Direct Electrochemistry of Blue Copper Proteins at Au Electrodes Modified with Promoters

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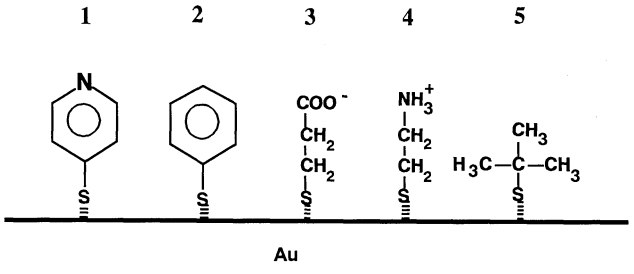
Cyclic voltammetry of six blue copper proteins, plastocyanin, azurin, umecyanin, plantacyanin, pseudoazurin, and stellacyanin was performed at Au electrodes modified with five promoters. A proper combination of non-covalent interactions such as hydrophobic interaction, hydrogen bonding, and electrostatic interaction realizes the access of the northern area of the protein molecules towards the electrode surface.

Blue copper protein is relatively small-sized protein whose molecular weight is 10000 - 20000. The type 1 copper (blue copper) is in the active site, being coordinated by one cysteine, two histidines, and one methionine (or glutamine).[1] According to the crystal structures reported hitherto, an imidazole edge of the ligand histidine at the "northern area" is exposed to solvent.[2-6] This imidazole group is the entry and exit of the electron-transfer, although in the case of plastocyanin a tyrosine residue adjacent to the acidic patch in the "south-west" is also suggested as the pathway of the electron-transfer.[7]

In spite of many structural and kinetic studies[1] blue copper proteins have not been widely studied electrochemically because successful direct electrochemistry of macromolecules have been limited to several small-sized proteins.[8] Recently, we realized direct electrochemistry of cucumber plantacyanin, lacquer stellacyanin, *Achromobacter cycloclastes* pseudoazurin, horse radish umecyanin, *Psedomonas aeruginosa* azurin, and cucumber plastocyanin at a bare glassy carbon electrode and at a bis(4-pyridyl) disulfide-modified Au electrode.[9,10] In the present study, direct electrochemistry of these six blue copper proteins is studied at Au electrodes modified with five promoters, bis(4-pyridyl) disulfide (**1**), diphenyl disulfide (**2**), 3,3'-dithiodipropionic acid (**3**), bis(2-aminoethyl) disulfide (**4**) and di-*t*-butyl disulfide (**5**) (halves of their structures were shown). Modifications of the electrode surface were performed according to literature.[11]



Cyclic voltammetry of six blue copper proteins using modified electrodes, **1**-**5** was performed (Table 1 and Figure 1 as examples). Measurement conditions were carefully selected as to be independent of protein concentration and sweep rate (A mode of diffusion is the classical linear one at less than ca. 2 x 10^{-4} mol dm^{-3} protein concentration. The electron transfer was diffusion-limited at sweep rate of 10 - 400 mV s^{-1}. Potassium

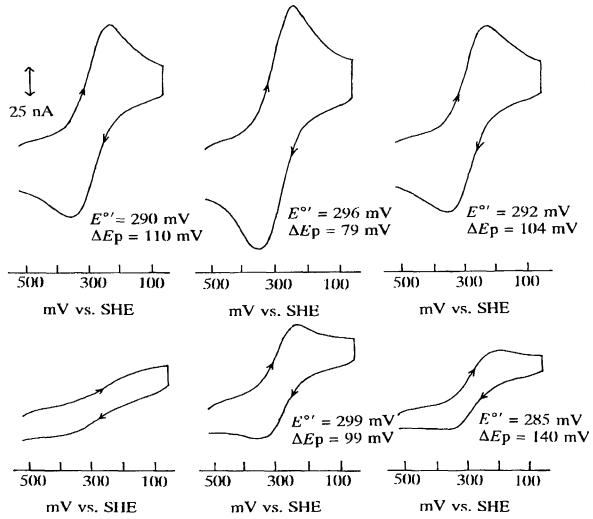


Figure 1. Cyclic voltammograms of plantacyanin at modified Au electrodes, **3** (left), **4** (center), and **2** (right). upper row, 1.04 x 10^{-4} mol dm^{-3} protein in 0.2 mol dm^{-3} phosphate buffer, lower row, 5.7 x 10^{-5} mol dm^{-3} protein in 5 x 10^{-3} mol dm^{-3} phosphate buffer. pH 6.0, 25 °C, 5mV s^{-1} sweep rate.

