BMB
reports

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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We measured the pH-induced spectral changes of yeast *iso*-1-cytochrome *c* on silver nanoparticle surfaces using surface-enhanced resonance Raman scattering (SERRS) at 457.9 nm. At a pH of ~3, the Met80 ligand in yeast *iso*-1-cytochrome *c* is assumed to dissociate, leading to a marked conformational change as evidenced by the vibrational spectral shifts. The Soret band at ~410 nm in the UV-Vis spectrum shifted to ~396 nm at pH~3, indicating a transition from a low spin state to a high spin state from a weak interaction with a water molecule. Thus, SERRS spectroscopy can measure the pH-induced denaturalization of cyt *c* adsorbed on metal nanoparticle surfaces at a lower concentration with a better sensitivity than ordinary resonance Raman spectroscopy. [BMB reports 2009; 42(4): 223-226]

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 from, 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

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Received 6 November 2008, Accepted 16 December 2008

Keywords: Ag nanoparticles, pH change, SERRS, UV-Vis absorption, Yeast *iso*-1-cytochrome *c*

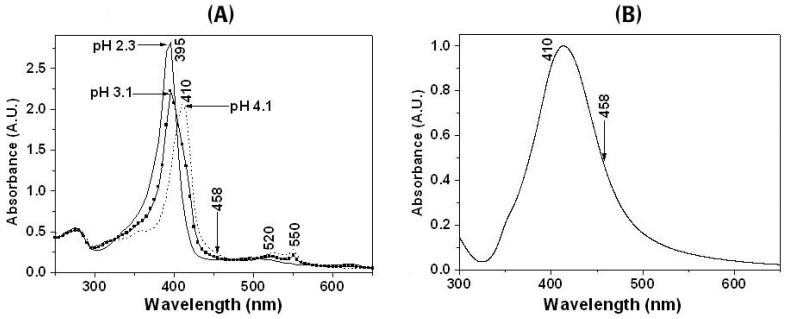

Fig. 1. (A) pH-induced UV-Vis absorbance spectra of ~10^{-5} M yeast *iso*-1-cytochrome *c* in aqueous solution. The Soret and Q bands were found at 410 and 523~550 nm, respectively. Arrows indicate the excitation wavelengths at 457.9 nm for Ag SERRS experiments. (B) UV-Vis absorbance spectral change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

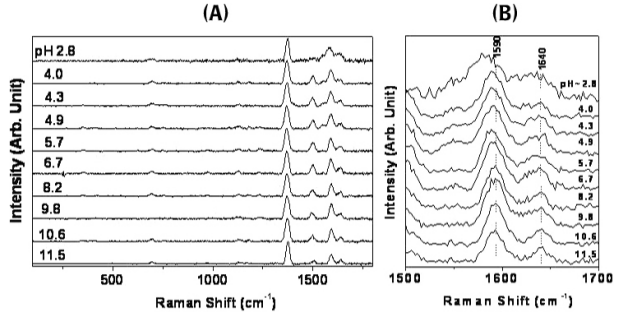

Fig. 2. (A) Resonance Raman (RR) spectra of ~10^{-3} M yeast *iso*-1-cytochrome *c* in distilled water upon irradiation using 457.9 nm at pH 2.8~11.5 in the spectral region between 300 and 1,800 cm^{-1}. (B) An expanded view of RR spectra of ~10^{-3} M yeast *iso*-1-cytochrome *c* between 1,500 and 1,700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

