Hindawi Publishing Corporation
Journal of Nanomaterials
Volume 2008, Article ID 391497, 9 pages
doi:10.1155/2008/391497

Research Article

Nanomaterials Enhanced Gene Expression in Yeast Cells

Su-Fang Chien,[1] Shi-Hui Chen,[1] and Chhiu-Tsu Lin[2]

[1] *Department of Chemistry and Graduate Institute of Life Science, College of Sciences, Tamkang University, Tamsui 25137, Taiwan*
[2] *Department of Chemistry and Biochemistry, College of Liberal Arts and Sciences, Northern Illinois University, Dekalb, IL 60115, USA*

Correspondence should be addressed to Chhiu-Tsu Lin, ctlin@niu.edu

Received 22 August 2007; Accepted 2 January 2008

Recommended by Jun Liu

Metal nanomaterials are shown to enhance gene expression for rice α-galactosidase gene (α-Gal) in yeast cells. Au and Ag nanoparticles and their nanocomposites, silica-Au and silica-Ag, were prepared and characterized by UV-vis spectroscopy and TEM technique. The rice α-galactosidase gene was cloned into the yeast chromosome, where the cloned cells were precultured and induced into a medium containing each of the testing nanomaterials. The nanomaterials were observed to incorporate inside the cells, and no cell death has been detected during the course of gene expression. The enzyme activity was determined by a synthetic substrate, p-nitrophenyl-α-D-galctopyranoside, and the yellow product yield was recorded in a spectrophotometer at 400 nm. When Au and Ag nanoparticles were incorporated with the culture, a 3–5 fold enhancement in α-galactosidase was observed for intracellular activity as well as the secreted activity into the medium. The secreted protein was analyzed to have a pure form and displayed as a single protein band in the SDS-gel electrophoresis. The effects of size and chemical nature of nanomaterials on gene expression for the rice α-galactosidase gene in yeast cells are discussed.

1. INTRODUCTION

The size and surface area of the nanoparticles together with their available functional groups and charges are crucial factors in targeting and the attachment of cell-specific ligands that can lead to an increased selectivity in delivery and expression of genes. Gold and silica nanoparticles have been employed to investigate gene expression from the unamplified total human RNA [1] and in vivo in the brain [2]. It has been known for a long time that the Indian eats silver powder and the Chinese mixes gold thin films in food. Gold nanoparticles were loaded and modified with oligonucleotide and employed as the intracellular gene regulation agents for controlling protein expression in cells [3]. With the aid of gold nanoparticles, these intracellular gene regulating agents exhibit more than 99% cellular uptake and can introduce oligonucleotides at a higher effective concentration than the conventional transfection agents [3]. By employing surface plasmon resonance spectra of Ag nanoparticles as the nanometer-size index probes, the real-time probing and imaging of membrane transport in living microbial cells have been demonstrated [4]. Moreover, the application of organically modified silica nanoparticles as a nonviral vector for efficient in vivo gene delivery has been communicated [2]. The in vivo gene delivery is an area of current research, where genetic materials (e.g., DNA, RNA, and oligonucleotides) could be used to inhibit undesirable gene expression or to synthesize therapeutic proteins [5, 6].

The rice α-galactosidase gene was isolated from the stem portion of taro (*Colocasia esculenta*) that has been demonstrated to have the capability of converting group B into group O red blood cells [7]. In this paper, we describe the use of oligonucleotide-loaded nanoparticles to enhance the expression of rice α-galactosidase gene in yeast cells. Au, Ag, silica-Au, and silica-Ag nanoparticles were synthesized and charaterized. The TEM images of nanomaterials transfected inside the cell membrane will be illustrated. The results will be used to address the following questions. Will the nanomaterials be toxic and kill micro-organisms, or be activated to enhance gene expression? Will these α-galactosidase activities be highly selective and specific, and also depend on the nature and type of nanomaterials? If the gene expression is enhanced, will the protein expression be limited only to the intracellular activity or included also the secreted activity

into medium? If the secreted activity is highly specific, will the protein expressed be a pure form and no further protein purification should be required?

2. EXPERIMENTAL

2.1. Materials

Tetraethyl orthosilicate (TEOS), hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), silver nitrate ($AgNO_3$), sodium citrate, and ammonium hydroxide solution (NH_4OH) were purchased from Sigma-Aldrich (St. Louis, MO 63195, USA) and used as received. Ethyl alcohol, 200 proof, was obtained from Pharmco (Shelbyville, KY 40065, USA). The water employed in all preparations was purified by a Milli-Q system (Millipore) (Bedford, MA, USA). Rice α-galactosidase gene was cloned from cDNA library. The yeast strain, *Pichia pastoris* SMD1168, and pPIC-9k plasmid were obtained from Invitrogen (Carlsbad, Calif, USA). The synthetic *p*NP-substrate, p-nitrophenyl-α-D-galactopyranoside were obtained from Sigma-Aldrich. Other materials for cell cultivation and cloning, such as agar plate, YPD plate, yeast extract, lactose, sodium carbonate, LB medium, SMGY medium, YNB medium, Eppendorf tube, and Pyrex Petri dishes were used from a conventional laboratory stock.

2.2. Preparation and characterization of nanomaterials

Silica colloidal particles were processed by following the method of Stöber et al. [8]. Briefly, it involved hydrolysis and successive condensation of tetraethylorthosilicate (TEOS) in ethanol/water mixture with ammonium hydroxide as a catalysis. In a typical formulation, a mixture of 0.6 mL of TEOS and 10 mL ethanol was sonicated for 30 minutes. While stirring the sol-gel precursor solution, a 0.1 mL of 3% NH_4OH solution was added dropwise. The silica colloidal solution turned turbidly white and allowed to stir for 3-4 hours for the reaction to be completed. The silica product solution was centrifuged at 15000 rpm for 30 minutes. The SiO_2 precipitates were collected, washed with water twice, and dried at 37°C. The silica nanoparticles were redispersed in water to form a 1 g/L colloidal solution for further use.

