**SUPPORTING INFORMATION**

**Title of Primary Research Paper**

# Further Insights into the Catalytical Properties of Deglycosylated Pyranose Dehydrogenase from *Agaricus meleagris* Recombinantly Expressed in *Pichia pastoris*

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**Abstract of Supporting Information**

This Supporting Information consists of experimental details added in Experimental Section describing: a) deglycosylation procedure; b) spectrophotometric determination of enzyme's activity; c) determination of protein MW with gel electrophoresis; d) tryptic preparation of proteins for MALDI-MS; and into Results and Discussion for: e) determination of optimal applied potential of gPDH/Os(dmbpy)PVI; dgPDH/Os(dmbpy)PVI; fdgPDH/Os(dmbpy)PVI and fdgPDH/Os(dmobpy)PVI-modified graphite electrodes (Figures S-1, S-2); f) characterisation of electrodes modified with gPDH/Os(dmbpy)PVI; dgPDH/Os(dmbpy)PVI and fdgPDH/Os(dmbpy)PVI using cyclic voltammetry; and g) characterisation of electrode's stability (Figure S-4).

## EXPERIMENTAL SECTION

**Deglycosylation with Endo Hf.** Deglycosylation was performed with Endo Hf according to a non-denaturing deglycosylation procedure. Briefly, gPDH (60 mg) was incubated with Endo Hf (80 NEB units, 10 NEB units equals to 1 IUB milliunit) in a 124 mM sodium citrate buffer (pH 5.5) at 37 °C for 6 h. The deglycosylated PDH was concentrated with 30 kDa Amicon ultrafiltration tubes (EMD Millipore Corporation, Billerica, MA, USA), washed with 50 mM sodium phosphate buffer (pH 6.5) containing 137 mM NaCl and purified with S300 Sephacryl size exclusion chromatography (GE Healthcare, Piscataway, New Jersey, USA) according to the manufacturer's recommendations. The resulting pool was concentrated with a 30 kDa Amicon tube and washed with 50 mM sodium phosphate buffer (pH 6.5). The deglycosylated enzyme had a protein content of 20.4 mg $mL^{-1}$ (Bradford assay) and a volumetric activity of 342 U $mL^{-1}$ (ferricenium assay, 20 °C).

**Enzyme Assay.** An aliquot of the enzyme was added to a mixture of 100 mM sodium phosphate buffer containing 137 mM NaCl (pH 7.4), 50 mM D-glucose and 0.4 mM $Fc^{+}PF_6^{-}$ (freshly prepared from 3.3 mg of salt dissolved in 5 mL of 5 mM HCl) in one milliliter cuvette and the decrease in absorbance was monitored for 3 min at 300 nm using a molar absorptivity ($\varepsilon$) 4.3 $mM^{-1}$ $cm^{-1}$. One unit of enzyme activity is equal to the amount of enzyme required for reduction of 2 μmol of $Fc^{+}$ per min at 20 °C.

**Gel Electrophoresis.** The gel slab was fixed in 50% ethanol and 5% acetic acid for 5 min, washed with two portions of 50% ethanol for 30 min and finally with deionised water for 15 min. The gel slab was then sensitised with 0.02% sodium thiosulfate for one min and washed twice with deionised water (one min per wash). Staining was performed with 0.1% (w/v) silver nitrate solution for 15 min under constant shaking followed by washing with deionised water (one minute per wash). In the final step a small volume of developing solution containing 2% sodium carbonate and 0.04% formaldehyde was added to the gel slab and shaken until the solution turned yellow. It was discarded and replaced with a fresh portion of developing solution. The reaction was allowed to take place under constant shaking until the bands reached satisfactory intensity and was then stopped with 5% acetic acid.

**Trypsin Digestion.** An aliquot of 100 μg of protein sample was solubilised with 5 μL of TFE in 100 μL of 100 mM ammonium bicarbonate buffer and reduced with 2.5 μL of 40 mM DTT for 1 h at 60 °C. The free sulfhydryl groups were then blocked with 10 μL of 40 mM IAA for

1 h in the dark at room temperature. A volume of 2.5 µL of 40 mM DTT was added in order to destroy excess IAA and the reaction was allowed to proceed for one more hour in the dark at RT. For trypsin digestion the pH of the mixture was raised to 7.5–8 by addition of 100 mM ammonium bicarbonate buffer and an aliquot of fresh trypsin solution (Promega, Madison, WI, USA, sequence grade) was added in a 1:50 trypsin:protein ratio. Enzymatic digest was carried out at 37 °C overnight and was stopped by addition of neat formic acid (2 µL). The digests were stored at -20 °C if not analysed directly.

**RESULTS AND DISCUSSION**

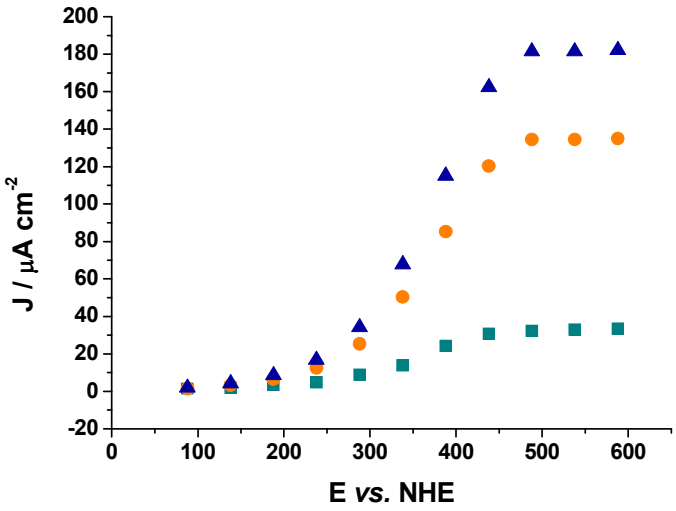


**Figure S-1.** Variation of the catalytic response of (■) gPDH/Os(dmbpy)PVI; (●) dgPDH/Os(dmbpy)PVI and (▲) fdgPDH/Os(dmbpy)PVI-modified graphite electrodes to 5 mM glucose with the applied potentials measured with the FIA system in 50 mM PBS pH 7.4 (137 mM NaCl) at a flow rate of 0.5 mL $min^{-1}$.

