

Detection of the Recombinant Proteins in Single Transgenic Microbial Cell Using Laser Tweezers and Raman Spectroscopy

Changan Xie,^{†,‡} Nhu Nguyen,[§] Yong Zhu,[§] and Yong-qing Li^{*†}

Department of Physics and Department of Biology, East Carolina University, Greenville, North Carolina 27858

Laser tweezers Raman spectroscopy (LTRS) has been used for the rapid detection of recombinant somatolactin protein produced in single *Escherichia coli* bacteria and *Pichia pastoris* yeast cell in the current study. A cDNA sequence encoding mature peptide of zebrafish somatolactin β was inserted into two different expression vectors and transfected into *E. coli* or *P. pastoris* yeast cells. We measured Raman spectra of single *E. coli* cells at different culture times following the induction with isopropyl β -D-1-thiogalactopyranoside, from which the amount of the generated somatolactin proteins was obtained by the projection of the entire cell's spectrum onto the spectrum of the pure somatolactin proteins or the dot product between these two spectral vectors. We found that the intensity of the somatolactin β protein-associated spectra from single *E. coli* cells increased as the function of the culture time, which correlates with the accumulation of recombinant proteins inside the cells. This spectral observation was supported by evidence obtained by conventional methods of sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting analyses. The increased intensities of recombinant protein-associated Raman bands were also observed in another expression system, *P. pastoris* yeast cells. These findings demonstrate that the LTRS is a useful method for rapid sensing of recombination production in single host microorganism in vivo.

Studying dynamic molecular reactions in a living cell is of particular interest and challenging to biologists and physicists. Much of our knowledge on molecular reactions in cells has come from traditional biochemistry techniques that depend on purification of the biomolecules from a group of nonsynchronized cells and examination of the molecules in vitro. Although this approach is successful, the information obtained from the in vitro experiments does not fully represent the milieu of a living cell. A dynamic biological reaction is often at different equilibrium states due to the interaction of multiple reactions in a living cell. It is difficult and almost impossible for any in vitro experiment to mimic the

complex environments within a cell. A population of cells usually has different physiological responses to a specific stimulus due to the heterogeneous internal environments within individual cells. The individualized physiological responses in different cells are often neglected and normalized on those in vitro experiments. Thus, noninvasive analysis and monitoring of the dynamic changes of molecules in a single living cell is important for understanding physiological changes of cells. Our aim for the present study is to determine whether confocal laser tweezers Raman spectroscopy (LTRS) can be used to monitor the dynamic expression of recombinant proteins in single transgenic microbial cells.

Recombinant proteins can be produced by various host cells transfected with genetically engineered expression vectors inserted with a desired gene. Biochemical, physiological, structural, and molecular characterization of the proteins usually requires large amounts of recombinant proteins due to low levels of expression in native tissues and where large amounts of native tissues are limited.^{1–4} The production of recombinant protein involves the construction of an expression vector, assessment of expression levels of the protein, scale-up production, and optimization of purification.⁵ Optimization of the entire process is usually time-consuming due to the unique expression of each recombinant protein and different requirements of purification. All protocols require screening of the various transfected clones in order to identify clones that have relatively stable and high levels of expression. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting are conventional methods for monitoring the production and expression of the recombinant protein, which requires a large amount of cells for detection. These procedures also require extensive labor, skilled researchers, and are time-consuming.⁶ Developing a new analytical method that is sensitive, rapid, and noninvasive could improve the monitoring, production, and purification of recombinant proteins.

The fluorescence spectroscopy and vibration spectroscopy^{7,8} have been recently developed for detection of recombinant protein,

* To whom correspondence should be addressed. Fax: 252-328-6314. E-mail: liy@ecu.edu.

[†] Department of Physics.

[‡] Current address: Department of Physics, University of Utah.

[§] Department of Biology.