The aqueous colloidal solution of Au and Ag, individually, was prepared following a chemical reduction method employed by Turkevich et al. [9] and Fu et al. [10]. First, an aqueous solution of 2 mM for each metal salt, $HAuCl_4$, and $AgNO_3$ was prepared. Second, to synthesize Au colloidal solution, a volume of 25 mL $HAuCl_4$ solution was heated to boil and stirred vigorously, and 2.5 mL of freshly prepared sodium citrate solution (38.8 mM in ethanol) were added dropwise until a wine-red color was observed. A similar procedure was used also for preparing Ag colloidal solution.

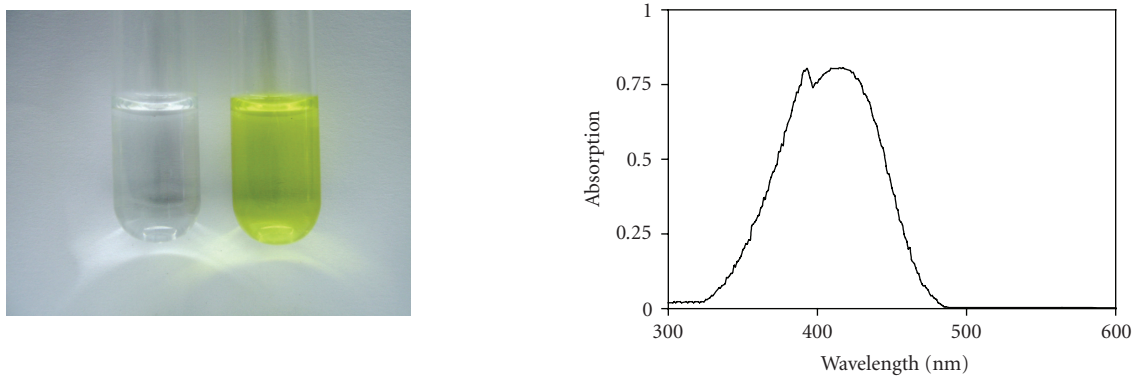
The composite nanomaterials, silica-Au and silica-Ag, were prepared by the procedure described previously [10, 11]. For a nanoparticle, silica-Ag as an example, a volume of 25 mL of 2 mM $AgNO_3$ solution and 0.2 mL of 1 g/L SiO_2 colloidal solution (as prepared above) was heated to boil and stirred vigorously, and 2.5 mL of freshly prepared sodium citrate solution (38.8 mM, in ethanol) were slowly added. Initially, a white/turbid solution of silica particles with silver nitrate turned pale yellow and after reacting for 30 minutes, the color of the solution turned golden yellow indicating the formation of silica-Ag nanocomposites. It is important to ensure that the silver and gold ions have been reduced completely by adding an excess amount of reducing agents. Moreover, the nanoparticles were normally centrifuged, washed, and resuspended in solution before use, in order to minimize the alcohol cosolvent and a possible existence of the trace amount of metal ions in solution. The size, shape, and distribution of the synthesized nanomaterials and also the α-Gal/nanomaterial complexes were characterized by a TEM (Jeol JSM-1200 Ex II, operated at 80 kV) and a UV-vis spectrophotometer (Hitachi U-2000).

2.3. Gene cloning and gene expression in yeast cells

Rice α-galactosidase gene was cloned in pPIC-9k plasmid (Invitrogen, Calif, USA) and transformed into SMD 1168 yeast strain chromosomal DNA by electroporation according to the Invitrogen protocol described in EasySelect (www.Invitrogen.com) and given by Higgins and Cregg [12]. The gene expression was done also by following the Invitrogen EasySelect procedure. The cloned SMD 1168-α-Gal yeast cells were cultured overnight from a single colony on YPD plate in 2 mL SMGY medium. The cells were harvested and the induction was carried out in 2 mL of a YNB medium containing amino acids, 2% of glycerol as carbon source, and 30% of the individual testing nanomaterials. For the preparation of control sample, the solution of nanomaterials dispersed in water was replaced by the same volume amount of pure water that was then used to carry out the control experiment for each type of nanomaterials tested [7, 13]. Finally, the expressed cells were induced by adding 1% of methanol everyday. The cell mass was measured spectrophotometrically by recording its optical density at 600 nm (OD600).

2.4. Determination of enzyme activity and enzyme assay

After each period of induction in the gene expression (24 hours, 48 hours, and up to 8 days), the enzyme activity was determined (in duplicate) by a synthetic *p*NP-substrate, p-nitrophenyl-α-D-galactopyranoside. Fifty μL of the induced yeast cells and 30 μL of 4 mM *p*NP-substrate were pipetted into an Eppendorf tube and incubated at 37°C for 10 minutes. After incubation, a 1.0 mL of 0.2 M Na_2CO_3, pH 9.8, was added to terminate the reaction. The final reaction mixture was then centrifuged at 8000 rpm for 15 minutes (as shown in the right test tube of Figure 1(a); the left test tube is the control), and the resulting clear yellow supernatant (i.e., the released *p*-nitrophenol, *p*NP, representing the secreted activity) was read in a spectrophotometer at 400 nm (as shown in Figure 1(b), a UV-visible spectrum from 300 nm to 600 nm) [7]. The intracellular enzyme activity was obtained by subtracting the secreted activity from the total activity. One unit of enzyme activity is defined as the amount of enzyme that can produce 1 μmol of *p*NP/min at 37°C.



(a) (b)

FIGURE 1: (a) Secreted recombinant α-galactosidase activity; see text for the details, and (b) OD at 400 nm of the secreted α-galactosidase supernatant, representing the production of *p*-nitrophenol.

The secreted proteins were analyzed by SDS-Polyacrylamide Gel Electrophoresis (PAGE) [14]. The SDS gel, containing 13.5% polyacrylamide with a 0.75 mm thickness, was prepared. After loading the samples, electrophoresis was carried out with 110 V and 20 mA in a continuousbuffer system for 2 hours. The gel was stained with Coomassie Brilliant Blue R-250 and destained until the background was clear. The question of a possible cell death during induction period, as may be caused by the nanomaterials toxicity, was monitored by cells staining with Trypan Blue [15] (4% solution from Sigma Adrich).

