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Structural and binding properties of laminarin revealed by analytical ultracentrifugation and calorimetric analyses

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ABSTRACT

One of the β-1,3-glucans, laminarin, has been widely used as a substrate for enzymes including endo-1,3-β-glucanase. To obtain quantitative information about the molecular interaction between laminarin and endo-1,3-β-glucanase, the structural properties of laminarin should be determined. The results from pioneering work using analytical ultracentrifugation for carbohydrate analysis showed that laminarin from *Laminaria digitata* predominantly exists as a single-chain species with approximately 5% of triple-helical species. Differential scanning calorimetry experiments did not show a peak assignable to the transition from triple-helix to single-chain, supporting the notion that a large proportion of laminarin is the single-chain species. The interaction of laminarin with an inactive variant of endo-1,3-β-glucanase from *Cellulosimicrobium cellulans*, E119A, was quantitatively analyzed using isothermal titration calorimetry. The binding was enthalpically driven and the binding affinity was approximately 10^6 M^{-1}. The results from binding stoichiometric analysis indicated that on average, E119A binds to laminarin in a 2:1 ratio. This seems to be reasonable, because laminarin mainly exists as a monomer, the apparent molecular mass of laminarin is 3.6 kDa, and E119A would have substrate-binding subsites corresponding to 6 glucose units. The analytical ultracentrifugation experiments could detect different complex species of laminarin and endo-1,3-β-glucanase.

1. Introduction

Glycoside hydrolase (GH) enzymes are the subject of intensive research, because they have pivotal roles in fundamental biological processes as well as in a wide range of industrial applications [1–4]. Bacterial endo-1,3-β-glucanases (EC 3.2.1.6 or 3.2.1.39) are classified as GH16 family enzymes, which are known to have β-sandwich architectures and to catalyze the hydrolysis of contiguous 1,3-β-linked glucosyl residues [5–8]. The enzymes are often produced as part of an extracellular complex of enzymes that hydrolyze the cell walls constituents of fungi, including β-1,3-glucans [9].

The substrates of the endo-1,3-β-glucanases, β-1,3-glucans, are found in both prokaryotes and eukaryotes. In a variety of eukaryotes, including brown algae and fungi, the β-1,3-glucans can be storage polysaccharides or are wall components [10]. The physicochemical properties of β-1,3-glucans depend on the degree of polymerization (DP), degree of branching, and higher-order structure such as triple-helix and random coil [11,12]. In some β-1,3-glucans, the thermal transition between triple-helix and random coil can be monitored in the absence and presence of DMSO or sodium hydroxide, by using differential scanning calorimetry (DSC) [13,14]. Because β-1,3-glucans are usually extracted from natural products such as seaweed and mushrooms, they tend to have polydispersity, leading to difficulty in the quantitative analysis of the carbohydrate binding with endo-1,3-β-glucanase. Although enzyme activities for various β-1,3-glucans have been studied [6,15,16], details of the binding mechanism have not yet been quantitatively described. Revealing these fine details is essential for deeper understanding of the carbohydrate recognition and should

Abbreviations: AUC, analytical ultracentrifugation; DP, degree of polymerization; DSC, differential scanning calorimetry; FRET, fluorescence resonance energy transfer; GH, glycoside hydrolase; ITC, isothermal titration calorimetry.

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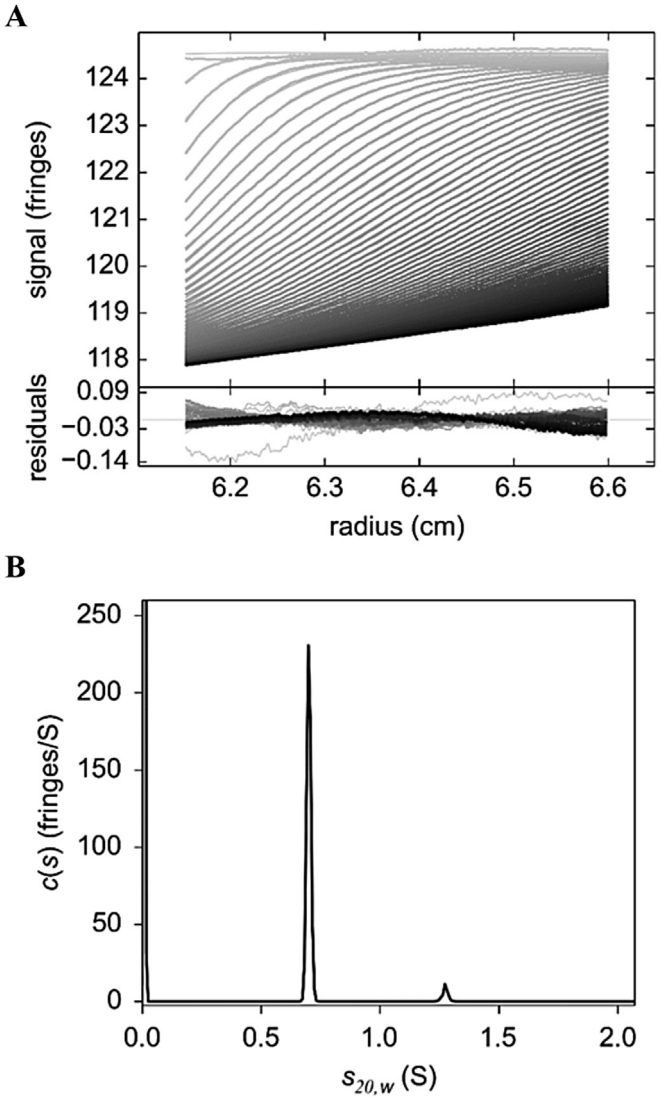

