Interactions in Plastocyanin−Lysine Peptide and Related Systems

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Keywords: Electron transfer / Proteins / Peptides / Structural change / Structure−activity relationships

The structure–function relationship of proteins is one of the major topics of protein studies. In spite of the importance of intermolecular interactions between proteins, which could control their functions, there are only a limited number of detailed studies on the protein structural changes induced by interaction with a protein or a peptide, because the associated changes in properties are small. Charged peptides, however, may be useful for studying the molecular recognition character of proteins and their interaction-induced structural changes, since they do not have any visible absorption. The active site Cu of plastocyanin (PC) exhibited a longer Cu–S(Cys) bond length and a higher redox potential on binding of a lysine peptide, suggesting that lysine peptides induce a structural change in PC to adapt the copper site for facile electron transfer. Structural changes were also observed for cytochrome (cyt) *f*, cyt *c*, and cytochrome *c* peroxidase on interaction with charged peptides.

1. Introduction

Structure−function relationship is one of the major topics in protein studies. In cells, each protein has to recognize its partner effectively, and thus the molecular recognition of proteins has been studied extensively. Intermolecular interactions between proteins and the induced structural changes, which may control their functions, are also important to understand the structure−function relationship of proteins. However, there are only a limited number of detailed studies on the structural changes induced by molecular interactions, because of the lack of a useful method.

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Electron-transfer proteins are indispensable for life. Many of them are charged on their surface and/or have a specific charged site for a specific protein−protein complex formation, and the electrostatic interaction is one of the main forces that guide them to form such a complex for electron transfer. For example, plastocyanin (PC), a mobile electron-transfer protein existing in the thylakoid lumen of photosynthetic organisms, is negatively charged at neutral pH for higher plant and green algae PCs, whereas PCs from cyanobacteria are characterized by high pI values. Higher plant and green algae PCs contain consecutive acidic residues (negative patch) located at the solvent-accessible site near the tyrosine residue remote from the Cu center.[1,2] PC receives an electron from cytochrome *f* (cyt *f*) of the cytochrome b_6f complex, which is an integral oligomeric membrane protein complex existing in the photosynthetic organism. By X-ray structural studies, a lysine (Lys) residue rich positively charged site (positive patch) has been revealed to exist at the solvent-exposed site of cyt *f*,[3] and the electrostatic interaction between the negative patch of PC and the positive patch of cyt *f* has been inferred to be the force for the formation of the cyt *f*·PC complex.

An NMR spectroscopic study on the Cd-substituted PC·cytochrome *c* (cyt *c*) complex indicated that the PC·cyt *c* complex consists of a highly dynamic ensemble of structures.[4] Theoretical docking models demonstrated three docked complexes between PC and cyt *f* and suggested that the negative patch of PC is not important in the active electron transfer complex, although it is important for the initial binding.[5,6] Kostić et al. proposed that PC and cyt *f* or cyt *c* bind and react with each other in different configurations resulting from the protein−protein interaction termed as the gating process for electron transfer,[7−9] showing possible configurations for the diprotein complex by computer simulation.[10,11] A paramagnetic NMR spectroscopy and restrained rigid-body molecular mechanics study also indicated that the electrostatic interactions guide PC and cyt *f* into a position that is optimal for electron transfer,[12] whereas electron transfer has been shown to occur via His87 to Cu in PC by mapping NMR chemical-shift changes.[13] However, kinetic evidence for multiple binary complexes has been obtained for the protein docking and gated electron-transfer reactions between zinc cyt *c* and a new PC from the fern *Dryopteris crassirhizoma*,[14] which has a very large acidic surface extending into the area that is hydrophobic in other PCs.[15]

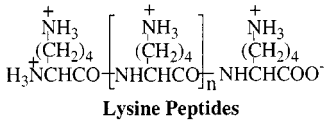
Cyt *c* is a well-known electron-transfer heme protein which is positively charged at neutral pH,[16,17] whereas cytochrome *c* peroxidase (C*c*P), an electron-transfer partner of cyt *c*, is negatively charged at neutral pH.[18] The cyt *c*·C*c*P complex was therefore predicted to be formed by the electrostatic interaction, which has been verified by the X-ray crystal structure analysis of the cyt *c*·C*c*P complex.[19]

Redox-inactive inorganic compounds have been utilized to study the molecular recognition character of electron-transfer proteins. NMR spectroscopic investigations on the complex formation between proteins and Cr^{III} compounds were made,[20,21] and $[Cr(NH_3)_6]^{3+}$ was shown to interact with both the hydrophobic and negative patches of PC,[21] whereas it bound to several sites on the surface of cyt *c*.[20] Sykes et al. showed that small redox-inactive inorganic compounds inhibit electron transfer between PC and cyt *c*,[22] cyt *f*,[23] or inorganic redox partners,[24−26] which has been explained by competitive inhibition due to interaction between the positive charge of the redox-inactive inorganic compound and the negative charge of PC.

