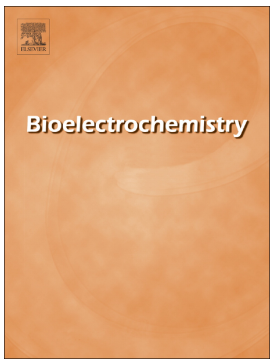

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Electroanalytical characterization of the direct *Marinobacter hydrocarbonoclasticus* nitric oxide reductase-catalysed nitric oxide and dioxygen reduction

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Abstract

Understanding the direct electron transfer processes between redox proteins and electrode surface is fundamental to understand the proteins mechanistic properties and for development of novel biosensors. In this study, nitric oxide reductase (NOR) extracted from *Marinobacter hydrocarbonoclasticus* bacteria was adsorbed onto a pyrolytic graphite electrode (PGE) to develop an unmediated enzymatic biosensor (PGE/NOR)) for characterization of NOR direct electrochemical behaviour and NOR electroanalytical features towards NO and O_2. Square-wave voltammetry showed the reduction potential of all the four NOR redox centers: 0.095±0.002, -0.108±0.008, -0.328±0.001 and -0.635±0.004 V vs. SCE for heme *c*, heme *b*, heme b_3 and non-heme Fe_B, respectively. The determined sensitivity ($-4.00\times10^{-8} \pm 1.84\times10^{-9}$ A/µM and $-2.71\times10^{-8}\pm1.44\times10^{-9}$ A/µM for NO and O_2, respectively), limit of detection (0.5 µM for NO and 1.0 µM for O_2) and the Michaelis Menten constant (2.1 and 7.0 µM for NO and O_2, respectively) corroborated the higher affinity of NOR for its natural substrate (NO). No significant interference on sensitivity towards NO was perceived in the presence of O_2, while the O_2 reduction was markedly and negatively impacted (3.6 times lower sensitivity) by the presence of NO. These results clearly demonstrate the high potential of NOR for the design of innovative NO biosensors.

Keywords:
Direct electron transfer; Nitric oxide reductase; Heme proteins; Nitric oxide bioelectrocatalysis; Dioxygen bioelectrocatalysis.