Artificial Modification of Bordered Pits in Softwoods

(1) Treatment with Sodium Hydroxide Solution

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Abstract——The object of this study was to catch the state of dissolution or destruction of tori using sodium hydroxide solution.

Fresh green samples of two conifer species, SUGI (*Cryptomeria japonica* D. DON) and KUROMATSU (*Pinus thunbergii* PARL.) were used, and each sample of species was classified into 3 groups and treated respectively by the different methods, that is, two drying methods (drying at 105°C and air drying at room temperature) and FAA treatment known as a fixing one, and again treated respectively with sodium hydroxide solution of four different concentrations (1, 0.5, 0.25 and 0.125%). And then, from all these samples treated, small block specimens were prepared according to the electron microscope technique, and sections of about 2 μ thickness were cut and observed under a phase contrast microscope.

From the results of this experiment, it was indicated that there was a possibility of dissolving or removing tori without large modification of the morphological features of cell walls or pit borders, and it was known that even a dilute solution of sodium hydroxide such as 0.125 % was also well available to dissolve the pectic material of tori, that the difference of concentration would be almost negligible within the limits of this experiment, and that no remarkable difference existed between the two drying treatments but only some general tendencies were recognized in the case of concentration differences and drying methods. As to SUGI wood, the comparatively remarkable effect of FAA was recognized, but similar result of FAA on KUROMATSU wood was not obtained. Then, the causes of balloon-like expansion of half-bordered pit membranes, and of curving pit borders in the direction to tracheid lumina were also discussed and presumed.

Introduction

Wood which is a natural material having porous structure may not always prove to have good permeability of fluid into wood in spite of its high porosity.^{1,2,3)} In the case of the treatments of preservatives, fire-proof agents and insecticides, wood permeability often comes into question.⁴⁾

In this report, the first object was to observe the states of dissolution or destruction of pit membranes especially tori, which would be the bottleneck for the penetration of fluid into wood, by a reagent that dissolves pectic material which has been already reported to be the main component of tori,^{5,6)} and consequently to suppose continuous progression of their dissolution or destruction, and finally,

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to clarify whether tori were selectively and preferentially attacked, and dissolved or removed without modifying the large morphological feature of cell walls and pit borders or not, and secondly to know the effects of two drying methods and FAA pretreatment on dissolution of tori using sodium hydroxide solution, to know the effects of four different concentrations of sodium hydroxide solution on dissolution of pit membranes especially tori, and to guess the continuous progression of their dissolution.

The reagent used, that is a solvent of pectic material, is not newly developed one but is usually used in many former studies.^{7,8,9)} But the concrete description on the progression of torus dissolution is unexpectedly scarce. It is because of the impossibility of dynamic observation of them, and it depends only on the way that is guessed from many static observations with a considerable difficulties.

Specimens examined were prepared according to the electron microscope technique, and the thickness of sections was about 2μ which was intermediate thickness between that for the optical microscopy and the electron microscopy, and these sections were observed with a phase contrast microscope. This was to be able to observe more details than by optical microscope because of the thinner sections, moreover, to observe larger range of view by the phase contrast microscope than electron microscope. This advantage seems to be the most important in the case of observing the details having largely variable elements because of the thickness or the condition of pit membranes including tori even within a same section.

Materials and Methods

Small green wood pieces were collected by a chisel from standing trees of sugi (*Cryptomeria japonica* D. Don) and KUROMATSU (*Pinus thunbergii* PARL.). In a series of experiment, they were dried by different conditions, that is air drying in a room for about 2 years and oven drying at 105° C for 3 days, and preserved in a desiccator, and in another series of experiment, they were fixed by FAA (90 parts of 50 % ethanol, 5 parts of glacial acetic acid and 5 parts of formalin).

Small blocks $(1 \times r \times t: ca. 3 \times 2 \times 2 \text{ mm})$ were prepared from these pieces in both series of experiments, soaked respectively in 1, 0.5, 0.25 and 0.125 % sodium hydroxide solutions for about 15 minutes at room temperature, and then washed in water in order to stop reaction. These specimens were dehydrated through ethanolpropylene oxide series, embedded with epoxy resin, and then the sections of about 2μ thickness were cut from them for observation by phase contrast microscope.