2. Plastocyanin−Lysine Peptide Interaction

2.1 Molecular Recognition

Since the electrostatic interaction is essential for complex formation between these electron transfer proteins, we came upon the idea of using charged peptides as models for the protein interacting sites (Figure 1). Positively charged peptides interact with the aspartyl (Asp) and glutamyl (Glu) residues of the negatively charged sites of proteins, whereas negatively charged peptides can interact with the Lys residues of the positively charged sites. Actually, positively charged lysine peptides interacted with the consecutive Asp and Glu residues of the negative patch of PC and competitively inhibited electron transfer from reduced cyt *f* or cyt *c* to oxidized PC (Figure 2).[27] The inhibitory effects of lysine peptides on electron transfer were explained as competitive inhibition due to neutralization of the PC negative patch by formation of PC·lysine peptide complexes (Figure 3). Upon decreasing the net charge of the PC negative patch by mutation, the electron transfer rates from reduced cyt *c* to oxidized PC and the inhibiting effect of lysine peptide decreased. These results strongly indicate that the negative patch of PC is the dominant cyt *c*/*f* recognition site.



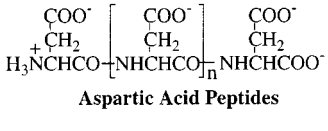


Figure 1. Chemical structures of lysine and aspartic acid peptides

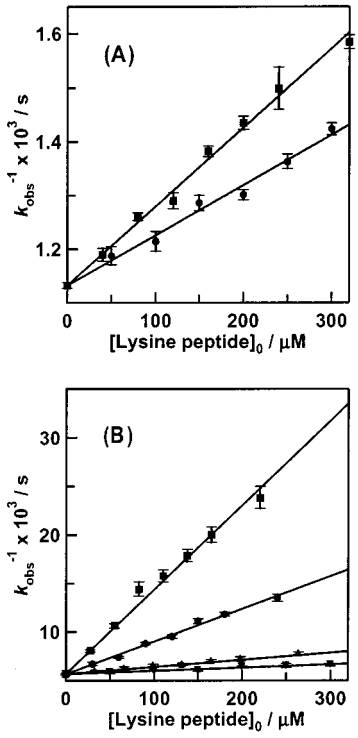


Figure 2. Plots of the reciprocal electron transfer rate constants ($1/k_{obs}$) from reduced cyt *f* or cyt *c* to oxidized PC vs. the initial concentrations of lysine peptides: *di*- (▼), *tri*- (▲), *tetra*- (•), and *penta*-Lys (■); (A) reduced cyt *f*-oxidized PC and (B) reduced cyt *c*-oxidized PC electron-transfer systems; protein concentrations: PC, 5 μM; cyt *f* and cyt *c*, 0.5 μM; Tris-HCl buffers (10 mM), pH = 7.3, containing 10 mM (A) and 60 mM NaCl (B), respectively, were used; modified from refs.[27,54]

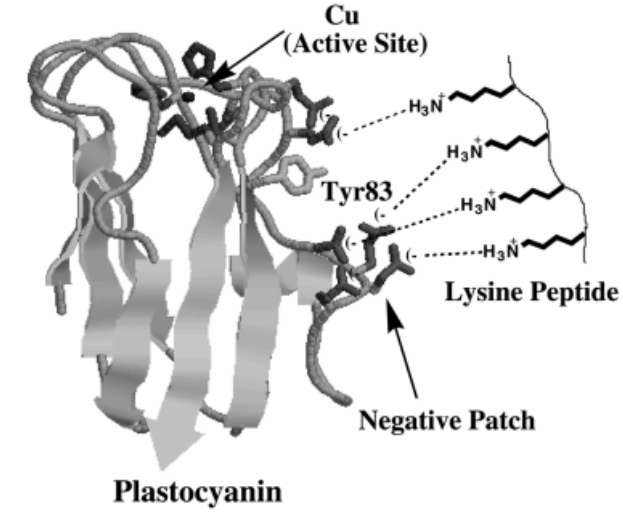


Figure 3. A schematic view of silene PC (PDB 1BYO, ref.[2]) interacting with *tetra*-Lys

The observed inhibition may be interpreted by considering formation of two complexes, a PC·cyt *c*/*f* complex where electron transfer subsequently occurs, and a PC·lysine peptide complex that competitively inhibits formation of the PC·cyt *c*/*f* complex and thus inhibits electron transfer. The complex formations are expressed by Equation (1) and Equation (2), where K_{OS} and K_i are the association constants for PC_{ox}·cyt c/f_{red} and PC_{ox}·lysine peptide complexes, respectively, and k_e represents the electron-transfer rate constant.

$$PC_{ox} + \text{cyt } f/c_{red} \underset{}{\overset{K_{OS}}{\rightleftharpoons}} (PC_{ox}\cdot \text{cyt } f/c_{red})_R \xrightarrow{k_e} (PC_{red}\cdot \text{cyt } f/c_{ox})_R \quad (1)$$

$$PC_{ox} + \text{lysine peptide} \overset{K_i}{\rightleftharpoons} (PC_{ox}\cdot\text{lysine peptide})_R \quad (2)$$