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in addition to traditional techniques such as electrophoresis,⁹ Western blotting,¹⁰ and mass spectroscopy.¹¹ These techniques require specific dyes and labeling or destruction of cells. In contrast, the Raman spectroscopic method has significant advantages as a nondestructive and reagentless procedure for spectral measurement. The amount of light scattered solely depends on the molecules found within the sample and the environment. Biological molecules such as nucleic acids, proteins, lipids, and carbohydrates all generate specific Raman spectrum. Therefore, Raman spectra of individual cells will provide "fingerprinted" information regarding the molecular composition, structure, and interactions of different classes of molecules within a single cell. Most recently, the Raman technique has been coupled with confocal light collection, which defines a small excitation/collection volume for probing local structure by imaging the scattered light through a pinhole.^{12,13} The high spatial resolution provided by confocal microscopy enables detection of a tiny space, even from single organelles inside living cells.

Another exciting development of Raman spectroscopy is utilizing optical tweezers for the analysis of moving cells in aqueous solution. Several recent studies have demonstrated the benefit of combining optical tweezers and confocal Raman spectroscopy for the study of a single biological cell in solution.^{14–16} Confocal LTRS has been used for the detection of bacteria spores,¹⁷ discrimination and sorting of microorganisms,¹⁸ and analysis of human lipoproteins^{19,20} and chromosomes.²¹ Recently, LTRS also has been applied to monitor the dynamic biological processes of single cells.^{17,22–24} Our current study demonstrates that confocal LTRS can also be used to detect the dynamic accumulation of recombinant protein produced in a single living cell.

We utilized *Escherichia coli* bacteria and *Pichia pastoris* yeast for the production of the recombinant somatotactin β protein.^{25,26}

E. coli bacteria and *P. pastoris* yeast are the most extensively used hosts because of the advantages of short production cycle, low cost, and relative ease of purification of the recombinant protein.^{27,28} They are the most widely studied microorganisms, and a large number of genetically well-defined strains and plasmids are commercially available. Somatotactin is a pituitary hormone belonging to the growth hormone/prolactin superfamily with two paralogous forms designated as somatotactin α and β forms, which was recently reported.²⁵ Physiological studies have generated controversial evidence that somatotactin may or may not be involved in reproduction, osmoregulation, light/dark adaptation, and metabolism. Physiological studies have generated controversial evidence that somatotactin may or may not be involved in reproduction, acid–base balance, background adaptation, and fasting. Therefore, different approaches including producing a large amount of recombinant somatotactin protein are required for further studies of somatotactin function. In this paper, we demonstrated that confocal LTRS could be used for the detection of recombinant somatotactin β protein in single *E. coli* and *P. pastoris* yeast. Intensity of a specific Raman spectrum was correlated with production of recombinant somatotactin protein in bacteria and yeast, and dot product vector analysis was shown to be a useful technique for estimating recombinant protein accumulation in the bacteria. The results obtained by confocal LTRS were supported by the conventional methods of SDS-PAGE electrophoresis and Western blotting.

MATERIALS AND METHODS

Host Microorganisms and Sample Preparation. *E. coli* bacteria were grown in 25 g/L of Luria Broth at 37 °C using a New Brunswick C24 shaker incubator. *P. pastoris* GS115 (Invitrogen) was maintained in YPD broth (10.0 g of yeast extract, 20.0 g of peptone, 20.0 g of dextrose/L). MD plates (13.4 g of yeast nitrogen base, 10.0 g of glucose, 0.4 mg of biotin, 100 mL of 1 M K₂HPO₄/KH₂PO₄, pH 6.0, and 20 g of agar/L) were used for plasmid selection. BMGH broth (1.34% yeast nitrogen base, 4 × 10⁻⁵% biotin, 1% glycerol, 100 mM potassium phosphate pH 6.0) was used for enrichment. BMMH broth (1.34% yeast nitrogen base, 4 × 10⁻⁵% biotin, 0.5% methanol, 100 mM potassium phosphate pH 6.0) was used for induction. Unless otherwise indicated, all the chemicals were purchased from Sigma. One 500- μ L aliquot of *E. coli* was removed prior to an OD₆₀₀ reading of 0.5 and at the following culture times: 4, 12, 20, and 24 h after isopropyl β -D-1-thiogalactopyranoside (IPTG) induction for Raman measurement (Figures 1 and 2a). Aliquots of 500 μ L of *P. pastoris* yeast were removed prior to induction and at 2, 4, 6, 10, 12, 17, 24, 48, and 72 h following the induction (with 0.5% v/v methanol) for Raman spectra accumulation (Figure 5). Raman spectra of the control groups (transfected host without induction) for two microorganisms were also collected for comparison. (Please refer to the Supporting Information for detailed information of the construction of expression plasmids, transformation and expressions, SDS-PAGE/Western blot, and Protein extraction.)

