Rapid Detection of the Recombinant Proteins Production in Single Transgenic Microbial Cells Using Raman-Tweezers Spectroscopy

SURPORTING INFORMATION

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Construction of Expression Plasmids: The coding region of zebrafish somatolactin β was amplified by PCR with forward and reverse primers respectively: 5'-CACCTCTCCAGTGGAGTG-3' and 5'-TCAAAAGAGGGAGCAGTTTTCC-3'. For the first five cycles of PCR, the conditions were 94 ºC for 30 sec, 50 ºC for 30 sec, and 68 ºC for 1 min after two minutes of denaturation at 94ºC. There was an additional 25 cycles with the following condition: 94 ºC for 30 sec, 55 ºC for 30 sec, and 68 ºC for 1 minute. The PCR products were gel purified, ligated into pET100 vector (Invitrogen) and transformed into BL21 *E. coli* cells. For the production of recombinant protein in *P. pastoris* yeast cells, the somatolactin β was amplified from a plasmid containing the cDNA sequence of zebrafish somatolactin β[1] by PCR using a forward primer: 5'-GGGAATTCTTTTGCGCCG and a reversed primer 5'-TTCTAGAAAGAGGGAGCAG. After a 2-minute denaturation at 94 ºC, the PCR cycle was repeated 30 times with the condition of 94 ºC for 30 seconds, 55 ºC for 30 seconds, and 72 ºC for 1 minute. The PCR products (ca 740 bp) were gel-purified, digested with *Eco*RI/XbaI enzymes for 2 hours at 37 °C

and ligated into pPICZaA (Invitrogen) previously digested with the same enzymes. Ten positive clones were selected following transformation of the vector in XL1-Blue (Stratagene) competent cells. Plasmid DNA was purified from bacterial cells using the QIAprep Spin Plasmid Kit (Qiagen). All 10 plasmid DNAs were sequenced with forward and reverse universal primers using Big-Dye Terminator kit and ABI Prism DNA sequencer 377 (Perkin-Elmer, CA).

Transformation and Expressions in *E. coli* and *P. pastoris*: pET100 expression vector containing the somatolactin β cDNA were transformed into BL21 *E. coli* cells by heat shock. BL21 cells were initially grown in 10 mL LB at 37ºC, then transferred to 500 mL of LB. One 500ul aliquot was removed prior to an OD_{600} reading of 0.5 and at the following times: 4, 12, 20, and 24 hours. pPICαA-derived plasmids containing the zebrafish somatoalctin β encoding sequences were linearized at the *Pme*I site for four hours at 37 °C. The linearized plasmids were then transformed into *P. pastoris* GS115 (5 μg/transformation) and the transformed strains were selected on MD plates following electroporation according to the manufacturer's instructions (Invitrogen). Clones able to grow in the absence of histidine were inoculated into sterile test tubes containing BMMG and incubated overnight at 30 °C. After centrifugation (750 g for 15 minutes), the BMMG medium was removed and the BMMH broth (containing methanol at 0.5% v/v) was added. Expression was allowed to take place in an orbital shaker (200 rpm) for 72 hours with addition of methanol (0.5% v/v) every 24 hours. Aliquots of 500 μl were removed prior to induction and 2, 4, 6, 10, 12, 17, 24, 48, 72 hours following the induction. Part of samples was analyzed immediately using optical Raman-tweezers spectroscopy. The remainder yeast cells were obtained by centrifugation and stored at -70 ^{0}C until analysis.

SDS-PAGE/Western Blotting: The yeast cells were sonciated in 200 μl of 1X sample loading buffer (0.125 M Tris, 4%SDS, 20% glycerol, 10% 2-mercaptoethanol) with 2-3 second short burst at power 3 using Sonic Dismembrator (Fisher Scientific). The supernatant containing recombinant zebrafish somatolactin proteins was obtained by centrifugation (12,000g, 10 minutes) of the mixture. The proteins were separated on a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and

transferred to a nitrocellulose membrane. The non-specific binding sites in the membrane were blocked with 10 ml of TBST block buffer (2.42g TrisBase, 8g NaCl, 1 ml Tween per liter) containing 5% nonfat dry milk. Thereafter, the membrane was incubated in 10 ml of the blocking buffer containing primary antibody (rabbit serum against red drum somatolactin, 1:5000 dilution) for overnight at 4^0C. Excess primary antibodies were washed away from the membrane using TBST (4 times, 5 minutes each). The membrane was then incubated for 1 hr at room temperature with a secondary antibody (goat anti- rabbit IgG, 1:2000, Pierce) conjugated to peroxidase. The membrane was developed using chemiluminescent substrate (SuperSignal West Dura Extend Duration Substrate, Pierce) for 5 minutes. The specific somatolactin protein was detected and quantified using an image system (Alpha Innotech, Flurochem 8900). Electrophoresis gels were stained by Coomassie Blue staining (62.5 ml R-250, 250 mL methanol, 100 mL acetic acid, and deionized water up to 500 mL). The same procedure was applied to *E coli* cells containing somatolactin β expression vector.