3. RESULTS AND DISCUSSION

3.1. Size and size distribution of nanomaterials

The surface plasmon absorption bands observed in the UV-visible spectra are commonly used for characterizing the size and shape of metallic nanoparticles. In particular, the gold and silver nanomateterials have been well documented. Normally, the UV-visible spectra of the synthesized nanomaterials in solution are recorded from 200 nm to 800 nm. Small gold nanoparticles of <5 nm diameter do not show any plasmon absorption, but gold nanoparticles of 5–50 nm show a sharp absorption band in the 520–530 nm region [16]. As the particles grow bigger, the absorption band broadens and covers the visible spectral range [17]. The absorption spectrum of the silver colloids of facetted silver nanocrystals (with a particle diameter of 40–60 nm) shows a surface plasmon absorption band with a maximum around 420 nm. For silver nanoparticles, a shift in the plasmon absorption band from 400 nm to 670 nm was reported as the particle shape changed from spherical to triangular prisms during visible light irradiation [18]. Under the experimental conditions described in Section 2. Figure 2 shows the individual plasmon resonance absorption band for each species of the prepared nanomaterials, recorded from 200 nm to 800 nm. The surface plasmon absorption band maximum is observed at Figure 2(a) to be 475 nm for Ag nanoparticle, Figure 2(b) 425 nm for silica-Ag nanocomposite, Figure 2(c) 525 nm for Au nanoparticle, and Figure 2(d) 550 nm for silica-Au nanocomposite. The plasmon bands are broad in all spectra suggesting a wide range of particle size distribution. Based on the observed spectral wavelength of surface plasmon bands, the particle size of Ag nanoparticle in Figure 2(a) should be ~60 nm whereas silica-Ag nanocomposite in Figure 2(b) should be in the range of 40–60 nm. For Au nanoparticle in Figure 2(c), the size should be within 5–50 nm. On the other hand, the size of the silica-Au nanocomposite in Figure 2(d) should be ~50 nm.

The particle size and size distribution of nanomaterials assigned from the observed surface plasmon bands in Figure 2 can also be verified by the TEM images as shown in Figure 3. In Figure 3(a), the size of Au nanoparticles is not very uniform but they are all in the ranges between 12 nm and 20 nm, as suggested by the observed surface plasmon band at 525 nm in the UV-visible spectrum as displayed in Figure 2(c). The size of nanocomposite of SiO_2 and Au, that is, silica-Au in TEM, Figure 3(b), is observed to be in the ranges between 40 nm and 60 nm in agreement with the assignment of UV-visible spectrum of Figure 2(d). The sizes of Ag particles in TEM, Figure 3(c), are also not very uniform ranging from 25 nm to 30 nm, and they tend to form some larger agglomerates. This may explain why the surface plasmon band in Figure 2(a) is observed at a longer wavelength of 475 nm. These agglomerates seem to disappear in TEM, Figure 3(d), when Ag nanoparticles form composite structure with SiO_2 particles, that is, the particle size of silica-Ag is observed to be in the ranges between 40 nm and 60 nm, as assigned in the UV-visible spectrum of Figure 2(b). It is worthwhile to mention that the size and size distribution of nanomaterials are critically important for gene delivery to effectively transport into cells interior and to enhance gene expression in cells, which will be demonstrated below.

3.2. α-galactosidase activity in cells assisted by nanomaterials

When gold nanoparticles were loaded and modified with oligonucleotide, the effectiveness of using gold nanoparticle-oligonucleotide complexes (via surface binding) as intracellular gene regulating agents for the control of protein expression in cells has been illustrated [3]. By chemically tailoring

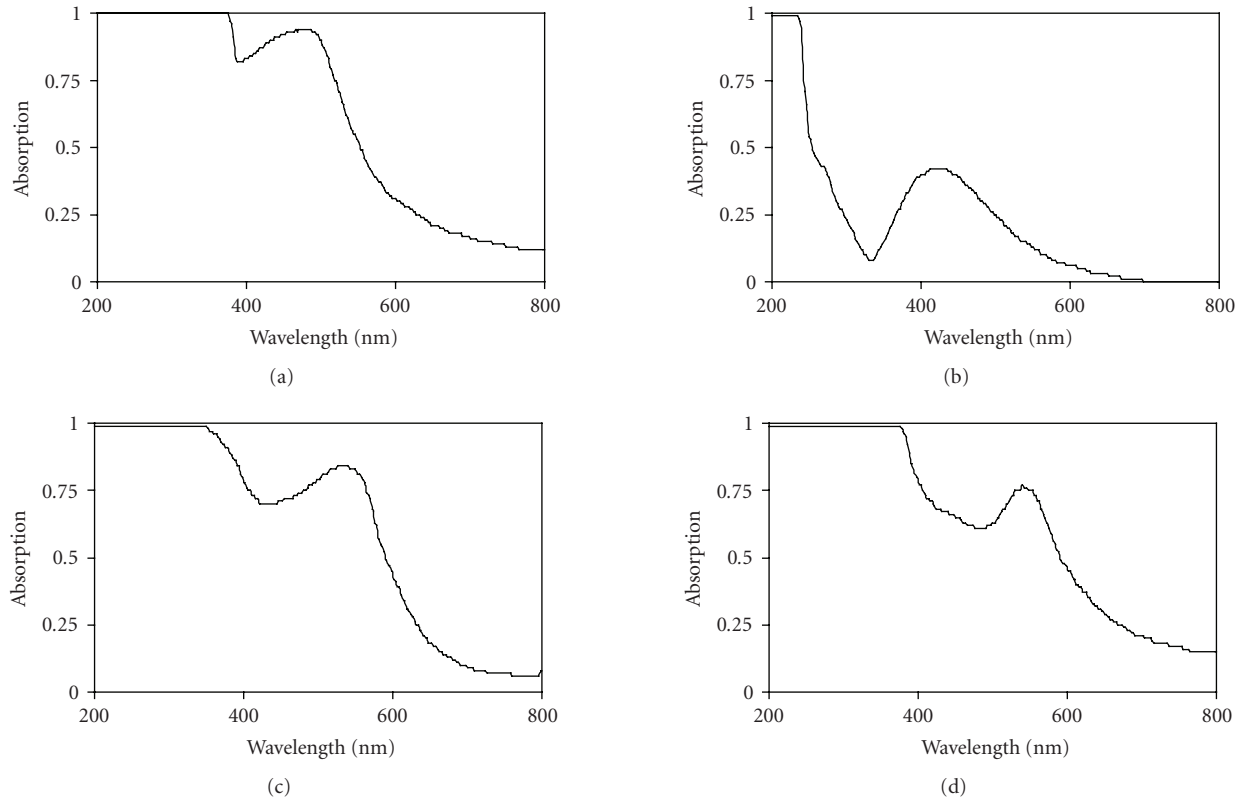


Figure 2: UV-visible spectra of surface plasmon band: (a) Ag at 475 nm, (b) silica-Ag at 425 nm, (c) Au at 525 nm, and (d) silica-Au at 550 nm.

