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Sho-ichi Tsujiyama

Differential scanning calorimetric analysis of the lignin–carbohydrate complex degraded by wood-rotting fungi

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Abstract Differential scanning calorimetric (DSC) analysis was applied to the lignin–carbohydrate complex (LCC) fractions degraded by the wood-rotting fungi *Tyromyces palustris* and *Coriolus versicolor*. Based on changes of the thermogram, it is assumed that the brown-rot fungus *T. palustris* split the hemicellulose fragment from the LCC during incubation. On the other hand, in the case of the white-rot fungus *C. versicolor*, the peak temperature of the LCC fraction became higher and then lower, indicating that this fungus first degraded the hemicellulose portion and then decomposed the lignin polymer. Furthermore, the appearance of a lignin-rich fraction was found in the fraction from *C. versicolor*, which was not determined by gel-permeation chromatographic analysis. These results demonstrated that DSC analysis can be used to analyze the decay mechanism of solid lignocellulosic samples.

Key words *Coriolus versicolor* · Differential scanning calorimetry · Lignin–carbohydrate complex · *Tyromyces palustris*

Introduction

Thermal analysis is convenient and highly reproducible, so it has been used to analyze lignocellulose materials.[1–10] Differential thermal analysis (DTA) and differential scanning calorimetry (DSC) were applied to decayed wood as useful analytical methods.[11–15] However, as assignments on the thermogram were not exact, the discussions in earlier reports were not comparable.

S. Tsujiyama (✉)
Department of Forest Science, Faculty of Agriculture, Kyoto Prefectural University, Kyoto 606-8522, Japan
Tel. +81-75-703-5648; Fax +81-75-703-5648
e-mail: s_tsuji@kpu.ac.jp

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Recently, we reported assignment of the DSC thermogram of wood and its components.[16] Our assignment proposed that the thermogram is affected by the interaction of each component. Therefore, the detectable peak by DSC analysis cannot be assigned to each component (e.g., cellulose, hemicellulose, and lignin) but appears as a complex material under the existing states. For example, lignin is determined not to be an independent component but to be a complex with hemicelluloses, as proposed by Kerr and Goring.[17] This observation reveals that DSC analysis has the advantage of detecting change in the lignin-hemicellulose matrix in the secondary cell wall. During the decay process, the cell wall matrix is decomposed accompanied by degradation of each component, so DSC is expected to be useful when analyzing wood decay.

In this study I applied DSC analysis to the lignin–carbohydrate complex (LCC) fractions degraded by wood-rotting fungi to determine the advantage of thermal analysis before studying decayed woods. As the decayed LCC fractions used in this study had been characterized by chemical and spectrophotometric analyses previously,[18,19] they were considered suitable for examining whether DSC analysis is applicable.

Materials and methods

Incubation of mycelia and preparation of degraded fractions

A brown-rot fungus, *Tyromyces palustris* (Burk. and Curt.) Murr. (FFPRI 0507), and a white-rot fungus, *Coriolus versicolor* (L.: Fr.) Quél. (FFPRI 1030), were incubated for 31 days in liquid culture medium containing a water-soluble lignin–carbohydrate complex (LCC-W) prepared from akamatsu (*Pinus densiflora* Sieb. et Zucc.) as the sole carbon source.[20]

Two degraded LCC fractions (P-1 and P-A) were prepared as described previously;[19,20] the P-1 fraction was extracted with 70% aqueous methanol from insoluble

materials precipitated by centrifuging the culture medium. The P-A fraction was prepared by acidifying the supernatant to pH 2–3 after centrifuging the culture medium.

DSC analysis of fractions degraded by wood-rotting fungi

A DSC analysis was carried out as follows: 1 mg of ground sample was placed on an aluminum pan and dried. Thermal analysis was carried out in an ambient atmosphere using a Seiko Industry DSC-220 at a constant heating rate of 10°C/min.

Results

DSC analysis of the P-1 fractions from *T. palustris* culture

The water-insoluble fraction P-1 was the major fraction in *T. palustris* culture. Little structural change in lignin was detected, and only the decomposition of hemicellulose was determined.[18] In the DSC thermogram of this fraction (Fig. 1), the main peak at around 490°C, which can be assigned to the complex of lignin and hemicelluloses,[16] shifted to 529°C (Fig. 2). The shape of the main peak became sharper, and a small peak was observed at around 350°C after 31 days' incubation. This new peak was due to hemicelluloses, determined by estimating its temperature. Considering that the lignin content of this fraction did not change much,[18] this thermographic change indicates that the hemicelluloses were peeled from LCC and depolymerized by a hemicellulolytic enzyme action, but the hemicellulose fragments maintained linkages to lignin. Consequently, the lignin polymer was purified by enzyme action and the main peak shifted to a higher temperature range.

