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Cloning, high level expression of human paraoxonase-3 in Sf9 cells and pharmacological characterization of its product

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Abstract

Human paraoxnase-3 (hPON3) (EC3.1.8.1) is a lipid-associated enzyme with antioxidant activity, and can inhibit the oxidation of low-density lipoprotein (LDL), thereby inhibiting early atherogenic process. In the present study, human *PON3* gene was cloned from Human Fetal Liver Marathon-Ready cDNA and expressed in insect cells using baculovirus vector. Twenty-eight milligrams of purified recombinant hPON3 (rhPON3) was obtained from 1 L Sf9 cells culture. The K_m and V_{max} values of rhPON3, with respective to phenylacetate hydrolysis were 7.46 ± 4.40 mM and 89 ± 10.54 U/mg ($n = 3$). The kinetic parameters of V_{max} and K_m for dihydrocoumarin hydrolysis by rhPON3 were 698 ± 248 U/mg and 0.84 ± 0.24 mM ($n = 3$). LDL oxidation assay indicated that rhPON3 could effectively protect LDL against copper-induced oxidation in vitro.

Keywords: Human paraoxonase-3; Baculovirus-mediated expression system; Sf9 cell; Gene expression; LDL oxidation; Dihydrocoumarin

1. Introduction

The human paraoxonase (hPON) gene family includes three members: *PON1, PON2* and *PON3*, located adjacent to each other on the long arm of chromosome 7 [1]. The three hPONs share approximately 65% identity at the amino acid level and 70% identity at the nucleotide level. In humans, hPON3 is primarily produced in liver, the expression of hPON3 is also present in kidney [1,2].

The PON3 enzyme (EC3.1.8.1) is a 40-kDa glycoprotein with calcium-dependent esterase activity being able to catalyze the hydrolysis of a broad range of substrates including aryl-esters, lactones and pharmacological agents. PON3 and PON1 reside in the cholesterol-carrying particles high-density lipoprotein (HDL) and are involved in the prevention of atherosclerosis. Studies have shown that PON1 and PON3 can inhibit lipid oxidation in low-density lipoprotein (LDL), thus reducing the levels of oxidized lipids that are involved in the initiation of atherosclerosis [2–6].

PON3 and PON1 share three conserved cysteine residues: Cys-41, Cys-283 and Cys-351, and possess similar properties in structure and activities. In contrast to PON1, PON3 has very limited arylesterase activity and no PON activity, but it can hydrolyze lactones rapidly [3–5]. Previous studies have suggested that both PON1 and PON3 are important in the prevention of atherosclerosis [3,6–13]. PON1 protects both LDL and HDL from oxidation, probably, due to their ability to hydrolyze specific oxidized phospholipids and cholesteryl esters with linoleate hydroperoxides [6–13]. Both rabbit PON1 and rabbit PON3 have the ability to protect LDL against in vitro copper-induced oxidation. However, it was reported that the rabbit PON3 purified from serum is approximately 100 times more potent than the rabbit PON1 in protecting LDL against oxidation [4]. Under oxidation stress, PON1's expression is down regulated while PON3's expression level keeps unchanged [2]. Thus, PON3 may act by a mechanism similar to that of PON1 and play a key role different from PON1 in the prevention of atherosclerosis. Its contribution against LDL oxidation and in the prevention of atherosclerosis might be as great as that of PON1. So, PON3 may have the potential to be a therapeutic agent to prevent atheroma.

Very recently, Draganov et al. [14] reported the expression of *PON1, PON2* and *PON3* from human tissue by a baculovirus-mediated system. Insect cells used in conjunction

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with the baculovirus expression vector system (BEVS) are gaining ground rapidly as a platform for recombinant gene expression, especially for proteins that are insoluble in *Escherichia coli* or are covalently modified. In this study, we amplified hPON3 cDNA from Human Fetal Liver Marathon-Ready cDNA and expressed it in baculovirus-mediated Sf9 cells at high level. The pharmacological properties of the rhPON3 were also studied. In contrast to the results from Draganov et al. [14], we found that the purified rhPON3 from Sf9 cells could inhibit LDL oxidation in vitro.