The suffixes ox and red refer to the oxidized and reduced states, and R denotes that the complex is formed at the Cu-remote negative patch of PC. If we write the observed rate constant as k_{obs}, $K_{OS} \times k_e$ as k, and the initial concentrations of PC and lysine peptide as $[PC]_0$ and $[\text{lysine peptide}]_0$, we obtain the relationship

$$\frac{1}{k_{obs}} = \frac{K_i}{k[PC]_0}[\text{lysine peptide}]_0 + \frac{1}{k[PC]_0} \quad (3)$$

Plots of $1/k_{obs}$ against $[\text{lysine peptide}]_0$ give straight lines, substantiating the validity of the assumptions leading to Equation (3). K_i values obtained from least-squares fitting of the data for the cyt *c*−PC electron-transfer system (Figure 2, B) with Equation (3) are listed in Table 1. The association constants for the PC·lysine peptide complexes increase as the length and thus the charge of the peptide increase, which shows that PC effectively recognizes the sites with four or more positive charges. The inhibitory effects demonstrate that these lysine peptides, especially *tetra*- and *penta*-Lys, function as models for the PC interacting site of proteins.

Table 1. Association constants (K_i) for PC−lysine peptide complexes obtained by least-squares fitting of the data in Figure 2 (B) with Equation (3)

Length of lysine peptide	K_i/M^{-1} [a]
di	550±130
tri	1400±200
tetra	5900±300
penta	15300±1000

[a] In 10 mM Tris-HCl buffer, pH = 7.3, containing 10 mM NaCl at 15 °C.

Interactions of lysine peptides with PC were also studied by measuring electron transfer between $[Fe(CN)_6]^{4-}$ and oxidized PC in the presence of the peptides.[28] Lysine peptides up to *penta*-Lys promoted electron transfer from $[Fe(CN)_6]^{4-}$ to PC.[28] The electron transfer promoting effects of lysine peptides may be due to formation of PC·lysine peptide or lysine peptide·$[Fe(CN)_6]^{4-}$ complexes, subsequently forming an electron transferring complex, PC·lysine peptide·$[Fe(CN)_6]^{4-}$, without repulsion of the negative charges.

Table 2. Absorption maximum wavelengths, average ν_{Cu-S} frequencies, EPR parameters, and midpoint redox potentials ($E_{midpoint}$) of wild-type and Tyr83 mutant PCs

Species	Wavelength[a] [nm]	ν_{Cu-S} [b] [cm^{-1}]	EPR parameters $g_{\|\|}$	$\|A_{\|\|}\|$	$E_{midpoint}$[c] [mV]
wild-type	597.5	418.4	2.242	6.2	344
Y83F	597.5	417.9	2.256	5.7	340
Y83S	595.5	420.6	2.239	6.5	325
Y83L	595.5	423.3	2.238	6.5	320
Y83H	598.0	417.0	2.246	6.2	382

[a] Error ±0.5 nm. – [b] Error ±1.0 cm^{-1} for the calculated values. – [c] Versus NHE; measured in 10 mM phosphate buffer, pH = 7.4; error ±5 mV.

2.2 Relationship Between the Spectroscopic and Electrochemical Properties of PC

PC is classified as a type 1 copper protein and contains one copper atom, which is coordinated to two histidine nitrogen atoms, one methionine sulfur atom, and one cysteine sulfur atom in a distorted tetrahedral geometry according to the crystal structures of its oxidized and reduced forms.[1,2,29,30] Oxidized type 1 copper proteins exhibit unique spectroscopic properties, such as a low energy ligand-to-metal charge transfer (LMCT) band near 600 nm in the absorption spectrum and a narrow hyperfine coupling constant ($|A_{||}| < 90{\cdot}10^{-4}$ cm^{-1}) in the electron paramagnetic resonance (EPR) spectrum.[31,32]

Studies on the relationship between the structural and spectroscopic properties of type 1 copper proteins have been reported.[32–42] The general relationship will be discussed along with the properties of wild-type and Tyr83 mutant PCs (Table 2, Figure 4).[43] The spectroscopic and electrochemical properties of the wild-type and Tyr83 mutant PCs were similar but slightly different. In the absorption spectra, an intense band around 600 nm has been assigned to the cysteine thiolate [S(Cys)]-to-Cu^{II} LMCT band (Figure 5).[31,32] Y83H mutant PC which has a longer wavelength peak for this 600-nm band, exhibits an increase and decrease in the intensities of the 460- and 700-nm bands, respectively, compared with the corresponding bands of other PCs.[43] The ratio of the intensity of the 460-nm absorption band to that of the 600-nm band has been found to correlate with the rhombicity of the EPR signal for type 1 copper proteins,[35] and the rhombic distortion has been suggested to be associated with a stronger binding of the axial ligand and a concomitant shift from a trigonal planar toward a tetrahedrally distorted Cu site geometry.[35–37] Actually, wild-type PC which showed a stronger 460-nm absorption band than that of the Y83S or Y83 L proteins, exhibited a more rhombic EPR spectrum with larger $g_{||}$ and smaller $|A_{||}|$ values (Table 2), suggesting a more tetrahedral geometry for the Cu^{II} site.

