Redox Potentials of NP4 and its Mutants by Spectroelectrochemical Method

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1. Introduction

Nitrophorin4 (NP4) is a one of four heme proteins from the salivary glands of a blood sucking insect, *Rhodnius prolixus*, and its function is to transport NO. NP4 heme must be stabilized in the Fe (Ⅲ) state, and thus, NP4 has more negative redox potential than that of the usual heme protein. In the present study, we will report the simple method to measure the redox potential of NP4, of wild type (WT) and of three mutants.

2. Experimental

The redox potential of WT and three mutants of NP4 were measured by spectroelectrochemical method in a glove box system under nitrogen atmosphere. An optically transparent In_2O_3 electrode was used as a working electrode. A Pt plate and an Ag/AgCl electrode were used as a counter and a reference electrode, respectively. 2-hydroxy-1, 4-naphthoquinone ($E^{o'}$ = -0.35 V), phenosafranin ($E^{o'}$ = -0.452 V) and benzyl viologen ($E^{o'}$ = -0.56 V) were used as mediators.

3. Results

Since the redox potential of NP4 was unknown, three kinds of mediators with different potential were put in the solution and were used as mediators. Visible spectra of NP4 (WT) at pH 5.0 as a function of applied potential are shown in Fig. 1. Clearly, an isosbestic point is observed. The spectral change was reversible over many cycles of positive and negative applied potentials. The Nernst plot using the absorption maximum at 430 nm for fully reduced NP4 (WT) is shown in an inset of Fig. 1. Since the number of electron (n) is 0.88, 1-electron reaction takes place for NP4 (WT). The redox potential of NP4 (WT) was –0.384 V vs. Ag/AgCl, which is negative compared with that of myoglobin (-0.14 V vs. Ag/AgCl) having the same proximal ligand structure (His-heme-H_2O). Three mutants of which amino acid near the heme was changed were prepared (D30N, D70N, and K125Q) (Fig. 2). Redox potentials were measured with the same conditions as that of WT. The potential of D70N and K125Q were close to that of WT, but the potential of D30N was shifted to the negative by 45 mV (Table. 1). By changing the charge of Asp30, the redox potential shifted due to change in hydrogen bonding network around heme.

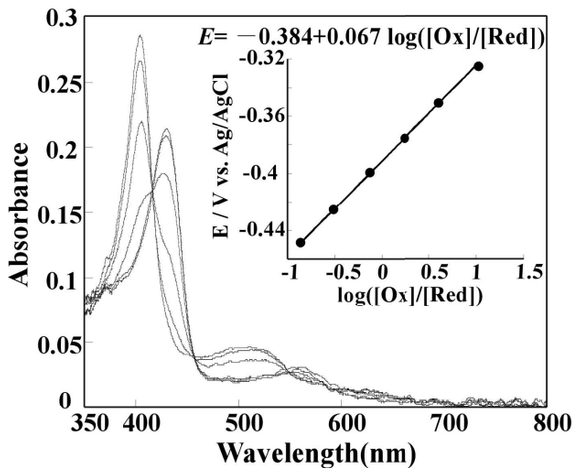

Fig. 1 Visible spectra of NP4 (WT) at pH 5.0 as a function of applied potential. Fully oxidized NP4 (WT); λ_{max} = 403 nm; fully reduced NP4; λ_{max} = 430 nm. Inset: The Nernst plot of the spectroelectrochemical data.

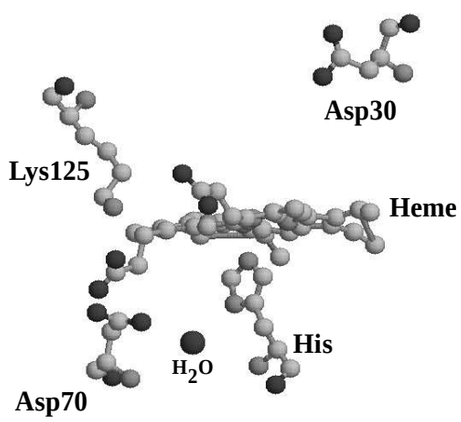

Fig. 2 Structure of active site of NP4 (WT)

Table. 1 Redox potentials of NP4 (WT) and mutants at pH5.0

Protein	Slope of the Nernst plot (V)	$E^{o'}$ (V vs. Ag/AgCl)
WT	**0.067**	**−0.384**
D30N	**0.079**	**−0.429**
D70N	**0.074**	**−0.390**
K125Q	**0.065**	**−0.410**