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Reduction of plastocyanin by tyrosine-containing oligopeptides

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Abstract

Oxidized plastocyanin (PC) was reduced with TyrTyrTyr and LysLysLysLysTyrTyrTyr (KKKKYYY) oligopeptides at neutral pH. The TyrTyrTyr site of the peptides provided an electron to the copper active site of PC, whereas the tetralysine site of KKKKYYY functioned as the recognition site for the negative patch of PC. The reciprocal initial rate constant ($1/k_{int}$) increased linearly with the reciprocal TyrTyrTyr concentration and proton concentration, although the electron transfer rate decreased gradually with time. The results showed that PC was reduced by the deprotonated species of TyrTyrTyr. A linear increase of log k_{int} with increase in the ionic strength was observed due to decrease in the electrostatic repulsion between negatively charged PC and deprotonated $(TyrTyrTyr)^-$. PC was reduced faster by an addition of KKKKYYY to the PC–TyrTyrTyr solution, although KKKKYYY could not reduce PC without TyrTyrTyr. The ESI-LCMS spectrum of the products from the reaction between PC and TyrTyrTyr showed molecular ion peaks at m/z 1015.7 and 1037.7, which suggested formation of a dimerized peptide that may be produced from the reaction of a tyrosyl radical. The results indicate that PC and the tyrosine-containing oligopeptides form an equilibrium, $PC_{ox}/(oligopeptide)^- \leftrightarrows PC_{red}/(oligopeptide)^{\bullet}$. The equilibrium is usually shifted to the left, but could shift to the right when the produced oligopeptide radical reacts with unreacted peptides. For the reaction of PC with KKKKYYY in the absence of TyrTyrTyr, the produced KKKK(YYY)$^{\bullet}$ radical peptide could not react with other KKKKYYY peptides, since they were positively charged. In the presence of both KKKKYYY and TyrTyrTyr, PC may interact effectively with KKKKYYY through its tetralysine site and receive an electron from its TyrTyrTyr site, where the produced KKKK(YYY)$^{\bullet}$ may interact with TyrTyrTyr peptides.

Keywords: Plastocyanin; Tyrosine-containing peptide; Lysine peptide; Protein–peptide interaction; Active site reduction

1. Introduction

Oxidation of tyrosine is frequently observed in proteins [1–5], and concomitant electron transfer is often coupled with the reduction of the metal site, which is indispensable for the enzymatic reaction. Oxidation of tyrosine produces a tyrosyl radical, which is highly reactive towards phenols [6]. For example, cross-linking tyrosines are formed to produce a hard fertilization membrane by oxidation of protein-bound tyrosyl residues in the presence of peroxidase [7–10]. These tyrosine-containing peptides have also been shown to polymerize by oxidation with compound I or compound II of horseradish peroxidase [11–14], lactoperoxidase [13], and myeloperoxidase [15,16]. Formation of *o*-tyrosine and 3,3′-dityrosine in proteins by metal-catalyzed oxidation with Cu^{II} and H_2O_2 has been observed, where dityrosine has been shown to accumulate in the protein upon the oxidation [17]. Oxidation of a tyrosine

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residue to a quinone by post-translational modification has also been observed in amine oxidase [18,19]. Since tyrosine oxidation may occur in proteins, it is important to understand the nature of the tyrosine oxidation process in proteins. Studies on the reactions of tyrosine-containing peptides with metalloproteins at neutral pH would provide such information.

Plastocyanin (PC) is a mobile copper protein existing in the thylakoid lumen of photosynthetic organisms. PC accepts an electron from cytochrome *f* (cyt *f*), a subunit of the cytochrome b_6f complex, and donates it to the reaction center chlorophyll in the photosystem I (PSI) complex [20–22]. PC is classified as a Type 1 copper protein, which exhibits a low energy ligand-to-metal charge transfer band near 600 nm in the absorption spectra and a narrow hyperfine coupling constant ($|A_{\|}| < 90 \times 10^{-4}\ \mathrm{cm}^{-1}$) in the electron paramagnetic resonance spectra [23,24]. Plant PC contains one copper atom with two histidine nitrogen atoms, one methionine sulfur atom, and one cysteine sulfur atom coordinated in a distorted tetrahedral geometry, which is revealed by the crystal structures of PC [25–29].