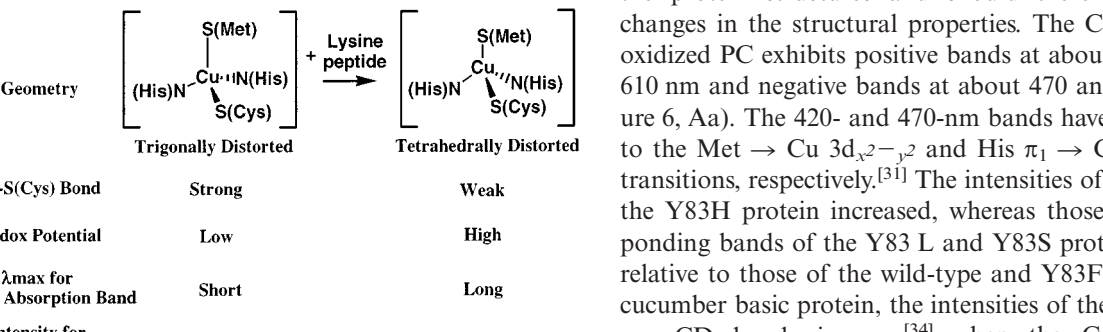


Figure 4. Relationship among the spectroscopic and electrochemical properties and the Cu active site geometry of oxidized PC

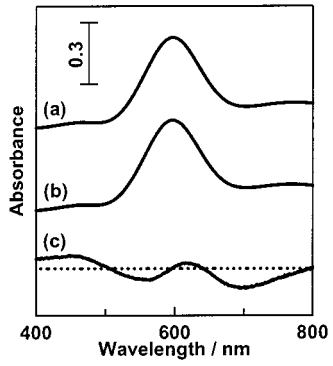


Figure 5. Absorption spectra of oxidized PC with (a) and without *penta*-Lys (b) and their difference spectrum (a – b) multiplied by 20 (c); the baseline of the difference spectrum is shown as a dotted line; concentrations of PC and *penta*-Lys were 100 and 330 μM, respectively; sodium phosphate buffer (10 mM), pH = 7.4, was used; modified from ref.[53]

The circular dichroism (CD) spectra are very sensitive to the protein structures and should therefore reflect the changes in the structural properties. The CD spectrum of oxidized PC exhibits positive bands at about 420, 560, and 610 nm and negative bands at about 470 and 760 nm (Figure 6, Aa). The 420- and 470-nm bands have been assigned to the Met → Cu $3d_{x^2-y^2}$ and His π_1 → Cu $3d_{x^2-y^2}$ CT transitions, respectively.[31] The intensities of these bands of the Y83H protein increased, whereas those of the corresponding bands of the Y83 L and Y83S proteins decreased, relative to those of the wild-type and Y83F proteins.[43] In cucumber basic protein, the intensities of the 420- and 470-nm CD bands increase[34] when the Cu–S(Cys) and Cu–S(Met) bond lengths are extended and shortened by 0.1 and 0.2 Å, respectively,[44] and the Cu–S(Cys) stretching (ν_{Cu-S}) frequency is lower [45] then that in PC.

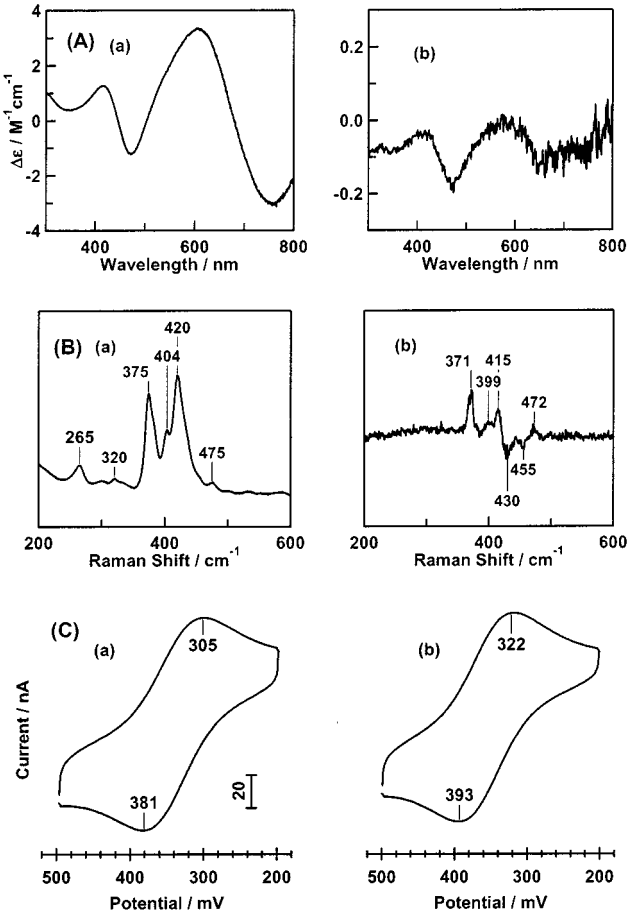


Figure 6. (A) CD spectra of oxidized PC without *tetra*-Lys (a) and the difference CD spectrum obtained by subtracting the CD spectrum from that with *tetra*-Lys (600 μM) (b); (B) RR spectra in the 200−600 cm^{-1} region for oxidized PC without *penta*-Lys (a) and the difference RR spectrum obtained by subtracting the RR spectrum from that with *penta*-Lys (660 μM) (b); (C) cyclic voltammograms of PC without (a) and with *penta*-Lys (260 μM) (b); concentration of PC was 70, 500, and 100 μM for A, B, and C, respectively; sodium phosphate buffer (10 mM), pH = 7.4, was used; modified from refs.[27,43]