2. Materials and methods

2.1. Materials

Human Fetal Liver Marathon-Ready cDNA was from CLONTEC (USA); restriction enzymes, ExTaq DNA polymerase and T4 DNA ligase were from Takara (Japan); lipofectin and FBS were from Gibicol-BRL (USA); DIG High Prime DNA Labeling and Detection Starter Kit was from Roche Applied Science (Germany); TNM-FH medium, goat anti-mouse IgG HRP conjugated, LDL, dihydrocoumarin and phenylacetate were from Sigma-Aldrich (USA); 6-Histidine (Epitope Tagging) AB-1 (Clone 4D11) mouse monoclonal antibody was from NEO MARKERS (USA); antibiotics, protein molecular weight markers, and NP-40 were from BBI Fermentas (USA); Chelating Sepharose™ high performance and anion chromatography media DEAE-Sepharose Fast Flow were from Amersham Biosciences (Sweden); primers were synthesized by Sangon (China). All other chemicals were of analytical grade. Water was purified in a Milli Q system from Millipore.

2.2. Plasmids

pMD18-T vector was from Takara (Japan); pET-28a vector was from Novagen (UK); transfer vector pVL1393 was from Invitrogen Corporation (USA).

2.3. Cells and viruses

Sf9 cell line was from Invitrogen Corporation (USA) and maintained in TNM-FH with 10% FBS at 27 °C. Wild-type AcNPV DNA was made by ourselves.

2.4. Cloning of human PON3 (hPON3) cDNA and construction of recombinant transfer vector

To amplify the human *PON3* cDNA from Human Fetal Liver Marathon-Ready cDNA, the forward primer (Primer 1: 5'-ATGGGGAAGCTCGTGGCGCTGGT-3') and the reverse primer (Primer 2: 5'-CTAGAGTCTAGATCTAGAGCTCACAGTACA-3') were used. PCR was carried out with 5 μl cDNA and 0.625 units of ExTaq DNA polymerase (TaKaRa) in a final volume of 25 μl using the following conditions: 94 °C for 4 min, 28 cycles (94 °C for 30 s, 68 °C for 30 s and 72 °C for 2 min) and a final extension of 72 °C for 7 min. The PCR product was purified by agarose gel electrophoresis and cloned into pMD18-T to yield pMD18-T-hPON3 plasmid. The plasmid pMD18-T-hPON3 was sequenced by Sangon (China).

The plasmid pMD18-T-hPON3 was used as a template for the construction of recombinant transfer vector. A sense primer (Primer 3: 5'-CGCATATGGGGAAGCTCGTGGCGCTGGT-3') and an anti-sense primer (Primer 4: 5'-CGGAATTCTTATCAGAGCTCACAGTACAGAGT-3') were used to amplify hPON3 cDNA. PCR was carried out with 1.25 units of ExTaq DNA polymerase in a final volume of 50 μl using the following conditions: 94 °C for 4 min, 30 cycles (94 °C for 30 s, 56 °C for 30 s and 72 °C for 60 s) and a final extension of 72 °C for 7 min. The PCR product was double digested with NdeI and EcoRI, purified by agarose gel electrophoresis, and cloned into pET-28a to yield pET-28-rhPON3 plasmid. The plasmid pET-28a-rhPON3 was digested using *Xba*l and *Eco*Rl, and the resultant fragment was subsequently inserted into pVL1393 transfer vector under the control of polyhedrin promoter. In pVL1393-rhPON3, a 6-Histidine tag was contained for the purification and detection of the expressed rhPON3.

2.5. Construction of recombinant baculovirus

The recombinant transfer vector pVL1393-rhPON3 (1 μg) and wild-type AcNPV DNA (1 μg) were co-transfected into Sf9 cells using lipofectin. The viral progeny was screened for the presence of recombinant virus in which the polyhedrin gene was inactivated by the insertion of rhPON3 cDNA. The recombinant virus was isolated by plaque purification method and identified by dot-blot hybridization using a digoxigenin-labeled fragment of hPON3 cDNA as a probe and activity analysis. After three cycles of plaque purification, the purified recombinant virus AcNPV-rhPON3 with the highest expression level was chosen for further study.

2.6. Recombinant hPON3 expression

Sf9 cells (2×10^6 /25 cm^2 flask) were seeded into cell culture flasks. After attachment of the cells, the medium was removed and the cells were infected with the recombinant virus AcNPV-rhPON3 at multiplicities of infection (MOI) of ~10. One hour later, the inoculum was replaced by 4 ml of fresh medium without FBS and incubated at 27 °C; the culture supernatant was collected at various intervals and invested for enzyme activity.

