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A model recognition switch. Electrochemical control and transduction of imidazole binding by electrode-immobilized microperoxidase-11

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Electrode-immobilized microperoxidase-11 exhibited a titratable potentiometric response to imidazole, demonstrating both molecular recognition and the capability for "switchable" changes in the affinity of an immobilized redox-receptor for a target ligand.

Biological macromolecules serve as useful structural recognition elements in biosensors. A desirable goal in biosensor design is the ability to control molecular recognition in a switchable ("on" / "off") fashion through means of an external stimulus. Recent solution studies have demonstrated the influence of oxidation state on the affinity and specificity of electroactive redox-receptors for target ligands.[1] We were motivated to investigate such effects with immobilized redox-receptors with the goal of imparting switchable recognition properties to biosensors. Described here are our results demonstrating electrochemical control of affinity of an electrode-immobilized model redox-receptor, microperoxidase-11 (MP-11), for imidazole, a model small molecule target.

Microperoxidases are electroactive peptides proteolytically derived from cytochrome c and comprised of an 8-11 amino acid sequence containing a covalently attached heme group (denoted as MP-8 through MP-11).[2] The heme-iron center of microperoxidase is ligated at four coordination sites within the heme group and has two free sites available for binding other ligands. In both MP-8 and MP-11, a histidine residue in the peptide sequence coordinates one of these two sites.[3] Prior to our investigation, it was recognized that the free coordination site of MP-8 in solution could bind a variety of weak base ligands, including imidazole, amino acids, and pyridine.[4] It was also recognized that electrode-immobilized MP-11 exhibits reversible electrochemistry of the heme (Fe^{II}/Fe^{III}) couple.[5] Based on these demonstrations and on the sensitivity of the heme (Fe^{II}/Fe^{III}) formal potential on ligation,[6] we were motivated to examine electrode-immobilized MP-11 as a model integrated recognition switch and transduction element—one that could bind a molecule with one of two binding constants depending upon electrode voltage and that could report binding of a target molecule by shift in formal potential.

Microperoxidase-11 (MP-11), shown in Scheme 1, was covalently immobilized on either freshly-cleaned gold wire or gold disc electrodes by cross-linking to amine-terminated self-assembled monolayers on gold, using a modification of the method of Lotzbeyer, *et al.*[7] The formal potential of immobilized MP-11, as measured by cyclic voltammetry, was −0.377 V (*vs.* Ag/AgCl), and is in agreement with previously published results.[8,9] With addition of imidazole, the formal potential of MP-11 decreased incrementally to a minimum value of −0.408V (*vs.* Ag/AgCl) at 10 mM imidazole. Fig. 1 shows cyclic voltammetry of electrode-immobilized MP-11 in the absence and presence of 4.7 mM imidazole. Fig. 2 shows the dependency of the formal potential of MP-11 on imidazole concentration. Control experiments showed that the shift in formal potential was not due to changes in ionic strength or repeated scanning of the MP-11-modified electrode.

Scheme 1 Amino acid sequence of MP-11.

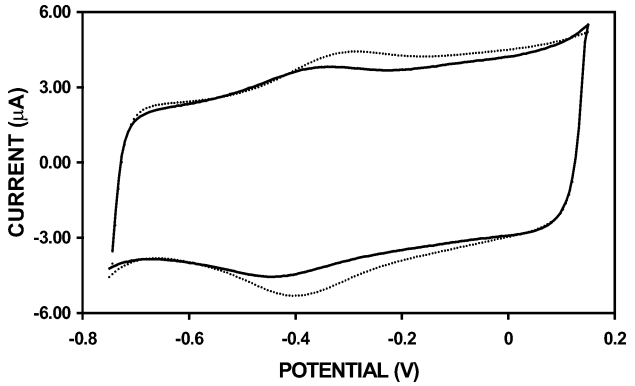

Fig. 1 Cyclic voltammetry of electrode-immobilized MP-11 in the absence (dotted line) and presence (solid line) of 4.7 mM imidazole (scan rate = 400 mV s^{-1}).

The negative shift in the formal potential indicates that the binding of imidazole stabilizes oxidized MP-11 and that MP-11 has a higher affinity for imidazole in the oxidized state than in the reduced state.[10] The ratio of binding constants of the oxidized and reduced forms of MP-11 for imidazole may be determined from the following equation:[10,11]

$$K_1/K_2 = \exp[-(nF/RT)(E_2 - E_1)] \quad (1)$$

where K_1 and K_2 represent the binding constants of oxidized and reduced forms of MP-11 for imidazole, and E_1 and E_2 represent the formal potentials for MP-11 in the absence and presence of 10 mM imidazole, respectively. For the observed −30.7mV (±5mV) shift, K_1/K_2 = 3.3 (2.7–4.0).