The reason why the small wood pieces were dried by the two drying conditions

was to observe the reactive difference of pectic material to sodium hydroxide solutions, and the aim that small wood pieces were treated with FAA was to recognize the effect of FAA on the sodium hydroxide reaction. From the four samples marked AK2, BS1, CK3 and DK2 in the subsequent description, that is 'Results and Discussions', no good evidences or results of the photomicrographs were obtained because of too much difference of hardness for cutting sections between the latewood and the embedding resin, and of too few pits in their sections.

By the way, heading symbol letters used, A, B, C, D and K, S, in subsequent 'Results and Discussions' column mean four grades of concentration of sodium hydroxide solution, 1, 0.5, 0.25, 0.125 % and KUROMATSU, SUGI respectively, and simillarly, heading figures, 1, 2 and 3 express the differences of the drying conditions and fixing treatment with FAA. That is, figure 1 shows drying at 105° C, 2 air drying and 3 fixing. For instance, the symbol AS1 shows that the green wood specimens of suGI are treated with 1% sodium hydroxide solution after drying at 105° C.

Results and Discussions

A. Treatment with 1% sodium hydroxide solution

AS 1. sugi wood dried at 105°C

The results, Photos 1-3, were obtained from the specimens treated with 1% sodium hydroxide solution after drying sugi green wood at 105° C.

As shown in Photo 1, pit membranes especially tori were almost dissolved and didn't remain as their traces, but in Photo 2 showed their faint traces leaning on pit borders. Although cell wall distortion or destruction might be expected because of such a rapid drying condition (105°C), the almost normal sections having no distortion as seen in these photos were also obtained according to the choice of cutting places. It was often found that even in such a same drying condition very wide difference was caused within a small block specimen. Photo 3 showed that pit borders were curving to the direction to tracheid lumina. The surface of pit borders in the side of pit chambers seemed to be attacked by sodium hydroxide solution and changed in dark colour under the observation of phase contrast microscope. This may mean the result that sodium hydroxide solution not only attacks the surface of pit borders but also permeates into the cell walls, and that the distortion of pit borders is caused by the resultant difference of sodium hydroxide distribution and of its swelling power in pit borders. The same result as Photo 3 was also obtained from the case of the treatment with 0.125 % (1/8 of 1%) sodium hydroxide solution (Photo 35). In Photos 1-3, the differences of the dis-



Photo 1. AS 1, $800 \times (x)$



Photo 2. AS 1, $800 \times (x)$



Photo 3. AS 1, $800 \times (t)$



Photo 4. AK 1, $800 \times (x)$



Photo 5. AK 1, $800 \times (x)$



Photo 6. AK 1, $800 \times (t)$

solution of pit membranes especially tori might show the sectional effect of pit borders at the strongest attack of this solution, and the progress of dissolution might be presumed to be follwed as Photo 2 to Photo 1. According to the morphological observation of pits and pit membranes especially tori, the latter was appeared to be dissolved more easily and quickly than cell walls, though the dissolution degree of cell walls was not discernible exactly. In short, serious change of cell walls was not observed at least on the morphological feature in this case.

AK 1. KUROMATSU wood dried at 105°C

As shown in Photo 5, cell walls and pit borders were considerably distorted perhaps because of the rapid drying $(105^{\circ}C)$. At the bottom of Photo 5 remained a faint trace of a pit membrane including torus in the pit chamber, and it was obvious that the dissolution of pit membrane especially torus was caused by the solution. Photo 4 seemed to show the condition that a pit membrane especially torus was dissolving and extending to the lumen outwards through a pit pore. In all cases, Photos 4 and 5, tori were comparatively well dissolved. In Photo 6, it was observed that half-bordered pit membranes between ray parenchyma cells and adjacent tracheids expanded like balloons, but it was uncertain whether this ballooning of half-bordered pit membranes was caused by sudden reduction of pressure or by dissolution swelling. The simillar ballooning of half-bordered pit membranes was also observed in Photo 20 (cf. BK 2).

AS 2. **SUGI** wood air dried

As shown in Photos 7 and 8, tori left their faint traces, but they would not be found without sufficient care even by the phase contrast microscope. This is considered to be the result that some materials with optically high densities are dissolved and removed from the tori. As compared with the case of suGI wood dried at 105° C (AS 1), it appears that, in this case (AS 2), the sample was attacked more weakly than that of suGI wood dried at 105° C but no remarkable difference existed between these two cases.

AK 2. KUROMATSU wood air dried

Lacking of available results because of the reason as above mentioned in the column of 'Materials and Methods.'

