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Biosensing with allosteric nano-biocatalysts: A reductase subunit of a bacterial hydroxylase as molecular example

Biosensing with allosteric nano-biocatalysts:
A reductase subunit of a bacterial hydroxylase as molecular example

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Abstract
p-Hydroxyphenylacetate (HPA) hydroxylase (HPAH) from Acinetobacter baumannii is a distinct double-subunit enzyme that capably exploits functional cooperation between its reductase (C1) and oxygenase (C2) segments for biocatalytic conversion of substrate HPA into product 3,4-dihydroxyphenylacetate. Earlier biochemical studies1 had shown that isolated C1 on its own is a potent converter of NADH into NAD+, with dissolved molecular oxygen (O2) used as partner for required enzyme redox recycling. Hydrogen peroxide (H2O2) is a well assessable by-product of cyclic C1/NADH/O2 interaction, which by allosteric stimulation is much intensified through HPA effector bonding to a matching C1 affinity site.
Reported here will be exploitation of H2O2 electroanalysis as novel non-biochemical assay for thorough inspection of favourable C1 allostery. The most common electrochemical H2O2 screen, namely anodic analyte detection at carbon or noble metal working electrodes with high enough positive polarization, was impractical here as variations in the electrolyte levels of C1 substrate NADH and C1 effector HPA produced H2O2 signal-interfering oxidation currents. Low-potential cathodic H2O2 detection at Prussian blue modified screen printed carbon electrodes could, on the other hand, measure H2O2 in suitable HPA/NADH interference free manner. When pre-dissolved C1 was challenged in electrolyte with NADH additions cathodic H2O2 amperometry was indeed sensitive enough to recognize the onset of substrate conversion activity of the nano-biocatalyst almost instantly and follow the enzymatic process until total NADH depletion with truthful current traces. Comparative amperometric trials in solutions without and with different levels of HPA verified electrochemically very well the pronounced concentration dependence of the allosteric acceleration of C1-driven NADH-to-NAD+ turnover due to an affinity capture of the stimulating phenolic compound. Accomplishment of the highly efficient electroanalysis of C1 allostery allowed further explorations as novel competitive analytical practice for trace NADH or HPA quantification. The potential of the latter compound as a urinary biomarker of, for instance, intestinal microbial overgrowth2 ('Dysbiosis') or brain defects such as Major Depressive Disorder and Parkinson Disease3 in mind, amperometric HPA valuation has advantages. Facilitation of the analytical assay by the nanoscopic biological machinery of allosteric enzyme catalysis bestows applicants with an exceptional analyte specificity while the simplicity and low prize of involved electrochemical apparatus is encouraging routine use of the methodology in country-wide communal clinical lab facilities.

Keywords: Nano-biocatalyst; enzyme allostery; cathodic H2O2 detection; amperometry

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