This change in thermal behavior is not in agreement with that for brown-rot fungus-infested wood reported by Reh et al.[12] (A brown-rot fungus *Piptoporus betulinus* shifted the peak at around 480°C to a lower temperature region.) The present result demonstrates that the peak temperature of LCC at around 490°C became higher in the brown-rot *T. palustris* culture. Considering our assignment[16] and the decay mechanism of brown-rot fungi, the present result is more reasonable because it supports the chemical and spectrophotometric data.[18] Hence, any different action between those in LCC and in wood might be present during the decay process.

DSC analysis of P-1 fractions from *C. versicolor* culture

Though the P-1 fraction is a minor fraction in *C. versicolor* culture, DSC analysis was carried out to examine the difference between *T. palustris* and *C. versicolor*. The P-1 fraction of the *C. versicolor* culture indicated two main peaks (Fig. 3). As the analysis was carried out under aerobic conditions, few endothermal peaks due to organic materials appeared at this temperature region. Furthermore, the endothermal peak due to inorganic materials was thought not to appear because there was little contamination by inorganic materials during preparation of this fraction. In earlier reports on decayed woods,[12,14] several peaks were observed at this temperature region, so the sharp peak in fraction P-1 was thought to be caused by the action of white-rot fungi.

The main peak A at around 450°C shifted slightly to a lower temperature range (Fig. 4). Probably, there was little action of *C. versicolor* against this fraction. Another sharp peak B was observed at around 500°C that was not seen with other fractions of both fungi. This peak appeared after 7 days of incubation and gradually shifted to a lower temperature region (Fig. 4). Based on our assignment,[16] this peak is thought to be lignin-rich. The presence of this lignin-

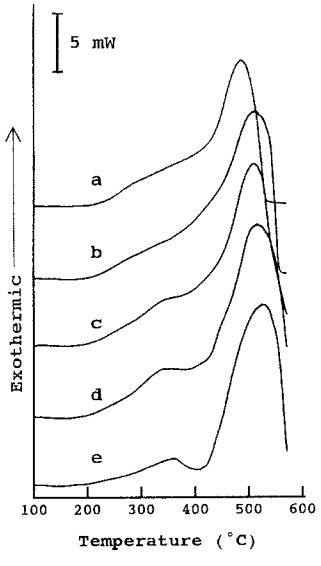

Fig. 1. Thermogram of fraction P-1 from a *Tyromyces palustris* culture. *a*, water-soluble lignin–carbohydrate complex (LCC-W); *b*, 10 days; *c*, 14 days; *d*, 21 days; *e*, 31 days of incubation

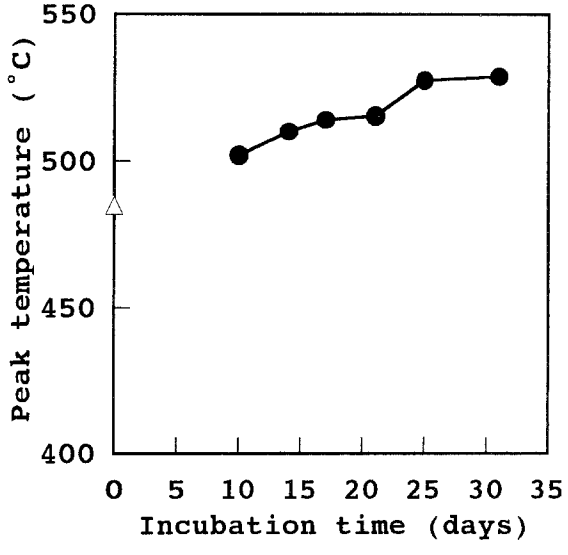

Fig. 2. Peak temperature change of fraction P-1 from a *T. palustris* culture. *Circles*, fraction P-1 of *T. palustris*; *triangle*, LCC-W