AS 3. SUGI wood treated with FAA

Photos 9 and 10 seemed to show that the degree of being attacked on tori was more weakly as compared with the wood dried at 105° C (AS1) or/and air-dried wood (AS2). It was rarely found that tori were strongly attacked and dissolved, but the conditions like Photos 9 and 10 were predominant in general.



Photo 7. AS 2, $800 \times (x)$



Photo 8. AS 2, $800 \times (x)$



Photo 9. AS 3, $800 \times (x)$



Photo 10. AS 3, $800 \times (x)$



AK 3. KUROMATSU wood treated with FAA

As shown in Photo 11, tori were perfectly dissolved and their traces were not observed in their regular position. However, a dark stick-like substance existed in a tracheid lumen, and it resembled to a central section of pit membrane including torus.

B. Treatment with 0.5 % sodium hydroxide solution

BS 1. **sugi wood dried at** 105°C Lacking of results.

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BK 1. KUROMATSU wood dried at 105°C

As shown in Photo 12, only gleaming spots were observed at the end of pit border sections at pit aperture but it was uncertain what the gleaming spots meant. In Photo 14, it would not to be decided that the bow-shaped substances in pit chambers of a series of bordered pits (bottom) were the traces of tori because of much transformation of them, though their shapes resembled to that of tori.

BS 2. sugi wood air dried

As shown in Photos 15 and 16, though the closed pit membranes were clearly observed, the contrast of these photos was not sufficient enough, so some substances having high optical density seemed to be dissolved from these pits. Photo 17 showed that the tori were dissolved to the degree as shown in it, but, for the most part, condition like Photos 15 and 16 was predominant.

BK 2. KUROMATSU wood air dried

As shown in Photos 18 and 19, it was easily known that the pit membranes including tori were attacked more strongly than that of airdried sugi wood (BS2). In Photo 19, small gleaming dots, existing in the pit chamber along its pit border were considered to be the traces of closed torus that were not perfectly dissolved. Photo 20 showed that half-bordered pit membranes in the cross sections of ray cells were dissolved, but it appeared that the tracheid lumen sides of the membranes were strongly expanded outwards, while the other sides of the membranes rather shrinked in ray cell lumina. If the former part of half-bordered pit membranes might expand by the pressure reduction, the latter part would have to expanded too, but actually this appeared to shrink. Therefore, the outside expansion of the former part of the membranes was presumed to be owing to the dissolution swelling caused by the action of sodium hydroxide solution.

BS 3. SUCI wood treated with FAA

As shown in Photo 21, small white particles in tracheid lumina were observed but their chemical property remained unknown. However, these particles were



Photo 12. BK 1, $800 \times (x)$











Photo 17. BS 2, $800 \times (t)$

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Photo 18. BK 2, $800 \times (x)$





Photo 20. BK 2, $800 \times (t)$



Photo 21. BS 3, $800 \times (x)$



Photo 22. BK 3, $800 \times (x)$

often observed in wood treated with FAA. In this photo, it appeared that tori didn't undergo a large change. In the case of treatment with 1% sodium hydroxide solution (AS 3), tori were considerably attacked, while they didn't undergo a large change in the case of the treatment of 0.5% sodium hydroxide solution (BS 3).

BK 3. KUROMATSU wood treated with FAA

As shown in Photo 22, it was considered that tori in late wood were almost not attacked.

C. Treatment with 0.25 % sodium hydroxide solution

CS 1. sugi wood dried at 105°C

When Photo 23 was carefully observed, it was found that the traces of tori were leaning on pit borders and considerably attacked.

CK 1. KUROMATSU wood dried at 105°C

Photo 24 showed that cell walls and pit borders were considerably distorted, and the traces of tori were not observed. However, in Photo 25, it was faintly observed that pit membranes were aspirated on the pit borders.

CS 2. SUGI wood air dried

Largely variable conditions of the torus dissolution were recognized, but the state of Photos 26 and 27 was predominant. The traces of tori were faintly recognized without being perfectly removed.

CK 2. KUROMATSU wood air dried

As shown in Photos 28 and 29, the traces of tori were almost dissolved, and not found even by the careful observation.

CS 3. SUGI wood treated with FAA

As shown in Photo 30, it appeared that tori were not attacked.

CK 3. KUROMATSU wood treated with FAA

Lacking of result.