Plant PC usually possesses two highly conserved sites which have been considered as molecular recognition sites for its redox partners, cyt *f* and PSI: One site is located at the solvent-accessible site containing the Cu-coordinated histidine (Cu-adjacent hydrophobic patch), and the other site is positioned at another solvent-accessible site including several acidic residues (Cu-remote negative patch) (Fig. 1). The Cu-remote negative patch of PC consists of two clusters: One lower cluster (Asp42/Glu43/Asp44/Glu45) and another upper cluster (Glu59/Glu60/Asp61) (Fig. 1). Both of these clusters have been indicated to be essential for the binding of PC to cyt *f* [30] and have been shown to interact with charged molecules and proteins [31,32]. The negative patch of PC and the positively charged site of cyt *f* interact through electrostatic interactions to form a PC–cyt *f* complex for electron transfer [33–43], whereas the hydrophobic patch of PC has also been shown to be crucial for the PC–cyt *f* complex by NMR and mutation studies [30,44–47]. It has been shown with the use of photoinduced zinc cytochrome *c* (cyt *c*) that PC and cyt *f* or cyt *c* react with each other in different configurations resulting from the protein–protein interaction termed as the gating process for electron transfer [48–50].

We have previously shown that oxidized cyt *c* can be reduced with a tyrosine-containing peptide, tyrosyltyrosylphenylalanine (TyrTyrPhe), producing an oxidized species of the tyrosine [51]. From the mass spectra of the reaction products, formation of quinone and tyrosine derivatives of the peptide was suggested. We proposed formation of a cyt c_{ox}/(TyrTyrPhe)$^-$ ⇆ cyt c_{red}/(TyrTyrPhe)$^{\cdot}$ equilibrium, which is usually shifted to the left but shifts to the right by the interaction of the tyrosyl radical with unreacted TyrTyrPhe peptides though hydrophobic interaction. It would be important to check whether tyrosine-containing peptides reduce other metalloproteins, to elucidate the mechanism of metalloprotein reduction by the peptides, and to design a more reactive peptide. We, therefore, investigated the reaction of oxidized PC with TyrTyrTyr and LysLysLysLysTyrTyrTyr (KKKKYYY), which possesses a molecular interaction site for PC. The present work shows that metalloproteins could be reduced effectively by tyrosine-containing oligopeptides.

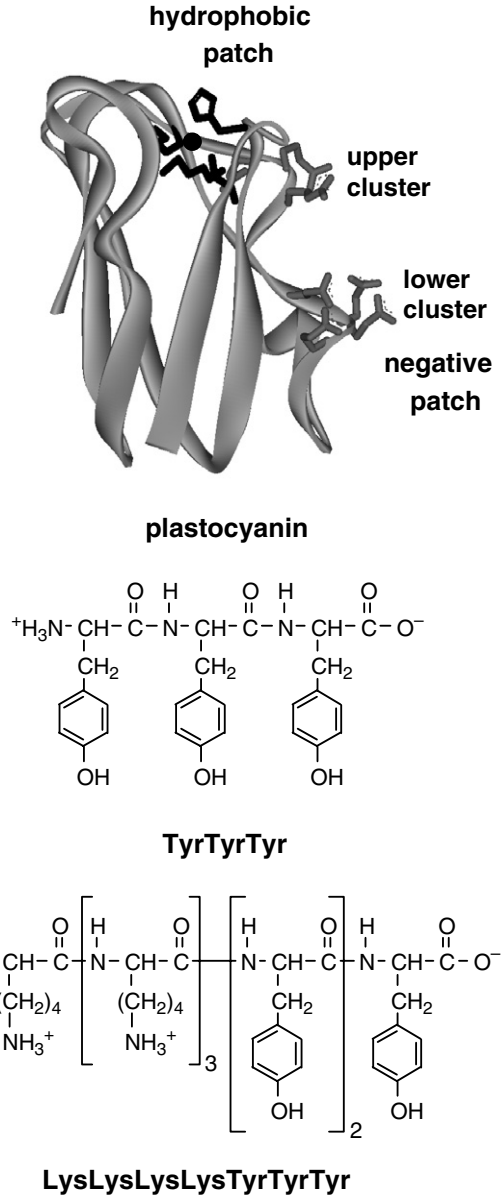

Fig. 1. Protein structure of silene PC (PDB entry, 1BYO) and chemical structures of the peptides used in this study.

2. Experimental

2.1. Sample preparation

Silene pratensis (white campion) PC was purified as described before [32,33]. Purified PC was dialyzed with 1 or 20 mM phosphate buffer, pH 6.4–7.5, before each measurement, and the protein concentration was adjusted by