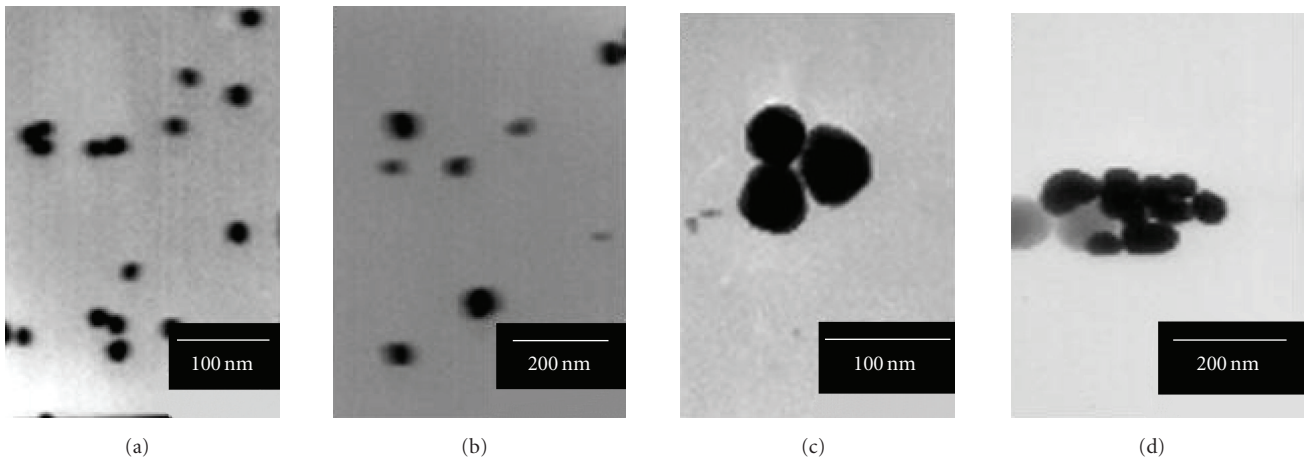


Figure 3: TEM images of the synthesized nanomaterials: (a) Au, (b) silica-Au, (c) Ag, and (d) silica-Ag.

the density of DNA bound to the surface of gold nanparticles, it was possible to introduce oligonucleotides at a higher effective concentration than conventional transfection agents, and remain nontoxic to the cells. In the present study, Rice α-galactosidase gene is expected to load on the surface of nanomaterials via electrostatic interactions. The nanomaterial modified α-galactosidase complexes can then transport effectively into yeast cells interior to enhance gene expression.

The TEM imaging technique was used to trace the location of α-Gal/nanomaterial complexes, either inside or outside the yeast cells to give the enhanced α-galactosidase activity. If the nanomaterials are located in the solution medium (or outside the yeast cells) as the unbounded or free nanomaterials, they will be easily washed off by the double deionized water. On the other hand, if α-Gal/nanomaterial complexes are incorporated into the yeast cells, then they will be remained inside the cells upon water washing. However, the

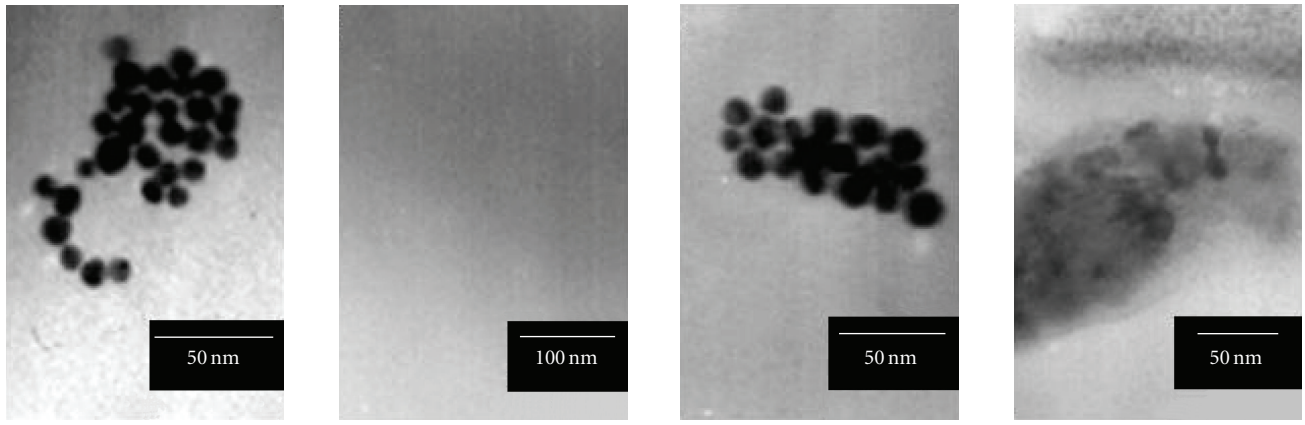


Figure 4: TEM images of α-Gal/nanomaterials incubated in yeast cells, after 5 days of induction: (a) Au nanparticles, (b) sample in (a) washed with royal water, (c) Ag nanoparticles, and (d) sample in (c) washed with double deionized water.

