Pressure effects on catalytic properties and structural stability of human paraoxonase

To cite this article: C Cléry-Barraud et al 2008 J. Phys.: Conf. Ser. 121 112001

View the article online for updates and enhancements.
Pressure effects on catalytic properties and structural stability of human paraoxonase

C Cléry-Barraud, J Leva, N El Bakdouri, F Renault, P Masson and D Rochu

Département de Toxicologie, Centre de Recherches du Service de Santé des Armées, BP 87, 38702 La Tronche cedex, France

E-mail : cclerybarraud@crssa.net

Abstract. Human paraoxonase (HuPON1) is a candidate as catalytic bioscavenger for pre-treatment and therapy of poisoning by organophosphate compounds. HuPON1 is a hydrophobic protein associated with a partner, the human phosphate binding protein (HPBP) in plasma high density lipoproteins. The relationship between the composition and the size of multimeric states of HuPON1 is not well understood. Moreover the effect of HPBP’s presence on enzyme catalytic mechanisms and stability is unclear. We investigated the effect of hydrostatic pressure and temperature on structural stability and activity of different PON1 preparations (hybrid recombinant PON1, natural HuPON1 free of its partner or in the presence of 50% w/w HPBP). We showed that PON1 exists under several multimeric forms and that the binding of HPBP amends the size of the hetero-oligomeric states and exerts a stabilizing effect on the activity of PON1.

1. Introduction

Human paraoxonase (HuPON1; EC 3.1.8.1) is a 354-amino acid calcium-dependent protein associated with high-density lipoprotein (HDL) in plasma. HuPON1 is also tightly associated with Human Phosphate Binding Protein (HPBP). Little progress have been made in identifying natural substrate(s) of HuPON1 and defining its physiological function(s). HuPON1 hydrolyses a broad range of esters such as arylesters, lactones and organophosphates (OP). This promiscuous enzyme with antiatherogenic and detoxification properties is considered as a promising pharmaceutical catalytic scavenger [1], and is the subject of intensive research. The three dimensional structure of HuPON1 is still unknown, but a monomeric crystal of a bacterially-expressed mouse-rat-rabbit-human PON1 chimera obtained through gene shuffling experiments recently provided the structure of a recombinant PON1 (rePON1) [2]. Besides, the X-ray structure of HPBP was also solved [3]. Recent experiments suggested that HuPON1 and HPBP exist in different oligomeric states, but the relevance of this association on mechanisms and stability is unclear. High pressure (HP) is a suitable and efficient tool for dissociating complex biological systems and perturbing macromolecule conformation [4]. In the present work, we have undertaken the study of HuPON1-HPBP molecular interactions using high pressure biochemical methods to determine the content of multimeric complexes of HuPON1, and to clarify the contribution of HPBP in activity/stability of HuPON1.
2. Materials and methods

2.1. Enzymes and chemicals
HuPON1 from pooled plasma was purified to obtain free HuPON1 or HuPON1 in the presence of 50% HPBP (HuPON1/HPBP) [5]. The enzyme was stored at –20 °C in 25 mM Tris/HCl, 1 mM CaCl₂, 0.1 % Triton-X100, pH 8. The recombinant PON1 (rePON1) dubbed G3C9-8His was kept at 4°C in Tris 50 mM, NaCl 50 mM, CaCl₂ 1 mM, tergitol 0.1%. The protein differs by ≈ 16% of amino acids from the human enzyme.

2.2. Structural stability studies
The equipment of electrophoresis under HP was described elsewhere [6]. The empiric relationship of Ferguson describes the protein relative mobility (m) vs acrylamide concentration (T%) in non-denaturing polyacrylamide gel electrophoresis (PAGE): log m = log m₀ – Kᵣ x T% where Kᵣ, the retardation coefficient, depends of the protein size and shape. For a spherical protein, Kᵣ is related to the equivalent molecular radius [7].

2.3. Functional stability studies
Enzymatic parameters (kₐₜ and Kₘ) with phenylacetate (arylesterase activity) andparaoxon (OP-hydrolase activity) were determined by Michaelis-Menten analysis. Kinetic data with coumaranone (lactonase activity) were fitted to the Michaelis-Menten equation for [S]<<Kₘ. We determined the apparent activation volume (ΔVₚ/kₐₜ/Kₘ) with ΔVₚ/kₐₜ/Kₘ = -RT x (δln (kₐₜ/Kₘ)/δP)ᵣ, where R is the gas constant (82 ml. atm. K⁻¹.mol⁻¹).

3. Results and discussion

3.1. Oligomeric states in PON1 preparations at atmospheric pressure
The estimation of the molecular weight (MW) of unknown samples referring to a calibration curve generated with standard proteins was obtained by plotting Ln (MW) versus Kᵣ/½. HuPON1 exists in multiple oligomeric forms and HPBP associates with HuPON1 to form large hetero-oligomers retaining arylesterase activity. At atmospheric pressure, dimeric, trimeric and tetrameric HuPON1-containing forms were present in free HuPON1 preparation, whereas dimeric, tetrameric and hexameric forms coexisted in HuPON1-HPBP sample.