Resonance Raman (RR) spectroscopy is a powerful method for investigating the character of the Cu−S(Cys) bond in type 1 copper proteins by exciting the Raman scattering near the S(Cys)-to-Cu^{II} CT band.[40,46−49] The ν_{Cu-S} frequency is shown to be a sensitive probe of the Cu−S(Cys) bond strength and copper coordination geometry for proteins with cysteine thiolate coordination.[39−42] It is closely related to the electron-transfer exothermicity for several type 1 copper proteins.[38] In practice, several bands are observed at 374−475 cm^{-1} for the RR spectrum of oxidized PC excited near its S(Cys)-to-Cu^{II} CT band (Figure 6, Ba), which is mainly due to the mixing of the Cu−S stretch with several angle bendings of the coordinated Cys side chain.[40,46−49] The weighted average frequency based on the S-isotope dependence was shown to correlate with the Cu−S(Cys) distance for azurin, another type 1 copper protein, and its mutants.[39] The average ν_{Cu-S} frequencies of wild-type and Tyr83 mutant PCs were therefore calculated from the intensities and frequencies of three major RR bands, which mainly contain contributions from the ν_{Cu-S} (Table 2).[50] The average ν_{Cu-S} frequency was in the order Y83 L > Y83S > Y83F ≈ wild-type > Y83H, suggesting a stronger Cu−S(Cys) bond in this order. Species exhibiting a more intense 460-nm absorption band exhibits a smaller average ν_{Cu-S}. A similar relationship has also been found by comparison of the properties of type 1 and type 2 cystein-coordinated copper proteins.[40]

As regards the redox potential, Y83F mutant PC exhibited a potential similar to that of wild-type PC, whereas the potentials of Y83S and Y83 L PCs shifted to lower values and the potential of Y83H PC shifted to a higher value relative to that of wild-type PC, where species with a higher ν_{Cu-S} frequency and a stronger Cu−S(Cys) bond exhibited a lower redox potential (Table 2).

The spectroscopic and electrochemical properties of PC may be summarized as follows. Species exhibiting a more intense 460-nm absorption band and a longer wavelength for the 600-nm absorption band show a more tetrahedral geometry for the Cu site, a smaller average ν_{Cu-S} frequency, and a higher redox potential. A weaker Cu−S(Cys) bond would create a weaker ligand field with the Cys ligand, and thus the energy of the S(Cys)-to-Cu^{II} CT band would decrease and the redox potential of Cu would shift to a higher value. In PC, the Cu−S(Met) bond is the longest metal−ligand bond,[1,2] and a stronger Cu−S(Met) axial bond has been suggested to cause a larger displacement of the Cu^{II} from the trigonal plane containing two histidine nitrogen atoms and one cysteine sulfur atom, shifting the active site coordination structure toward a tetrahedral geometry.[35] Comparison between several type 1 copper proteins showed that proteins with a higher energy ligand field associated with rotation of the Cu $d_{x^2-y^2}$ half-filled HOMO exhibit longer Cu−S(Cys) and shorter Cu−S(Met) bonds with a more tetrahedral geometry.[34,35] A weaker Cu−S(Cys) bond would therefore induce a structural change with a shorter Cu−S(Met) bond and a more tetrahedral Cu^{II} geometry, which has been detected by comparison of the Y83H protein with the Y83S and Y83 L proteins (Table 2).

2.3 Active Site Structural Change Induced by Interaction with Lysine Peptide

Redox-induced conformational changes in PC have been studied by infrared spectroscopy, and protonation of (an) Asp or Glu side chain(s) upon PC oxidation, at acidic pH, has been indicated.[51] Recently, Ag- and Cd-substituted PC were used as models for native Cu^{I} and Cu^{II} PCs, respectively, and the metal site structure depending on the charge of the metal ion has been indicated to influence the dissociation between PC and photosystem I.[52] Lysine peptides are very useful for investigating the molecular interaction-induced structural changes of metalloproteins having absorption bands in the visible region, since the peptides do not have any absorptions in the visible region. In this section, we focus on the active site structural changes of oxid-

ized PC by interaction with lysine peptide at its negative patch (Figure 3).

