Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Dynamics of the Activated State of NAD-dependent Dehydrogenase Investigated by the Weighted Histogram Analysis Semi-Empirical Method

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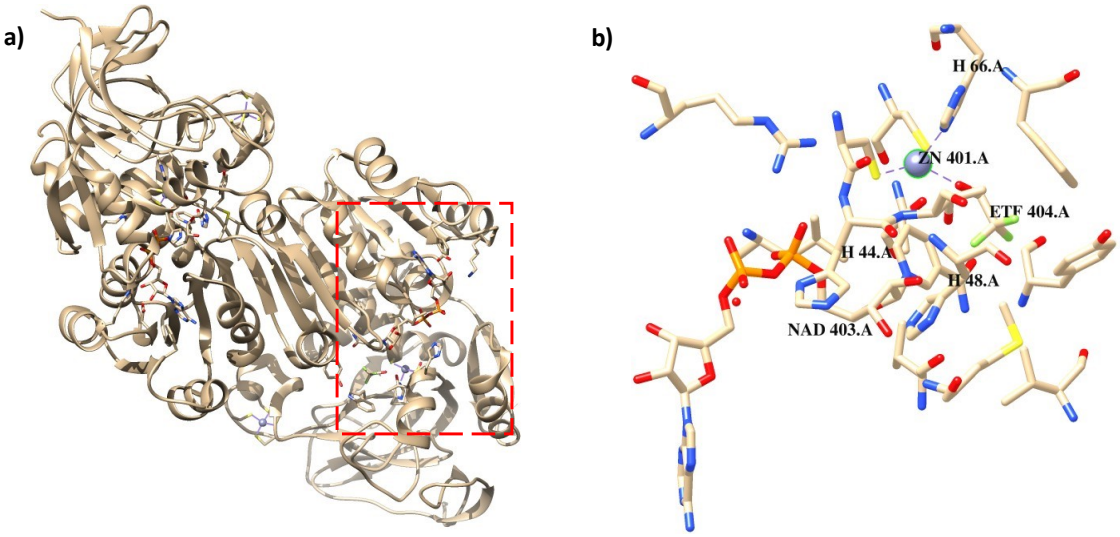

Figure S1. a) Structure of the yeast ADH from PDB 5ENV, the highlighted region corresponds to the active site. b) Structure details for the active site of ADH. Amino acids residues, the coenzyme NAD, and the substrate trifluoroethanol are highlighted in stick mode and colored as CPK code. The only amino acid residues presenting code are histidines, due to their importance in the hydride transfer. Zinc ions are shown as gray spheres, and the dashed lines indicate interactions between zinc ions and amino acids involved in the active site.

Figure S2 presents a plot of square root of scan rate versus current density that results in a linear fit. There is evinced that this electrochemical reaction is diffusion-controlled, thus the process is in agreement with the Randles-Sevcik equation:

$$i_p = 0.443\, nFAC\left(\frac{nFvD}{RT}\right)^{\frac{1}{2}} \quad (1)$$

where, i_p is the peak current, n is the number of electrons transferred in the redox event, F is the Faraday's constant, A is the electrode area, C is the concentration of the electroactive specie, v is the scan rate of the CV, D is the diffusion coefficient, R is the universal constant of gases, and T is the temperature. At 25 °C the equation can be simplified in;

$$i = \left(268{,}000\, n^{\frac{3}{2}} A\, D^{\frac{1}{2}} C\right) v^{\frac{1}{2}} \quad (2)$$

therefore, by plotting the peak current against $v^{\frac{1}{2}}$, a plot of density current peak (in mA cm^2) versus the square root of the scan rate is a straight line provides a slope proportional to $n^{\frac{3}{2}} D^{\frac{1}{2}}$, the value of slope of the figure S2 is 3.14 10^{-6}.

The generated current comes from the oxidation of NADH, this reaction at neutral pH is well established, this process involve two electrons and one proton that is an electron transfer-chemical step-electron transfer type (ECE), which can be described by:

$$NADH \rightarrow NAD^{\bullet +} + e^- \quad (3)$$

$$NAD^{\bullet +} \rightarrow NAD^{\bullet} + H^+ \quad (4)$$

$$NAD^{\bullet} \rightleftharpoons NAD^+ + e^- \quad (5)$$

As the oxidation reaction of NADH as well the bioelectrooxidation of ethanol provides two electrons by equation 2, it is possible to calculate the diffusion coefficient, which is 8.9 10^{-6} cm^2, this value is close for NADH diffusion coefficient values, which were reported.[1]

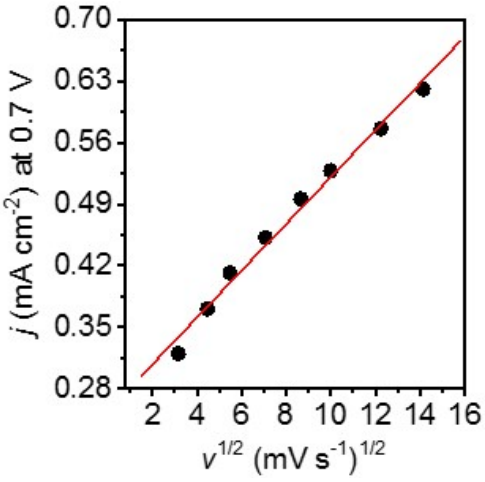

Figure S2. The dependence of peak current at 0.7 V on root square of scan rate. Supporting electrolyte: Phosphate buffer 0.1 mol L^{-1}, pH 7.5. NAD^+ 0.3 mmol L^{-1}. Ethanol 0.86 mol L^{-1}. T 25 ºC.

Chemicals

Alcohol dehydrogenase from *Saccharomyces cerevisiae* and absolute ethanol were purchased from Sigma-Aldrich. FCF electrodes were extracted from a flexible carbon cloth (CCS200) from Delpho Instruments Brazil. NaH_2PO_4/Na_2HPO_4 salts utilized for the preparation of buffer solution, sulfuric acid and potassium permanganate were purchased from Synth. Nicotinamide adenine dinucleotide free acid was purchased from Merck.

References:

1. Rajaram, R., Anandhakumar, S., & Mathiyarasu, J. (2015). Electrocatalytic oxidation of NADH at low overpotential using nanoporous poly (3, 4)-ethylenedioxythiophene modified glassy carbon electrode. *Journal of Electroanalytical Chemistry*, *746*, 75-81.