2.7. Enzyme assay

Arylesterase activity towards phenylacetate was measured spectrophotometrically at 270 nm. The E_{270} for the reaction was 1310 M^{-1} cm^{-1}. One unit of arylesterase

activity is equal to 1 μmol of phenylacetate hydrolyzed/ml/min. Reaction mixtures contained 50 mM Tris/HAc (pH 8.0), 1 mM $CaCl_2$, 10 mM substrate (from a 800 mM stock, dissolved in ethanol) and 20–50 μl enzyme in a total volume of 1 ml. The reaction was carried out for 10 min under 37 °C and stopped by adding 100 μl 0.1 M EDTA.

Lactonase activity towards dihydrocoumarin was measured spectrophotometrically at 270 nm. The E_{270} for the reaction was 1295 M^{-1} cm^{-1}. One unit of lactonase activity is equal to 1 μmol of dihydrocoumarin hydrolyzed/ml/min. In a typical experiment, a cuvette contained 1 mM substrate (from a 100 mM stock, dissolved in methanol) in 50 mM Tris/HCl (pH 8.0), 2 mM $CaCl_2$, and 20–50 μl enzyme in a total volume of 1 ml. The reaction was carried out for 10 min under 37 °C and stopped by adding 100 μl 0.1 M EDTA.

2.8. SDS–polyacrylamide gel (PAGE) and Western blotting analysis

2×10^6 Sf9 cells were resuspended in 200 μl ice cold 50 mM Tris–HCl (pH 8.0) and sonicated on ice shortly. After centrifugation at 12,000 rpm for 10 min, 25 μl supernatant was taken for Western blotting analysis. The culture medium was dialyzed against ddH_2O and lyophilized. The resulted powder was dissolved in 200 μl 50 mM Tris–HCl (pH 8.0) and 25 μl solution was taken for Western blotting analysis. All protein samples were diluted into SDS–PAGE sample buffer (50 mM Tris/HCl (pH 6.8), 2% SDS, 10% glycerol, 15 mM 2-mercaptoethanol and 0.25% bromphenol blue) and electrophoresis in 12% denaturing polyacrylamide gel. Proteins were electrophoretically transferred to nitrocellulose membrane and processed for immunodetection using specific 6-Histidine mouse monoclonal antibody. The immunoblotting was carried out using the protocol recommended by Bio-Rad with HRP-conjugated anti-mouse IgG as the detecting reagent.

2.9. Oxidation of LDL by Cu^{2+}

LDL (0.1 mg protein/ml) oxidation was induced with 10 μM $CuSO_4$ in phosphate-buffered saline (pH 7.4) in the presence of PON3 for 3 h at 37 °C. Blank consisted of LDL without copper and control consisted of LDL incubated with copper alone for the same period. Lipid peroxide accumulation was estimated by monitoring conjugated dienes formation measured as an increase of absorbance at 234 nm of the sample at different time points.

3. Results

3.1. Cloning of hPON3 and construction of recombinant baculovirus AcNPV-rhPON3

The hPON3 full-length coding region was amplified from human fetal liver cDNA by PCR and inserted into the baculovirus transfer vector pVL1393 as described in Section 2. The strategies were shown in Fig. 1. After PCR, the amplified fragment was cloned into pMD18-T vector and sequenced to confirm the coding region. Then, the cDNA fragment was cloned into pET-28a plasmid to yield a new DNA fragment, in which 6xHis tag encoding sequence was fused with hPON3 coding region at its 5' end.

The recombinant plasmid pVL1393-rhPON3 was co-transfected with wild-type AcNPV DNA into Sf9 cells. After three rounds of plaque purification, one of the positive recombinant viruses (AcNPV-rhPON3) with the highest biological activity was chosen for further study.

3.2. Expression of rhPON3 in Sf9 cells

The Sf9 cells were infected with the purified recombinant virus AcNPV-rhPON3 for the expression of hPON3 as described in Section 2. Arylesterase activity and lactonase activity were detected, respectively, 24 h after the infection. A time-course study showed that the highest activity of rhPON3 in the supernatant was observed at 72 bpi. The

