

Electrodes for redox-active membrane proteins

Steve Evans, Richard Bushby, Simon Connell, Peter Henderson and Lars Jeuken

Introduction

Redox proteins, which are estimated to account for a quarter of all proteins, perform a myriad of functions in biology. They shuttle electrons and catalyse redox reactions in many vital processes, including photosynthesis and metabolism. Dynamic electrochemical techniques have proven to be powerful tools to study these proteins. The thermodynamics and kinetics can be studied in detail if they are electrochemically connected or 'wired' to the electrode surface. The main challenge is to adsorb proteins in their native state on the electrode while efficiently exchanging electrons. Because membrane proteins are more difficult to manipulate experimentally than globular proteins, less work has been reported on the electrochemistry of these proteins. Here, we report a novel approach to link membrane proteins to an electrode surface.

Cholesterol tethers to ‘wire’ membrane proteins

We have used cholesterol tethers to bind crude membrane extracts (membrane vesicles) of *B. subtilis* to a gold electrode surface. Atomic force microscopy (AFM, Fig. 1), including force measurements, electrochemical impedance spectroscopy (EIS) and surface plasmon resonance (SPR) revealed that the membrane vesicles are ‘flattened’ upon adsorption, but otherwise remain intact. The natural co-enzyme (i.e., menaquinone-7 [MQ-7]), which is located in these vesicles, can be oxidised and reduced electrochemically. The membrane protein, succinate menaquinone oxidoreductase (SQR), reduces fumarate using MQ-7 as mediator (Fig. 2). The cat-

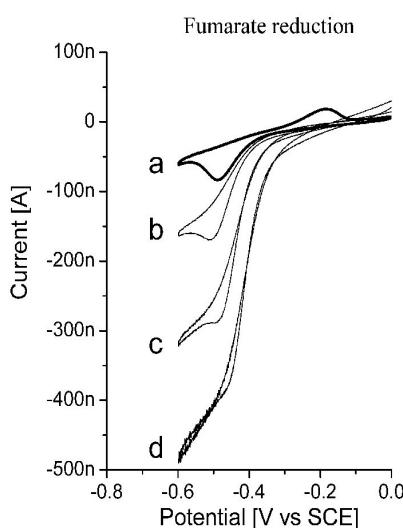


Fig. 2. CVs (1 mV/s) of a cholesterol-modified gold electrode with adsorbed membrane vesicles in the absence (a) and presence (b - d) of 2 mM fumarate at different temperatures, (a-b) 20, (c) 30 and (d) 40°C

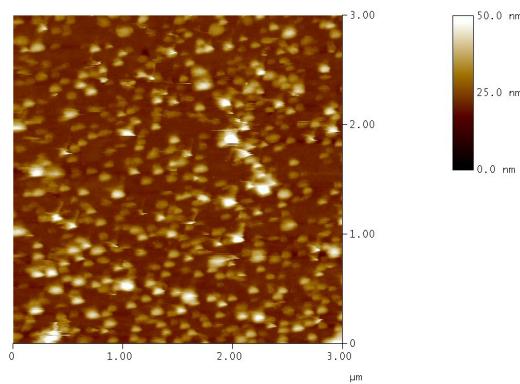


Fig 1. AFM image of a cholesterol-modified gold electrode after adsorption of membrane vesicles of *B. subtilis*

se (SQR), remains in the vesicles and is able to reduce g. 2). The catalysis of the reverse reaction (oxidation of succinate), which is the natural catalytic function of SQR, is almost absent with MQ-7. However, adding the co-enzyme ubiquinone, which has a reduction potential that is about 0.2 V higher, restores the succinate oxidation activity. These results corroborate previous reports that *B. subtilis* uses transmembrane electrical and proton gradients to supply additional energy to oxidise succinate using MQ-7 as electron acceptor.

Publications

Jeuken, L.J.C., Connell, S.D., Nurnabi, M., O'Reilly, J., Henderson, P.J.F., Evans, S.D. and Bushby, R.J. (2005) Direct electrochemical interaction between a modified gold electrode and a bacterial membrane extract, *Langmuir*, In Press.

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