Supporting Information

Inhibition assay of yeast cell walls by plasmon resonance Rayleigh scattering and surface-enhanced Raman scattering imaging

Manikantan Syamala Kiran,[1] Hiroko Abe,[1] Yasuko Fujita,[1] Kazuya Tomimoto,[1] Vasudevanpillai Biju,[1] Mitsuru Ishikawa,[1] Yukihiro Ozaki,[2] Tamitake Itoh[1,*]

[1]*Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Takamatsu, Kagawa 761-0395, Japan*

[2]*Department of Chemistry, School of Science and Technology, Kwansei Gakuin University, Sanda, Hyogo 669-1337, Japan*

*Corresponding author's email: tamitake-itou@aist.go.jp

Detailed Experimental Section

Materials and chemicals

Yeast cell strains (*Saccharomyces cerevisiae* W303-1A), β 1,3 glucanases, β 1,4 glucanases, hydrofluoric acid (HF), SDS, NaOH, HCl, and Silver nitrate (Sigma Aldrich, MO, USA) were high quality reagents.

Sample preparation

A colloidal solution of Ag NPs was prepared by the method described elsewhere.[1] Briefly 90 mg of Ag nitrate was dissolved in 500 ml of deionized water and the solution was heated to boiling. Then 10 ml of a 1% trisodium citrate aqueous solution was added into the boiling Ag nitrate solution accompanied by vigorous stirring. The solution was kept boiling for further 10 min. Finally, a green-gray Ag colloid was obtained, which was stable for several days or weeks. The concentration of 1×10^{-11} M and the average NPs size 40 nm were observed for the colloidal solution of Ag NPs. For PRRS and SERS imaging analysis yeast cells in water containing PBS (phosphate buffer saline) in the ratio 1:10 (PBS:Water) were mixed with an equivalent amount of (~500 µL) colloidal solution of Ag NPs. An aliquot of the incubated yeast cells and Ag NPs solution was dropped on a glass slide prior to PRRS and SERS imaging measurement.

Deleted: The concentration of PBS used was so negligible to aggregate colloidal solution of Ag NPs.

Deleted: he presence of PBS is not inevitable for binding of Ag NPs to the cell wall. The yeast cell will also take up Ag NPs in the absence of PBS but the incubation time should be increased to 10 to 15 minutes at room temperature.

Differential treatment for removing components of yeast cell wall

Removal of non-covalently attached proteins: To understand the contribution of non-covalently attached proteins on the cell wall to PRRS and SERS images we specifically removed these proteins by boiling the cells with 0.1% SDS as described earlier.[2] Briefly, the cells from exponential phase were pelleted down by centrifugation from culture medium and washed thoroughly in doubly distilled water. About 100 mg wet weight cells were transferred to two 1.5 mL micro centrifuge tubes (Eppendorff, Hamburg, Germany). To each tube ~500 µL (0.1% SDS w/v) was added and then the cells were boiled for 5 and 10 min in a boiling water bath. Parallel to this procedure control cells were also treated

similarly but without SDS. The cells were then washed to remove the SDS and unbound non-covalently attached proteins. The cells were then finally collected in 1 mL doubly distilled water. About 200 µL of the cell sample was treated with an equivalent amount of Ag NPs and subjected to PRRS and SERS imaging measurement.

Treatment with Hydroflouric acid to remove GPI anchored protein: Yeast cells from exponential phase were treated with 2.5 and 5% hydrofluoric (HF) acid at room temperature (25 °C) for 3h to specifically remove GPI anchored proteins associated with the cell wall.[3] The cells were then washed thoroughly with doubly distilled water and collected in 500 µL doubly distilled water. Equivalent amounts of colloidal solution of Ag NPs and the cell samples were mixed and then the samples were subjected to PRRS and SERS imaging measurement. Appropriate controls without HF treated were also analyzed and the results were compared with the untreated controls.

Removal of β 1,3 glucan using β 1,3 glucanases: Yeast cells were treated with β 1,3 glucanase to remove the β 1,3 glucan frame work.[4] The cells from the exponential phase (100 mg wet weight) was treated with β 1,3 glucanases 0.5 units dissolved in 0.01 M sodium acetate buffer pH 5.0 at 37 °C for 3 h. The cells were then collected by centrifugation and washed thoroughly with doubly distilled water. The cells were then collected in 500 µL doubly distilled water. An aliquot of 250 µL cell suspension was treated with equal volume of colloidal solution of Ag NPs and subjected to PRRS and SERS imaging measurement.