encapsulated nanomaterials inside the yeast cells may be dissolved away if the "royal water"—aqua regia was used for washing. The TEM images of α-Gal/nanomaterials incubated in yeast cells, after 5-day induction, are shown in Figure 4, Figure 4(a) Au nanparticles, and Figure 4(b) Ag nanoparticles. It is observed that α-Gal/nanomaterials have a compatible size as those of free nanomaterials shown in the TEM images of Figure 3, except that α-Gal/nanomaterials are shown to encapsulate in a unique shape of a cell (Figure 4(a) and 4(c)). When these incubated samples were washed with double deionized water, the nanomaterials remained as shown in Figure 4(d) for α-Gal/Ag complexes (the same is also obtained for α-Gal/Au complexes). This result indicates that α-Gal/nanomaterials are not simply dispersed in the solution medium as free nanoparticles, in fact they are encapsulated inside the yeast cells. Furthermore, when an aqua regia solution was used to wash the incubated samples, the α-Gal/Au complexes were shown to dissolve away as seen in the TEM images of Figure 4(b) (the same is also obtained for α-Gal/Ag complexes). The TEM images in Figure 4 have provided the illustrations that the transfection of α-Gal/nanomaterials were indeed inside the yeast cells to give the observed enhancement in gene expression.

The nonviral transfer of DNA into cells (or transfection) is a routine procedure in modern biochemistry [19]. Following the protocol of the transfection mechanism, nanoparticles with DNA are given to the cell culture medium and incorporated into the cells by endocytosis. Inside the cytoplasm, the nanoparticles finally reach the nucleus and insert the DNA into the nucleus. This is the location in the cell where DNA molecules are copied for translation into proteins or are multiplied for cell division. Xu et al. [4] studied the real-time probing of membrane transport in living microbial cells (*Pseudomonas aeruginosa)* using single nanoparticle (silver nanoparticle) optics. They showed that Ag nanoparticles with sizes ranging up to 80 nm were accumulated in living microbial cells. This observation demonstrated that the Ag nanoparticles with size of 80 nm or smaller can transport through the inner or outer membrane of the *P. aeruginosa cells.* This particle size, 80 nm or less as shown in TEM images of Figure 3 for the nanomaterials synthesized, would be used as the particle size reference for our works on nanomaterials enhanced gene expression in yeast cells.

In this study, the gene expression experiments in yeast (SMD1168/α-Gal) are carried out by adding nanomaterials (30% of the total volume of the medium) to 2 mL of the cell cultures for every 24 hours of induction. Figure 5 shows the enhanced gene expression, α-galactosidase activity in yeast cells by adding Au (12 nm–20 nm) and silica-Au (40 nm–60 nm) nanomaterials. The intracellular and secreted enzyme activities resulting from day 1 to day 4 inductions are plotted in Figures 5(a) and 5(b), respectively. The day 1 activity was measured for a 24-hour induction after each nanomaterial was added. The α-galactosidase activity is plotted in yellow line (-▲-), pink line (-■-), and blue line (-♦-), separately for the addition of Au, silica-Au, and the control samples (no nanomaterial added). It shows that both Au and silica-Au nanomaterials are not toxic to yeast cells; rather, they act to increase the transfection activity. The enhancement of gene expression is higher for Au nanoparticle than for silica-Au nanocomposite. It is noted that, one unit of enzyme activity is defined as the amount of enzyme that can produce 1 μmol of *p*NP/min at 37°C. From Figure 5, the intracellular α-galactosidase activity is shown to be about 5–7 times higher than the secreted (extracellular) activity in the medium, that is, about 20% of enzyme activity was secreted in the meduim [13]. It should be noted that, in our experiment, the testing samples and control sample have the same amount of cells. After induction (day 1 to day 5), the control sample was shown to express a basal level of α-galactosidase both in the medium and inside the cells. With the addition of nanomaterials, the secreted level of enzyme displayed a 3-4 fold enhancement.

In Figure 5, the intracelluar activity increases from day 1 to day 5 induction, and then it starts to decrease after the day 5 induction (including the control sample without the nanomaterials added), while the extracellular activity increases

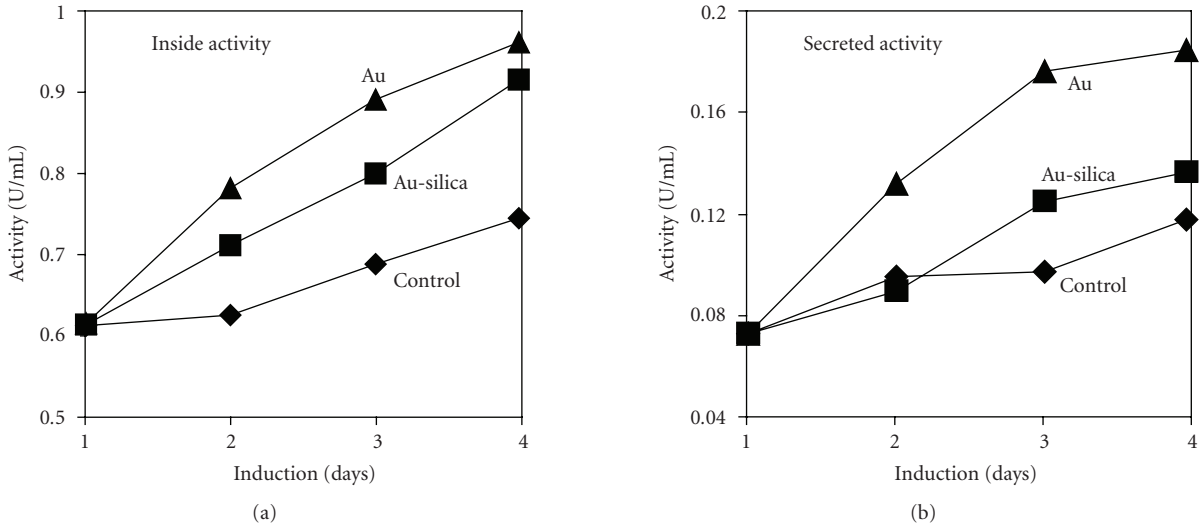


Figure 5: Gene expression enhanced by Au (-▲-) and silica-Au (-■-), and the control sample (-♦-). (a) intracellular activity and (b) extracellular (secreted) activity.

