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Raman Spectroscopy and Density Functional Theory Calculations of β-Glucans and Chitins in Fungal Cell Walls

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Fungi play an environmentally important role in indoor air pollution and in building-related illnesses.[1-5] The fungal wall is a heterogeneous structure consisting of lipids and carbohydrates such as chitin, 1,3-β-glucan and 1,6-β-glucan. As observed by spectroscopic analysis, the composition of the cell wall frequently varies in a noticeable way between species of fungi. (Figure 1) depicts diagrammatic representations of parts of fungal cell walls.[6] It was expected that strong peaks from either glucans and chitins would appear in the vibrational spectra of fungal cell walls.

Among fungal cellular components, airborne 1,3-β-glucan levels are reportedly related to the extent of respiratory sick-building symptoms.[7] The detection of airborne glucan by Raman spectroscopy may be helpful to monitor the indoor glucan levels. The chitin system often forms characteristic structural components in exoskeletons of insects.[8] Phylogenetic results suggest that the chitin compositions are different for fungal species.[9,10] It is thus possible to differentiate the certain fungal species by carefully examining the Raman spectra of chitins and glucans.

Density functional theory (DFT) has been used to study carbohydrate compounds.[11] In many cases, the DFT calculations may be useful to assign the complicated vibrational spectra. There has been few reports on the Raman analysis of β-glucan[12] and chitin.[13,14] In this work we performed a combined study of DFT theory and Raman spectroscopy to better understand the composition of fungal cell walls. Our work may be helpful in indentifying the fungal species by means of Raman spectroscopy.

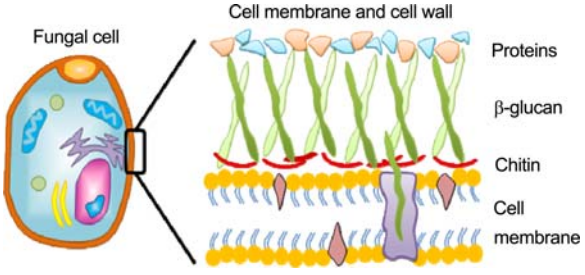

Figure 1. Diagram of fungal cell wall.

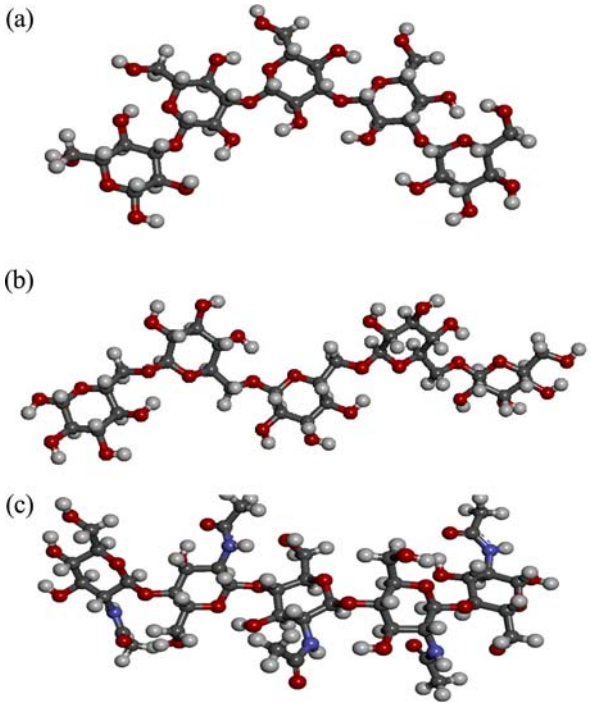

Figure 2. Optimized geometries of (a) 1,3-β-glucan, (b) 1,6-β-glucan, and (c) chitin.

Figure 2 shows optimized geometries of 1,3-β-glucan, 1,6-β-glucan, and chitin from our DFT calculations. Due to the size limitation of molecules to be treated with the DFT theory, only the 5-mer molecules for each case are considered. Geometry optimization with an empirical force field was performed with 50,000 randomly generated conformers for each molecule. Among them, the five most stable conformers were selected to calculate the vibrational frequencies using DFT calculations with the B3LYP/6-31G**. The structures and vibrational frequencies of the most stable conformers for each molecule are reported here. It is also possible to generate the vibrational band positions and

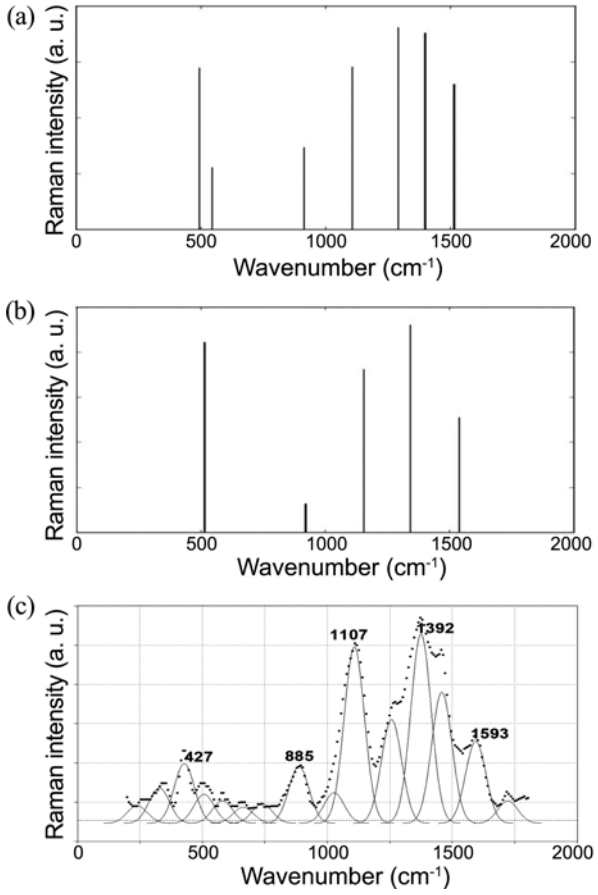

Figure 3. Calculated spectra of (a) 1,3-β-glucan and (b) 1,6-β-glucan. (c) Experimental spectra of β-glucans from black yeast. Weak spectral lines from the DFT calculations were omitted for a better comparison with the experimental values. The overlapping experimental Raman spectral bands (dotted lines) were decovoluted using the Peakfit software.

