Supporting Information for

Direct Assay of Enzymes in Heme Biosynthesis for the Detection of Porphyrias by Tandem Mass Spectrometry. Part 2. Porphobilinogen Deaminase

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Oxidation Measurements

Experiment A: Uroporphyrinogen + Blood + blank assay + Incubation at 37 °C for 30 minutes.

PBGD assay buffer (50 μL; 100 mM Tris-HCl, pH 8.2) was added to a 1.5 mL polypropylene microfuge tube followed by 10 μl uroporphyrinogen I (225 μM), 60 μl (~ 2 mg hemoglobin) of diluted red blood cell lysates, and 130 μL distilled water. The tube was capped and incubated in a water bath at 37 °C for 30 minutes. The water bath chamber was covered with foil to exclude light. After incubation, the reaction was quenched in an ice bath. Ammonium formate buffer solution (150 μL; 1.0 M, pH 3.17) was added to adjust pH to the appropriate range for extraction, followed by *n*-butanol (400 μL), and heptacarboxylporphyrin I (15 μL, 20 μM, 1 M HCl) as internal standard. The mixture was placed on a vortex mixer for 30 seconds before and then centrifuged at 15,000 g for 3 minutes. The top 300 μL of supernatant was transferred using a pipettor with a polypropylene tip to a new 1.5 mL polyprolyene microfuge tube containing 150

μL ammonium formate (20 mM, pH 3.17). The mixture was placed on a vortex mixer for 30 sec and centrifuged again for 3 min. The top 150 μL butanol layer was transferred to another microfuge tube for ESI-MSMS analysis.

Experiment B: Uroporphyrinogen + Blood + blank assay, without incubation;

Same as A (without incubation), only different sample processing step. 10 μl Uroporphyrinogen I (225 μM), 60 μl (~ 2 mg hemoglobin) of diluted red blood cell lysates were added to the blank assay tube, and sample processing step was carried out immediately, without incubation step.

Experiment C: Uroporphyrinogen + blank assay, without incubation.

10 μl Uroporphyrinogen I (225 μM) was added to the blank assay tube, and the sample processing step was carried out immediately, without incubation step.

Recovery Measurements

The recovery yield was measured by comparison of MS signals after extraction using standard sample additions that were added before and after extraction. One more additional washing step compared to the normal sample processing procedure was used to avoid salt matrix effects when adding standards after extraction. Half amount of standards was added after extraction for half amount of butanol solvent was used at the last step. Direct injection was used in MS analysis. Recovery Yield: 90%.

Experiment A: 7.5 μL uroporphyrinogen I (1 M HCl) and 7.5 μL heptaporphyrin I (1M HCl) were added to 1.5 mL polypropylene microfuge tube with PBGD assay buffer (50 μL; 100 mM

Tris-HCl, pH 8.2) and 200 μL water. Ammonium formic buffer solution (150 μL; 1.0 M, pH 3.17) was added to adjust pH to the appropriate range for extraction, followed by *n*-butanol (400 μL). The mixture was placed on a vortex mixture for 30 seconds before being centrifuged at 15,000 g for 3 minutes. The top 300 μL of supernatant was transferred using a pipettor with a polypropylene tip to a new 1.5 mL polyprolyene microfuge tube containing 150 μL ammonium formate (20 mM, pH 3.17). The mixture was placed on a vortex mixer for 30 sec and centrifuged again for 3 min. The top 200 μL butanol layer was transferred to another microfuge tube, followed by adding 150 μL ammonium formate (20 mM, pH 3.17) and 7.**5** μL HCl (1 M). The mixture was placed on a vortex mixture for 30 seconds before being centrifuged at 15,000 g for 3 minutes. The top 100 μL was used for MS analysis.

Experiment B: 15 μL HCl (1 M) was added to 1.5 mL polypropylene microfuge tube with PBGD assay buffer (50 μL; 100 mM Tris-HCl, pH 8.2) and 200 μL water. Sample processing step was the same as Experiment A. 3.75 μL uroporphyrinogen I (1 M HCl) and 3.75 μL heptaporphyrin I (1M HCl) were added instead of 7.5 μL HCl (1 M) at the last washing step.

