

## Functional Analysis of Expansin with Infrared Spectroscopy

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Expansin is cell wall protein that plays an important role for expansion growth of the plant cell and remodeling of cell wall. It is thought that expansin relaxes the cross-link by hydrogen bonds between cellulose and hemicellulose/cellulose in cell wall, which results in loosening and allowing the wall to be expanded by turgor pressure. Interestingly, molecule of expansin is a glycosylhydrolase of GH-45 family although it does not have no hydrolyzing activity of glucan. To understand the mechanism of expansin, we have used infrared spectroscopy together with intracrystalline deuterated cellulose as a substrate.

### Experiment

In order to selectively see the hydrogen bonding in cellulose crystal, we prepared deuterated cellulose by hydrothermal treatment with NaOD/D<sub>2</sub>O system. Cellulose from marine algae *Valonia* was selected as its large crystallite size allows spectroscopic changes to be easily detected when hydrogen bonding is disturbed in light water. A bacterial homologue of expansin EXLX1 is heterogeneously expressed with hexa-histidine tag fused at C-terminal by *E. coli*. The recombinant EXLX1 was purified by Ni<sup>2+</sup> affinity chromatography. The deuterated cellulose was precisely measured to prepare 1 mg/mL suspension in 50 mM citrate buffer (pH4.8), and treated by 0.2 mg/mL of the purified EXLX1 at 45°C for 24h. For clearly seeing OD/OH exchange in cellulose, EXLX1 was thoroughly removed by washing with 0.1 N NaOH solution. FT-IR measurement was done with microscopic mode (Figure 1), and about 200 spectra were collected from different places in each specimen. The ratio between OD- and OH-signal (OD/OH) was calculated from the spectra, and compared with each other by statistical test.

### Results

The author showed that a bacterial homologue of expansin EXLX1 decreased OD/OH ratio in the deuterated crystalline cellulose. This can mean OD/OH exchange in crystalline cellulose as previously hypothesized<sup>1)</sup>. As well however, it was shown that BSA also decreased the ratio although smaller change. Then the author conducted the same experiment with lost-function mutant of EXLX1. When the residue of EXLX1 corresponding to catalytic acid in HiCel45A (endoglucanase V of *Humicola insolens*)<sup>2)</sup> is mutated to Asn, the decrease of OD/OH was not observed. This result supports that (i) the observed decrease of OD/OH in crystalline cellulose results from the action of expansin itself and (ii) the mechanism of this activity may be substantially same as that of inverting-type hydrolases. Although the author tried to correlate this spectral change with the degree of enhancement of cellulase reaction, the consistent interpretation seems difficult. More detailed studies are definitely necessary to know what the decrease of OD/OH means and subsequently understand the mechanism of expansin for cellulose/hemicelluloses or cell wall.

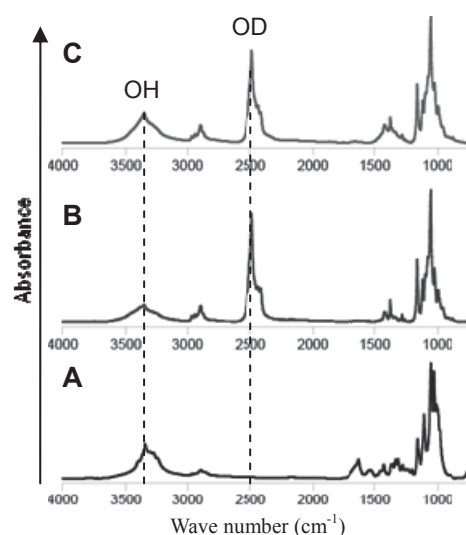


Figure 1 Infrared spectra from purified *Valonia* cellulose (A), its intracrystalline deuterated cellulose (B) and EXLX1-treated deuterated cellulose (C).

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### References

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