phosphate buffer was used throughout measurements except for plastocyanin. In Table 1, results in 0.2 mol dm^{-3} and 0.005 mol dm^{-3} buffer were shown as examples typical in high concentration buffer and in low concentration buffer, respectively. The clear voltammetric responses with relatively narrow cathodic and anodic peak separation (ΔEp < ca. 110 mV) was obtained for nearly half of measurements (denoted as "good" in the table). In some cases ΔEp was relatively wide (ca. 100 - 180 mV) and the results were denoted as "fair". When the peak current was extremely weak and when current did not flow, the results were noted as "poor" and "bad", respectively. Voltammetry of plastocyanin was performed in 0.05 mol dm^{-3} sodium acetate (pH 6.0) containing 0.02 mol dm^{-3} NaCl and 0.1 mol dm^{-3} $MgCl_2$. When concentrations of NaCl and $MgCl_2$ were low, voltammetric response was unsatisfactory, and accordingly highly concentrated inorganic salts are indispensable to promote redox waves of plastocyanin at modified Au electrodes in contrast to the direct electrochemistry at an edge-oriented pyrolytic graphite.[8]

The isoelectric point of the proteins and the overall charge at pH 6.0 were shown in Table 1 in order to understand how directelectrochemistry of blue copper proteins is realized. When **1** with pyridyl group was used as promoter, satisfactory voltammograms were obtained by appropriately choosing phosphate buffer concentration. The reason why this promoter is best among five promoters will be that the pyridyl group is able to directly interact with the coordinated imidazole group through the hydrogen bonding. Although it is not certain whether the electron-transfer is performed through this hydrogen bond or not,

Table 1. Voltammetric response of blue copper proteins

Protein	Isoelectric Point	Overall Charge (pH 6)[a]	Buffer Concentration / mol dm^{-3}	Electrode 1	2	3	4	5
Plastocyanin	4.0	-7.8	[b]	fair	poor	fair	poor	bad
Azurin	5.4	-0.9	0.2	good	fair	poor	fair	bad
			0.005	fair	fair	bad	fair	bad
Umecyanin	5.8	+0.4	0.002	good	good	good	good	bad
			0.005	fair	good	good	good	bad
Pseudoazurin	8.4	+0.7	0.2	good	bad	good	fair	bad
			0.005	fair	fair	good	good	bad
Stellacyanin	9.9	+6.1	0.2	good	good	fair	good	fair
			0.005	good	good	fair	fair	fair
Plantacyanin	10.6	+6.0	0.2	good	good	good	good	fair
			0.005	good	fair	poor	good	fair

[a]Calculated from amino acid content. [b] In 0.05 mol dm^{-3} sodium acetate (pH 6.0) containing 0.02 mol dm^{-3} NaCl and 0.1 mol dm^{-3} $MgCl_2$.

this interaction play a crucial role for the ligand histidine to orient towards the electrode surface. Moreover, this promoter has hydrophobic character, which assists the protein molecules to take the orientation favorable for the electron-transfer by interacting with the hydrophobic region encircling the ligand histidine imidazole group. The fact that direct electrochemistry of azurin, umecyanin, and pseudoazurin at **1** was better in a highly concentrated buffer supports that the hydrophobic interaction is important.

On the other hand, **2** to give the only hydrophobic interaction was also effective in many cases, indicating that the northern area around the ligand histidine[2-6] hydrophobically interacts with this promoter. However, *t*-butyl group, **5** did not operate as an effective promoter. The Au electrode might not be properly modified with this promoter because of inhomogeneity of di-*t*-butyl disulfide in aqueous solution. Interesting are results using promoters with positive charge and negative charge, **3** and **4**. Voltammetry of the highly basic proteins such as plantacyanin and stellacyanin was apparently better at the positively charged promoter than at the negatively charged promoter. The electric repulsion at the side or bottom of the protein molecules increases the chance that the northern area of the protein molecules orients toward the electrode.[2-6] On the other hand, direct electrochemistry of the almost neutral proteins, umecyanin and pseudoazurin was preferred at both **3** and **4**. The direct electrochemistry of highly acidic plastocyanin was better at **3** than at **4**, indicating that the electric repulsion is also an important factor to promote the well-resolved redox waves of the highly charged protein. Although a local electrostatic interaction might be also effective in some cases, for example it seems to be reflected in the case of pseudoazurin, it will not be the dominant factor to achieve direct electrochemistry of blue copper proteins. The successful direct electrochemistry of blue copper proteins at an amorphous glassy carbon[9, 10] and a pyrolytic graphite[12] would have been realized because of the highly hydrophobic character of the carbon electrodes.

In conclusion, the integrated effect including hydrophobic interaction and hydrogen bonding between promoter and protein molecule is important to promote well-resolved redox waves of blue copper proteins. When the interaction involving areas apart from the electron-transfer pathway is predominant, the electric communication between protein and electrode becomes poor. The present result furnishes a general strategy to realize direct electrochemistry of macromolecules.

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