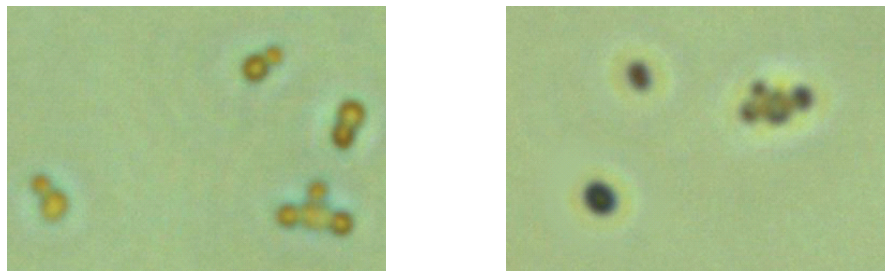


Figure 6: Optical micrographs of the stained yeast cells: (a) the cells after induction for 5 days containing α-Gal/nanomaterial complexes, and (b) the cells treated with 100% methanol (i.e., dead cell).

continuously from day 1 to day 8 induction (not shown in Figure 5). One of the possible explanations for this observation is the cell death, where the continuously increased in extracellular activity after day 5 induction might come from the decreasing intracellular activity, that is, partially, due to the enzyme leakage from inside. In order to verify that cell death was not the case, a 4% trypan blue solution was used to stain the cells. Figure 6 shows an optical micrograph of the stained yeast cells: Figure 6(a) the cells after induction for 5 days containing α-Gal/nanomaterial complexes, and Figure 7(b) the cells treated with 100% methanol (i.e., dead cell). The dead cells turned blue color, whereas no cell death was observed for the yeast cells after induction for 5 days with α-Gal/nanomaterials.

Figure 7 shows (a) the intracellualr and (b) secreted α-galactosidase activity assisted by adding different amount of silica-Ag, 450 μL (yellow line, -▲-), 225 μL (pink line, -■-), and control (blue line, -♦-, no nanomaterial added). The induction period was carried out from day 1 to day 8. The activity was labeled in unit/mL, where one unit of enzyme activity was defined as the amount of enzyme that can release 1 μmol of *p*NP/min at 37°C. For intracellular activity, the activity at day 5 induction was measured to be 0.595, 0.66, and 0.74 unit/mL for the controlled sample, 225 μL added silica-Ag sample, and 450 μL added silica-Ag sample, respectively. This represents 11% and 24% enhancements in enzyme activity for the 225 μL and 450 μL added silica-Ag samples, respectively, as compared to the control sample. The results indicate that cells incubated with silica-Ag enhance the intracellular activity; while a double dose of silica-Ag gives a double enzyme activity. After day 5 induction, the enzyme activity went down, where the control sample lost the α-galactosidase activity faster (i.e., the nanomaterial-α-galactosidase complexes are less susceptible to degradation). At day 8 induction, the intracellular activity was changed to 0.18, 0.55, and 0.68 unit/mL for the controlled sample, 225 μL added silica-Ag sample, and 450 μL added silica-Ag sample, respectively. This corresponds to 206% and 278% enhancements in enzyme activity for the 225 μL and 450 μL added silica-Ag samples, respectively, as compared to the control sample. It is noted that the amount of enzyme activity in unit/mL is about 4-5 times smaller in the secreted medium (Figure 7(b)) as compared to that inside the yeast cells (Figure 7(a)). This is again in agreement with ~20% of

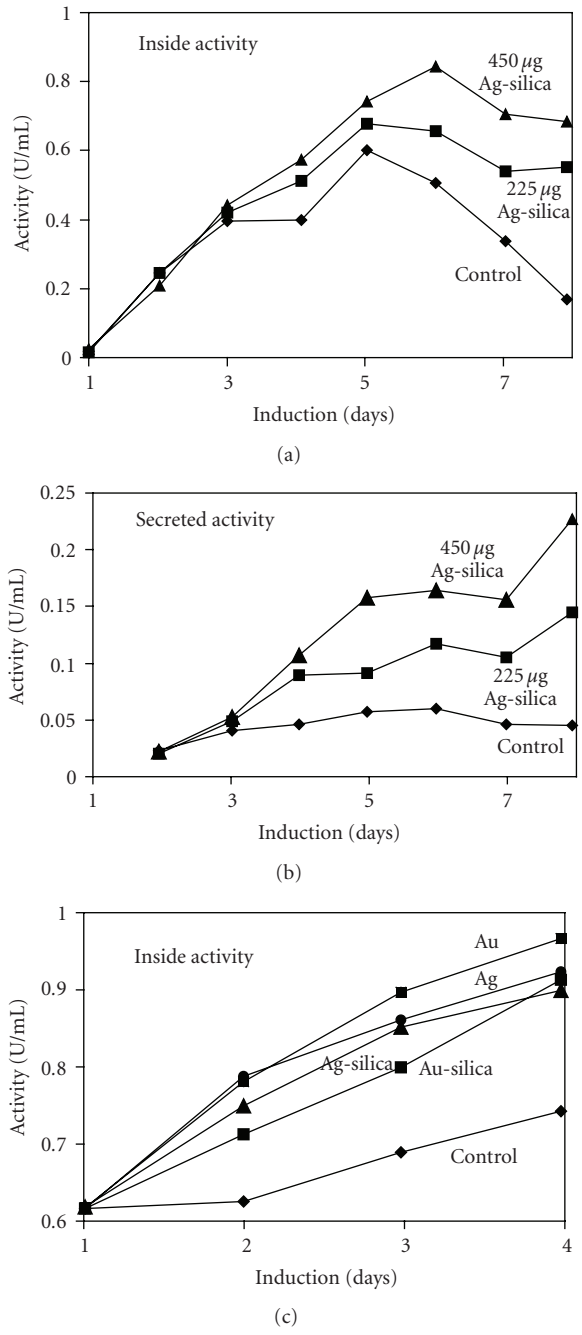


Figure 7: Gene expression enhanced by (a) and (b) different doses of silica-Ag nanocomposites, and by (c) different types of nanomaterials. (a) and (c) intracellular activity and (b) extracellular (secreted) activity. See text for details.

enzyme activity secreted in the medium reported previously [13]. However, the percent enhancement of extracellular activity assisted by silica-Ag is much larger than that shown in the intracellular activity. Unlike the intracellular activity, the secreted activity is shown to increase from day 1 to day 8 inductions. Again, this observation is not due to the cell death during the induction period as shown in Figure 6. At the day 8 induction, 233% and 423% enhancements in extracellular activity were observed for the 225 μL and 450 μL added silica-Ag samples, respectively, as compared to the control sample. In short, a double enzyme activity has been expressed when a double dose of silica-Ag was given to the yeast cells.