Laser Tweezers and Raman Spectroscopy. The experimental setup for Raman signal acquisition is similar to previously described methods.^{14,18} Raman spectra were acquired using a

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Raman spectrograph equipped with a liquid nitrogen-cooled CCD detector. Raman signal was collected in the spectral interval from 430 to 2140 cm^{-1} with the resolution of 6 cm^{-1} . The experiments were performed with a collection time of 60 s with 100 mW (Sacher Lasertechnik GmbH) at 790 nm for *E. coli* bacteria and 60 s with 16 mW (HL7852, Hitachi America, Dallas Tex.) at 785 nm for yeast cells. In order to reduce the fluorescence and Rayleigh scattering light coming from the cover slide, the cell was captured and elevated $\sim 10 \mu\text{m}$ from the cover glass. The Raman spectra of 20 cells were collected. After the measurement of 10 cells, a fresh diluted sample was refilled for new accumulation. The background was recorded with the same acquisition condition without the trapped cells.

Vector Analysis of Recombinant Protein Concentration.

Vector algebra is an objective method to extract the contribution of pure recombinant somatolactin protein from entire microorganism's Raman spectrum.²⁹ In mathematical terms, a Raman spectrum of a measured microbial cell consists of n data points on the base of wavenumbers ν , which can be regarded as a vector in an n -dimensional space. In this n -dimensional space, the collected spectrum of the i th bacterial cell can be expressed as

$$\bar{S}_i(\nu) = \eta_i \bar{P}(\nu) + \bar{O}_i(\nu) \quad (1)$$

where $\bar{P}(\nu)$ is the measured Raman signal coming from the pure somatolactin protein, $\bar{O}_i(\nu)$ is the measured signal coming from all of other molecules of the cell, and η_i is a constant that is proportional to the accumulation of somatolactin proteins in the bacterial cell body. Note that the other cellular molecules (other than the somatolactin proteins) might have the same or similar contributions to the whole cell's spectra. In order to subtract the contribution coming from those molecules, the Raman spectrum at 0 h (or right before induction) of the cells was collected as control and then subtracted from the whole-cell spectra collected at the subsequent times after induction, which contain the signals of increased somatolactin protein accumulation as well as the change in other molecules. The subtracted spectrum of the i th cell can be expressed as

$$\Delta \bar{S}_i(\nu) = \alpha_i \bar{P}(\nu) + \bar{Q}'_i(\nu) \quad (2)$$

where the item $\bar{Q}'_i(\nu)$ denotes spectral change in the other molecule after induction and α_i is a constant that represents the increase in somatolactin proteins compared to that at 0 h. Our objective is to measure α_i (proportional to the amount of somatolactin proteins produced in single cells). Let α_i to make the following item minimized:

$$\sum_i [\Delta \bar{S}_i(\nu) - \alpha_i \bar{P}(\nu)]^2 = \sum_i \bar{O}'_i(\nu)^2 \quad (3)$$

where the sum is over all individual cells detected at a specific culture time after induction. Take the derivative on both side of eq 3 on α_i , we have

$$\frac{\partial}{\partial \alpha_i} \left\{ \sum_i [\Delta \bar{S}_i(\nu) - \alpha_i \bar{P}(\nu)]^2 \right\} = 0 \quad (4)$$

This results in

$$\alpha_i = \frac{\Delta \bar{S}_i(\nu) \cdot \bar{P}(\nu)}{|\bar{P}(\nu)|^2} \quad (5)$$

Equation 5 implies that α_i is the projection of the difference spectrum $\Delta \bar{S}_i(\nu)$ on the vector $\bar{P}(\nu)$, normalized by the quadratic of pure somatolactin protein vector's scale. Apparently, the average value of α_i is the function of the culture time after the induction.