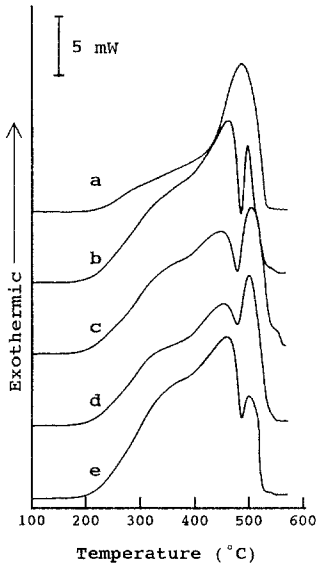

Fig. 3. Thermogram of fraction P-1 from a *Coriolus versicolor* culture. *a*, LCC-W; *b*, 10 days; *c*, 14 days; *d*, 21 days; *e*, 31 days of incubation

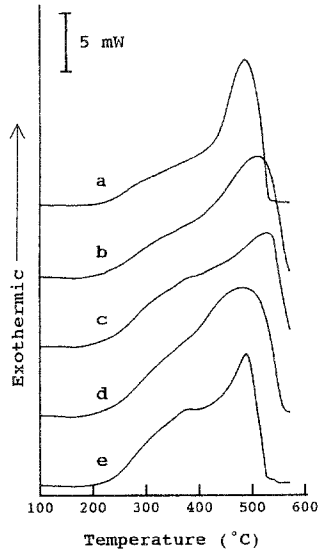

Fig. 5. Thermogram of fraction P-A from a *C. versicolor* culture. *a*, LCC-W; *b*, 10 days; *c*, 14 days; *d*, 21 days; *e*, 31 days of incubation

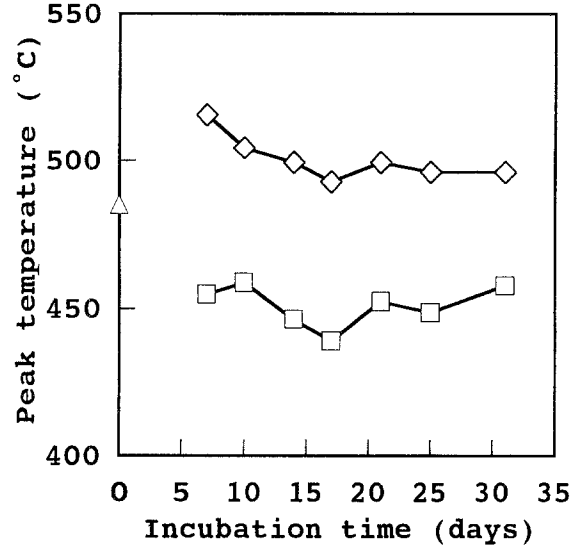

Fig. 4. Peak temperature change of fraction P-1 from a culture of *C. versicolor* culture. *Squares*, peak A in fraction P-1 of *C. versicolor*; *diamonds*, peak B in fraction P-1 of *C. versicolor*; *triangle*, LCC-W

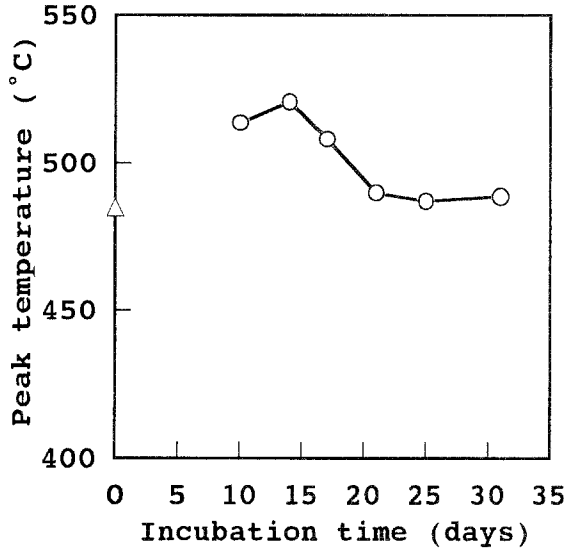

Fig. 6. Peak temperature change of fraction P-A from a *C. versicolor* culture. *Circles*, fraction P-1 of *C. versicolor*; *triangle*, LCC-W

rich fragment was not clearly observed by gel-permeation chromatography (GPC).[18] Probably such a lignin-rich portion was insoluble or poorly soluble, so it would be cut off by the membrane filter during GPC analysis. It suggests that DSC has the potential to detect a solid fraction in which the lignin/hemicellulose ratio is different.

Measurement of the glass transition temperature (Tg) using DSC determined the transition point due to lignin in this LCC sample.[21] This phenomenon is caused by formation of this lignin-rich fragment, but the reason it occurred could not be clarified on the Tg thermogram. The present DSC analysis provided the information that the lignin-rich fragment formed, so thermal decomposition analysis is more useful than the Tg measurement.

