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Enzyme-catalysed nitrate reduction—themes and variations as revealed by protein film voltammetry

Julea N. Butt [a,*], Lee J. Anderson [a], Luis M. Rubio [b,1], David J. Richardson [c], Enrique Flores [b], Antonia Herrero [b]

[a] *Centre for Metalloprotein Spectroscopy and Biology, School of Chemical Sciences, University of East Anglia, Norwich NR4 7TJ, UK*
[b] *Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Centro de Investigaciones Científicas Isla de la Cartuja, Avda, Américo Vespucio sln, E-40192 Seville, Spain*
[c] *School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK*

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Abstract

Protein film voltammetry has been used to define the catalytic performance of two nitrate reductases: the respiratory nitrate reductase, NarGH, from *Paracoccus pantotrophus* and the assimilatory nitrate reductase, NarB, from *Synechococcus* sp. PCC 7942. NarGH and NarB present distinct 'fingerprints' of catalytic activity when viewed in this way. Potentials that provide insufficient driving force for significant rates of nitrate reduction by NarB result in appreciable rates of nitrate reduction by NarGH. However, both enzymes display complex modulations in their rate of substrate reduction when viewed across the electrochemical potential domain.

Keywords: Nitrate reductase; Protein film voltammetry; Molybdenum; Iron–sulphur cluster; Electrochemistry

1. Introduction

Nitrate represents a widely available source of inorganic nitrogen which biology utilises in a number of important cellular processes [1]. For example, nitrate provides a source of nitrogen for assimilation into amino acids and nucleotides but can also be exploited as a respiratory substrate during growth in micro-aerobic or anaerobic environments. An essential step in the biological processing of nitrate is the two-electron reduction of nitrate to nitrite. The respiratory nitrate reductase, NarGH, from *Paracoccus pantotrophus* and the assimilatory nitrate reductase, NarB, from *Synechococcus* sp. PCC 7942 contain the Mo-*bis*-molybdopterin guanine dinucleotide (Mo-*bis*-MGD) cofactor associated with the site of nitrate reduction [2,3]. However, these enzymes differ in the number and type of [Fe–S] clusters which accompany the active site. NarGH contains one [3Fe–4S] and three [4Fe–4S] clusters while NarB contains one [4Fe–4S] cluster. We have previously reported the protein film voltammetry of NarGH that reveals complex, reversible modulations of catalytic activity in the electrochemical potential domain [2,4]. It was therefore of interest to compare the catalytic behaviour of NarGH with that of the structurally simpler NarB enzyme.

2. Experimental

Protein film voltammetry was performed with a three-electrode cell configuration housed in an anaerobic chamber as described previously [4]. All potentials are reported with respect to the standard hydrogen electrode (SHE). Films of NarGH and NarB were prepared by coating freshly polished pyrolytic graphite edge (PGE) electrodes with a sub-microliter quantity of ca. 30 μM enzyme containing 2 mM neomycin.

3. Results and discussion

A typical steady state voltammogram obtained from a film in 4 μM nitrate is shown in Fig. 1. Catalytic activity is

* Corresponding author. Tel.: +44-1603-593-877; fax: +44-1603-592-003.
E-mail address: j.butt@uea.ac.uk (J.N. Butt).
[1] Present address: Department of Biochemistry, University of Wisconsin, Madison, WI 53706, USA.

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detected below 150 mV and the wave shows a local activity maximum at 20 mV. Aside from a slow decrease in signal amplitude, the response is invariant during repeated cyclic voltammetry. When the substrate concentration is increased, the catalytic wave adopts an increasingly sigmoidal form and experiences displacement towards slightly more negative potentials to approach a limiting position with the wave centred on ca. 0 mV [2]. The position of the catalytic waveform for a given substrate concentration was found to be essentially independent of pH over the range 5–8.5. Representative film voltammetry from NarB in 250 μM nitrate is shown in Fig. 2. The large reduction currents observed below ca. −300 mV can be attributed to NarB-catalysed nitrate reduction since these currents were not observed in control experiments performed in the absence of either NarB or nitrate. The catalytic response from NarB shows a local activity maximum like that observed for NarGH but with an activity maximum at −480 mV.