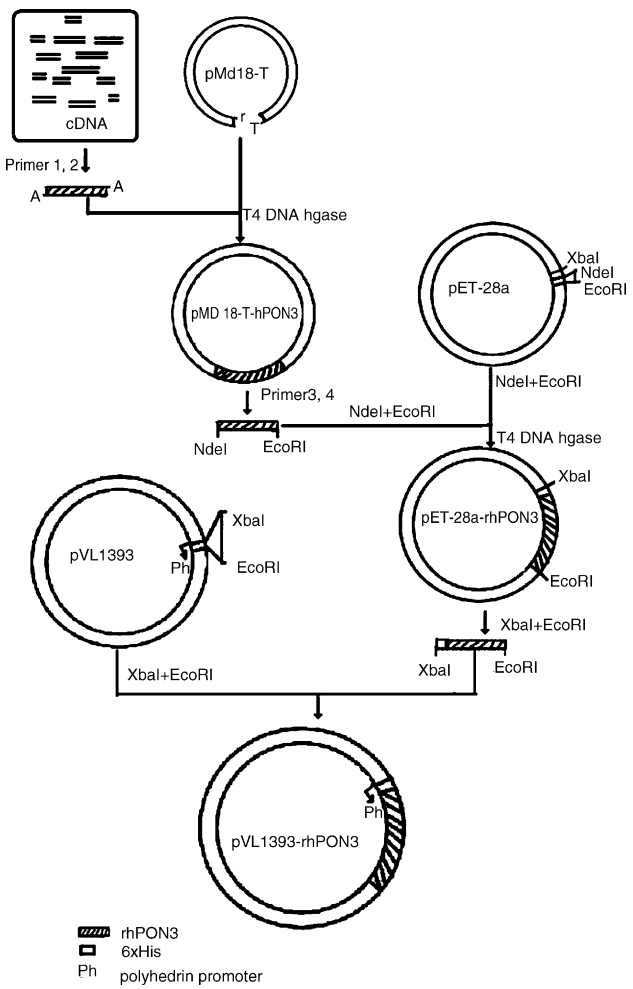

Fig. 1. Cloning of hPON3 gene and construction of recombinant transfer vector pVL1393-rhPON3.

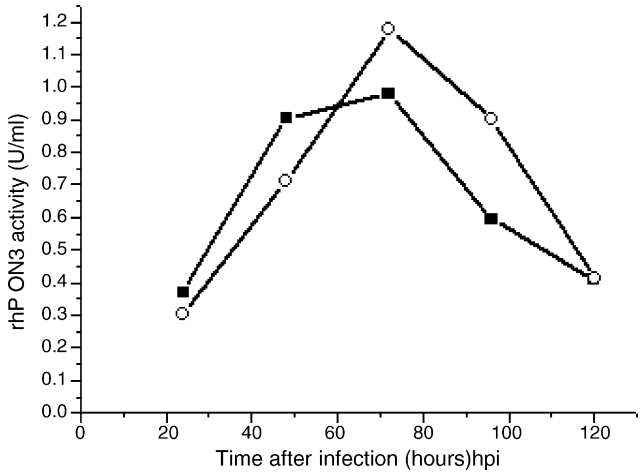

Fig. 2. Time-course of rhPON3 expression in Sf9 cells in cell culture. After AcNPV-rhPON3 recombinant virus infection, the arylesterase activity of rhPON3 towards phenyl acetate (◆) and the lactonase activity of rhPON3 towards dihydrocoumarin (○) in the supernatant of cell culture media were assayed at 24-h intervals.

highest arylesterase activity of rhPON3 towards phenyl acetate was 0.98 U/ml and the highest lactonase activity of rhPON3 towards dihydrocoumarin was 1.18 U/ml (Fig. 2). The Sf9 cells infected with the recombinant virus AcNPV-rhPON3 were sonicated on ice and the supernatant was obtained by centrifuging at 12,000 rpm. The supernatant and the culture medium were harvested and applied to purify rhPON3. After purification by DEAE-Sepharose fast flow anion exchange chromatography followed by Ni^{2+}-charged affinity chromatography, about 28 mg of purified rhPON3 could be obtained from 1 L Sf9 cells culture.

Western blotting and SDS–PAGE analysis of the purified rhPON3 indicated that besides the expected 43–45 kDa rhPON3 bands, there was another band with molecular weight of approximately 90 kDa (Fig. 3B and C). The high molecular weight band mainly existed in the culture medium (Fig. 3A). The product of high molecular weight also appeared in rhPON3 products expressed in *E. coli* (data not shown). The eluted high molecular weight protein had activities similar to those of 45 kDa protein (data not shown) and could react with 6-Histidine monoclonal antibody in Western blotting. On the basis of molecular mass and bioactivity assay, it was likely that the high molecular weight band might be a dimer of rhPON3 which was not held by intermolecular disulfide bond since it could not be dissolved by DTT or 2-mercaptoethanol (data not shown).