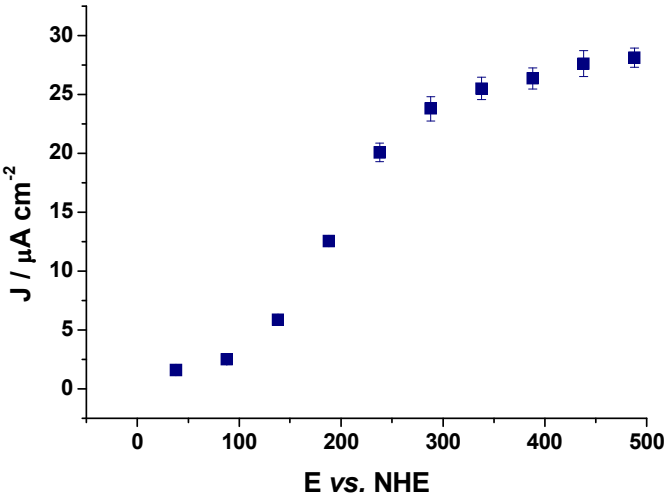


**Figure S-2.** Variation of the catalytic response of fdgPDH/Os(dmobpy)PVI-modified graphite electrodes to 5 mM glucose with the applied potential measured with the FIA system in 50 mM PBS pH 7.4 (137 mM NaCl) at a flow rate of 0.5 mL $min^{-1}$.

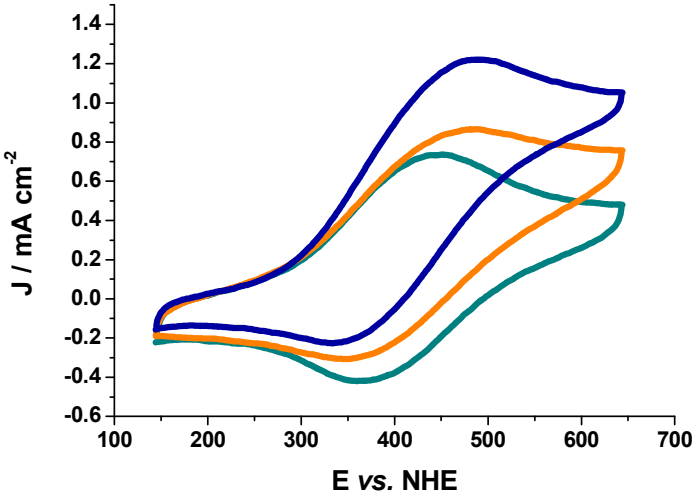


**Figure S-3.** Cyclic voltammograms of (lower curve) gPDH/Os(dmbpy)PVI; (middle curve) dgPDH/Os(dmbpy)PVI and (upper curve) fdgPDH/Os(dmbpy)PVI-modified graphite electrodes in the presence of 10 mM glucose measured in 50 mM PBS pH 7.4 (137 mM NaCl) at a scan rate of 50 mV $sec^{-1}$.

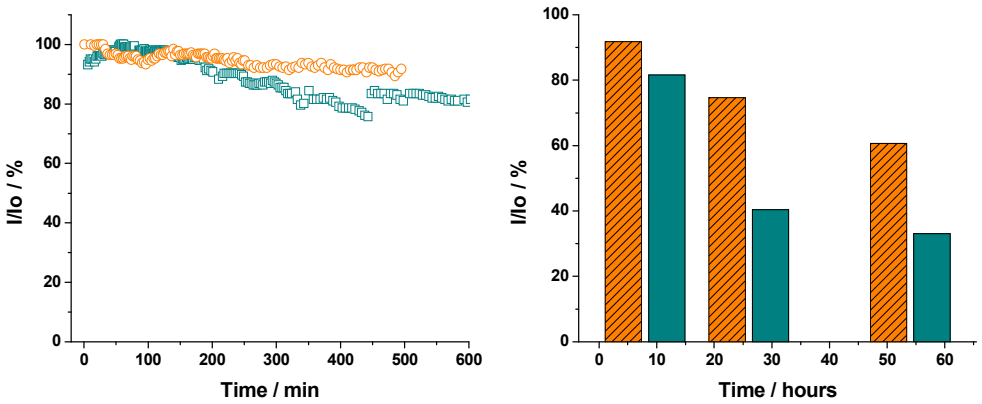


**Figure S-4.** Stability versus time of (○) fdgPDH/Os(dmbpy)PVI and (□) fdgPDH/Os(dmobpy)PVI-modified electrodes measured in the flow-injection mode with 5 mM glucose as substrate. Operational stability is depicted in the left graph; long-term stability is depicted in the right graph. For other conditions see Fig. 4.