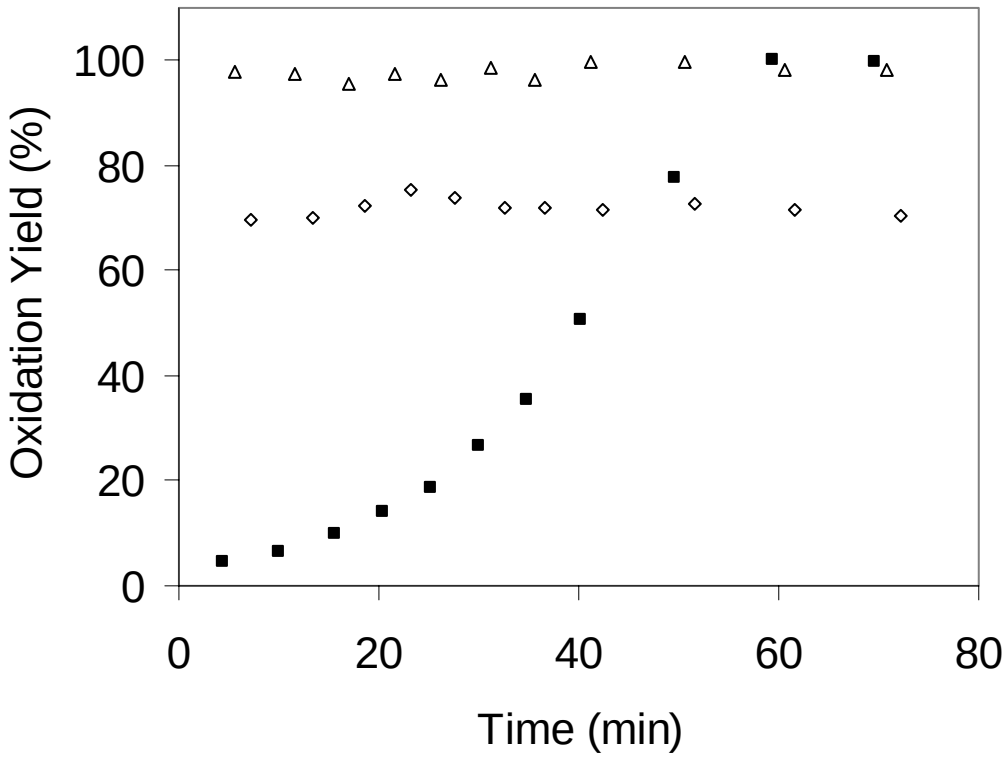

Figure S1. Oxidation and recovery experiments. Diamonds: Experiment A; triangles: Experiment B; solid squares: Experiment C.

