Development of New Analytical Tools for Monitoring of Cardiovascular Disease Markers - Towards the Detection of Homocysteine-Thiolactone

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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 paraxonase 1 (PON1) [2] results in an electroactive product, our novel strategy relies on developing a sensing device for Hcy-TL that couples PON1 to an electrochemical transducer.

Our main objective is to demonstrate the feasibility of the transducing scheme, by monitoring the catalytic conversion of paraoxon into p-nitrophenol by PON1 in human plasma, using square wave voltammetry (SWV).

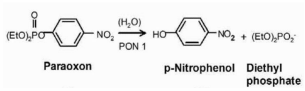

Fig. 1 – Catalytic conversion of paraoxon into p-nitrophenol by PON1 [2].

RESULTS

SWV: PARAOXON & P-NITROPHENOL

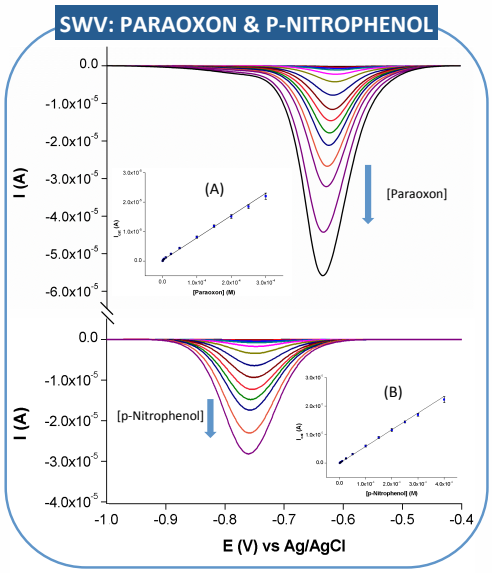

Fig. 2 – SWV characterization (freq. 25 Hz, amp. 20 mV, step 2 mV) of paraoxon and p-nitrophenol (1–500 µM) on glassy carbon electrode, in 2 mM $CaCl_2$, 200 mM KCl, 100 mM Tris-HCl buffer (pH 7.6, 37 °C). Insets: (A) paraoxon calibration curve (y=7.58x10^{-2} A.M^{-1} + 2.79x10^{-7} A, R^2=0.995, n=3); (B) p-nitrophenol calibration curve (y=5.85x10^{-2} A.M^{-1} + 2.74x10^{-8} A, R^2=0.996, n=3).

CV: HCY-TL & HCY

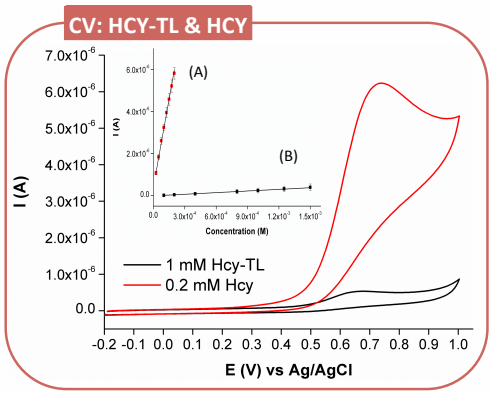

Fig. 5 – Cyclic voltammetry characterization (scan rate 50 mV.s^{-1}, step 5 mV) of homocysteine-thiolactone (black line) and homocysteine (red line) on pyrolitic graphite electrode, in 200 mM KCl, 100 mM Tris-HCl buffer (pH 7.6). Inset: (A) homocysteine calibration curve (y=2.79x10^{-2} A.M^{-1} + 4.18x10^{-7} A, R^2=0.998, n=3); (B) homocysteine-thiolactone calibration curve (y=2.60x10^{-4} A.M^{-1} - 1.40x10^{-8} A, R^2=0.997, n=3).

ENZYMATIC REACTION

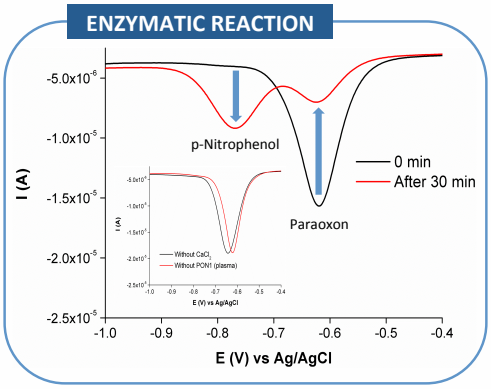

Fig. 3 – Enzimatic conversion of paraoxon into p-nitrophenol by PON1 (human plasma) in 2 mM $CaCl_2$, 200 mM KCl, 100 mM Tris-HCl buffer (pH 7.6, 37 °C). Inset: controls performed in the absence of $CaCl_2$ (black) and plasma (red); no conversion of paraoxon into p-nitrophenol is observed after 30 minutes.

KINETICS

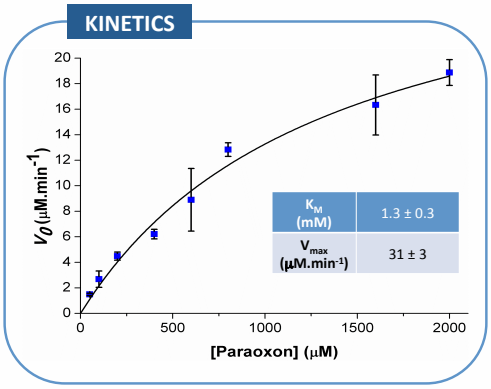

Fig. 4 – Kinetic curve of the enzimatic conversion of paraoxon into p-nitrophenol by PON1 (human plasma) in 2 mM $CaCl_2$, 200 mM KCl, 100 mM Tris-HCl buffer (pH 7.6, 37 °C), n=3. Inset: calculated kinetic parameters.

CONCLUSIONS AND FUTURE WORK

- ❑ Paraoxon conversion into p-nitrophenol in the presence of PON1 is easily monitored by SWV.
- ❑ The present methodology will be applied to the monitorization of the enzymatic conversion of homocysteine-thiolactone into homocysteine, by PON1.

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[2] Richter, R. J., Jarvik, G. P., Furlong, C. E. (2009) Paraoxonase 1 (PON1) status and substrate hydrolysis, *Toxicol Appl Pharmacol*, 235(1): 1–9.