The interaction of PC with lysine peptides was investigated by absorption, CD, and RR spectroscopy, and electrochemical measurements, which revealed changes in the active site Cu coordination geometry upon binding of a lysine peptide at the negative patch.[27,43,53] In the difference absorption spectrum between the spectra with and without *penta*-Lys, peaks and troughs were detected at about 460 and 630 nm and at about 560 and 700 nm, respectively (Figure 5, c). These peaks revealed that the 600-nm absorption band shifts to a longer wavelength and the intensities of the 460- and 700-nm absorption bands increase and decrease, respectively, on interaction with a lysine peptide. As discussed in section 2.2, the intensity increase of the 460-nm absorption band and the shift of the 600-nm absorption band to a longer wavelength can be associated with the lengthening of the Cu−S(Cys) bond and the change from a trigonal planar toward a more tetrahedral Cu site geometry.[35−37] The Cu−S(Cys) bond should therefore be elongated and the Cu site should become more tetrahedral on interaction with the lysine peptide. These structural changes at the Cu site are caused by an electrostatic interaction of the PC negative patch with the lysine peptide, since the intensities of the peaks observed in the difference spectra decreased when NaCl was added to the solution. Moreover, the observed spectral changes decreased when the charges at the negative patch were decreased by site-directed mutagenesis.[27]

The intensities of the peaks and troughs in the difference spectra increased with the concentration of the lysine peptide and became saturated at a certain peptide concentration (Figure 7).[53] The observed intensity changes in the absorption spectra can be interpreted by considering the formation of a 1:1 PC·lysine peptide complex according to Equation (2). If we write the difference between the observed absorption changes at 630 and 560 nm upon lysine peptide binding as ΔΔAbs, the difference between the corresponding absorption coefficient changes as ΔΔε, the concentrations of PC and lysine peptide as [PC] and [lysine peptide], respectively, and the cell length used for the measurements as l, we obtain the relationship of Equation (4).

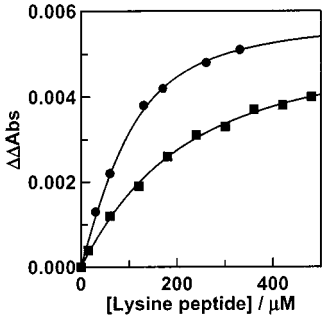


Figure 7. Plots of ΔΔAbs vs. [lysine peptide], together with least-squares fitted lines according to Equation (4); ■ = *tetra*- and • = *penta*-Lys; concentration of PC was 100 μM; modified from ref.[43]

$$\Delta\Delta Abs = \frac{\Delta\Delta\varepsilon \times l}{2}\left\{\left([PC] + [\text{lysine peptide}] + \frac{1}{K_i}\right) - \left[\left([PC] + [\text{lysine peptide}] + \frac{1}{K_i}\right)^2 - 4\,[PC]\,[\text{lysine peptide}]\right]^{1/2}\right\} \quad (4)$$

The K_i values (8000 ± 4000 and 24000 ± 5000 M^{-1} for *tetra*- and *penta*-Lys, respectively) were obtained by least-squares fitting of the experimental data with Equation (4) (Figure 7). Since the fitting was successful, it is reasonable to assume that only one lysine peptide is bound to the negative patch of PC, which is in agreement with the previous conclusion derived from the inhibitory character of lysine peptide on electron transfer from cyt *c* or cyt *f* to PC (see section 2.1).[27,54] The association constants between PC and lysine peptide obtained from the difference absorption spectra were larger than those obtained from electron transfer experiments,[27] which is probably due to NaCl added to the solution to make the electron transfer rate detectable. Absorption spectral studies with various lysine peptides showed that PC interacts effectively only with lysine peptides equal to or longer than *tetra*-Lys, due to the increase in the charges and stronger electrostatic interaction, where a similar conclusion was drawn from the inhibitory effect on electron transfer (see section 2.1). In connection with the absorption change by lysine peptide binding, the association constants between PC and cyt *f* or cyt *c* have been obtained by measuring the increase of the Soret band intensity of cyt *f* or cyt *c* on PC binding.[55−57]

The difference CD spectra of oxidized wild-type and Tyr83 mutant PCs in the presence and absence of *tetra*-Lys are shown in Figure 6, Ab. A positive peak at about 420 nm and a negative peak at about 470 nm were detected in the difference spectra, which demonstrate changes in their CD spectra and thus in the active site structure on interaction with *tetra*-Lys. Since the intensities of the 420- and 470-nm CD bands increase, the Cu−S(Cys) and Cu−S(Met) bond lengths should become longer and shorter, respectively, for PC by interaction with lysine peptides (see section 2.2). When *tetra*-Lys was added to PC, the Raman bands at 375−475 cm^{-1} shifted slightly to lower frequencies with a concomitant slight increase in the intensities of some lower frequency bands in this region, especially the ν_{Cu-S} related 375- and 422-cm^{-1} bands. These results indicate that the Cu−S(Cys) bond was weakened on addition of a lysine peptide. Interestingly, the RR spectral changes of PC were the same for PC−cyt *c* and PC−lysine peptide interactions, indicating that lysine peptides are excellent models for the PC recognition site of proteins. However, ESR spectroscopic measurements on oxidized PC gave no significant change on the Cu^{2+} signal upon addition of lysine peptides.[27,43] The lack of EPR spectroscopic detection of the structural change of PC on addition of lysine peptides could be due to the fact that EPR spectroscopy is more sensitive to the Cu−N bond geometry, while the 375−475-cm^{-1} RR bands are related to the Cu-coordinated Cys residue. In line with this, the 265-cm^{-1} RR band that is as-

signed to the Cu−His stretching mode did not show any change on interaction with lysine peptides.