Figure 7(c) compares the intracellular enzyme activity assisted by the same amount of different nanomaterials, Au (purple line, -∗-), Ag (yellow line, -▲-), silica-Au (light blue, -x-), silica-Ag (pink line, -■-), and control (blue line, -◆-, no nanomaterial added). The Au (12–20 nm) and Ag (25–30 nm) nanoparticles give a higher enhancement in enzyme activity than the corresponding silica-Au and silica-Ag nanocomposites (with a particle size of 40–60 nm) that presumably are due to the difference in their particle sizes. The results indicate that the highest enhancement in intracellular enzyme activity goes to Au nanoparticles assisted gene expression, followed by Ag nanoparticles, silica-Au nanocomposites, and then silica-Ag nanocomposites. It is also shown that the α-galactosidase activity is a function of the type and proportional to the amount of nanomaterials incorporated.

The use of nanomaterials to enhance transfection by physical concentration of DNA at the cell surface is essential for increasing gene expression and protein production. The DNA/nanomaterial complex cannot only be brought into the cytoplasm, but can also make its way to the cell nucleus where DNA molecules are copied for translation into protein. The ability of synthesizing therapeutic proteins via nanomaterials-assisted gene expression in cells is a great challenge, in particular, if the enhancement in protein yields is selective, specific, and obtainable in a commercial scale. The recombinant enzyme proteins in yeast cells and in the secreted medium, resulting from the nanomaterials-assisted gene expression, that is, α-galactosidase activity are subjected to the SDS-gel electrophoresis analysis. In order to show that the α-galactosidase was really produced by the cells, SDS-PAGE analysis [14] was carried out for each testing sample, dried medium, and cells. For cell sample, about 10 μL of the 150 μL suspension cells were used. For medium sample, the dried sample was dissolved in 15 μL of water before use. The results are shown in Figure 8, where α-galactosidase shows a single protein band at 46 kDa [13, 20]. Based on having 363 amino acid residues, the enzyme could exist as a monomer [13]. The S't and BSA represents Bio Rad low protein standard and bovine serum albumin, respectively. In Figure 8(a), the experiments were done with Au and silica-Au, whereas those carried out with Ag and silica-Ag are listed in Figure 8(b). The α-galactosidase was found in the secreted medium in Figures 8(a) lane 4, and 8(b) lane 7 and Lane 8, as well as in the cellular materials in Figure 8(b) lane 1 through lane 4. The secreted protein was shown to have a pure form, and it should be easier for any further protein purification if needed. When lane 4 was compared with lane 1 in Figure 8(a), Au nanoparticles were shown to enhance about four times of the protein expression in the medium. When lane 7 and lane 8 in Figure 8(b) were compared, both Ag and silica-Ag were shown to increase about 2-3 times of the protein expression under the same experimental conditions.

The nanomaterials-mediated transfer mechanism of α-galactosidase gene into yeast cells should be function of the characteristic of nanomaterials used (type, size, size distribution, interaction, etc.). The results in Figures 5 and 7

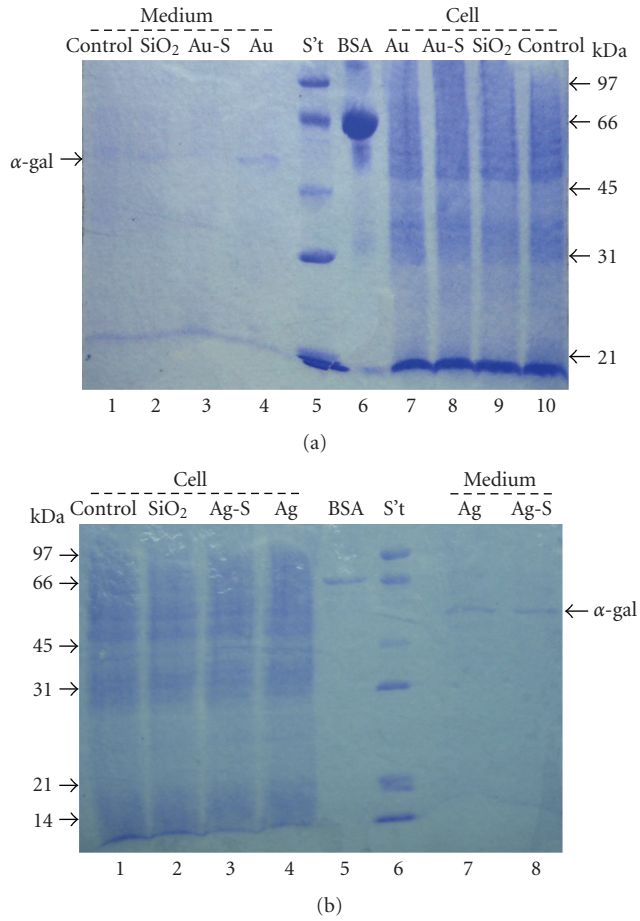


Figure 8: The SDS-polyacrylamide gel electrophoresis for the recombinant enzyme proteins obtained from SMD1168/α-Gal cells and its medium concentrates. See text for details.

have demonstrated that Ag and Au nanoparticles gave a higher enzyme activity than that assisted by silica-Ag and silica-Au nanocomposites. The synthesized Au and Ag nanoparticles are smaller in size (12–30 nm) as compared to that of silica-Ag and silica-Au nanocomposites (40–60 nm). It is suggested that the smaller nanoparticles are more effective in reaching the cell surface, penetrating into the cytoplasm via endocytosis, and making their ways to the cell nucleus. When Au and Ag nanoparticles were added to and incubated in the yeast culture, a 3–5 fold enhancement in α-galactosidase activity was observed for the intracellular activity as well as in the secreted medium as compared to those of the control group. The secreted protein in the medium was shown to have a pure form and displayed as a single protein band in the SDS-gel electrophoresis.

