DIRECT MEASUREMENT OF THE TUNNELING BARRIER OF AZURIN BY ELECTROCHEMICAL TUNNELLING SPECTROSCOPY

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Several electrochemical and imaging studies of azurin have been recently reported using Electrochemical Scanning Tunnelling Microscopy (ECSTM). Azurin is a metalloprotein involved in the respiratory chain of bacteria and a model for the study of electron transfer in blue copper redox proteins, and it has also arisen interest due to potential applications in nanotechnology as single molecule nanobiodevices. ECSTM allows studying the topography of electrode surfaces and the electrochemical processes occurring therein. It provides high spatial resolution and bipotentistatic, independent control of the electrochemical potentials of sample (Us) and STM probe (Ut) in solution.

Samples were prepared by direct covalent binding of the metalloprotein azurin on a previously annealed gold (111) surface and studied by ECSTM in 50 mM ammonium acetate buffer solution (pH 4.55) using homemade nanoprobes. High resolution images were obtained at different Ut and Us and single molecule profiles were analysed in order to determine individual heights. Spectroscopic I(z) curves were obtained by recording tunneling current as function of tip to sample separation at different Ut and Us potentials. Experimental curves were fitted to an exponential function with good regression rates in order to calculate the tunneling barrier height (TBH). Ut and Us potential values were evenly spaced in order to evaluate the effect of bias. A clean gold (111) surface was used in control experiments.

We have measured changes in the apparent height of single azurin proteins. In all cases, the height of monatomic gold steps remained constant over a wide range of potentials, while the apparent height of azurin changed significantly. We also report for the first time current-distance plots on azurin films at different Us and Ut values, which allow calculating the apparent TBH. Barrier values change significantly between 0.3eV and 1.3eV for different Ut and Us potentials, and also in comparison to the bare gold surface. The dependence of azurin apparent height and tunneling barrier on the azurin redox state, Ut and bias (Ut-Us) will be discussed in terms of the local density of states and a simple electron transfer model.