RESULTS AND DISCUSSION

Monitoring of Somatolactin Proteins Produced in Single

E. coli Cells. *E. coli* bacteria have been widely used for the production of recombinant proteins due to well-studied genomes, controlled expression, short production cycle, and low cost. In bacterial cells that contain expression vector, the protein synthesis will be switched from synthesizing bacterial proteins to the production of the recombinant protein, somatolactin β , following the induction with IPTG.³⁰ In our experiment, the induction was initiated when the batch culture growth of bacteria reached to late log phase or close to the stationary phase. The majority of overexpressed recombinant protein is prone to be accumulated as inclusion bodies in the bacteria due to rapid synthesis and misfolding of the recombinant proteins. The average Raman spectrum was obtained from 20 randomly chosen bacterial cells at 0 h (Figure 1, curve A) and 24 h after induction (Figure 1, curve B) from bacteria containing a somatolactin expression vector, with or without induction. The intensities of Raman spectra at positions 1004, 1451, and 1665 cm^{-1} showed apparent increases for the cells that were sampled at 24 h after IPTG induction and had produced recombinant somatolactin β , comparing to those cells sampled at 0 h prior to IPTG induction (Figure 1, curve C). These intensity increases in Raman bands might correlate to the accumulation of expressed recombinant proteins in bacterial cells after induction or changes of other molecules during the expression-related culturing process. Surprisingly, the relative intensity of Raman bands representing the genetic materials (e.g., 783, 1099, and 1573 cm^{-1}) decreased at 24 h after induction. After a lag period following induction, the main biochemical processes in transfected *E. coli* will be the synthesis of the recombinant proteins. The observed decrease in Raman intensities in 783-, 1099-, and 1573- cm^{-1} bands might be due to the reduction of RNA molecules or structure confirmation switching in nucleic acids materials (e.g., DNA) induced by changes of microenvironments inside the bacterial cells, although the amount of genetic materials should not have obvious changes. As expected, the difference spectrum (curve C in Figure 1) between the average spectra of single cells observed at 24 h after induction and of single cells observed at 0 h prior to the induction showed a spectrum similar to that obtained from the pure crystal-like somatolactin β protein suspended in

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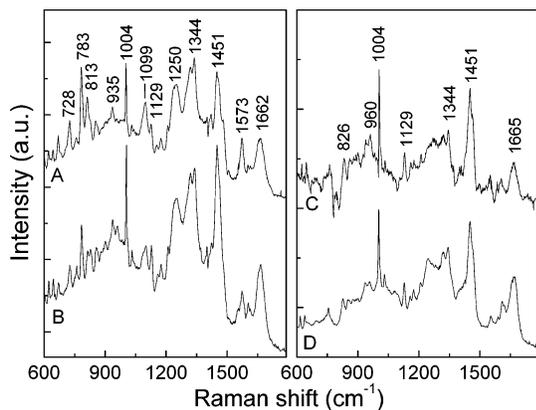


Figure 1. Near-infrared Raman spectra of *E. coli* bacteria obtained prior to the induction of recombinant somatolactin β production at 0 h (curve A) and for 24 h after induction and accumulation of somatolactin β protein (curve B). The spectra were averaged with 20 individual *E. coli* cells sampled at 0 and 24 h after IPTG induction, respectively, and each cell was measured with an acquisition time of 60 s and a power of 100 mW at 790 nm. Curve C was the difference spectrum (B - A), and curve D was the Raman spectrum of a saturated solution of pure somatolactin protein suspended in distilled water with an acquisition time of 60 s and 20 mW power.

distilled water (curve D in Figure 1). This may indicate the production and accumulation of recombinant somatolactin β proteins inside the cell bodies during the culturing process after induction. The spectrum range from 900 to 1800 cm^{-1} will be used for dot product analysis in following discussion. The dimension of the excitation volume is about 1 μm in diameter and 2 μm in length in our experiments. An *E. coli* bacterium almost filled the entire excitation volume when it was captured in the focused laser beam. Therefore, the difference spectrum represents the molecular changes inside the single *E. coli* bacterium before and after the induction. The curve D was accumulated with crystal-like protein (pure somatolactin protein in a saturated solution) that fills the entire excitation volume. The intensity of the difference spectrum (curve C) at the 1004- cm^{-1} band (phenylalanine) is $\sim 23.6\%$ of the intensity of curve D (pure protein) after calibrating the intensities of curves C and D with the excitation power and accumulation time. This suggests that the accumulated somatolactin protein might occupy up to 23.6% in volume inside an *E. coli* bacterium body after induced and cultured for 24 h.

