New PON1-based biosensor for the detection of homocysteine-thiolactone in human plasma

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INTRODUCTION

Elevated plasma levels of homocysteine (Hcy), and its conversion into the reactive metabolite Hcy-thiolactone (Hcy-TL) are linked to the progression of Cardiovascular Diseases (CVD). Providing a novel point-of-care test for Hcy-TL can represent a major breakthrough in CVD risk assessment [1]. Since the detoxification of Hcy-TL by human HDL-associated enzyme paraoxonase 1 (PON1) [2] can generate electroactive products, our novel strategy relies on developing a sensing device for Hcy-TL that couples PON1 to an electrochemical transducer.

Herein, we analyzed the catalytic activities of human PON1 (recombinant G3C9 *vs* plasma) – paraoxonase (Fig. 1) and lactonase (Fig. 2) by electrochemical techniques aiming at developing a first generation electrochemical biosensor based on this biorecognition element.

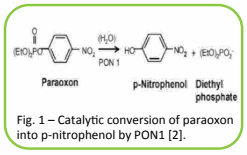

Fig. 1 – Catalytic conversion of paraoxon into p-nitrophenol by PON1 [2].

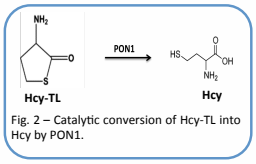

Fig. 2 – Catalytic conversion of Hcy-TL into Hcy by PON1.

RESULTS

PARAOXONASE ACTIVITY

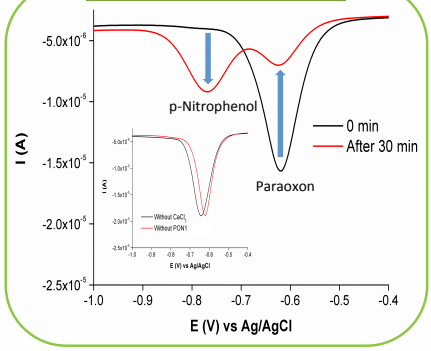

Fig. 3 – Enzimatic conversion of paraoxon into p-nitrophenol, monitored by square wave voltammetry (freq. 25 Hz). Supporting electrolyte: 2 mM $CaCl_2$, 200 mM KCl, 100 mM Tris-HCl buffer (pH 7.6, 37 °C). Inset: controls performed in the absence of $CaCl_2$ (black) and enzyme (red); no conversion of paraoxon into p-nitrophenol is observed after 30 minutes.

LACTONASE ACTIVITY

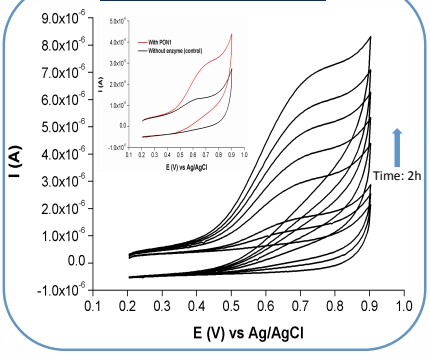

Fig. 4 – Enzymatic conversion of 1 mM Hcy-TL into Hcy by recombinant PON1-G3C9, monitored by cyclic voltammetry (scan rate 50 mV.s^{-1}). Supporting electrolyte: 2 mM $CaCl_2$, 200 mM KCl, 100 mM Tris-HCl buffer (pH 7.6, 37 °C). Inset: electrode's response after 30 min in the presence (red) and absence (black) of enzyme.

KINETICS

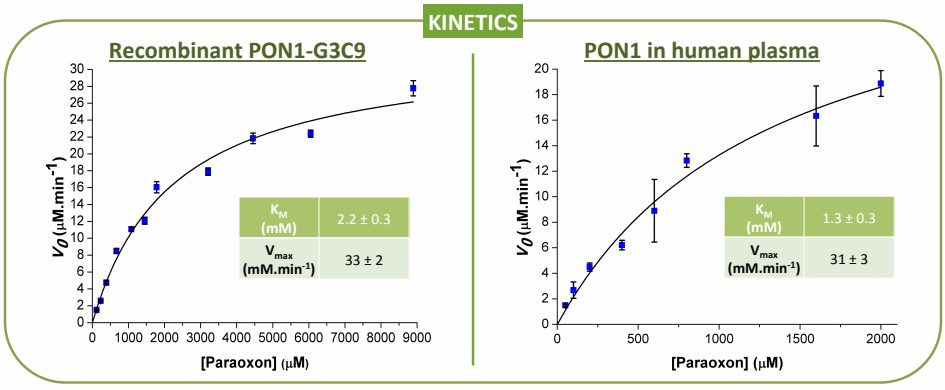

Fig. 5 – Enzyme activities as a function of paraoxon concentration in 2 mM $CaCl_2$, 200 mM KCl, 100 mM Tris-HCl pH 7.6 supporting electrolyte, 37 °C. Solid lines represent the Michaelis-Menten simulations of the enzyme kinetics.

CONCLUSIONS AND FUTURE WORK

- A novel electrochemical methodology was developed for the measurement of human PON1 activity in plasma.
- The lactonase activity from the recombinant PON1-G3C9 was monitored by cyclic voltammetry, and will be further optimized.
- The paraoxonase activity of recombinant PON1-G3C9 is higher than its lactonase activity.
- In the near future, other recombinant PON1 with higher activity towards lactones will be tested for the detection of homocysteine-thiolactone.

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