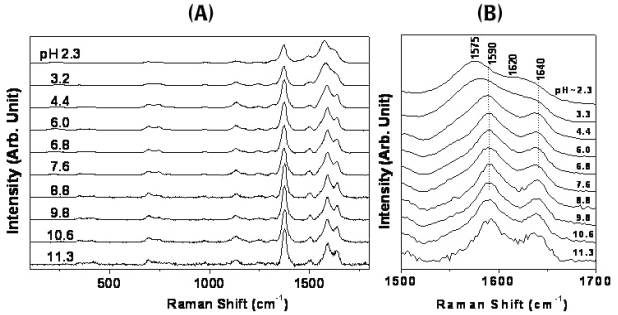

Fig. 3. (A) SERRS spectra of ~10^{-4} M yeast *iso*-1-cytochrome *c* on Ag nanoparticle surfaces upon irradiation using 457.9 nm at pH 2.3~11.3 in the spectral region between 300 and 1,800 cm^{-1}. (B) An expanded view of SERRS spectra of ~10^{-4} M yeast *iso*-1-cytochrome *c* region between 1,500 and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

spectral region between 300 and 1,800 cm^{-1} at the excitation wavelength of 457.9 nm. The spectral features appeared to depend on the excitation wavelengths of 457.9, 514.5, and 632.8 nm, presumably due to the different resonance enhancements for a specific vibrational band (data not shown; 11). Fig. 2 also compares resonance Raman (RR) spectra of ~10^{-3} M yeast *iso*-1-cytochrome c at pH values of 2.8~11.5 in aqueous solutions. We analyzed the Raman spectrum referring to earlier vibrational assignments described in the literature (11, 24, 25). In the RR spectrum, the spectral positions did not change significantly by varying pH above pH 3. An expanded view for the ν_{19} (or ν_{11}) and ν_{10} bands in the 1,500~1,700 cm^{-1} region is reproduced in Fig. 2B for a better comparison.

Surface-enhanced resonance Raman spectra of yeast *iso*-1-cytochrome *c* on Ag nanoparticle surfaces

Fig. 3 compares surface-enhanced resonance Raman scattering (SERRS) spectra of ~10^{-4} M yeast *iso*-1-cytochrome *c* on Ag nanoparticles at pH values of 2.3~11.3 in aqueous solutions. The SERRS spectra at ~10^{-4} M in Fig. 3 appeared to be more enhanced than those at ~10^{-3} M in the resonance Raman (RR) spectrum of Fig. 2. The bandwidths were broadened after adsorption on Ag, indicating an interaction between Ag nanoparticles and yeast *iso*-1-cytochrome *c*. At neutral alkaline pH values, the marker bands ν_4, ν_3, ν_{19} (or ν_{11}), and ν_{10} were observed at 1,375, 1,498, 1,592, and 1,640 cm^{-1}, respectively. These bands were red-shifted to 1,372, 1,489, 1,571, and 1,620 cm^{-1}, respectively, by lowering pH below ~3, suggesting a structural change (Table 1). An expanded view of the ν_{19} (or ν_{11}) and ν_{10} bands in the 1,500~1,700 cm^{-1} region is reproduced in Fig. 3B for a better comparison.

The Raman enhancements on Au nanoparticles were smaller than on Ag nanoparticles (data not shown). Raman spectra on Au nanoparticles were examined at pH values of 2.8~10.5. The marker bands ν_{11} and ν_{10} were observed at 1,550 and 1,625 cm^{-1}, respectively. These bands were red-shifted to 1,545 and 1,610 cm^{-1}, respectively, by lowering pH. Au nanoparticles showed a conformational change at pH~6, higher than Ag nanoparticles at pH~3 (15), suggesting greater stability when the protein is adsorbed to Ag nanoparticles (26). As nanoparticles are added to yeast *iso*-1-cytochrome *c* solution, the peak starts to shift at higher pH, indicating protein destabilization. Thus, SERRS and SERS can measure the pH-induced conformational changes of yeast *iso*-1-cytochrome *c* adsorbed on metal nanoparticle surfaces at a lower concentration with a better sensitivity.

