyme to hydrolyze and inactivate a variety of organophosphorous (OP) compounds [7]. Thus, the level and the activity of PON1 in blood have a major impact on the individual's susceptibility to OP-poisoning and various disease conditions.

In humans, PON1 is synthesized primarily in the liver and secreted into the plasma where it is associated exclusively with a category of HDL particles [8]. The association of PON1 with HDL not only stimulates the catalytic activities of the enzyme but also stabilizes the enzyme and provides protection against its inactivation [8,9]. It is proposed that HDL also serve as a shuttle for the distribution of PON1 from the site of its synthesis to the distal sites of lipid damage and other regions in the body [8]. HDL particles are made up of lipid and protein constituents and apoA-I is a major apolipoprotein constituent of HDL that plays an important role in facilitating the numerous biological activities of HDL [10].

In various disease conditions, the normal composition of the constituents of the HDL particles is found to be 'altered' as a result of which the HDL particles become dysfunctional [11–14]. These dysfunctional HDL particles have less athero-protective activities and are pro-atherogenic and pro-inflammatory in nature [11–14]. Many of the anti-atherogenic functions of HDL are now attributed to the HDL-associated PON1 and various activities of PON1 in the subjects having increased dysfunctional HDL particles are found to be considerably lower than in the normal subjects [11–14]. An increase in the Ox-PL content of HDL is one of the reasons why HDL becomes dysfunctional and numerous reports suggest an inverse correlation between the activity of the HDL-associated PON1 and the oxidation of the lipoprotein particles [5,11,12,15,16]. However, the molecular details of the inhibitory effect of Ox-PL containing HDL particles on the properties of PON1 are not clear.

In this study, we have compared the effect of control-rHDL (which contains no Ox-PL) and Ox-rHDL-II (which contains Ox-PLs) on the structure and function of ^{13}C-labeled recombinant PON1 (^{13}C-rPON1) by employing attenuated total reflectance Fourier transformed infrared (ATR-FTIR) spectroscopy and enzyme assay, respectively. Our results

Abbreviations: apoA-I, apolipoprotein A-I; ATR-FTIR, attenuated total reflectance Fourier transformed infrared; Chol, cholesterol; CVD, cardiovascular disease; Ox-PLs, oxidized phospholipids; Ox-PAPC, oxidized 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphocholine; PAPC, 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; rPON1, recombinant paraoxonase 1; rHDL, reconstituted HDL

☆ Conflict of interest: None.

* Corresponding author: Tel.: +91 172 2214 682; fax: +91 172 2214 692.
E-mail addresses: apande@niper.ac.in, abbupande@yahoo.co.in (A.H. Pande).

[1] Both authors contributed equally to this work.

[2] Present address: Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN.

[3] Present address: School of Biosciences, Apeejay Stya University, Sohna-Palwal Road, Sohna- 122103, Haryana, India.

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show that the presence of Ox-PLs in the rHDL particles alters the secondary structure of rPON1 and decreases the lactonase activity of the enzyme.

2. Materials and methods

2.1. Purification of ^{13}C-rPON1

The prokaryotic expression vector (pET32-trx) containing a His-tagged rPON1 (rPON1-G3C9) was a kind gift from Prof. Dan S. Tawfik, Weizmann Institute of Science, Israel [17]. This vector was transformed into competent Origami BDE3 cells (Novagen) using the $CaCl_2$ method and the glycerol stocks of the transformed cell were stored at −80 °C. ^{13}C-labeled rPON1 was generated by following a protocol previously reported [18,19], with some modifications. Briefly, expression of rPON1 was carried out in M9 minimal medium supplemented with $^{13}C_6$-D-glucose as a sole carbon source [20]. Glycerol stock of the transformed *E. coli* cells was streaked on M9 agar plate and the cells were allowed to grow at 37 °C for 20–24 h. A single bacterial colony was picked from the plate and inoculated into M9 minimal media and the culture was incubated at 37 °C for 22–24 h (starter culture). Five milliliters of the starter culture was inoculated into 500 ml of M9 minimal media and the resulting culture was allowed to grow further for 8–10 h at 30 °C followed by 32 h at 20 °C. The culture was then aseptically (in a Biological Safty Cabinet) transferred into autoclaved centrifuge-tubes and centrifuged (5000 ×*g* for 10 min at 4 °C). The supernatant ($^{13}C_6$-D-glucose containing media) was recollected in 2 l flasks and the cell pellets were stored at −80 °C in the same centrifuge tubes. To the collected media, filter-sterilized $CaCl_2$, $MgSO_4$ and antibiotics were added freshly and the media was further inoculated with fresh 5 ml starter culture (grown separately) for the second time. This culture was further allowed to grow for a total of 40 h, as described above. Again the cell pellets were collected by centrifugation. The cell pellets were pooled and used for the purification of ^{13}C-rPON1.

