International Journal of Biological Macromolecules 112 (2018) 591–597

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: https://www.journals.elsevier.com/ijbiomac

Acid-enhanced conformation changes of yeast cytochrome *c* coated onto gold nanoparticles, a FT-IR spectroscopic analysis

Aichun Dong *, Corina Brown, Shufeng Bai [1], Jian Dong [2]

Department of Chemistry and Biochemistry, University of Northern Colorado, Greeley, CO 80639, United States

ARTICLE INFO

Article history:
Received 4 January 2018
Received in revised form 20 January 2018
Accepted 30 January 2018
Available online 01 February 2018

Keywords:
Acid-enhanced conformational change
Cytochrome *c* coated gold nanoparticles
FT-IR spectroscopy

ABSTRACT

Under conditions with or without linker molecules, the effects of acidic pH on the conformation of yeast iso-1-cytochrome *c* coated onto gold nanoparticles (AuNPs) in correlation with color changes of a Cyt *c*-coated AuNPs solution/suspension were examined by Fourier transform infrared (FT-IR) spectroscopy and correlated to color change. The results of detailed secondary structural analysis revealed that although the color changes coincide with acid-induced conformational changes in Cyt *c* coated onto AuNPs, the pH-related conformational unfolding of Cyt *c* coated onto AuNPs differed dramatically from that of its counterpart in solution. For Cyt *c* free in solution, the acid-induced unfolding did not occur until the pH was below 3.0, whereas for Cyt *c* coated onto AuNPs via C102 coordination near the C-terminal, a partial unfolding was observed even at near neutral pH which continuously intensified as pH decreased. Insertion of a short alkanethiol (3-mercaptoproprionic acid, 3-MPA) molecule between Cyt *c* and AuNP, which changes the interaction mode from a thiol coordination between Cyt *c* and AuNP to an electrostatic interaction between Cyt *c* and 3-MPA, which stabilized the conformation of Cyt *c* significantly, but did not prevent the acid-induced aggregation of Cyt *c*-3MPA-AuNPs.

1. Introduction

Thiol or non-thiol proteins such as plasma proteins, enzymes and antibodies bound to gold and/or other metal nanoparticles to form protein corona are of great interest of researchers in the fields of nanotechnology and biopharmaceuticals, due to their potential applications in biosensory, diagnostic tools and drug delivery [1–5]. Among them, a potential biosensor for detecting acid-induced protein unfolding via pH-related color changes in a cytochrome *c* gold nanoparticles (AuNPs) model system has been reported [1]. Using Atomic Force Microscopy (AFM), confocal-laser-scanning microscopy (CFLSM), and transmission electron microscopy (TEM), Shukla and colleagues [6] studied the biocompatibility, cytotoxicity and immunogenic effect of AuNPs correlating with endocytotic uptake of the nanoparticles. Their results indicated that AuNPs are noncytotoxic and biocompatible to model macrophage cells and do not elicit secretion of proinflammatory cytokines TNF-α and IL1-β, making them suitable candidates for nanomedicine [6].

Despite a tremendous amount of effort focused on exploring the conformation of protein coated metal nanoparticles in recent years, the studies on the effects of binding to nanoparticles on the conformation of the proteins are limited to few model proteins [3,5,7]. The lack of studies in this area is due primarily to technical difficulties in protein conformational analysis under these conditions. For example, there is little direct evidence to correlate the color change of Cyt *c*-coated AuNPs solution/suspension to the conformational change of the protein. Several attempts by using FT-IR [3,4] and Circular Dichroism [7,8 and references cited therein] spectroscopies have been directed toward obtaining conformational information for proteins coated onto metal nanoparticles with some details. In view of the rapid development of protein immobilization technologies in areas of biosensors, cancer treatment, diagnostic tools and others, it is extremely important to understand how immobilization of protein molecules onto Au, Ag, or other metal nanoparticles would affect their conformation and stability.