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

RR[a]	Ag SERRS at each pH										Tentative assignment[b]
	2.3	3.2	4.4	6.0	6.8	7.6	8.8	9.8	10.6	11.3	
	234	241	236	228	231	226					Ag-S
354	345	349	349	352	348	350	349	352	349	346	ν_{51} δ $(C_\beta\text{-}C_1)_{asym}$
		413		408	415	418		416	412	418	ν_4 δ $(C_\beta\text{-}C_c\text{-}C_d)$
696	692	695	696	696	695	693	694	694	695	693	ν (C-S)
753	754	746	747	746	747	745	741	745	744		ν_{15} (pyr breathing)
	978	977	973	975	975		968		971	978	δ_{46} $(\text{pyr deform})_{asym}$
1,133	1,132	1,132	1,133	1,133	1,128	1,130	1,133	1,129	1,132	1,133	ν_{22} $(\text{pyr half-ring})_{asym}$
1,181				1,172	1,174	1,170		1,175			ν_{30} $(\text{pyr half-ring})_{sym}$
1,235	1,243	1,245	1,246	1,245	1,243	1,247	1,252	1,246	1,249		δ_{13} $(C_m\text{-}H)$
			1,321	1,315	1,315	1,315	1,315	1,315	1,318		δ_{22} $(C_m\text{-}H)$
1,370	1,372	1,372	1,374	1,375	1,373	1,372	1,375	1,375	1,377	1,375	ν_4 $(\text{pyr half-ring})_{sym}$
1,502	1,493	1,500	1,501	1,500	1,499	1,499	1,502	1,503	1,503	1,507	ν_3 ν $(C_\alpha\text{-}C_m)_{sym}$
1,592	1,577	1,580	1,589	1,591	1,591	1,591	1,591	1,590	1,591	1,593	ν_{11} ν (C-C)
											ν_{19} ν $(C_\alpha\text{-}C_m)_{asym}$
1,638	1,618	1,620	1,637	1,638	1,638	1,638	1,640	1,638	1,638	1,635	ν_{10} ν $(C_\alpha\text{-}C_m)_{asym}$

[a]Resonance Raman measurements at pH ~5.7. [b]Based on refs. 11,24, and 25.

DISCUSSION

The pH-induced structural changes of yeast *iso*-1-cytochrome *c* on silver nanoparticle surfaces were investigated using SERRS. 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, the oxidation marker bands ν_4, ν_3, ν_{19} (or ν_{11}), and ν_{10} were observed at 1,375, 1,498, 1,592, and 1,640 cm^{-1}, respectively, indicating yeast *iso*-1-cytochrome *c* with a heme Fe^{3+} in a low-spin state. These bands were red shifted to 1,372, 1,489, 1,571, and 1,620 cm^{-1}, respectively, by lowering pH below 3, suggesting a rupture of the Met80 and His18 ligand of yeast iso-1-cytochrome with a high-spin state. This structural transition occurs at a pH of ~3 for silver nanoparticles.

MATERIALS AND METHODS

Sample preparation

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 $AgNO_3$ (~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.

Instrumental measurements

Raman spectra were obtained using a Renishaw Raman confocal system Model 1,000 spectrometer equipped with an integral microscope (Leica DM LM). The 457.9 irradiation was from a 50 mW air-cooled Ar ion laser (Melles Griot Model LAP 431) (28). The 632.8 nm radiation was from and a 35 mW air-cooled He-Ne laser (Melles Griot Model 25 LHP 928) with a plasma line rejection filter used as the excitation sources for the SERS experiments on Ag nanoparticles. UV-Vis absorbance spectra were obtained using a Mecasys Optizen 3220 spectrophotometer. The pH values of Ag nanoparticle solutions were measured using a Thermoelectron Orion 3 star bench top pH meter (29). pH control was performed by the addition of 1 M NaOH or 1 M HCl stock solutions. For low pH measurements, the 1 M HCl stock solution was diluted to 1-50 mM. A microdroplet of each HCl solution was added to the cytochrome *c*-adsorbed Ag nanoparticle solution, in a volume of ~300 μL, to lower the pH values. The pH was similarly increased by NaOH.

Acknowledgments

Sang-Woo Joo would like to thank Mr. Hyoung-Woo Choi for helping with experiments. This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2007-314-C00146). This work was supported by the Soongsil University Research Fund. This research was supported by the Nano R&D program through the Korea Science and Engineering Foundation funded by the Ministry of Education, Science and Technology (2008-04308).

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