3.3. Arylesterase and lactonase activities of rhPON3

We found that the existence of nonionic detergent and calcium was crucial for rhPON3 to maintain its activity during the process of purification. When purified rhPON3 was kept under 4 °C without detergent and calcium, its activity decreased quickly (data not shown). The arylesterase activity and lactonase activity of rhPON3 could be inhibited by 5–10 μM EDTA or 1 mM PMSF (data not shown). In this study, the kinetic parameters of V_{max} and K_m for phenylacetate hydrolysis by rhPON3 were

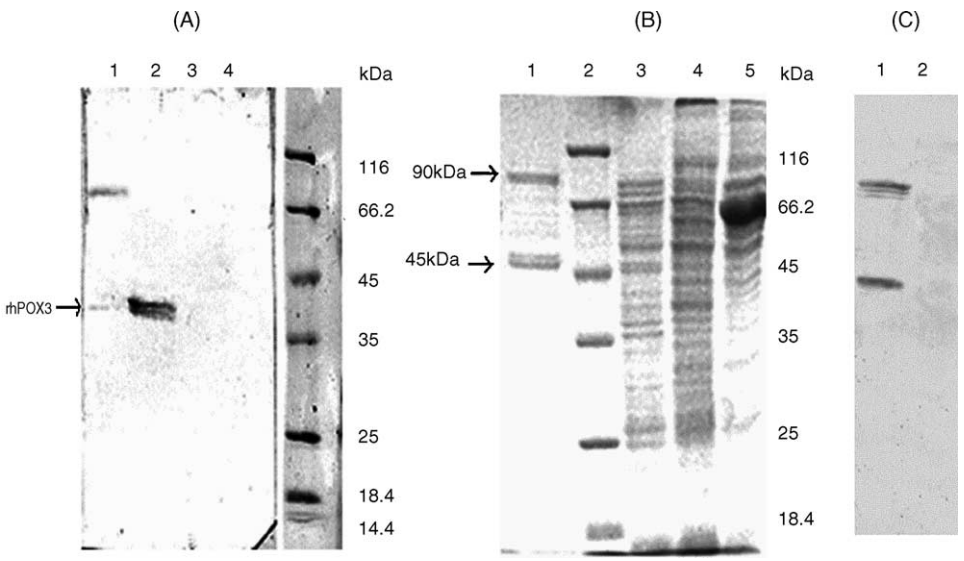

Fig. 3. Analysis of rhPON3 expressed by Sf9 cells. (A) Western blotting analysis of rhPON3 expressed by Sf9 cells. Sf9 cells and the culture medium were collected after 72 hpi. Sf9 cells lysate and culture medium were prepared as described in Section 2. Equal aliquots of cell lysate and culture medium were electrophoresed on denaturing 12.5% SDS–PAGE and protein was transferred to nitrocellulose for Western blotting analysis. Lane 1, culture medium from recombinant virus infected cells. Lane 2, lysate of recombinant virus infected Sf9 cells. Lane 3, culture medium from wild-type virus infected cells. Lane 4, lysate of wild-type virus infected Sf9 cells. (B) SDS–PAGE analysis. Samples were electrophoresed on denaturing 12.5% SDS–PAGE and the gel was Coomassie Blue stained after electrophoresis. Lane 1, partially purified rhPON3. Lane 2, protein molecular weight markers. Lane 3, total cell lysate of Sf9 cells. Lane 4, total cell lysate of recombinant virus infected Sf9 cells. Lane 5, culture medium from recombinant virus infected Sf9 cells. (C) Western blotting analysis of rhPON3 purified by affinity chromatography. Lane 1, partially purified rhPON3. Lane 2, flow-through.