In this study, we employed an experimental procedure that we had developed earlier [9] to precipitate/concentrate Cyt *c*-coated AuNPs and studied the acid-enhanced conformational changes using FT-IR spectroscopy, correlating the acid titration-induced color changes and detailed conformational changes of Cyt *c* coated onto AuNPs. Our results indicate that the use of acid-induced color change in Cyt *c*-coated AuNPs as a biosensor for detecting acid-induced protein unfolding [1]

Abbreviations: AFM, Atomic Force Microscopy; AuNPs, gold nanoparticles; Cyt *c*, cytochrome *c*; DTT, dithiothreitol; FT-IR spectroscopy, Fourier transform infrared spectroscopy; 3-MPA, 3-mercaptopropionic acid.

* Corresponding author.
E-mail address: aichun.dong@unco.edu (A. Dong).
[1] Current address: Alkermes Wilmington Inc., 265 Olinger Circle, Wilmington, OH 45177, United States.
[2] 2016 Summer Intern; current address: Dartmouth College, Hanover, NH 03755, United States.

https://doi.org/10.1016/j.ijbiomac.2018.01.202
0141-8130/

is unfeasible, because the phenomenon is artificially enhanced by the protein-AuNPs interaction, not a true acid-induced protein unfolding.

2. Materials and methods

2.1. Materials

Yeast iso-1-cytochrome *c* (*Saccharomyces cerevisiae*, C2436), Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$ 99.9%), DL-dithiothreitol (D0632), 3-MPA ($HOOC(CH_2)_2S$) and Brilliant blue R (C.I. 42,660; Coomassie Brilliant Blue R-250) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide (S318–500) was purchased from Fisher Scientific (Denver, CO). Hydrochloric acid (H0033) was purchased from Flinn Scientific (Batavia, IL). NuPAGE®12% Bis-Tris precast gel and electrophoresis kit were the products of Invitrogen (Thermo Fisher Scientific, USA). Anhydrous methyl alcohol and glacial acetic acid were purchased from Mallinckrodt Baker (Phillipsburg, NJ).

2.2. Preparation of Cyt c stock solution

Yeast Iso-1-Cyt *c* stock solution was prepared by dissolving 10 mg of lyophilized Cyt *c* powder into 0.5 ml of 10 mM potassium phosphate buffer, pH 7.2. The concentration of Cyt *c* was determined by an Agilent 8453A UV–Visible Spectrophotometer (Agilent Technologies, CA) using the extinction coefficient $\varepsilon_{410} = 106.1 \times 10^3\ M^{-1}\ cm^{-1}$ [10]. The Cyt *c* stock solution was temporarily stored in a 4 °C cold cabinet until use.

2.3. Gel electrophoresis

To assess the molecular state of commercial source yeast cytochrome *c*, PAGE gel electrophoresis was carried out using a NuPAGE®12% Bis-Tris precast gel (Thermo Fisher Scientific) and the protocol provided by the manufacturer. After electrophoresis, the NuPAGE®12% Bis-Tris precast gel was stained with Coomassie Brilliant Blue.

2.4. Preparation of gold nanoparticles

In this study, two different procedures were followed for preparing gold nanoparticles with diameters in ~13 and ~20 nm ranges using hydrogen tetrachloroaurate (III) trihydrate and sodium citrate respectively, as described by Keating et al. [11] and by Handley [12]. The particle sizes of AuNPs were verified by UV–Vis spectroscopy with absorbance maxima (λ_{max}) at 518 and 525 nm (Fig. 1) respectively, which were consistent with the reported values [10,11]. Only the AuNPs solution with UV–Vis absorption maxima (λ_{max}) at 525 nm (~20 nm in size) w ere used for Cyt *c*-coated AuNPs studies.