4. CONCLUSION

We have cloned rice α-galactosidase gene into yeast chromosome cells. Using different nanomaterials, the gene expression of α-galactosidase activity as the parameter for monitoring the effectiveness of particle's catalytic activity has been investigated. Au and Ag nanoparticles have shown to enhance gene expression in yeast cells with the cloned rice α-galactosidase gene. Under our experimental conditions, Ag, Au, silica-Ag, and silica-Au nanomaterials are not toxic, and they are not shown to kill the yeast cells. On the contrary, they act to enhance gene expression inside the yeast cells and also secrete into the cultural medium. The Ag and Au nanoparticles have a smaller size ranging from 12 nm to 30 nm that may be responsible for the effectiveness of making their ways to the cell nucleus. It is suggested that SMD 1168-α-Gal yeast cells may be a choice of model system for studying other nonviral transfer of gene or drug into cells assisted by nanomaterials. It is further indicated that the size and uniform distribution of nanoparticle are important for a controllable transfection study.

ACKNOWLEDGMENT

The authors thank Dr. Jin-Pei Deng, Department of Chemistry, Tamkang University for making TEM images of nanomaterials and α-Gal/nanomaterial complexes.

REFERENCES

[1] M. Huber, T.-F. Wei, U. R. Müller, P. A. Lefebvre, S. S. Marla, and Y. Paul Bao, "Gold nanoparticle probe-based gene expression analysis with unamplified total human RNA," *Nucleic Acids Research*, vol. 32, no. 18, p. e137, 2004.

[2] D. J. Bharali, I. Klejbor, E. K. Stachowiak, et al., "Organically modified silica nanoparticles: a nonviral vector for *in vivo* gene delivery and expression in the brain," *Proceedings of the National Academy of Sciences*, vol. 102, no. 32, pp. 11539–11544, 2005.

[3] N. L. Rosi, D. A. Giljohann, C. S. Thaxton, A. K. R. Lytton-Jean, M. S. Han, and C. A. Mirkin, "Oligonucleotide-modified gold nanoparticles for intracellular gene regulation," *Sceince*, vol. 312, no. 5776, pp. 1027–1030, 2006.

[4] X.-H. N. Xu, W. J. Brownlow, S. Kyriacou, Q. Wan, and J. J. Viola, "Real-time probing of membrane transport in living microbial cells using single nanoparticle optics and living cell imaging," *Biochemistry*, vol. 43, no. 32, pp. 10400–10413, 2004.

[5] S. S. Davis, "Biomédical applications of nanotechnology—implications for drug targeting and gene therapy ," *Trends in Biotechnology*, vol. 15, no. 6, pp. 217–224, 1997.

[6] W. F. Anderson, E. U. Canning, and B. Okamura, "A triploblast origin for Myxozoa?" *Nature*, vol. 392, no. 6674, pp. 346–347, 1998.

[7] S.-F. Chien, "The conversion of group B red blood cells into group O by an α-D- galactosidase from taro (*Colocasia esculenta*)," *Carbohydrate Research*, vol. 217, pp. 191–200, 1991.

[8] W. Stöber, A. Fink, and E. J. Bohn, "Controlled growth of mono-disperse silica spheres in the micron size range," *Journal of Colloid and Interface Science*, vol. 26, pp. 62–69, 1968.

[9] J. Turkevich, P. C. Stevenson, and J. Hillier, "A study of the nucleation and growth processes in the synthesis of colloidal gold ," *Discussions of the Faraday Society*, vol. 11, pp. 55–75, 1951.

[10] G. Fu, P. S. Vary, and C.-T. Lin, "Anatase TiO_2 nanocomposites for antimicrobial coatings," *Journal of Physical Chemistry B*, vol. 109, no. 18, pp. 8889–8898, 2005.

[11] S. A. Kalele, S. S. Ashtaputre, N. Y. Hebalkar, et al., "Optical detection of antibody using silica—silver core—shell particles," *Chemical Physics Letters*, vol. 404, no. 1–3, pp. 136–141, 2005.

[12] D. R. Higgins and J. M. Cregg, "Pichia protocols," in *Methods in Molecular Biology*, pp. 1–72, Humana Press, Totowa, NJ, USA, 1998.

[13] S. F. Chien, C. C. Lin, and S. H. Chen, "Molecular cloning and expression of rice α-galactosidase in *Pichia pastoris*," *Plant Molecular Biology Reporter*, submitted.

[14] B. D. Hames and D. Rickwood, *Gel Electrophoresis of Protein: A Practical Approach*, IRL Press, Arlington, Va, USA, 1st edition, 1981.

[15] C.-M. Cao, W.-Y. Yan, Y. Liu, et al., "Attenuation of mitochondrial, but not cytosolic, Ca^{2+} overload reduces myocardial injury induced by ischemia and reperfusion," *Acta Pharmacologica Sinica*, vol. 27, no. 7, pp. 911–918, 2006.

[16] A. Henglein and D. Meisel, "Radiolytic control of the size of colloidal gold nanoparticles," *Langmuir*, vol. 14, no. 26, pp. 7392–7396, 1998.

[17] S. Chen, R. S. Ingram, M. J. Hostetler, et al., "Gold nanoelectrodes of varied size: transition to molecule-like charging," *Scinece*, vol. 280, no. 5372, pp. 2098–2101, 1998.

[18] R. Jin, Y. Cao, C. A. Mirkin, K. L. Kelley, G. C. Schatz, and J. G. Zheng, "Photoinduced conversion of silver nanospheres to nanoprisms," *Science*, vol. 294, no. 5548, pp. 1901–1903, 2001.

[19] F. L. Graham and A. J. van der Eb, "A new technique for the assay of infectivity of human adenovirus 5 DNA," *Virology*, vol. 52, no. 2, pp. 456–467, 1973.

[20] N. Overbeeke, A. J. Fellinger, M. Y. Toonen, D. van Wassenaar, and C. T. Verrips, "Cloning and nucleotide sequence of the α-galactosidase cDNA from Cyamopsis tetragonoloba (guar)," *Plant Molecular Biology*, vol. 13, no. 5, pp. 541–550, 1989.