Electrochemical Investigations of Nitric Oxide Synthase and its Cofactors

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The accepted mechanism of nitric oxide synthase (NOS) involves the tetrahydropterin cofactor (BH4) as a single electron donor/acceptor. This transfer of a single electron contrasts with the obligate two-electron/two-proton interconversion of BH4 and dihydrobiopterin typically observed in solution. The electrochemical properties of pterin compounds in solution have been investigated previously by voltammetry, with marked differences in behavior reported for different types of electrodes employed. To address this systematically, we have investigated the solution electrochemistry of tetrahydrobiopterin at a variety of electrode surfaces (gold, graphite, and chemically modified variants of each). The trend observed is that changing the interfacial electron transfer (ET) rate at an electrode surface by passivation with oxide or adsorbants leads to a change in the reversibility of the observed two-electron voltammetric response. This can be shown for a glassy carbon electrode polished aerobically before introduction to the electrochemical cell in comparison to the original oxide-free surface (Figure A). Furthermore, the asymmetric shifting of these peaks reveals that a kinetic bias against the removal of a second electron from BH4 underlies the thermodynamic cooperativity. We are also investigating the direct electrochemistry of NOS proteins from a variety of bacteria (*B. subtilis, B. stearothermophilus, D. radiodurans*). Initial attempts have employed immobilization of purified enzyme on a carbon electrode using an adsorbed lipid film. Overall, the voltammetry we observe is indistinguishable from that of free hemin control experiments (Figure B). To address this issue further we are probing the stability of the protein under different conditions with the aim of finding an electrode environment that provides the direct electrochemistry of competent enzyme.

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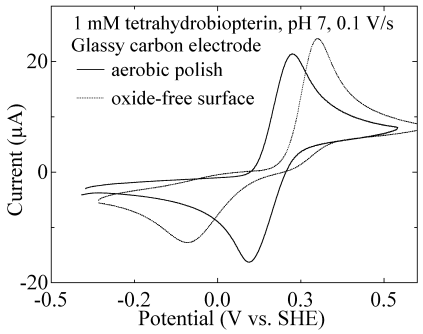

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