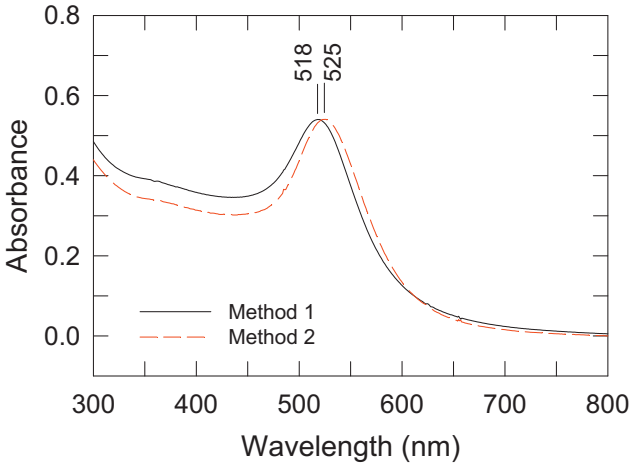

Fig. 1. The UV–Vis spectra of the gold nanoparticles preparations. The methods were described in Materials and methods. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.5. Atomic Force Microscopy

We also recorded an AFM image of bare gold nanoparticles placed on a clean mica plate after proper dilution with deionized water and dried in a desiccator (Fig. 2). The AFM image was recorded using a Veeco Nanoscope IIIa SPM Atomic Force Microscope (Bruker, CA) in tapping mode.

2.6. Preparation of Cyt c coated gold nanoparticles

A 3.5 μM concentration of yeast iso-1-Cyt *c* solution was prepared from the stock solution and used for AuNPs coating as described previously [1]. Cyt *c* was coated onto AuNPs in two procedures of minor difference. The first procedure was carried out using Cyt *c* as received from Sigma as described by Chah et al. [1] and the second procedure was slightly modified, in which Cyt *c* was fully reduced by adding a small excess of DTT prior to mixing with the AuNPs solution. An optimum volume ratio of 1:30 between Cyt *c* (3.5 μM) and AuNPs solution was used and the pH of AuNP solution was adjusted to 11.0 before mixing with Cyt *c* solution to prevent Cyt *c* from being acid denatured during self-assembly [1]. The resultant Cyt *c*/AuNPs mixture was vigorously mixed and then left overnight for full saturation.

After overnight incubation, the pH of the 1st preparation (Cyt *c* as received) of Cyt *c*-AuNPs was adjusted to 10.0, 8.0, 7.0, 6.5, 6.0, 5.0, 4.0 and 3.0 by drop-wise addition of HCl solution. The pH of the 2nd preparation (Cyt *c* as DTT reduced) of Cyt *c*-AuNPs was adjusted to 7.0, 6.5, 6.0, 5.0, 4.0 and 3.0 by drop-wise addition of HCl solution.

2.7. Preparation of Cyt c–3MPA–AuNP complex

The AuNPs were first coated with a monolayer of 3-MPA by addition of 2.5 μl of 3-MPA to 12 ml of AuNP solution (pH adjusted to 11.0) with vigorous stirring, then the samples were incubated overnight at room temperature. After 24 h, a 0.4 ml Cyt *c* solution (3.5 μM in 10 mM

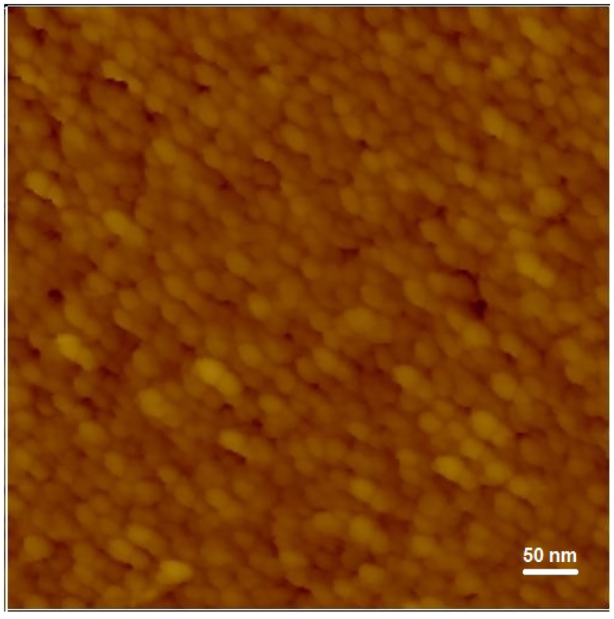

Fig. 2. Atomic Force Microscopy (AFM) image of gold nanoparticles (~20 nm). The image was obtained with AuNPs placed on a clean mica disc after proper dilution and dried inside a desiccator. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)