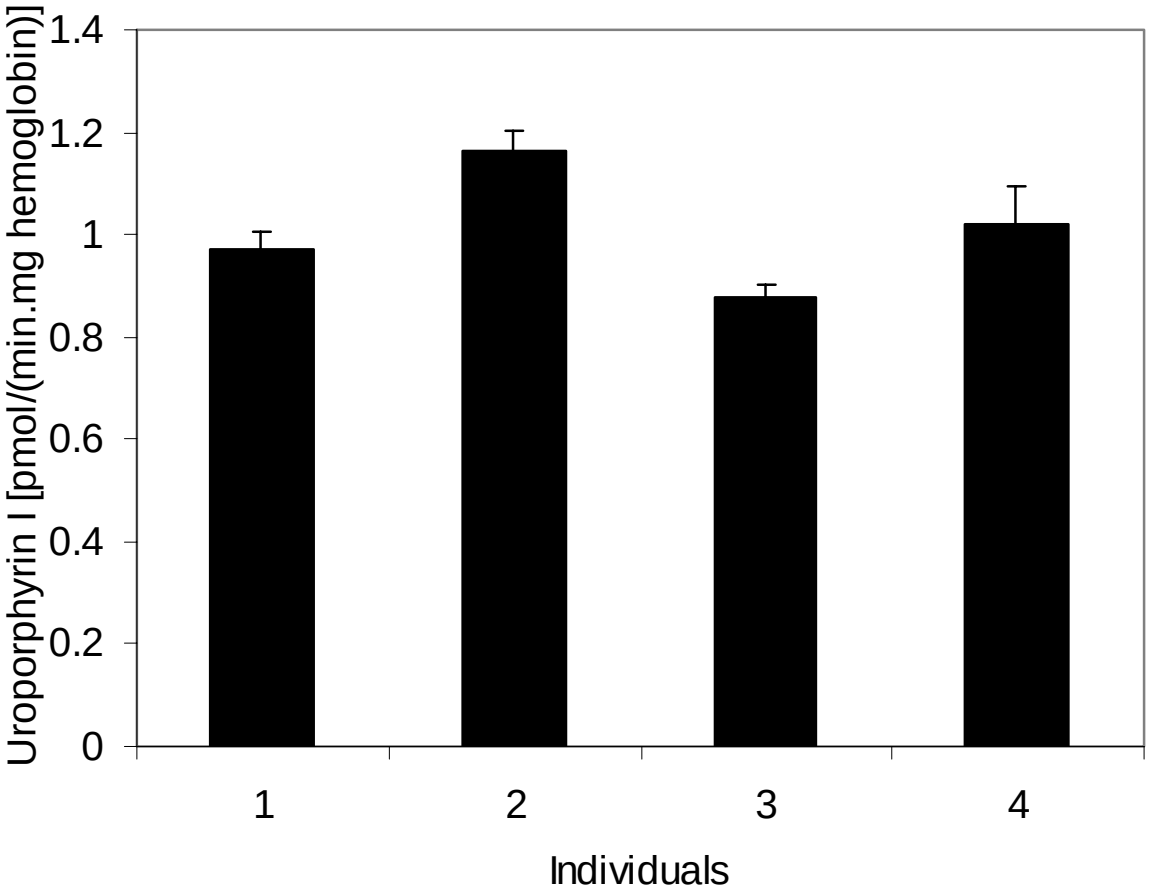

Figure S2. PBGD activity in four healthy individuals. The error bars are standard deviations from triplicate measurements.