Purification of ^{13}C-rPON1 was carried out by following the procedure previously reported [21]. Briefly, the cell pellets were re-suspended in 10–15 ml of ice-cold lysis buffer (50 mM TrisHCl, pH 8.0, containing 1 mM $CaCl_2$, 50 mM NaCl, 0.1 mM DTT and 50 μl of protease inhibitor cocktail). The cell suspension was passed through a syringe with a needle to break the cells and the sample was then subjected to sonication. To the sonicated sample 0.1% tergitol (final concentration) was added and the sample was incubated at 4 °C for 2 h to facilitate proper refolding of the recombinant protein. The sample was then centrifuged (10,000 ×*g* for 20 min at 4 °C) and the clear supernatant was used further. The supernatant was loaded onto a Q-Sepharose column equilibrated with Tris buffer (50 mM TrisHCl, pH 8.0, containing 50 mM NaCl, 1 mM $CaCl_2$, and 0.1% tergitol). The column was then washed with the same buffer, and the bound proteins were specifically eluted by increasing the concentration of NaCl in the same buffer. The activity was checked in the eluted fractions using paraoxon as a substrate [21]. Fractions showing good paraoxonase activity were pooled and were subjected to affinity chromatography using Ni-Sepharose 6 resin-containing column (HisPrep FF 16/10; GE Healthcare Bio-Sciences Ltd.). After washing the column to remove the unbound protein, the bound protein was specifically eluted using the Tris buffer containing 150 mM imidazole. The fractions containing proteins were pooled, dialyzed against the Tris buffer and were concentrated using an Amicon concentrator (5 kDa cutoff). The purity of the preparations at various stages of purification was checked by 4–20% SDS-PAGE and Western blot analysis using monoclonal mouse anti-HuPON1 antibody as the primary antibody [22].

2.2. Preparation of rHDL particles

Ox-PLs were prepared by the air-oxidation of PAPC as previously reported [21]. Briefly, the desired amount of PAPC dissolved in chloroform was taken in a glass tube and the organic solvent was evaporated using a stream of argon. The dried PAPC film was incubated at room temperature for 72 h to auto-oxidize in air. Ox-PAPC was then suspended in chloroform and the extent of the lipid oxidation in the preparation was measured directly by the thiobarbituric acid-reactive substance (TBARS) assay. Ox-PAPC thus prepared was used as a source of Ox-PLs.

The rHDL containing human apoA-I, cholesterol, POPC (±Ox-PL) was prepared by a cholate dialysis method and was purified by using size-exclusion chromatography as previously reported [21].

2.3. Preparation of detergent-free ^{13}C-rPON1 and incubation with rHDL particles

Detergent-free ^{13}C-rPON1 was prepared by using Extracti-Gel® D detergent-removing column [21] and the detergent-free ^{13}C-rPON1 was used within 1 day. To study the effect of rHDL on the properties of the enzyme, detergent-free ^{13}C-rPON1 was mixed with excess of the purified rHDL in the activity buffer (20 mM TrisHCl, pH 8.0, and containing 150 mM NaCl and 2 mM $CaCl_2$) to obtain the desired enzyme:apoA-I ratio and the mixture was incubated at 37 °C for 2 h. The enzyme:rHDL mixture was then used in the enzymatic and structural assays.

2.4. Lactonase activity measurement

Lactonase activity of the enzyme was measured by a pH-indicator colorimetric assay using δ-valerolactone as substrate [9]. The reaction was carried out in a 96-well microtiter plate in the bicine buffer (2.5 mM bicine, pH 8.3, containing 0.2 M NaCl, and 1 mM $CaCl_2$) containing 0.1 mM of *m*-cresol purple as indicator [9]. Detergent-free ^{13}C-rPON1 was pre-incubated for 2 h at 37 °C in either activity buffer alone, or activity buffer containing 0.1% tergitol or activity buffer containing rHDL particles. The final concentrations of rPON1 and apoA-I in the incubation mixture were 0.3 μM and 30 μM, respectively. Aliquots of these mixtures were added to the wells of the microtiter plate containing the bicine buffer and 0.1 mM of *m*-cresol purple. The reaction was initiated by adding the substrate (1 mM δ-valerolactone) to the wells and the decrease in the absorbance at 577 nm was monitored as a function of time. Enzyme activity was calculated from the HCl standard curve and corrected for the non-enzymatic hydrolysis of the substrate. The initial slope of the traces (first 30 min data) was used to calculate the specific activity of the enzyme.

2.5. ATR-FTIR spectroscopy

ATR-FTIR spectra of the samples were recorded on a Tensor 27 FTIR spectrometer (Bruker Optics, Germany) as previously reported [21]. Briefly, the sample (rHDL or ^{13}C-rPON1:rHDL mixture) was deposited on the ZnSe crystal of the BioATRCell™ I chamber and a thin semi-dried film was prepared by slow evaporation of the aqueous portion of the samples under a gentle stream of N_2 gas. This way of sample preparation is known to yield multilayers of sample molecules containing residual water [23]. The chamber containing the BioATRCell™ I was then sealed and the spectra were recorded at 25 °C using both parallel (||) and perpendicular (⊥) polarized incidence light with respect to a normal of the ATR plate. For each sample 512 interferograms were accumulated to improve the signal/noise ratio at a spectral resolution of 2 cm^{-1}. Each spectrum was cut in the desired range, baseline-corrected and smoothed using a five-point Savitzky–Golay function. Second derivatization of the spectra was done to identify the peak frequencies of spectral components in the amide I region. These peak frequencies were used to compute the secondary structural components of the proteins by least squares iterative curve fitting (Levenberg–Marquardt curve fitting method) as previously reported [18,19,21].