Supporting Information

Binding behaviour of a 12-mer peptide and its tandem dimer to gymnospermae and angiospermae lignins

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Materials and methods

1. Preparation of peptide samples

Peptides were synthesized and purified, using the Fmoc solid phase method and reverse-phase high performance liquid chromatography (HPLC), by a custom peptide synthesis service (Thermo Fisher Scientific, Tokyo, Japan). The molecular weight and purity of the synthesized peptides were confirmed via mass spectroscopy and HPLC by the manufacturer. The purity of the synthesized peptides was >95%. Concentration of the peptides was determined by UV absorbance at 257 nm derived from phenylalanine residues.

2. Preparation of milled-wood lignin (MWL)

MWLs were prepared from the ball-milled biomass of softwood *Cryptomeria japonica* (CMWL) and hardwood *Eucalyptus globulus* (EMWL) following the methods of Björkman and Lundquist.[1, 2]

3. Surface plasmon resonance (SPR)

The affinity of peptides and MWLs was determined by SPR measurements using a SPR02

instrument (Optoquest, Saitama, Japan). Self-assembled monolayer (SAM) formation and immobilization of MWLs on the SPR sensor chip was conducted following the method described by Yamaguchi, *et al*.[3] In brief, 1 mM methoxy polyethylene glycol thiol (mPEG-SH; average molecular weight: 5,000, Creative PEGWorks, Chapel Hill, NC, USA) was mounted to both the sample cell and the blank cell of the SPR sensor chip for making SAMs on the gold surfaces of the sensor chip. After the formation of SAMs on the sensor chip, 10 mg/ml of MWLs dissolved in dimethyl sulfoxide (DMSO) were immobilized on the sample cell.

All measurements were performed in PBS (pH 7.0) as a running buffer. In the association phase, 6.25 to 300 μM of the C416 peptide, or 1.56 to 100 μM of the C416 dimer peptide, was injected for 240 seconds, and the running buffer was injected for 180 seconds in the dissociation phase. After the dissociation phase, 10 mM Glycine–HCl (pH 2.0) was injected for sensor-chip regeneration. During the measurements, the flow rate was set at 20 μl/min. The sensorgram for the calculation of dissociation constant (K_D) was obtained as follows: Subtraction of the blank cell sensorgram from that of the sample cell and the subtraction of the 0 μM peptide (=running buffer) sensorgram from the sensorgrams was measured for each peptide concentration.

The calculation of K_D was performed by a non–linear regression curve fit using the Solver add–in of Microsoft Excel. Equation 1, described in below, was used for the calculation.

$$R = \frac{R_{max} \cdot C}{K_D + C} \quad (1)$$

where K_D is the dissociation constant, R is the SPR angle value, R_{max} is the maximum SPR angle value and C is the peptide concentration.

4. Circular dichroism (CD) spectroscopy

The far-UV (190–250 nm) CD spectrum of 75 μM C416 peptide and 30 μM C416 dimer peptide in 10 mM sodium phosphate (pH 7.0) were measured using a Jasco J-715 instrument (JASCO, Tokyo, Japan). The measurement conditions were as follows: temperature, 20 °C; cell length, 0.1 cm; scan speed, 20 nm/min; bandlength, 1 nm and cumulative number, 4.

5. ATR–FTIR spectroscopy

The ATR–FTIR spectra were recorded on a Spectrum Two™ instrument equipped with an UATR diamond/ZnSe ATR (PerkinElmer, Waltham, Massachusetts, USA). Samples (2 μl), containing 10 mM C416 peptide (or 5 mM C416 dimer peptide) and 1 mg/ml CMWL (or EMWL) in PBS (pH 7.0), were deposited onto the surface of the diamond element. Data within

the 4000 to 450 cm^{-1} range were recorded and 20 scans were averaged for each spectrum with a resolution of 4 cm^{-1}. The spectra were corrected using an ATR correction algorithm. The spectra derived from the peptides were obtained by subtracting the solvent spectra and MWLs spectra from the sample. The absorbance value was normalized and the second derivative spectra were calculated using the Savizky–Golay algorithm (five smoothing points). The second derivative spectra in the amide I region (1600–1700 cm^{-1}) were used for analysis of the peptide secondary structure.

References

1 A. Björkman, *Sven. papperstidning*, 1956, **59**, 477–485.

2 K. Lundquist and R. Simonson, *Sven. papperstidning*, 1975, **78**, 390–391.

3 A. Yamaguchi, K. Isozaki, M. Nakamura, H. Takaya and T. Watanabe, *Sci. Rep.*, 2016, **6**, 21833.