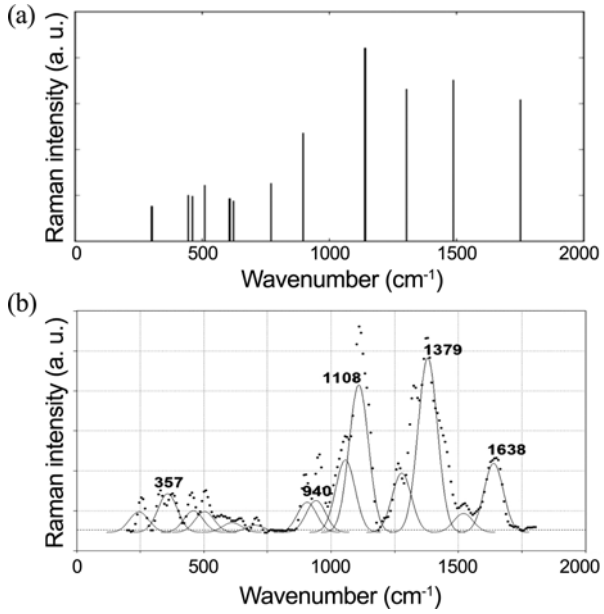

Figure 4. (a) Calculated and (b) experimental spectra of chitin.

intensities from these optimized geometries.

Figure 3 and 4 compare the experimental Raman spectra with the predicted vibrational bands from the DFT calculations. The DFT calculations yielded more peaks than those shown in Figures 3 and 4. The peaks showing weaker intensities were eliminated in the figures for a better comparison with the experimental values. Considering that the glucan sample was obtained from black yeast, this is supposed to contain both 1,3-β-glucan and 1,6-β-glucan. In fact our calculated spectral lines could correspond to the vibrational bands of both 1,3-β-glucan and 1,6-β-glucan. Since many spectral lines were overlapped in a certain congested region due to numerous vibrational modes of glucans and chitins, we performed a deconvolution analysis as shown in Figure 3(c) and Figure 4(b). Quite a few weak spectral lines from the DFT calculations had to be omitted for a better presentation. After the simplifying the theoretical results, our result indicates that the calculated vibrational spectra could still be matched to the DFT calculations.

Based on the previous assignments, the vibrational bands at 1108, 1379, and 1638 cm^{-1} for chitin were mainly ascribed to the ν_{sym} (COC) glycosidic, $\delta(CH_2)$, and amide I (C=O) bands in the previous report.[14] The glucan bands at 1107 and 1392 cm^{-1} can also be ascribed to the ν_{sym} (COC) glycosidic and $\delta(CH_2)$ bands, respectively. Considering that chitins are a polymer of *N*-acetylglucosamine, the vibrational band at 1638 cm^{-1} may be strongly correlated to the C=O band. This band was absent in the Raman spectra of β-glucans. In the Raman spectra of both β-glucans and chitins, the strong bands of the ν_{sym}(COC) glycosidic bands appeared at 1107-1108 cm^{-1}. The strongest peak of β-glucans at 1392 cm^{-1} may also be ascribed to the $\delta(CH_2)$ bending vibrational modes as in the case of those of chitins. The strong bands of chitin and β-glucans appeared in the quite overlapping regions except the C=O band at 1638 cm^{-1}. It is not absolutely certain whether we could differentiate the Raman peaks of 1,3-β-glucan from those of 1,6-β-glucan. The positions of the glycosidic bands may affect the polarizability of their Raman modes. Although we could observe dissimilar spectral features between 1,3-β-glucan and 1,6-β-glucan, it is problematic to identify their differences due to many congested spectral lines. Considering that our DFT calculations are limited to the 5-mer molecules, there may be a little discrepancy from the real polymer sample. It is however instructive that our DFT approach can produce quite similar spectral patterns to the experimental values. Despite the congested spectral lines, the DFT calculations are useful in leading a vibrational analysis of the fungal cell wall components. Our future work will focus on applying the theoretical values in the identification of fungal cell walls.

Experimental and Calculations

Glucan from black yeast (Catalogue # G0331) and chitin (Catalogue # 1398-61-4) were purchased from Tokyo Kasei. Raman spectra were obtained using a Bruker FT-Raman

spectrometer. Deconvolution analysis of Raman spectra was carried out using a SeaSolve software PeakFit version 4.12. Since we could not obtain strong Raman signals for glucans and chitins using the visible irradiation at 632.8 nm, we had to use the near-infrared excitation. For the computational part, only 5-mer molecules are considered for each case (Figure 2). Geometry optimization with an MMFF[15] empirical force field was performed with 50,000 randomly generated candidate conformers for each molecule. The five most stable conformers were selected based on the energies from the MMFF empirical force field. With the selected conformers, geometry optimizations have been performed with the B3LYP/6-31G** basis sets using Gaussian 03[16] have been done to the 5 conformers for each case. Based on the DFT energy, most stable conformers are selected and the vibrational frequencies are calculated using the same DFT calculations. We did not use any scale factors to match the calculated values with the observed band positions.

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