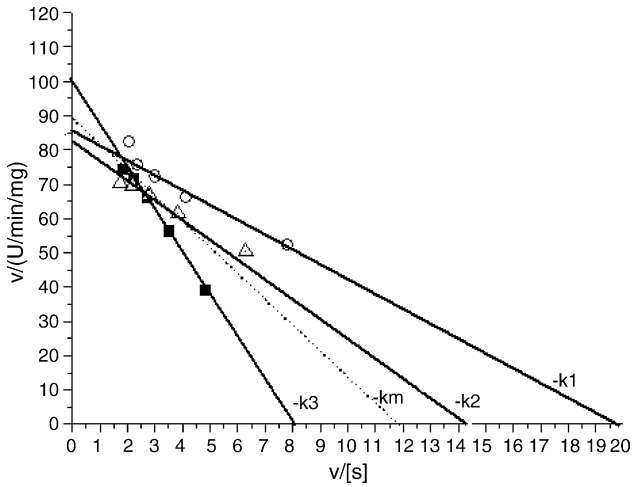

Fig. 4. Kinetic analysis of phenylacetate hydrolysis by rhPON3. The reactions were carried out under pH 8.0 and 37 °C. Phenylacetate hydrolysis was measured by monitoring the changes of A_{270} in the first 2 min of reaction. K_1, K_2 and K_3 represent the K_m values obtained in three independent experiments. In this study, the calculated V_{max} and K_m for phenylacetate hydrolysis were 89 ± 10.54 U/mg and 7.46 ± 4.40 mM ($n = 3$), respectively indicated by the dashed.

89 ± 10.54 U/mg and 7.46 ± 4.40 mM, respectively, calculated from the three independent experiments (Fig. 4). The kinetic parameters of V_{max} and K_m for dihydrocoumarin hydrolysis by rhPON3 were 698 ± 248 U/mg and 0.84 ± 0.24 mM, respectively, calculated from the three independent experiments (Fig. 5). The kinetic parameters of rhPON3 and rhPON1Q (prepared from Sf9 cells by ourselves, data not shown) were summarized in Table 1. The lactonase activity of rhPON3 from Sf9 cells was quite stable at pH 4.5–9.0 and the maximum activity was obtained at pH 8.5 (Fig. 6).

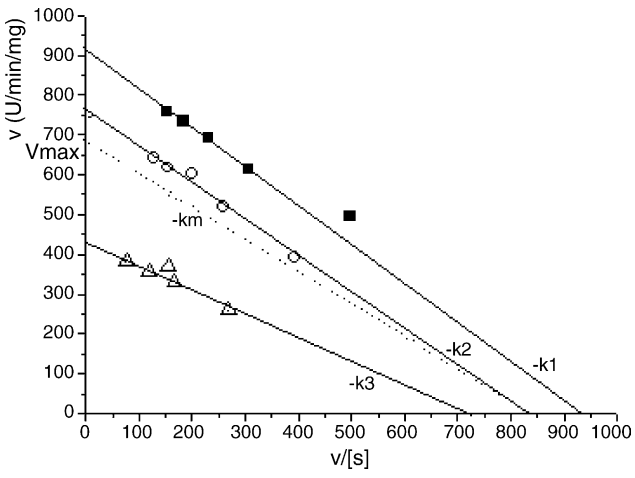

Fig. 5. Kinetic analysis of dihydrocoumarin hydrolysis by rhPON3. The reactions were carried out under pH 8.0 and 37 °C. Dihydrocoumarin hydrolysis was measured by monitoring the changes of A_{270} in the first 2 min of the reaction. K_1, K_2 and K_3 represent the K_m values obtained in three independent experiments. In this study, the calculated V_{max} and K_m for dihydrocoumarin hydrolysis were 698 ± 248 U/mg and 0.84 ± 0.24 mM ($n = 3$), respectively indicated by the dashed.

Table 1
Summary of kinetic parameters for recombinant human PON1Q and human PON3

	Arylesterase activity[a]		Lactonase activity[a]	
	K_m[b]	V_{max}[c]	K_m[b]	V_{max}[c]
rhPON1Q[d]	3.8 ± 1.8	1160 ± 38.78	3.5 ± 1.0	515 ± 77.56
rhPON3	7.46 ± 4.4	89 ± 10.54	0.84 ± 0.24	698 ± 248

[a] All values represent means ± S.D., $n = 3$. Kinetic parameters were determined by linear regression analysis of Eadic–Hoftstee plots. Arylesterase activity was measured with phenylacetate as substrate and lactonase activity was measured with dehydrocoumarin as described in Section 2.

[b] K_m, millimolar concentration.

[c] V_{max}, units per milligram of protein (1 unit = 1 μmol of substrate hydrolyzed per minute per milligram).

[d] rhPON1Q was produced in Sf9 cells and purified by ourselves (data not shown).