To extract unique binding constants for the oxidized and reduced forms of MP-11 for imidazole, the observed formal potentials in Fig. 2 were compared with formal potentials obtained from electrochemical simulations using DigiSim

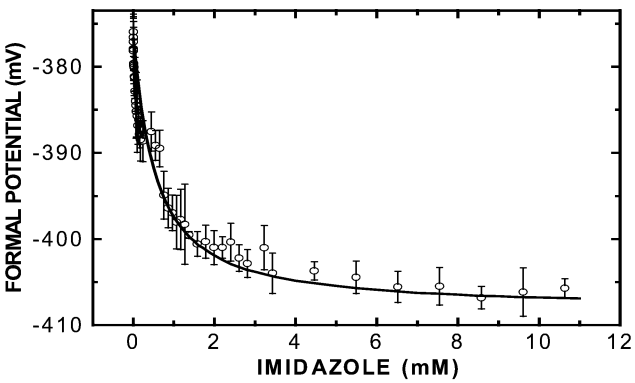

Fig. 2 Experimental voltammetric titration curve of electrode-immobilized MP-11 with imidazole. Observed formal potentials shown with standard deviation of measurements (n = 3). Solid line shows fit obtained from simulation using K_1 = 6250 M^{-1} and K_2 = 1890 M^{-1}.

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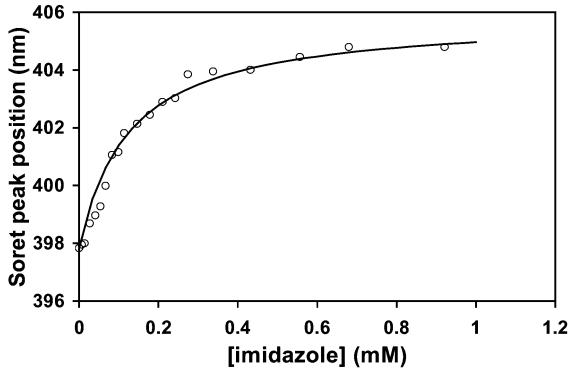

Fig. 3 Soret peak position of oxidized MP-11 ([MP-11] = 0.1 μM) as a function of increasing amounts of imidazole.

software.[12] The best fits to the observed data were obtained with K_1 = 6250 M^{-1} (±700 M^{-1}) and K_2 = 1890 M^{-1} (±150 M^{-1}) (see Fig. 2). K_1, the value of the affinity constant of the oxidized form of MP-11, was compared to the binding constant obtained from spectrophotometric titration of oxidized MP-11 in solution. Oxidized microperoxidase exhibits a strong Soret absorbance (~400 nm) that is red-shifted in the presence of ligands, and this parameter has been used to quantitatively assess its affinity for a variety of ligands.[13] Fig. 3 shows the position of the Soret peak of oxidized MP-11 as a function of increasing amounts of imidazole.[14]

From this titration, the affinity constant of the oxidized form of MP-11 for imidazole in solution was 7690 M^{-1} (7140 – 8330 M^{-1}). The close agreement between the affinity constants for electrode-immobilized MP-11 and MP-11 in solution suggests that the binding of imidazole at the electrode surface is proceeding through the coordination of imidazole to surface-accessible "vacant" ligand sites on MP-11. The above results are the first account of the electrochemical detection of a ligand by electrode-immobilized microperoxidase and furthermore demonstrate the capability to switch the affinity of an electrode-immobilized redox-receptor for a ligand by addressing the oxidation state of the receptor. We are currently investigating the effects of differing immobilization strategies and covalent modifications of MP-11 for the specific recognition of target analytes.

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9 Electrochemical behavior of immobilized MP-11 was assessed using cyclic voltammetry in a 3-electrode cell under continuous argon purge. Gold electrodes were placed in 10mM HEPES buffer (pH 7.5) and oxidation and reduction potentials were observed by scanning between −0.75 and +0.15V (*vs*. Ag/AgCl). Imidazole solutions were prepared in 10 mM HEPES buffer (pH 7.5). Imidazole titrations were performed by adding aliquots of imidazole solutions to 2 mL of buffer in the electrode sample cell. The sample cell was purged thoroughly and imidazole additions were allowed to equilibrate for 1 minute in between sequential additions.

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12 M. Rudolph, *J. Electroanal. Chem*., 1991, **314**, 13; M. Rudolph, *J. Electroanal. Chem*., 1992, **338**, 85; Computer simulations were performed using DigiSim v.2.1 software (Bioanalytical Systems, Inc., West Lafayette, IN). Covalently immobilized MP-11 was modeled as having finite diffusion with closed boundary conditions and diffusion rates of 1×10^{-10} cm^2 s^{-1} (*i.e*., essentially immobile and surface bound). Imidazole diffusion was modeled as open diffusion with a diffusion rate of 1×10^{-5} cm^2 s^{-1}. Butler-Volmer kinetics of electron transfer were assumed with a charge transfer coefficient of $\alpha = 0.5$. The heterogeneous electron transfer rate was set to 1×10^4 cm s^{-1} for each reaction. Each simulated titration curve was generated by entering the formal potential for MP-11 alone (−376.6mV) and assigning values to K_1 and K_2, such that K_1/K_2 = 3.3 (2.7–4.0). The software was then used to calculate the formal potential at different imidazole concentrations. A family of simulated titration curves was generated using different values for K_1 and K_2 and the best fit was obtained by comparison to observed formal potential values (shown in Fig. 2).

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14 To minimize the presence of dimers of MP-11 in solution (see ref. 4), the imidazole titration was performed with [MP-11] = 0.1 μM. Because of the experimental difficulty of maintaining MP-11 in a reduced state in solution, spectrophotometric titration of the reduced form was not examined.