The recombinant protein generally expressed and accumulated over a period of time, which in turn should change characteristics of the spectrum. The induction was initiated when the batch culture growth of bacteria reached the late log phase or close to stationary phase. The intensity of the average spectrum of transfected *E. coli* bacteria increased with the culture time at 0, 4, 12, 20, and 24 h after the induction. However, the pattern of the difference spectrum remained similar over the entire experimental period (Figure 2a). The averaged Raman spectra of *E. coli* bacteria transfected with somatolactin β cDNA at 0 h prior to the induction were accumulated as control. Curves A–D are the difference spectra of 20 measurements of single bacteria cell at designated duration (4, 12, 20, and 24 h) following induction with an IPTG-subtracted average spectrum obtained at 0 h. Curve E is the Raman spectrum of pure somatolactin protein in distilled water. The increase in the intensity of the spectra was observed at 4 (Figure 2a, curve A) and 12 h (Figure 2a, curve B) following

the induction. The intensities of Raman bands apparently increased in the bacteria at 20 or 24 h following the induction, which correlates well with dramatic increase of recombinant proteins in the bacterial cells analyzed by SDS-PAGE (Figure 2b). A unique band of recombinant SL β (Figure 2b, indicated by the arrow) at molecular mass 28 kDa was observed in total proteins extracted from bacterial cells transformed with expression vector in comparison to control bacterial cells without a vector. The additional thick band present underneath the recombinant SLB band represents a housekeeping gene produced by the *E. coli* cells.²⁶ The identity of the arrow-marked band was confirmed as recombinant somatolactin proteins by Western blotting analysis (data not shown). The relative amounts of recombinant protein produced in the bacteria were low at 4 and 12 h and increased dramatically at 20 or 24 h following the induction.

Effect of Batch Culture Process. The production of recombinant somatolactin protein usually takes ~ 24 h, a relatively long period of time. The control *E. coli* bacteria transfected with somatolactin β expression vector without induction did not produce recombinant somatolactin β protein (the data were not shown). The Raman spectra did not show an obvious difference in the first 12 h following induction from that at 0 h. The difference in Raman spectrum increases at 20–24 h after the induction. The results obtained from Raman spectra were confirmed by SDS-PAGE electrophoresis analysis: no accumulation of bacterial proteins in the first 12 h was found with the coomassie blue staining of the polyacrylamide gel. The staining of the gel became stronger in bacteria sampled between 12 and 24 h, which suggests that some bacterial proteins were accumulated in the bacterial cells. As shown in Figure 2a, the production of recombinant protein could be observed in the LTRS spectrum at 4 h after induction in the bacteria cells transfected with the expression vector. In contrast, the accumulation of protein for the bacteria without induction was not apparent in the first 12 h, although small amount bacterial proteins increases were also observed at 20 and 24 h after induction.

Quantitative Analysis of Somatolactin Protein Produced in *E. coli* Bacteria. The difference in Raman spectra offers a qualitative method for estimating protein accumulated in bacterial cells after induction. We have employed the vector analysis to estimate somatolactin protein produced in bacterial cells. For the control group (the bacteria with exactly the same expression vector but with no induction), the difference spectra were nearly unchanged within 20 h (time-lapse spectra not shown), which suggest no or very low-level accumulation of somatolactin protein in the cell body. By using dot product analysis, which is multiplication between the spectrum obtained from pure somatolactin protein and the spectrum obtained from bacteria that produce recombinant protein, only the recombinant somatolactin proteins will have a none zero value due to spectral correlation between the pure protein and recombinant protein produced in the bacteria. The result indicated that the projected coefficient α_i (proportional to the amount of the recombinant proteins) increased when the culture time increased (Figure 3A). Therefore, the vector analysis offers a plausible method to quantify the levels of somatolactin protein inside the bacteria cell. The value of α_i did not increase significantly in the first 8 h after the induction; however, the α_i value increases rapidly in the period of 12–24 h.