Removal of β 1,6 glucan using β 1, 6 glucanases: Yeast cells were treated with β 1,3 glucanase to remove the β 1,6 glucan frame work.[5] The cells from the exponential phase

(100 mg wet weight) was treated with β 1,6 glucanases 0.5 units dissolved in 0.01 M sodium acetate buffer pH 5.0 and maintained at 37 °C for 3 h. The cells were then collected by centrifugation and washed thoroughly with doubly distilled water. The cells were then collected in 500 µL doubly distilled water. An aliquot of cell suspension (250 µL) was treated with an equal volume of colloidal solution of Ag NPs and subjected to PRRS and SERS imaging measurement.

Removal of alkali soluble cell wall proteins: Yeast cells from the exponential phase (100 mg wet weight) were treated with 50 mM NaOH at 37 °C for 3 h.[6] The cells were then collected by centrifugation, washed thoroughly with doubly distilled water and suspended in doubly distilled water (500 µL). An aliquot of the cell suspension (250 µL) was mixed with an equal volume of colloidal solution of Ag NPs and subjected to PRRS and SERS imaging measurement. The NaOH extract was collected and neutralized with 1.0 M HCl. The neutralized extract was also subjected to SERS analysis.

Effect of pH on cell wall proteins: Yeast cells were maintained at pH 1.5, 7.0, and 12 for 1 h. The cells were then washed three times with doubly distilled water and collected in 500 µL doubly distilled water. Colloidal solution of Ag NPs (500 µL) and the yeast cell suspension (500 µL) were mixed well and an aliquot from this mixture was subjected to PRRS and SERS imaging measurement.

Construction of Pir1 over expressing yeast cells

The plasmid pAB8 was constructed as follows. The *gma12* fragment of the plasmid pAB4 was cleaved with *Sal* I.[7] This fragment of the remainder was self-circularized at the *Sal* I

site, thereby constructing pAB8 containing Pir1-HA fragment. The plasmid was inserted into the Saccharomyces cerevisiae W303-1A strain for generating the Pir1-HA overexpressing cells.

Affinity assay for Pir Protein for Ag NPs

The Pir1 proteins from the surfaces of saccromycetes *cerevesia* W3031A strain overexpressing Pir1 protein were isolated by incubating the cells overnight with 30 mM NaOH. The extract was separated from cell debris by centrifugation. The pH of the cell extract was then neutralized with HCl and mixed with an equal volume of (100 µL) concentrated colloidal solution Ag NPs. This mixture was kept at room temperature (25°C) for 3 h. The Ag NPs were then isolated by centrifugation, washed three times with doubly distilled water. The Ag NPs and the supernatant solution were subjected to western blot analysis to analyze the amount of Pir1 protein bound to Ag NPs.

Construction of *PIR1* gene disrupt yeast cells

For *PIR1* gene disruption, the disruption cassette was amplified by PCR (polymerase chain reaction) using the plasmid YEp352GAP-II containing *URA3* gene as the template, and hybrid oligonucleotides containing both the sequence of the *PIR4* gene to be disrupted and the sequence of *URA3* gene. The oligonucleotides used were: 5'-TTAGCTGCCTATGCTCCAAAGGACCCGTGGTCCACCGAGGCATATTTATGGTG-3' and 5'-CTTTATGTTTTCATGCGACTATGAGAGGTAAACTTCATTACGACCGAGATTCC -3'. The *PIR1* disruption strain was obtained by transformation using the disruption cassette. This strain was screened by PCR using three sets of primers. Set 1 of primer is 5'-

GCATAGTTGTCCTATC-3' (PIR1-F) and 5'- TCCAAGGCTAAGAGAG -3' (PIR1-R). Set 2 of primer is 5'- TCCAAGGCTAAGAGAG -3' (PIR1-F2) and 5'- CCGTCAGTCAGGATAC -3' (PIR1-R2). Set 3 of primer is 5'- GGTAGAGGGTGAACGTTAC -3' (URA-CF) and PIR1-R. The 1455-bp DNA fragment from *pir1Δ* strain and the 1203-bp DNA fragment from wild-type strain are amplified by a primer set 1. The 610-bp DNA fragment from wild-type strain is amplified by a primer set 2, but DNA fragment from *pir1Δ* strain is not amplified. The 218-bp DNA fragment from *pir1Δ* strain is amplified by a primer set 3, but DNA fragment from wild-type strain is not amplified.