Electrochemical measurements showed that the redox potential of PC shifted to a higher potential upon lysine peptide binding, which suggests that the structural change induced by binding of lysine peptides to PC adapts the copper site for facile electron transfer (Figure 6, C). This structural change might be related to the report by Kostić et al., who proposed that PC and cyt *c* or cyt *f* bind and react with each other in different configurations resulting from the protein−protein interaction termed as the gating process for electron transfer,[7−9,58] showing possible configurations for the diprotein complex by computer simulation.[10,11]

For the electron transfer from cyt *f* to PC, Tyr83 which is located near the negative patch of PC has been proposed to be involved in the electron transfer pathway, since the reaction rate decreased upon replacement of Tyr with other amino acid residues.[56,57] Ullmann et al. demonstrated by molecular dynamics the possible occurrence of a cation−π interaction at the Tyr83 site, which might be important for electron transfer from cyt *f* to PC.[11] On the other hand, Tyr83 mutants showed minor effects on the interaction between PC and PSI, and electron transfer from PC to $P700^+$ was proposed to occur at the hydrophobic patch.[59−61] Recently, electron transfer from cyt *f* to PC was inferred by NMR spectroscopic measurements to occur via His87 of the hydrophobic patch.[13] Since the structural changes observed for wild-type and Tyr83 mutant PCs by complex formation with lysine peptides were similar,[43] the structural change due to binding of lysine peptides to PC is concluded not to be transmitted through the path of Tyr83−Cys84−copper by a cation−π interaction.

In summary, when wild-type PC interacted with lysine peptide, the intensity of the 460-nm absorption band increased, the maximum wavelength for the 600-nm absorption band became longer, the average ν_{Cu-S} frequency became lower, and the redox potential shifted to a higher value. All these changes may be associated with a slightly more tetrahedral geometry for the Cu site, further supporting the proposal that PC suffers a structural change on interaction with its redox partner, cyt *f*, for facile electron transfer from it.

3. Other Protein−Charged Peptide Interactions

3.1 Interaction of Cyt *f* and Cyt *c* with Aspartic Acid Peptides

Positively charged aspartic acid peptides up to *penta*-Asp (Figure 1) served as competitive inhibitors of electron transfer from cyt *c* or cyt *f* to PC.[54] *Tetra*- and *penta*-Asp also served as competitive inhibitors of electron transfer from $[Fe(CN)_6]^{4-}$ to oxidized cyt *c*.[28] A cyt *c*/*f*·aspartic acid peptide complex is formed by the electrostatic interaction between the aspartic acid peptides and the Lys residues at the surface of cyt *c* or cyt *f*, and the electron-transfer inhibitory effects of aspartic acid peptides are ascribed to formation of the cyt *c*/*f*·aspartic acid peptide complex, similar to that of the PC·lysine peptide complex.

Changes in the absorption spectrum of cyt *c* in the Soret region were detected when aspartic acid peptides, up to *penta*-Asp, were added to the cyt *c* solution (Figure 8, A).[54] These changes were the same as those observed when cyt *c* interacted with PC, indicating that aspartic acid peptides interacted with cyt *c* in the same way as PC. Conformational changes of cyt *c* due to interaction with aspartic acid peptides observed by RR spectroscopy (Figure 8, B) were also similar to those reported for cyt *c* bound to its native partner, cytochrome *c* oxidase,[62] which indicates that aspartic acid peptides interact with cyt *c* in the same way as cytochrome *c* oxidase and serve as good models for the protein interacting sites. Electrochemical measurements showed that the redox potential of cyt *c* and cyt *f* shifts to lower potentials by 7−20 mV upon aspartic acid peptide binding, indicating the enhancement in the electron donor ability of both cyt *c* and cyt *f* upon complex formation with the aspartic acid peptide.[54] The changes in the absorption spectrum and redox potential increased with the length and concentration of the aspartic acid peptides used. The observed structural and redox changes of cyt *c* and cyt *f* are therefore attributed to adduct formations with aspartic acid peptides by electrostatic interactions.

