THE ELECTROCHEMICAL PROPERTIES OF CHEMICALLY-MODIFIED NITRITE REDUCTASE

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Introduction

Nitrite reductase from *Achromobacter cycloclastes* (AcNIR) is a homo trimer multi copper protein, which contains two kinds of copper, type I and type II, in each monomer. AcNIR catalyzes reduction of nitrite ion to nitrogen monoxide by receiving an electron from its natural redox partner, pseudoazurin (pAz). Because of its specificity on the catalytic reaction, AcNIR is a candidate for a sensing device. To immobilize AcNIR on the electrode, we prepared chemically-modified AcNIR which could be enhanced its stability on the electrode. Triethyleneglycol (TEG) and polyethyleneoxide (PEO) were used to modify amino groups of NIR. In this study, we investigate modification effects on the electron transfer reaction and the activity of AcNIR.

Experimental

AcNIR and activated TEG (10 times molar amount of amino groups for AcNIR) were dissolved in borate buffer (50 mM, pH 9.0). The mixture was stirred for 1 hour. The reaction solution was ultrafiltrated to remove the excess activated TEG, and then freeze-dried. The average number of modified amino groups on TEG-NIR was determined by the titration method with 2,4,6-trinitrobenzensulfonic acid. Same experiments were done with activated PEOs of molecular weight 350 (PEO_{350}) and 750 (PEO_{750}). Cyclic voltammetry (CV) of pAz (100 μM) under the presence of TEG-NIR (1 μM) and nitrite ion (50 mM) in potassium phosphate buffer (100 μM, pH 7.0) was carried out in a three-electrode cell with Pt wire as a counter electrode, Ag/AgCl as a reference electrode, and Au modified with bis-4-pyridyl disulfide as a working electrode at sweep rate of 2 mV/s.

Results and Discussion

The average number of TEG bound to one molecule of AcNIR was estimated to be 44 out of 54 amino groups. It is known that the visible absorption spectrum of native-NIR shows three peaks at 400, 460, and 589 nm. The absorption spectrum of TEG-NIR is remarkably similar to that of native-NIR. The EPR spectrum of TEG-NIR is also similar to that of the native-NIR. Based on these results, it is suggested that modification of TEG does not cause any structure change around both sites of the type I copper and type II copper of AcNIR. The catalytic response of pAz observed in the presence of TEG-NIR and nitrite ion is shown in Fig. 1. The catalytic current of TEG-NIR was 68 % compared to that of native-NIR. This result suggests that modification of TEG to AcNIR influences on both functions of type I copper and type II copper slightly.

The average numbers of PEO_{350} and PEO_{750} bound to one molecule of AcNIR were 34 and 31, respectively. As the molecular weight of modifying reagent increases, the number of modified amino groups decreases. The catalytic responses of pAz were also observed in the presence of each PEO modified NIR and nitrite ion. The effect of molecular weight in total of the modifying reagent on the catalytic current is shown in Fig. 2. It is shown that decrease in the catalytic current seems to be due to the steric hindrance of PEO rather than the change of electrostatic interaction.

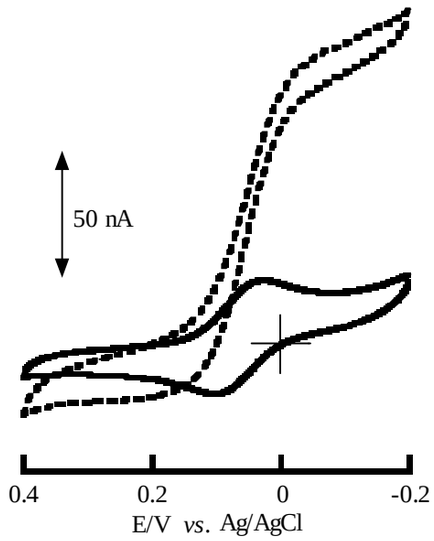

Fig. 1 Cyclic voltammograms of pAz (solid line) and after addition of TEG-NIR and NO_2^- (broken line).

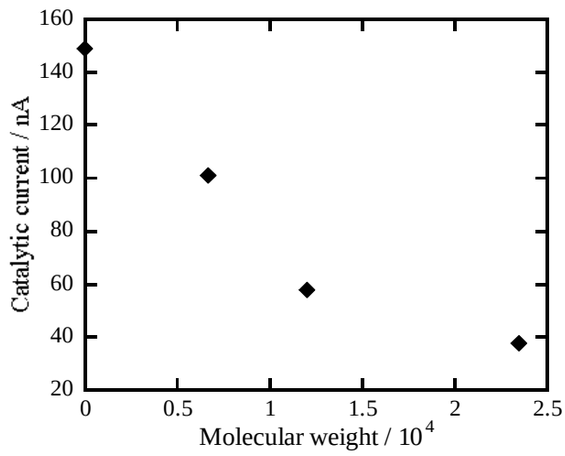

Fig. 2 Effect of molecular weight of modifying reagent on the catalytic current.