Fig. 1. Analytical ultracentrifugation of 2.0 mg/ml laminarin. Sedimentation velocity pattern and best fit result (A). *c*(*s*) distribution (B).

ultimately lead to the development of valuable enzymes used in industry.

Among the β-1,3-glucans, laminarin from *Laminaria digitata* is water-soluble and contains small but significant levels of β-1,6-linked branches [17,18]. The mean DP value and the polydispersity have been reported to be 25–36 and 1.12, respectively [17–19]. The high solubility and low polydispersity of laminarin are an advantage when using it as an experimental reagent. In fact, laminarin has been widely used as a substrate for enzyme activity assay and as a biological response modifier [6,16,20–22]. Despite its wide usage, the higher-order structure of laminarin is still controversial. Young et al. developed a useful fluorescence resonance energy transfer (FRET) technique and suggested the conformational change of laminarin between closed and partially opened triple-helices [23]. On the other hand, the molecular mass of laminarin is below the minimum of known triple helical structures [24]. Further structural information will be quite useful for evaluating the laminarin binding capacity of endo-1,3-β-glucanase and the requisites for immunologic studies. Its immunostimulating activity seems to be correlated with its higher-order structure [24].

In the present study, we used analytical ultracentrifugation (AUC) and DSC to investigate the molecular mass distribution and the higher-order structure of laminarin. The results indicated that laminarin exists mainly as a single-chain species, which was also supported by the binding stoichiometry with bacterial endo-1,3-β-glucanase determined by AUC and isothermal titration calorimetry (ITC). Elucidating the structure of laminarin made it possible to quantitatively analyze the binding thermodynamics with endo-1,3-β-glucanase. In addition, we have shown that AUC is useful to define the molecular property of a carbohydrate and its binding to a protein.

2. Results

2.1. Molecular mass distribution of laminarin

The molecular mass distribution of laminarin was analyzed using AUC to investigate its higher-order structure (Fig. 1). Because laminarin has no absorbance at 280 nm, the data were acquired using a Rayleigh interference optical system. In the sedimentation velocity method, the apparent molecular mass of the main peak

Table 1
Apparent molecular mass and percentage of each peak obtained in AUC experiments.

Concentration	Apparent molecular mass[a] (kDa)	Percentage[b] (%)	$s_{20,w}$[c]
Laminarin			
2.0 mg/ml (556 μM)	3.6 (3.55, 3.75)[d]	94.9	0.7 (0.69, 0.73)[d]
	10.3	5.1	1.3[e]
E119A glucanase			
16 μM	27.0 (26.2, 27.2)[d]	100.0	2.7 (2.62, 2.72)[d]
70 μM	27.0 (26.3, 27.1)[d]	100.0	2.7 (2.63, 2.71)[d]
150 μM	27.6 (25.8, 27.8)[d]	100.0	2.8 (2.62, 2.82)[d]
Laminarin/E119A glucanase			
27 μM/16 μM	25.0 (23.2, 25.5)[d]	53.2	3.3 (3.06, 3.37)[d]
	39.7 (39.0, 41.9)[d]	35.8	4.4 (4.32, 4.64)[d]
	59.8	11.0	5.7[e]
133 μM/16 μM	32.7 (31.1, 34.5)[d]	97.4	3.1 (2.95, 3.27)[d]
	66.8 (63.3, 67.6)[d]	2.6	5.0 (4.74, 5.06)[d]
533 μM/16 μM	37.1 (35.1, 39.1)[d]	100.0	2.9 (2.74, 3.06)[d]
12 μM/70 μM	21.5 (20.5, 22.7)[d]	7.8	3.1 (2.95, 3.27)[d]
	59.3 (58.3, 61.3)[d]	92.2	6.0 (5.90, 6.20)[d]
1.2 μM/70 μM	26.8 (25.7, 27.7)[d]	86.3	2.7 (2.59, 2.79)[d]
	66.3	1.9	4.7[e]
	96.7 (95.9, 98.8)[d]	11.8	6.3 (6.25, 6.44)[d]

[a] Under the assumption that unbound E119A and existing complex have similar frictional coefficient ratio in *c*(*s*) analysis, molecular masses for each peak were estimated.
[b] A trace (less than 1%) of a content was removed from calculation.
[c] The sedimentation coefficient, $s_{20,w}$, was converted into those in H_2O at 20 °C, using the software, SEDNTERP [37].
[d] The 68% confidence intervals calculated using F-statistics are shown in parentheses.
[e] These species were not considered significant by F-statistics.