The redox potential of silene PC and *Brassica komatsuna* cyt *f* are 343 and 364 mV, respectively, when no charged

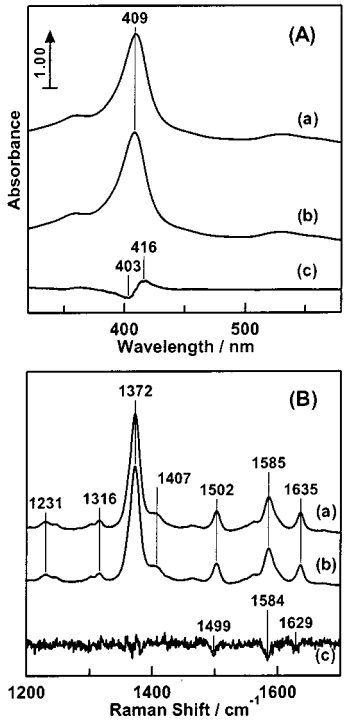


Figure 8. (A) Absorption spectra of oxidized cyt *c* with (a) and without *tetra*-Asp (b) and their difference spectrum (spectrum a − spectrum b) multiplied by 30 (c); (B) resonance Raman spectra in the 1200−1700 cm^{-1} region for cyt *c* with (a) and without *penta*-Asp (b) and their difference spectrum (spectrum a − spectrum b) multiplied by 15 (c); concentration of cyt *c* was 100 and 50 μM for A and B, respectively; Tris-HCl buffer (10 mM), pH = 7.4, was used; modified from ref.[54]

peptides are bound to them,[28,54] and considering their redox potentials, it appears unlikely that efficient electron transfer would occur between these proteins. Our observation that the redox potential of PC shifts to a higher potential and that of cyt *f* to a lower one, upon binding of charged peptides, suggests that redox potential shifts due to complex formation facilitate electron transfer between these proteins of non-physiological interaction and could also facilitate it between their physiological partners.

3.2 Cyt *c*−Cytochrome *c* Peroxidase System

The structural change of oxidized C*c*P due to interaction with lysine peptides was studied by measurements on electron transfer between reduced cyt *c* and C*c*P^{IV}=O (Trp191$^{\cdot+}$) and the absorption spectra in the presence of lysine peptides.[63] Lysine peptides competitively inhibited electron transfer from cyt *c* to C*c*P, which indicated that they interacted with C*c*P at the same site where cyt *c* binds and would be effective models for the C*c*P interacting site of cyt *c*.

Peaks were observed in the difference absorption spectrum of C*c*P between the spectra in the presence and absence of lysine peptides, demonstrating a structural perturbation of C*c*P, at least at its heme site, also occurs on interaction with the lysine peptides (Figure 9). This interaction was also proved to be electrostatic, since no significant peak was detected in the difference absorption spectrum measured in the presence of NaCl.

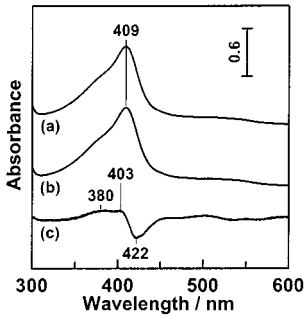


Figure 9. Absorption spectra of oxidized C*c*P with (a) and without *tetra*-Lys (b) and their difference spectrum multiplied by 20 (c); concentrations of C*c*P and *tetra*-Lys were 10 and 100 μM, respectively; Tris-HCl buffer (10 mM), pH = 7.8, was used; modified from ref.[63]

4. Conclusion

We propose that charged peptides are useful for studying the molecular recognition character of proteins and their structural changes induced by the molecular interaction. In particular, charged peptides which are good models of the protein interaction sites could be used for investigating the molecular interaction induced structural changes of metalloproteins which have absorption bands in the visible region, since the peptides have no visible absorption.

Positively charged lysine peptides inhibited electron transfer from reduced cyt *f* or cyt *c* to oxidized PC. Likewise, aspartic acid peptides served as competitive inhibitors of electron transfer from cyt *c* or cyt *f* to PC. They also served as inhibitors of electron transfer from $[Fe(CN)_6]^{4-}$ to oxidized cyt *c*. These inhibitory characters are ascribable to the formation of protein−peptide complexes which inhibit formation of the electron transfer complexes.

Changes in the absorption spectrum which were observed on addition of lysine peptides to the PC solution have been ascribed by RR spectroscopic studies to the change in the active site Cu−Cys geometry upon binding of lysine peptide to the PC negative patch. Similar absorption changes were observed for the PC−cyt *c* interaction, demonstrating that cyt *c* and lysine peptides interact with PC in a similar way. Electrochemical measurements showed that the redox potential of PC shifts to a higher potential upon lysine peptide binding, which suggested that lysine peptides induce a structural change in PC to adapt the copper site for facile electron transfer.

Changes in the absorption spectrum of cyt *c* in the Soret region were also detected when aspartic acid peptides were added to the cyt *c* solution. These changes were similar to those observed when cyt *c* interacted with PC, indicating that PC and aspartic acid peptides interact with cyt *c* in a similar way. Conformational changes of cyt *c* due to interaction with aspartic acid peptides observed in the RR spectra were also similar to those reported for cyt *c* when bound with its native partner, cytochrome *c* oxidase. Electrochemical measurements showed that the redox potential of cyt *c* and cyt *f* shifted to lower potentials upon aspartic acid peptide binding, showing the enhancement in the electron donor ability of both cyt *c* and cyt *f* upon complex formation with the aspartic acid peptide.

Acknowledgments

We are deeply indebted to the co-workers whose names are listed in the papers cited in the references for their contribution and enthusiasm. The support of the research by the Ministry of Education, Culture, Sports, Science and Technology of Japan is gratefully acknowledged.

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Received April 12, 2001
[I01132]