Note

Alkali Extraction of β-D-Glucans from the Sclerotia of Corticium rolfsii

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Received July 22, 1983

The sclerotium is a hard mass of hyphae formed by some fungi in their life cycle. It is not only physically solid but also very resistant to chemical or biological attack. Thus, formation of sclerotia helps the fungi to survive unfavorable conditions. Corticium rolfsii, a pathogenic fungus responsible for so called “Shirakinu (white silk) disease,” is one of the most typical sclerotia forming fungi.

Although sclerotia are very resistant to chemical or biological attack, no satisfactory explanation has yet been offered to account for this resistivity. It can be suggested that some special structural feature of the cell wall components is responsible. Alternatively, some special arrangement of the components may be required to preserve the rigidity of sclerotia. It is known that β-D-glucan is a main structural component of fungal cell walls. It is probable, therefore, that β-glucans play an important role in the resistivity of sclerotia. However, no detailed structural analysis of the sclerotial β-D-glucans has been made except for that of Sclerotinia libertia.1

In an attempt to obtain evidence for the contribution of β-D-glucans to the resistivity of sclerotia, we started a systematic examination of the polysaccharides in the sclerotia of Corticium rolfsii. Isolation and characterization of a glycogen from the sclerotia of this strain has been reported in a previous paper.2 The present paper described structural analysis of the β-D-glucans isolated from sclerotia of Corticium rolfsii by fractional extraction with alkali.

β-D-Glucans were prepared from the sclerotia as previously reported.2 The β-D-glucan-rich fractions (7A-S: 5560.9 mg, 14A-S: 941.8 mg and 21A-S: 291.5 mg) were further fractionated by DEAE-Sephadex A-25 column chromatography (borate type) as previously reported.3 Fractions eluted with 0.5 M sodium borate and 0.5 M NaOH were collected from each sample. These fractions were shown to contain no appreciable amounts of glycogen as checked by the Krisman iodine staining method3 and α-amylase hydrolysis. Seven β-D-glucan fractions were finally obtained: 1 and 2. Two fractions, extracted with 7% NaOH, and eluted with 0.5 M sodium borate (7A-S-I: 1751.9 mg, [α]D = +18°) and 0.5 M NaOH (7A-S-II: 1547.8 mg, [α]D = +14°), respectively. 3 and 4. Two fractions, extracted with 14% NaOH, and eluted with 0.5 M sodium borate (14A-S-I: 188.1 mg, [α]D = +34°) and 0.5 M NaOH (14A-S-II: 206.3 mg, [α]D = +53°), respectively. 5 and 6. Two fractions, extracted with 21% NaOH, and eluted with 0.5 M sodium borate (21A-S-I: 34.5 mg, [α]D = 0°) and 0.5 M NaOH (21A-S-II: 34.5 mg, [α]D = 0°), respectively. 7. Residue after 21% NaOH extraction (R-S: 1300.5 mg).

Low specific rotation and the characteristic IR absorption at 890 cm⁻¹ (data not shown) indicated β-linkages for all glucan preparations. Results of methylation analysis of the seven β-D-glucan fractions are shown in Table I. All showed short average chain lengths (ACL) ranging from 3 to 5 indicating highly branched structures. Moreover, they were classified into three groups with respect to average chain length, namely the fraction eluted with sodium borate (A-S-I, ACL = 3), the fraction eluted with NaOH (A-S-II, ACL = 4) and the alkali insoluble residue (R-S, ACL = 5).

Furthermore, by increasing the concentration of alkali, methylation analysis was carried out by the Hakomori method4 as described by Lindberg.5

Abbreviation: ACL, average chain length.
the content of 1,6-linkages in the extracted β-D-glucan increased.

Gel filtration patterns of β-D-glucan fractions prepared by extraction with various concentrations of alkali showed a gradual shift in molecular size (Fig. 1). With increasing concentrations of alkali, the size of the extracted β-D-glucan decreased.

Therefore, the cell walls of sclerotia of C. rolfsii appear to be constituted of three types of β-1, 3-D-glucans: An insoluble glucan relatively enriched in 1,6-linkages and 1,6-linked branches compared to the alkali soluble β-D-glucan and alkali soluble glucans further separable into two types with respect to their average chain lengths. The localization of the three types of β-1,3-D-glucan in the sclerotia is not known.

REFERENCES


FIG. 1. Sepharose CL-2B Column Chromatography of A-S-I and A-S-II.

Column size, 1.4 x 66.5 cm; solvent, 0.1 N NaOH; Vo, elution position of native dextran from Leuconostoc mesenteroides strain NRRL B1299; Vi, elution position of glucose.

1.0 mg of sample was applied to the column. Carbohydrate was assayed by the phenol–sulfuric method.6)