

Supporting information

S1.1. Experimental procedures for determination of ChiW-CD properties

The enzyme assays were performed according to the method used for ChiW-SLHd (Itoh *et al.*, 2014) by monitoring the increasing in absorbance at 405 nm after the enzymatic reaction was terminated, which corresponds to the releasing *p*-nitro phenol (pNP) from pNP-*N,N'*-diacetyl-D-chitobiose [pNP-(GlcNAc)₂] (Seikagaku Kogyo, Tokyo, Japan). The reaction was performed at 310 K with 0.2 M of sodium acetate buffer at pH 5.5, 0.1 mM pNP-(GlcNAc)₂ and 10 nM enzyme, and was terminated by mixing the solution with 0.5 M NaOH. One unit of enzyme activity was equivalent to the amount of enzyme required to release 1 μ mol of pNP in 1 min.

After the electrophoretic transfer to a PVDF membrane (Immobilon-PSQ; Millipore, Billerica, MA, USA), the N-terminal amino acid sequence of the recombinant ChiW-CD was determined by Edman degradation using the Procise 491HT protein sequencing system (Life Technologies, Carlsbad, CA, USA).

Structural conformation of purified ChiW-CD was also evaluated by far-UV circular dichroism spectroscopy using a Jasco J-805 spectropolarimeter at 190–250 nm with a quartz cell having a 1 mm path length. The enzyme (1 μ M) was prepared in a buffer containing 10 mM sodium phosphate at pH 7.4. The quadruplicate measurements were performed at room temperature under nitrogen flow. The results were expressed as ellipticity [θ] (deg cm² dmol⁻¹) and were analyzed for assessing the secondary structure content of ChiW-CD by using the *CDPro* analysis software and the *SELCON3* program (Sreerama *et al.*, 1999).

S1.2. Results of enzymatic activity, N-terminal sequencing analysis and secondary structural contents of ChiW-CD

The activity (0.74 U mg⁻¹) of ChiW-CD for pNP-(GlcNAc)₂ was nearly equivalent to that of ChiW-SLHd (0.71 U mg⁻¹) (Itoh *et al.*, 2014). The molecular mass of the enzyme was estimated at approximately 95 kDa (Fig. 1*b*), which was a little smaller than the calculated molecular mass based on its deduced amino acid sequence (98 kDa). The N-terminal amino acid sequence of the protein was determined to be NH₂-MNHKVH, which matched to the predicted sequence. The circular dichroism spectrum (Fig. S1) exhibited a negative peak around 220 nm, indicating that the recombinant ChiW was folded with some α -helix and β -strand structures. The estimated of α -helix and β -strand content of ChiW-CD was 21.4 and 27.0%, respectively. The amino acid sequences of ChiW-CD catalytic

domains were similar to that of chitinase A1 from *Bacillus circulans* WL-12 with approximately 42% identity (PDB: 1ITX; Matsumoto *et al.*, 1999). The chitinase A1 TIM-fold consisted of approximately 35% α -helix and 18% β -strand structures. Therefore, the remaining regions of ChiW-CD should be almost exclusively β -stranded structures.

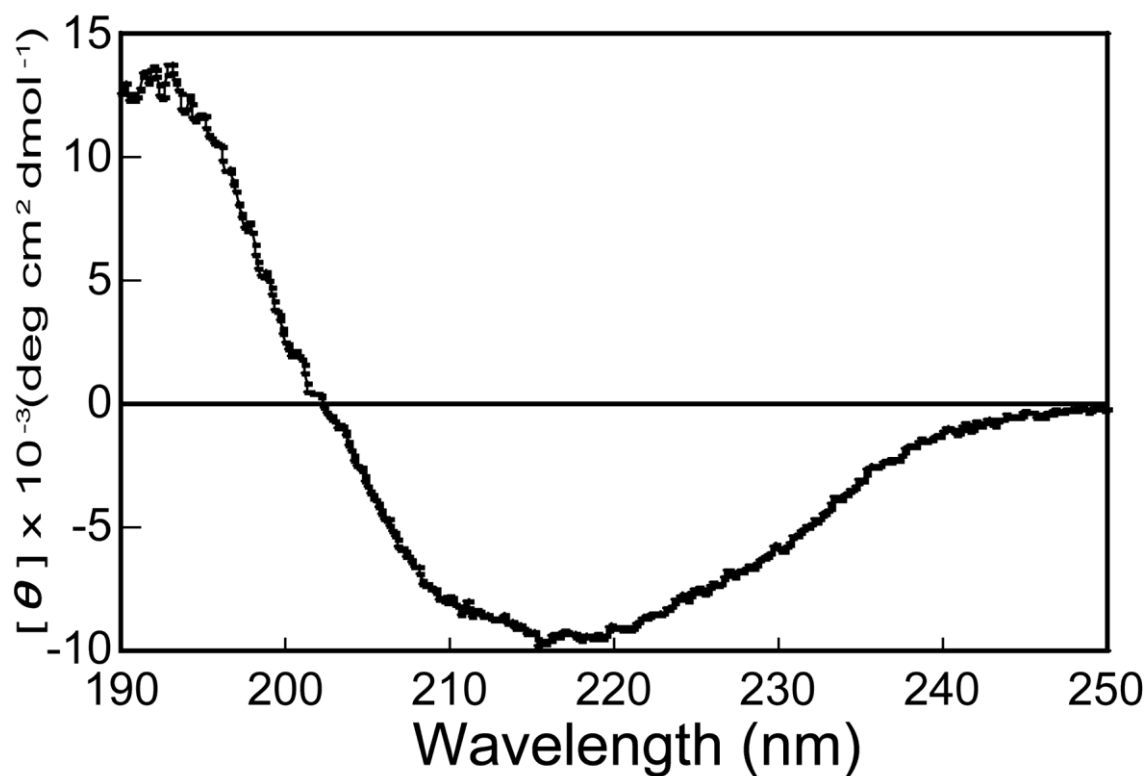


Figure S1 Circular dichroism spectra of ChiW-CD. The path-length of the cell was 1 mm for measurements from 250 to 190 nm. The enzyme (1 μM protein concentration) was prepared in a buffer containing 10 mM sodium phosphate at pH 7.4.

References

- Itoh, T., Sugimoto, I., Hibi, T., Suzuki, F., Taketo, A., Fujii, Y. & Kimoto, H. (2014). *Biosci. Biotechnol. Biochem.* In the Press.
- Sreerama, N., Venyaminov, S.Y. & Woody, R.W. (1999). *Protein Science*, **8**, 370–380.
- Matsumoto, T., Nonaka, T., Hashimoto, M., Watanabe, T. & Mitsui, Y. (1999). *Proc. Jpn. Acad. Ser. B*, **75**, 269–274.