Protein Extraction: Recombinant somatolactin β proteins were produced by inoculating SLB/pET100 plasmids transformed in BL21 *E. coli* cells into 10 mL Luria Broth (LB) containing 10 ul of ampicillin and incubated at 37 ºC overnight. The entire 10 mL was then inoculated into 500 mL LB and incubated at 37 ºC for 2-4 hours until OD_{600} reads between 0.5-0.8. 1M IPTG was used to induce the cells for expression of the recombinant proteins. Cells were collected and resuspended in phosphate-buffered saline (1 mM KH_2PO_4- 10 mM Na_2HPO_4-137 mM NaCl- 2.7 mM KCl at pH 7.4). Next, the cells were sonicated in 20 mM Tris-HCl- 5 mM EDTA-1 mM PMSF- 1% Triton X-100 at pH 8 followed by centrifugation at 3,000 g for 30 minutes. The resulting lysate was shucked overnight at 4 C in 20 mM Tris-HCl- 5 mM EDTA- 4% Triton X-100 at pH 8. The pellets were then washed four times for 1 hour in 20 mM Tris-HCl- 5 mM EDTA at pH 8. Finally, the pellets were stirred at room temperature in 20 mM Tris-HCl- 6 M guanidinium HCl- 1% β-mercaptoethanol for two days.

(1) Zhu, Y.; Stiller, J. W.; Shaner, M. P.; Baldini, A.; Scemama, J. L.; and Capehart, A. A. *J. Endocrinol*. **2004**, *182*, 509-518.

(2) Nguyen N, Sugimoto M, Zhu Y. Gen. Comp. Endocrinl. 2006, 145, 182-187.

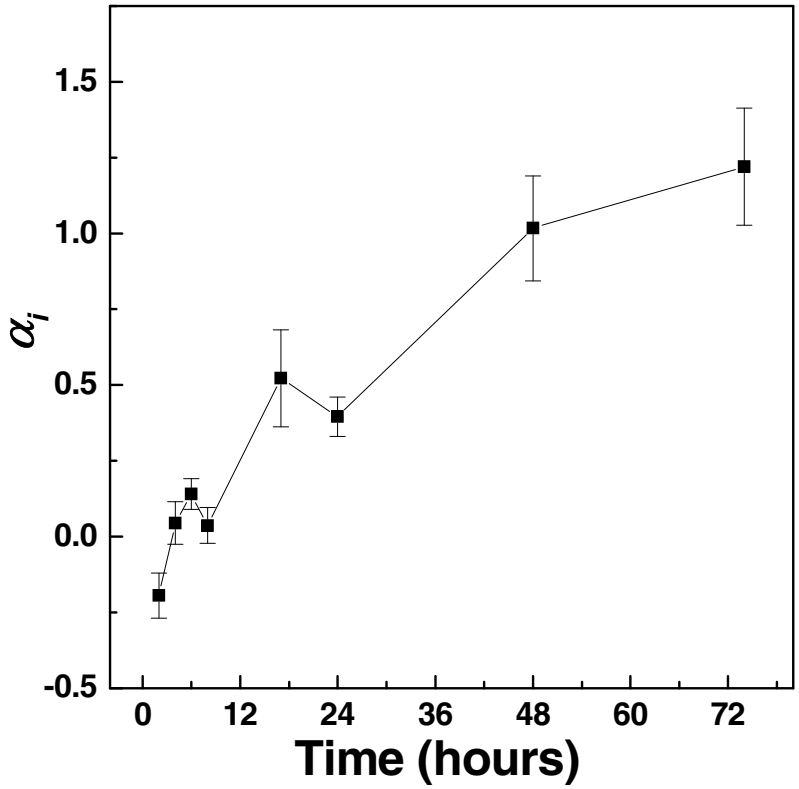

Figure 1. The projection α_i values (proportional to the amount of somatolactin β proteins) as the function of culture time for the induced group of transgenic yeast cells after induction with 0.5% v/v methanol. The error bar is standard error.