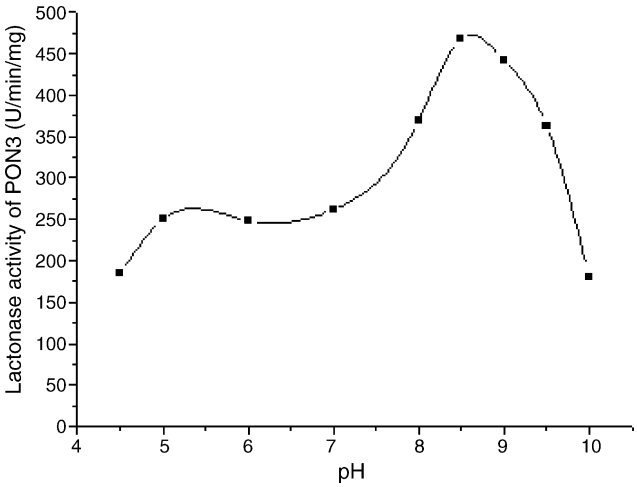

Fig. 6. Effect of pH on lactonase activity of rhPON3. The hydrolysis of dihydrocoumarin by purified rhPON3 derived from Sf9 cells was determined over a pH range of 4.5–10.0. Data are shown from one experiment representative of the three experiments performed.

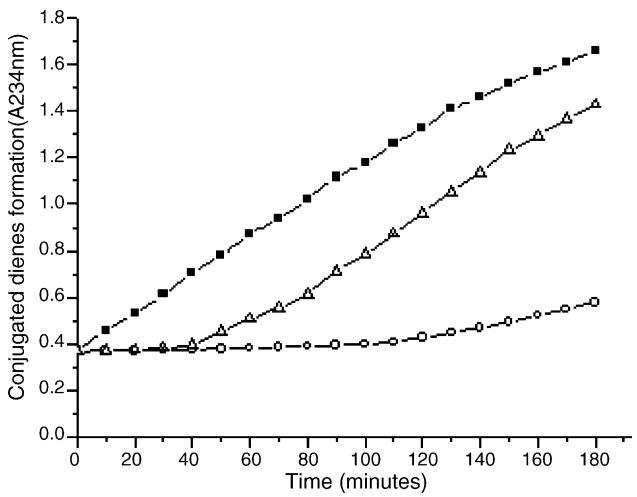

Fig. 7. Effect of rhPON3 on in vitro copper-induced LDL oxidation, LDL (0.1 mg/ml) was incubated with $CuSO_4$ (10 μmol/L) at 37 °C for 3 h in the absence (◆) or presence of 2.5 μg (△) and 25 μg purified rhPON3 (○). Lipid peroxidation was determined by the accumulation of conjugated dienes measured as an increase in the absorbance of the samples at 234 nm after addition of Cu^{2+}.

3.4. Effect of protection against oxidation in vitro by rhPON3

LDL oxidation was investigated by incubating lipoprotein with copper ions (10 μM $CuSO_4$) in the absence or presence of rhPON3 at different doses for 3 h at 37 °C. The accumulation of lipid peroxide was estimated by measuring the accumulation of conjugated dienes which leads to an increase in the absorbance of the sample at 234 nm after addition of Cu^{2+}. The results of this experiment showed that rhPON3 could delay and inhibit LDL oxidation in a dose-dependent manner. When 2.5 μg or 25 μg purified rhPON3 was added to the incubation mixture, the lipid peroxide accumulation was reduced about 25% or 80%, respectively (Fig. 7).

4. Discussion

Serum hPON3 enzyme (EC3.1.8.1) is an enzyme with antioxygenic property thereby inhibiting the oxidation of LDL. Human PON3 is an HDL-related protein and shows a high similarity in structure and functions with those of human PON1. Mature hPON3 and hPON1 contain the N-terminal hydrophobic peptide and share three conserved cysteine residues: Cys-41, Cys-283 and Cys-351. Residues Cys-41 and Cys-351 form an intramolecular disulfide bond and residue Cys-283 is free in active PONs. The free sulfhydryl group is not required for PON1's arylesterase activity but is very important for its antioxidant activity [15]. Previous reports show that PON3 and PON1 possess similar antioxidant properties and can hydrolyze a variety of lactones and cyclic carbonate esters, which may be important in the prevention of atherosclerosis [2,4,5,7].