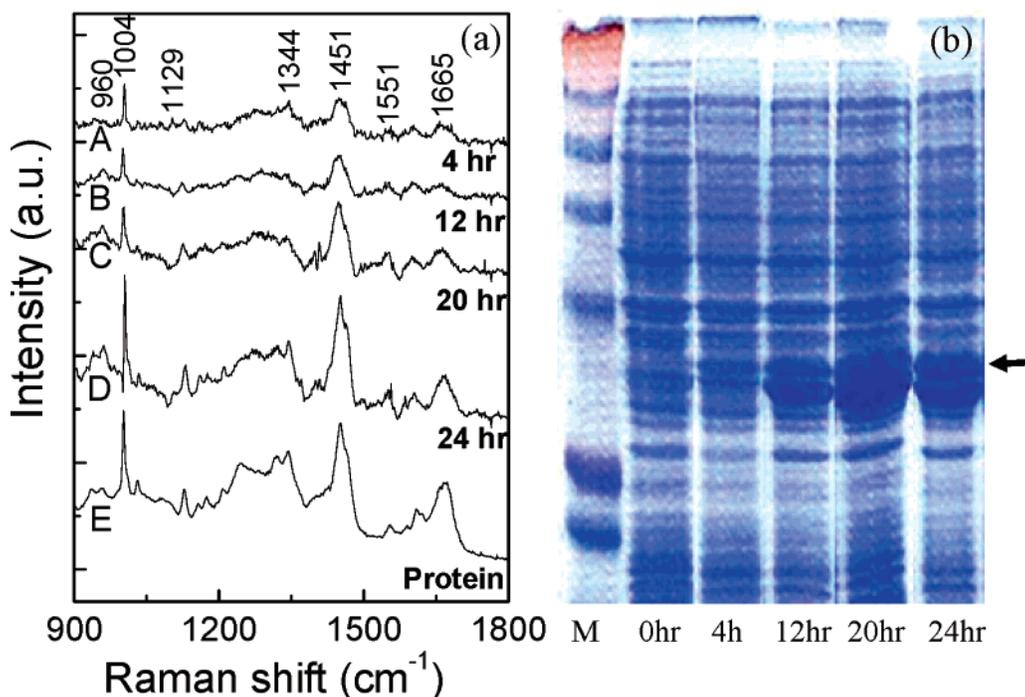


Figure 2. (a) Difference Raman spectra of *E. coli* bacteria sampled at 4 (curve A), 12 (curve B), 20 (curve C), and 24 h (curve D), and Raman spectrum of pure protein (curve E). The Raman spectrum of *E. coli* bacteria sampled at 0 h was subtracted in the difference spectra. (b) SDS polyacrylamide gel electrophoresis analyses of the total proteins extracted from bacteria prior to the IPTG induction (0 h), 4, 12, 20, and 24 h following the IPTG induction. The recombinant somatolactin β protein (indicated with arrows) appeared at 12 h following the induction.

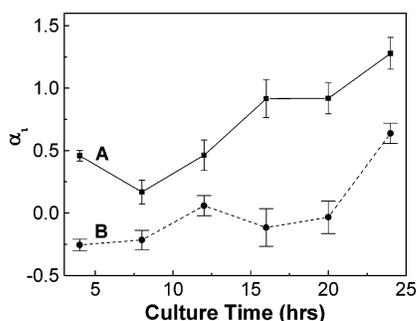


Figure 3. Projection α_i values (proportional to the amount of somatolactin β proteins) as the function of culture time for (A) the induced group with IPTG induction and (B) the control group that was without IPTG induction. The error bar is standard error.

In contrast, in control bacteria (with no induction), the α_i value did not increase in the first 20 h of culture, although a notable increase in α_i was also observed between 20 and 24 h (Figure 3B) but its values were well below the values of the bacteria with induction. The small amount of proteins (α_i value) in transfected bacteria without induction is known as the contribution of residual production of the somatolactin protein as reported previously.²⁶

Production of Somatolactin Protein in *P. pastoris* Yeast Cells. *P. pastoris* is a eukaryotic cell capable of growing at very high density and secreting large amounts of recombinant protein. Unlike the microbial expression system of *E. coli*, *P. pastoris* is capable of many of the posttranslational modifications including proteolytic processing, folding, disulfide bond formation, and glycosylation. Thus, many proteins are produced as biological active molecules in *P. pastoris*.³¹ Figure 4 gives the averaged Raman spectra of a control group and a transgenic group of yeast