Detailed description of the correlation between PRRS and SERS imaging and size of daughter cells

We have demonstrated that proteins linked to β1,3 glucan on cell wall are involved in PRRS and SERS spots. However the dynamics of expression of the proteins in daughter cell walls during cell division is not clarified. As shown in Fig. S1(a) PRRS and SERS spots do not appear on daughter cells when daughter cells are smaller than parent cells. However, as shown in Fig. S1(b,c) PRRS and SERS spots appear on the tip of the bud with growing of daughter cells. The appearance is consistent with earlier reports that newly synthesized Pir proteins are inserted at the tip of newly formed bud.[8,9] As shown in Fig. S1(d) PRRS and SERS spots are observed at regions other than the tip of the bud when size of daughter cells are comparable to parent ones possibly just before the septum formation and cytokinesis. These results suggest that the proteins involving PRRS and SERS spots

may be due to the expression of Pir proteins. The result indicates that SERS microscopy can be employed for understanding the temporal expression of protein during cell wall remodeling.

References

1. Lee, P.C.; Meisel, D. J. Phys. Chem. **1982**, *86*, 3391-3395.

2. Valentin, E.; Herrero, W.; Pastor, J. F. I.; Sentandreu, R. J. Gen. Microbiol. **1984**, *130*, 1419-1428.

3. Ferguson, M.A. In Lipid Modification of Proteins. A Practical Approach; Tumer, A.J., Hooper, N., Ed.; IRL Press: Oxford, **1992**; pp 191-230.

4. Montijn, R.C.; Van Rinsum, J.; Van Schagen, F.A.; Klis, F.M. J. Biol. Chem. **1994**, *269*, 19338-19342.

5. De La Cruz, J.; Pintor-Toro, J.A.; Benitez, T.; Llobell, A. J. Bacteriol. **1995**, *177*, 1864-1871.

6. Mrsa, V.; Tanner, W. Yeast **1999**, *15*, 813-820.

7. Abe, H.; Shimma, Y.; Jigami, Y. Glycobiology. **2003**, *13*, 87-95.

8. Sumita T.; Yoh-ichi. Y.; Jigami. Y.; Eukaryot. Cell **2005**, *4*, 1872.

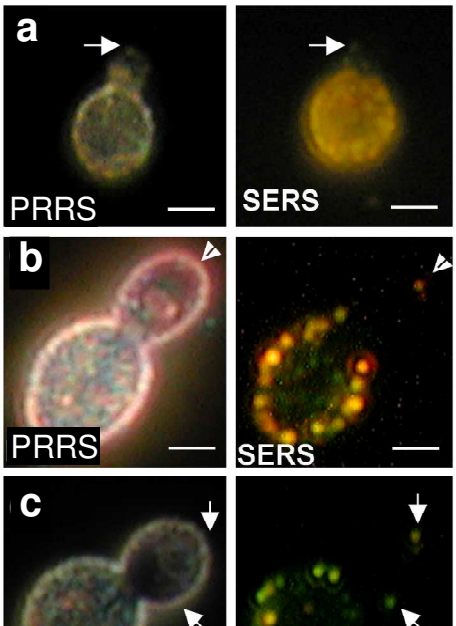
9. Tkacz, J.S.; Lampen, J.O. *J. Gen. Microbiol.* **1972**, *72*, 243.

Supplementary Figure

Figure S1. PRRS and SERS images of yeast cell undergoing cell division. (a) PRRS and SERS images of yeast cells when daughter cells are enough smaller than parent ones, (b,c)

PRRS and SERS images of yeast cells when daughter cells are rather smaller than parent ones, (d) PRRS and SERS images of yeast cells when daughter cell size is comparable to than parent ones. Each scale bar denotes 10 μm. Arrow head points to SERS spots in daughter cells.

Fig. S1



1

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17