3.2. Pressure and temperature effects on structural stability of PON1
As shown in figure 1, PON1 structure was insensitive to pressure (up to 180-200 MPa) and temperature (up to 65°C) in all preparations (except free HuPON1 above 40°C). Stabilizing role of HPBP towards heat, already shown by capillary electrophoresis at varying temperature and differential scanning calorimetry [8], was confirmed by studying the pressure/temperature effects. Pressure (from 50 MPa) (2C) and temperature (10-65°C) (2F) promoted dissociation of homo-oligomers into the dimeric PON1 form, but not into monomer. There was no pressure-induced dissociation of HuPON1/HPBP complex up to 200 MPa under these experimental conditions. Kᵣ values showed large fluctuations in the presence of HPBP. This suggests that HPBP is either more sensitive to pressure and/or that interface interactions are sensitive to pressure.
3.3. Determination of kinetic parameters at atmospheric pressure

The catalytic efficiency parameters ($k_{cat}/K_m$) obtained at atmospheric pressure are presented in table 1.

| Activity           | HuPON1   | HuPON1/HPBP | rePON1 (G3C9) |
|--------------------|----------|-------------|---------------|
| Arylesterase activity | ($3 \pm 0.4) \times 10^6$ | ($6 \pm 0.3) \times 10^6$ | ($8 \pm 0.5) \times 10^6$ |
| Lactonase activity  | ND*      | ($0.8 \pm 0.1) \times 10^6$ | ($7.9 \pm 0.4) \times 10^6$ |
| OP-hydrolase activity | ND*     | ($2 \pm 0.2) \times 10^7$ | ($9 \pm 0.7) \times 10^7$ |

ND*: not determined. Activity buffer was 50 mM tris, 1 mM NaCl, pH 8 at 25°C and atmospheric pressure. (mean values ± S. E., results in triplicate).

The catalytic efficiency for hydrolysis of arylester was of the same order for the three PON1 preparations ($\sim 10^6$ min$^{-1}$.M$^{-1}$). The fact that the catalytic efficiency for both hydrolysis of lactone and OP was higher for rePON1 than for HuPON1/HPBP suggests that the numerous mutations at the surface and near the active site of rePON1 favor the enzyme activity/stability. The HuPON1 kinetic parameters for these activities could not be determined due to a too low enzyme response and a loss in stability. In this respect, rePON1 proved to be a satisfactory model to mimic the “physiological” HuPON1, i.e., in the presence of HPBP.

3.4. Pressure effect on functional stability of PON1

Figure 2 shows the pressure dependence of Ln ($k_{cat}/K_m$) of arylesterase, lactonase and OP-hydrolase activities for the three PON1 preparations. For both arylesterase and lactonase activities, plots were biphasic, quite similar and allowed the determination of two activation volumes ($\Delta V^\beta$), depending of the pressure range. The three PON1 preparations were slightly affected by pressure, up to the pressure break ($P_t$), corresponding to an associated $\Delta V^\beta$ value close to zero ml.mol$^{-1}$. Beyond this pressure break, the $\Delta V^\beta$ value was positive for both activities, suggesting a sudden pressure-induced inhibition of the reaction.

The pressure dependence of Ln ($k_{cat}/K_m$) for OP-hydrolase activity was quite different for both rePON1 and HuPON1/HPBP. The $\Delta V^\beta$ value determined for rePON1 was close to zero up to 250 MPa, suggesting no pressure-induced unfolding of the enzyme. Moreover, the pressure...
dependence of $k_{cat}/K_m$ for HuPON1/HPBP OP-hydrolase activity was linear up to 200 MPa. The observed negative $\Delta V^\circ$ value indicated a reaction widely favored by pressure (5-fold more active at 200 MPa than at atmospheric pressure).

**Figure 2.** Activation volume ($\Delta V^\circ$) associated with arylesterase (A), lactonase (B) and OP-hydrolase (C) activities by HuPON1 (■), HuPON/HPBP (▲) and rePON1 (●).

**Conclusion**

All these results provide evidence that there was no pressure-induced unfolding of PON1. The non-linear pressure dependence of $k_{cat}/K_m$ could be caused by a change in the rate determining step and/or local modification of the enzyme active site. This assumption is strengthened by the fact that the pressure effect on PON1 was similar for phenylacetate and coumaranone hydrolysis, but was different for paraoxon hydrolysis. This suggests that the arylester and the lactone are similarly positioned in the active site, whereas the OP binds on another subsit. This corroborates Tawfik’s statement [9]. Further studies are in progress to determine catalytic mechanisms of PON1.

**Acknowledgments**

This work was supported by DGA/DSP/STTC PEA n°010807 to P.M. and German Bundesministerium der Verteidigung (M/SAB/1/7A004) to D.R.

**References**

[1] Rochu D, Chabrière E and Masson P 2007 Toxicology 233 47
[2] Harel M et al. 2004 Nat. Struct. Mol. Biol. 11 412
[3] Morales R et al. 2006 Structure 14 601
[4] Balny C, Masson P and Heremans H 2002 Frontiers in high pressure biochemistry and biophysic, eds C Balny, P Masson and K Heremans (Amsterdam, Elsevier) 3
[5] Renault F, Chabrière E, Andrieu J-P, Dublet B, Masson P and Rochu D 2006 J. Chromatogr. B 836 15
[6] Masson P 1997 in High Pressure Techniques in Chemistry and Physics : a practical approach. Holzapfel W B & Isaacs N S IRL Press, Oxford pp353.Ferguson K A 1964 Metabolism 13 985
[7] Rochu D, Renault F, Cléry-Barraud C, Chabrière E, Masson P 2007 Biochim. Biophys. Acta 1774 874
[8] Kersonsky O and Tawfik D S 2005 Biochemistry 44 6371