Catalytic film voltammetry from NarGH and NarB clearly reveals distinct operating potentials for these enzymes. Potentials that provide insufficient driving force for significant rates of nitrate reduction by NarB result in appreciable rates of nitrate reduction by NarGH. This behaviour must originate from the distinct thermodynamic and kinetic parameters describing the catalytic cycle of each enzyme. Both enzymes display a local maximum in catalytic activity that may arise from electrochemical transformations of the Mo-*bis*-MGD centre or the [Fe–S] clusters [2]. Interestingly, the operating potential of each nitrate reductase appears well matched with the reduction potential of their respective physiological electron donors: the membrane-bound quinol pool for NarGH and low potential flavodoxin or ferredoxin for NarB [1,3]. Thus, the electrochemical modulations of nitrate reductase activity revealed by protein film voltammetry could be exploited in vivo for finely tuned regulation of the activity of these important enzymes.

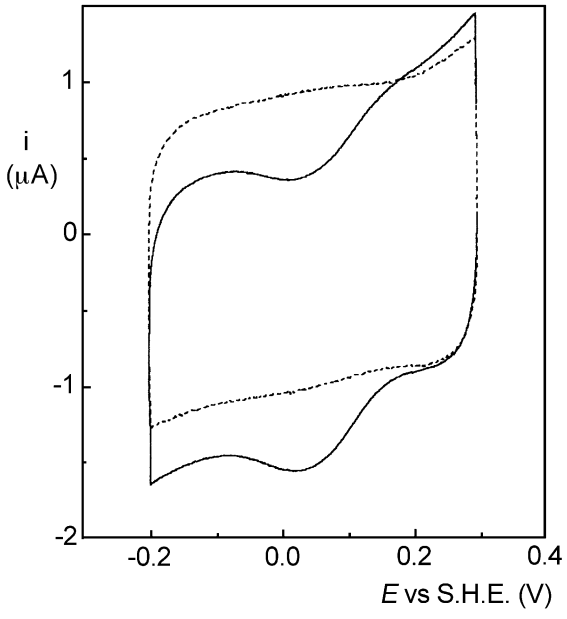

Fig. 1. Typical steady state voltammogram obtained from a film of *P. pantotrophus* NarGH in 4 μM nitrate (solid line) and in the absence of nitrate (broken line). The buffer-electrolyte was 2 mM neomycin, 10 mM each of acetate, Mes, Epps and Ches, pH 6.8, 25 °C. Electrode rotation rate = 3000 rpm. Scan rate = 10 mV s^{-1}.

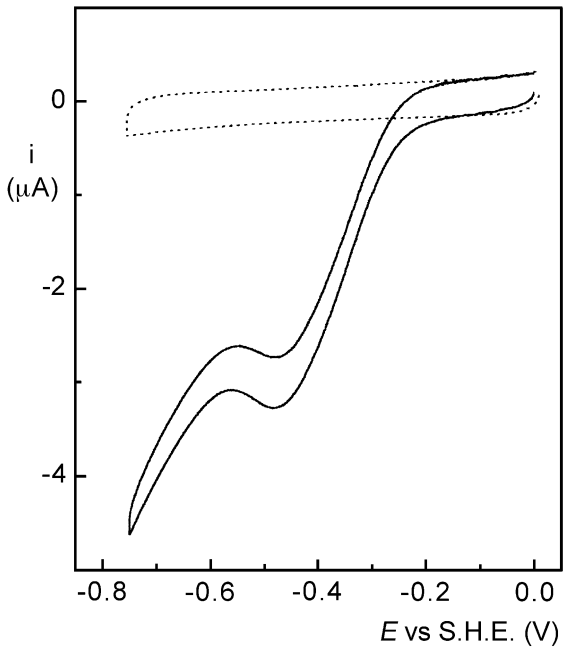

Fig. 2. Typical steady state voltammogram from a film of *Synechococcus* NarB in 250 μM nitrate (solid line) and in the absence of nitrate (broken line). The buffer-electrolyte was 2 mM neomycin, 100 μM EGTA, 50 mM Hepes, pH 8.0, 30 °C. Electrode rotation rate = 3000 rpm. Scan rate = 20 mV s^{-1}.

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