pH-Dependent surface-enhanced resonance Raman scattering of yeast iso-1-cytochrome c adsorbed on silver nanoparticle surfaces under denaturing conditions at pH < 3

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INTRODUCTION

Conformational changes in proteins are important in understanding biological interactions (1, 2). Cytochrome c regulates mitochondrial electron transfer activity, apoptosis, and biological activity (1-6). A conformational transition between β-sheet aggregation and disordered structure occurs in cytochrome c (7). In the ferric form, the heme iron is axially coordinated by two internal ligands, histidine and methionine, leading to a six-coordinated low spin (6cLS) configuration (8). Yeast iso-1-cytochrome c isolated from Saccharomyces cerevisiae is a soluble 12.6 kDa monoheme protein (9, 10). In the native state of yeast iso-1-cytochrome c, the heme group in the protein is covalently attached to the His 18 and Met 80 residue. During protein unfolding, the methionine ligand is dissociated from the heme and replaced by the other histidine group or water molecules, leading to a high spin state (9-11). Yeast iso-1-cytochrome c can be covalently bound to the metal surface by a thiol group in the cysteine residue (12-14). Yeast iso-1-cytochrome c bound on gold nanoparticles can be a colorimetric sensor because it unfolds at low pH and refolds at high pH 8 (15).

Silver nanoparticles have attracted much attention in the past decade due to their stability and optical properties (16). Biological applications focus on the effect of size, shape, biocompatibility, uptake, and sub-cellular distribution of silver nanoparticles. Colloidal silver nanoparticles are popular platforms for surface enhanced Raman scattering (SERS) (17).

SERS is an ultra-sensitive spectroscopic tool for interface studies as chemical sensors in biophysical chemistry (18). Chemically-specific information is provided by unique vibrational modes of target adsorbates, which depends on the metal substrates (19-23). However, the detailed adsorption characteristics on metal surfaces have not been fully clarified. In this study, we examined the adsorption behaviors of yeast iso-1-cytochrome c on Ag nanoparticle surfaces using SERS to better understand the pH-induced conformational changes on metal surfaces.

RESULTS

UV-Vis absorbance spectra

Fig. 1A shows the pH-induced UV-Vis absorbance spectra of ~10^-5 M yeast iso-1-cytochrome c in aqueous solution. The Soret and Q transitions bands were found at 410 and 523-550 nm, respectively. The arrows indicate the excitation wavelengths at 457.9 nm for Ag SERRS experiments. Our excitation wavelength lies between the Soret and Q band. Fig. 1B shows UV-Vis absorbance spectra of citrate-reduced Ag nanoparticles. The excitation wavelengths at 457.9 nm should lie close to the surface plasmon resonance band of the silver nanoparticles, which may produce a strong enhancement in the SERRS experiments.

Resonance Raman spectra of yeast iso-1-cytochrome c

Fig. 2 shows Raman spectra of yeast iso-1-cytochrome c in the
Fig. 1. (A) pH-induced UV-Vis absorbance spectra of \(\sim 10^{-5} \text{ M yeast iso-1-cytochrome c}\) in aqueous solution. The Soret and Q bands were found at 410 and 523–550 nm, respectively. Arrows indicate the excitation wavelengths at 457.9 nm for Ag SERRS experiments. (B) UV-Vis absorbance spectral change in citrate reduced-Ag colloidal nanoparticles. The arrows indicate the excitation wavelengths at 457.9 nm for Ag SERRS experiments.

Fig. 2. (A) Resonance Raman (RR) spectra of \(\sim 10^{-3} \text{ M yeast iso-1-cytochrome c}\) in distilled water upon irradiation using 457.9 nm at pH 2.8–11.5 in the spectral region between 300 and 1,800 cm\(^{-1}\). (B) An expanded view of RR spectra of \(\sim 10^{-3} \text{ M yeast iso-1-cytochrome c}\) between 1,500 and 1,700 cm\(^{-1}\).

Fig. 3. (A) SERRS spectra of \(\sim 10^{-4} \text{ M yeast iso-1-cytochrome c}\) on Ag nanoparticle surfaces upon irradiation using 457.9 nm at pH 2.3–11.3 in the spectral region between 300 and 1,800 cm\(^{-1}\). (B) An expanded view of SERRS spectra of \(\sim 10^{-4} \text{ M yeast iso-1-cytochrome c}\) region between 1,500 and 1,700 cm\(^{-1}\).
**DISCUSSION**

The pH-induced structural changes of yeast iso-1-cytochrome c on silver nanoparticle surfaces were investigated using SERS. At pH below ~3, the methionine or histidine ligand in yeast iso-1-cytochrome c dissociates, causing a marked change in the conformation of the molecule. At neutral alkaline potentials, was used as a reducing agent (27). A portion of AgNO₃ (~90 procedures reported in the literature, wherein sodium citrate Colloidal silver nanoparticles were prepared according to the purchased from Sigma and used without further purification.

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Yeast iso-1-cytochrome c from Saccharomyces cerevisiae was purchased from Sigma and used without further purification. Colloidal silver nanoparticles were prepared according to the procedures reported in the literature, wherein sodium citrate was used as a reducing agent (27). A portion of AgNO₃ (~90 mg) was dissolved in ~500 mL of distilled water, brought to boiling, and a solution of ~1% sodium citrate (10 mL) was added and boiled for ca. 1 h. All chemicals used were reagent-grade unless otherwise specified. Triply-distilled water of resistivity greater than 18.0 MΩ · cm was used in making aqueous solutions.

Table 1. Spectral data and vibrational assignments for yeast iso-1-cytochrome

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aResonance Raman measurements at pH ~5.7. bBased on refs. 11, 24, and 25.

**REFERENCES**