DSC analysis of P-A fractions from *C. versicolor* culture

The P-A fraction, which was water-soluble and acid-precipitable, was the main fraction of *C. versicolor* culture but formed after 10 days of incubation.[19] Figure 5 shows the change of its thermograms. The change in this fraction was quite different from that of the main fraction of *T. palustris* (P-1). The peak temperature was high during the formation of this fragment, and became gradually lower (Fig. 6). This indicates that fraction P-A contained higher amounts of lignin despite the fact that it is water-soluble; and its lignin polymer was gradually decomposed. Compared with fraction P-1 of *T. palustris*, the peak width was larger, demonstrating that *C. versicolor*

decomposed lignin polymer in LCC without cutting off the hemicelluloses.

The behavior of both fractions (P-1 and P-A) in *C. versicolor* culture was different from that in the white-rotted woods reported by Reh et al.[12,14] Although Reh et al. reported that thermograms of white-rotted woods indicated complicated patterns, the present results suggest two patterns: white-rot fungi mainly degrade lignin polymer and cause a slight coupling reaction of lignin in LCC. Fractionation of the LCC sample might clarify the degradation mode.

Discussion

The DSC analysis was applied to lignocellulosic materials in earlier studies. The procedure for Tg measurement was used to analyze wood polymeric components[22–24] and to detect the characteristic patterns of LCC.[25] I earlier reported on the application of Tg analysis to decayed LCC and determination of the different decay modes of brown rot and white rot; I also noted the splitting of bonds between lignin and hemicelluloses.[21] This method is available for LCC samples but is not useful for decayed wood because the Tg points due to components could not be clearly detected. In earlier reports, Tg measurement was applied mainly to isolated components and was not reported for solid wood. Probably wood is a complex of three characteristic polymers and would not include free volumes indicating clear Tg points. Even in the case of LCC samples, because the miscibility prevents separation of the Tg peaks due to lignin and hemicelluloses, the appropriate preparation procedure for DSC analysis was needed to avoid the miscibility of lignin and hemicelluloses (unpublished data).

Compared with Tg measurements, thermal decomposition analysis was widely applied to woods and component polymers and has detected characteristic patterns for various samples.[1–15] We were able to make exact assignments and determined that detectable peaks are not independent thermochemically and that they interact each other.[16] For example, different thermograms were observed in two cases of the mixture and impregnation with holocellulose and lignin, indicating that differences in the states of components caused the different interaction of components. Alternatively, complex materials have corresponding patterns based on the thermal interaction of components. Wood is a complex material, so its thermogram indicates not distinctive components but complicated patterns due to interactions among the components. Therefore, the thermal decomposition analysis reported in this study has possible application for decayed wood in the solid state, rather than Tg measurement.

A further advantage of DSC analysis is that it is a direct analysis, not requiring pretreatment. Though GPC analysis is suitable for determining the state of polymers, pretreatment to remove insoluble materials is necessary; GPC can analyze only the soluble portion, so the lignin-rich portion with poor solubility is overlooked (as in this LCC sample the P-1 fraction of *C. versicolor*). In practice, decayed LCC samples that passed through a membrane filter (pore size 0.45 μm) revealed a thermal pattern quite different from that before passing through the filter (data not shown). Direct analysis of solid materials demonstrates the possibility of analyzing wood samples, and it is expected to trace the wood deterioration process not only of decay but of weathering.

Present results differ at many points from those of the decayed wood samples reported by Reh et al.[12,14] The behavior of brown-rotted samples was especially different. Reh et al. reported that brown-rot fungi shift the peak at around 490°C to a lower temperature, but this study proposes that brown-rotted LCC was shifted to a higher temperature because of the decomposition of hemicelluloses and the residue of polymeric lignin. The reason for the difference is not clear: whether it was caused by the difference in substrates between solid woods and water-soluble LCC, or the degradation process of solid woods is different from that of the LCC. For example, as interaction between components is not independent during thermal analysis, it is not clear how decomposed cellulose would have any interaction with lignin and hemicelluloses or how thermogram would change with decomposition of crystal and amorphous regions. In addition, as the cellulose crystal region can be decomposed by ·OH,[26] these changed processes of the cellulose moiety in wood might be reflected in the DSC thermograms. Further study of decayed wood is needed on the basis of degraded LCC.

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