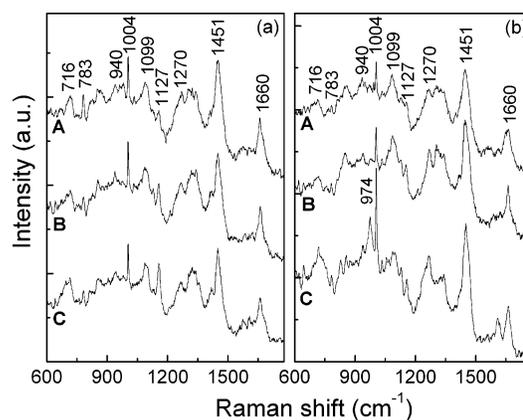


Figure 4. Raman spectra of single yeast cells of (a) the control group without the insert of zebrafish somatolactin β coding sequence and (b) the transgenic group that contained zebrafish somatolactin β coding sequence. Raman spectra of both groups were measured at 0 h prior to the induction (curve A), at 24-h incubation without induction (curve B), or at 48 h after methanol induction (curve C). The laser excitation power for each cell was 16 mW at 785 nm, and the acquisition time was 60 s.

cells cultured for different times, and Table 1 gives the tentative assignments of typical Raman bands. Figure 4a is the Raman spectra of yeast cells in the control group without zebrafish somatolactin gene inserted under different physiological states. Curve A shows the averaged Raman spectrum of 20 control yeast

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Table 1. Raman Bands of Single Yeast Cells and Tentative Assignments^{32–34}

Band (cm ⁻¹)	assigned to ^a
1660	amide I
1608	Tyr, Phe, Trp
1451	lipids/p: -CH
1340	nucleic acids (A, G)
1306	amide III (def)
1270	amide III/adenine
1155	p: C–C/C–N str
1099	DNA: O–P–O ⁻
1004	Phe
974	Unknown
940	DNA: bk/p: (α)
853	Tyr
783	C, T
716	adenine

^a Abbreviations: A, adenine; G, guanine; C, cytosine; T, thymine; def, deformation; Tyr, tyrosine; Phe, phenylalanine; Trp, tryptophan; str, stretching; bk, backbone; p, proteins.

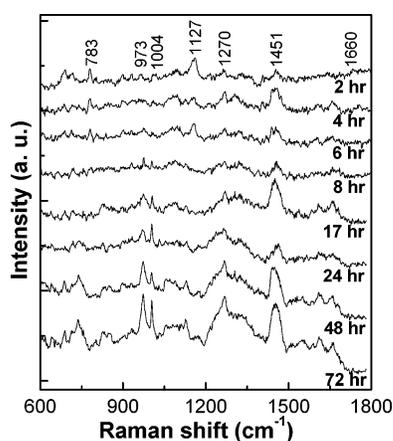


Figure 5. Difference Raman spectra of transgenic yeast cells cultured after methanol induction at different times: 2, 4, 6, 8, 17, 24, 48, and 72 h. The average spectrum acquired at 0 h was subtracted in the difference spectra.

cells prior to induction, curve B is for the yeast cells cultured for 24 h without induction, and curve C is for the yeast cells cultured for 48 h in simulated environment (after induction). From curves A–C, it reveals that, for the control yeast cells (without zebrafish somatolactin gene inserted), there is no difference in Raman spectra before and after induction. The dynamic molecular changes or recombinant protein accumulation inside the control yeast cells is negligible. However, the molecular changes inside transgenic yeast cells are quite obvious after induction, as shown in the averaged Raman spectra of 20 yeast cells at the same three physiological states (Figure 4b). The dynamic molecular changes were also demonstrated in the time-lapse difference spectra of transgenic yeast cells cultured at different times after induction; see Figure 5. In these measurements, the Raman spectrum of each yeast cell was accumulated with an excitation power of 16 mW at 785 nm, and the acquisition time was 60 s. The difference spectra were obtained by subtracting the Raman spectrum at 0 h from those measured at different times after induction. The most significant change is in a marker band at 974 cm⁻¹, the intensity of which was increased for the yeast cells inserted with somatolactin gene following induction. In addition, the intensities of the Raman