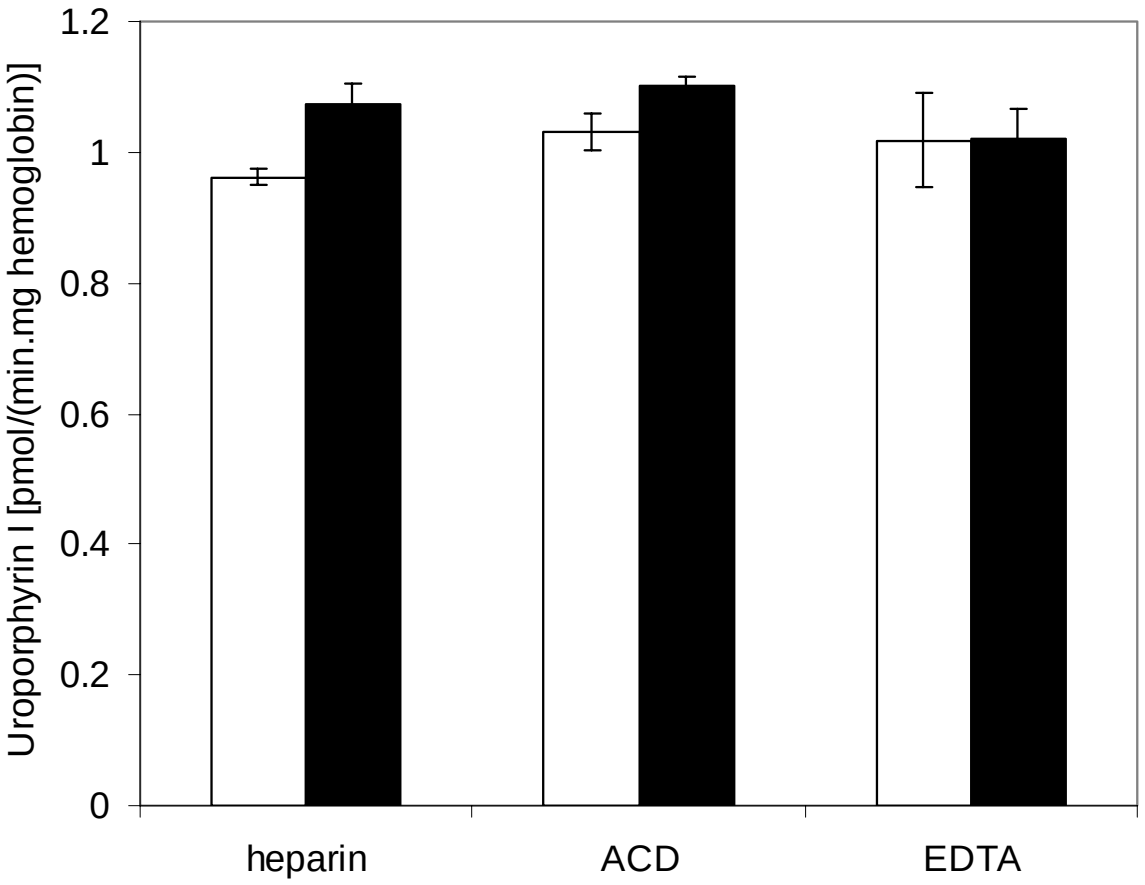

Figure S3. PBGD activity using different anticoagulants. White bar: fresh blood; solid bar: blood stored at 4 °C for 2 days. For abbreviations see the main text.

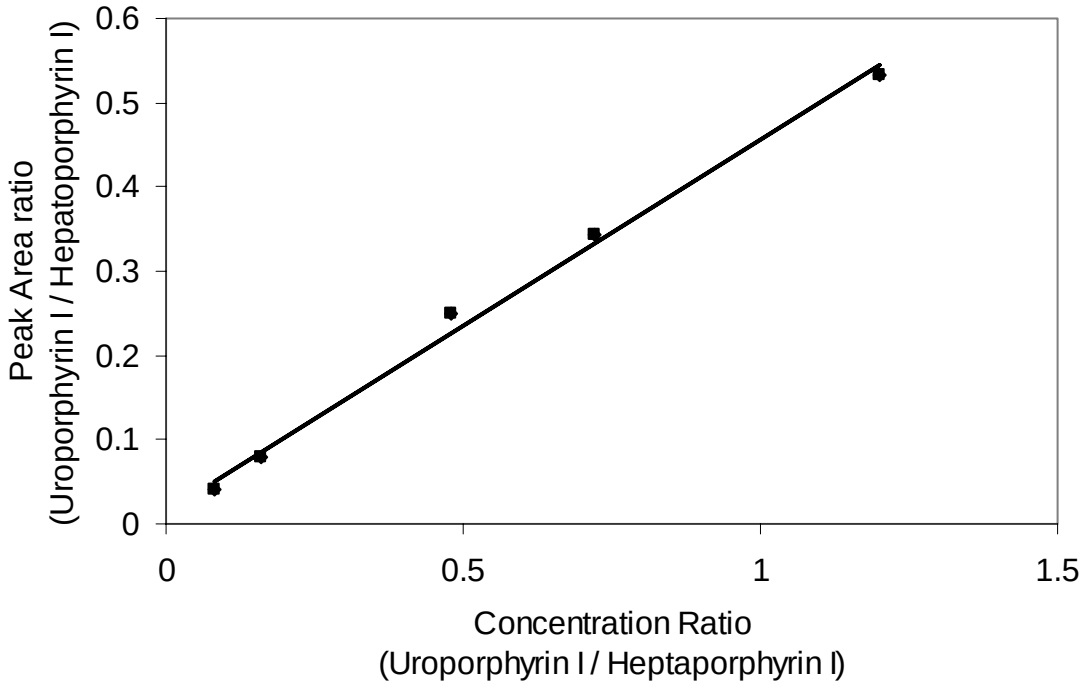

Figure S4. Calibration Curve (heptacarboxylporphyrin I: 300 pmol), and calibration curve was prepared according to the standard sample processing procedure after incubation.