PON3 may act by a mechanism similar to that of PON1 in the prevention of atherosclerosis, but rabbit PON3 showed much more potential than rabbit PON1 in protecting LDL against oxidation in vitro [4]. This anti-oxidative ability of PON3 has generated considerable interest in studying the structure and function of hPON3. In order to get enough rhPON3 for structure-functional study, we initially tried to express rhPON3 in *E. coli*. However, most of the expressed product existed as inactive inclusion bodies (data not shown). Baculovirus-infected cells perform many post-translational modifications as other higher eukaryotes do, and the recombinant proteins are soluble with antigenicities and immunogenicities, and their functional properties are similar to those of their native counterparts. In this study, a baculovirus-mediated rhPON3 expression system was able to produce relative large quantities of rhPON3 (28 mg purified rhPON3/L cell culture) in two weeks time.

In our present study, two bands with molecular weight about 90 and 45 kDa, respectively, were detected in purified rhPON3. The product of 90 kDa, which existed mainly in the culture medium, could also bind Ni^{2+} charged resin and were eluted with 20 mM imidazole (data not shown). The higher molecular weight protein had similar activities to those of monomeric rhPON3 and could also react with 6-His Ab-1 mouse monoclonal antibody in Western blotting (Fig. 3C). On the basis of molecular mass and bioactivity assay, it was likely that the high molecular weight band might be a dimer of rhPON3. It might not be held by intermolecular disulfide bond, since it could not be dissolved by DTT. It was reported that PON1 existed as oligomers or dimers in the presence of detergent [16]. The higher molecular weight protein was also detected when hPON1 was expressed in insect cells [17]. It will be of interest for further research to reveal the mechanism by which the dimer has been formed.

We found that the rhPON3 derived from insect cells hydrolyzed dihydrocoumarin at similar rate as rabbit serum PON3 did [4]. The activity of rhPON3 in hydrolyzing phenylacetate which was consistent with that of rabbit PON3 was very limited. The activities of rhPON3 were quite stable with the existence of nonionic detergent NP-40 and calcium and decreased rapidly without NP-40 or calcium. Purified rhPON3 could maintain its activities for at least one month under 4 °C (data not shown). Enzyme assays also showed that the lactonase activity of rhPON3 was kept well at pH 6.0–9.0 and the maximum activity was obtained at pH 8.5.

Oxidized LDL has a central proatherogenic role [18]. The three PON family members, *PON1, PON2* and *PON3*, appear to be capable of preventing the oxidation of lipids in LDL and HDL. They can inactivate oxidized lipids in LDL by hydrolyzing biologically active oxidized phospholipids and destroying lipid hydroperoxides in oxidized LDL. It was reported that rabbit PON3 had much more potential for protecting LDL against oxidation than rabbit PON1 did [4], by which the precise mechanism is not known. Our results showed that the insect-cells-derived-rhPON3 could also effectively delay and reduce the oxidation of LDL induced by copper in does-dependant manner. Very recently, Draganov et al. [14] reported that the purified recombinant PONs, which were expressed in insect cells, failed to protect human LDL against copper-induced oxidation. In a previous report [19], they also demonstrated that the purified human serum PON1 did not protect LDL against oxidation in the in vitro assays and that the antioxidant activity of serum PON1 was actually attributable to a low molecular mass contaminant (3 kDa) as well as to the detergent present in the preparations. In our experiments, the final rhPON3 fractions after affinity chromatography were dialyzed against 20 mM Tris–HCl buffer containing a detergent before they were applied to the copper-induced LDL oxidation assays. The detergent also failed to exert such antioxidant activity in control experiments. Thus, the possibility that low molecular mass contaminants or detergent contributed to the antioxidant activity should be excluded. Different protein environments in LDL oxidation assays between our study and above mentioned report

could be the possible reason resulting in the contradicted conclusions. As suggested by Draganov et al., copper-induced in vitro LDL oxidation is an inappropriate method to study the antioxidant properties of PON3.

The antioxidative property of PON3 that can protect LDL from lipid peroxidation, prevents the formation of mildly oxidized LDL and inactivates preformed mildly oxidized LDL, has many anticipated secondary consequences. For example, it can reduce macrophage chemotaxis attribute to oxidized phospholipids and foam cells formation, thereby attenuating atherosclerosis developing. This suggests that rhPON3 may have the potential to be a new therapeutic agent to prevent atherosclerotic disease in clinical trial.

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