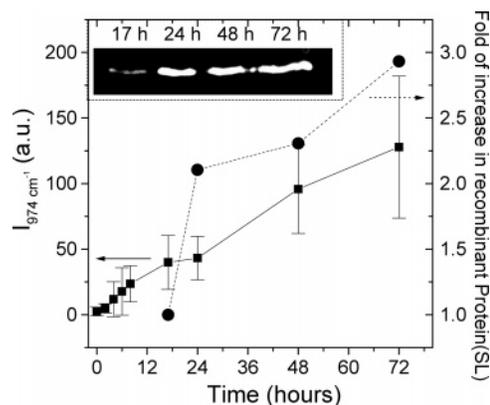


Figure 6. Raman intensities of the 974-cm⁻¹ band (left scale, solid line) and the relative levels of recombinant somatolactin β proteins (right scale, dot line) in yeast cells following methanol induction. Relative protein levels were normalized to those at 17 h, which was scaled with a value of 1.0. Inset: Western blotting analysis of recombinant somatolactin β proteins.

bands at 1004, 1449, and 1610 cm⁻¹ (all associated with proteins) increased significantly after induction. The relative amount of somatolactin proteins accumulated inside the transfect yeast cells after induction was calculated and is shown in the Supporting Information (Figure 1). Note that a new band at 974 cm⁻¹ appears for transgenic yeast cells after induction although the molecular correspondence is not yet identified. The appearance of this band should have direct or indirect relation with the production of recombinant proteins, which is likely resulted from the associated molecules produced and accumulated during the process of recombinant protein expression.

The results obtained with the LTRS method was verified with the Western blotting method. The Raman intensity at 974 cm⁻¹, I_{974} , was plotted as the function of culture time in Figure 6. It increases linearly with culture time, even after a few hours following the induction. Also shown in Figure 6 are the relative levels of recombinant protein produced in yeast cells measured with the Western blotting technique, in which the recombinant somatolactin protein was found to appear at 17 h after induction and increased with the incubation time. Due to the detection limitation of Western blotting, the production of somatolactin protein was not observable in the first 17 h. The result obtained with the Western blotting technique was consistent with that of Raman measurement. However, the sensitivity of Raman spectroscopy clearly showed the appearance of 974-cm⁻¹ band before 17 h after induction. The cells in the control group (without the gene insertion) were cultured and measured under the same conditions. No observable changes were found in Raman band I_{974} , and no production of recombinant protein was observed in the Western blotting method.

CONCLUSION

We have demonstrated rapid detection of the production of recombinant protein in a single transgenic cell in aqueous solution using the combined techniques of laser tweezers and Raman spectroscopy. The single living *E. coli* bacteria or *P. pastoris* yeast cell can be held in the focus of the laser beam for Raman analysis with high sensitivity. Changes of spectral intensity as a result of the progress of culture time serve as an indicator for the

accumulation of recombinant protein induced in *E. coli* bacterial cells. Mathematical prediction using two vectors, the difference among spectrum measured at different time points following induction and the Raman spectrum of pure protein, enabled us to estimate concentration of recombinant protein quantitatively in the transfected *E. coli* bacterial cells. The accumulated inclusion bodies of the recombinant somatolactin proteins occupied up to 23.6% of the bacteria cells. The production of recombinant protein with the *P. pastoris* microbial system was also studied. Following the induction, the recombinant somatolactin protein increased linearly in transfected yeast cells as the culture time progressed. Difference intensity in Raman spectra observed even within a few hours of culture suggests confocal LTRS is more sensitive than the traditional Western blotting method. Taken together, our results demonstrated that confocal LTRS is useful for rapid sensing of recombination protein production in single host microorganisms.

ACKNOWLEDGMENT

This work was supported in part by the National Science Foundation Grant IBN-0315349 (Y.Z.), College Research Award, and the Research/Creative Activity Grant from East Carolina University (Y.Z; Y.-q.L).

SUPPORTING INFORMATION AVAILABLE

Detail information of the construction of expression plasmids, transformation and expressions, SDS-PAGE/Western blot, and protein extraction. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Received for review May 21, 2007. Accepted October 3, 2007.

AC0710329