D. Treatment with 0.125 % sodium hydroxide solution

DS 1. SUGI wood dried at 105°C

As shown in Photo 31, the traces of tori could not be observed. When the treating effect of 1% sodium hydroxide solution on tori was compared even with that of 0.125% solution, that is one 8th of 1%, their reactive difference existed scarcely between these two concentrations each other. This was, of course, restricted only on very near surface of a small wood block specimen. As shown in Photo 31, it was considered that the darkish substance margining or sticking along







Photo 24. CK 1, $800 \times (x)$





Photo 26. CS 2, $800 \times (x)$



Photo 28. CK 2, $800 \times (x)$



the cell wall surfaces and within the pit chambers was a resultant material which was dissolved even with 0.125 % sodium hydroxide solution, and such a substance was easily discernible by the own tone of darkish colour.

DK 1. KUROMATSU dried at 105°C

Photo 32 showed that the trace of torus was not observed.

DS 2. sugi wood air dried

Photos 33 and 34 showed the condition of dissolving tori, and it was known that cell membranes and tori were more strongly attacked in the case of Photo 35 than that of Photo 36.

DK 2. KUROMATSU wood air dried

Lacking of result.

DS 3. SUGI wood treated with FAA

Photo 37 showed that a nucleus existed in a ray cell, and retained its shape without being ruptured. It was observed that a few substance having fairly high optical density (light in colour) existed in the lumina of some tracheids, but its chemical property was unknown. Furthermore, it was also uncertain whether this substance in Photo 37 differed or not from that in Photo 38 which had been glittering in the pit chambers or so. Chemical property of this substance in Photo 38 was also uncertain, but it was obvious from Photos 38 and 39 that the substance had a high optical density. Photo 39 was a microphotograph under an ordinary light microscope, and showed the same field as that of Photo 38.

DK 3. **KUROMATSU** wood treated with FAA

Photos 40 and 41 differed very much from that which tori of late wood in Photo 22 were not dissolved, but agreed well with the result in Photo 11 treated with 1% sodium hydroxide solution (AK 3).

As is stated above, the results on the dissolution of pit membranes especially





Photo 32. DK 1, $800 \times (x)$





Photo 34. DS 2, $800 \times (x)$





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Photo 38. DS 3, $800 \times (x)$



Photo 39. DS 3, $800 \times (x)$







Photo 41. DK 3, $800 \times (x)$

tori are discussed. However, theoretically, in order to improve the permeability of wood, it is necessary that the sodium hydroxide solution is to attack selectively pit membranes especially tori, to dissolve or remove them, and to permeate preferentially and deeply into wood. But this experiment was restricted to use only the surface layer of small wood blocks because it had been experienced that the reactivity of sodium hydroxide solution in the surface of wood differed from that in the inside one. Accordingly, the activity of sodium hydroxide solution into the inside of the larger specimens should be continuously investigated precisely furthermore. By the way, the concentration of sodium hydroxide solution showed almost no actual difference of reactivity for pit membrane especially torus dissolution within the range of this experiment.

Conclusions

1. It was possible to dissolve and remove pit membranes especially tori without largely modifying the morphological feature of cell walls and pit borders (Photos 13 and 28). Distortion and rupture observed on cell walls and pit borders (Photo 5) were considered to be caused by rapid drying.

2. Balloon-like expansions of half-bordered pit membranes concerning ray cells that have been caused by dissolution swelling were observed (Photos 6 and 20). However, it was uncertain that the outward expansion of half-bordered pit membranes was owing to the pressure difference between ray cells and adjacent tracheids.

3. The cause that pit borders often curved to the direction of tracheid lumina (Photos 3 and 35) was presumed that the pit chamber side of the borders was attacked more strongly than the other side of them, that is tracheid lumen side.

4. As to the aspirated, closed bordered pit pairs, the contact places of pit borders and pit membranes including tori may resist to the dissolving action of the solution, and accordingly, their traces often remained as some dots along pit borders in the pit chambers after treatment with sodium hydroxide solution as shown in Photo 19.

5. In the case of sugi wood, the effect of FAA treatment on dissolving action of tori with sodium hydroxide solution was clearly observed. Pretreatment of FAA resists to the dissolution of tori. However, in the case of KUROMATSU wood, similarly consistent result was not obtained.

6. The concentration difference of treating solution, sodium hydroxide, for the dissolving effects of pi tmembranes especially tori was almost negligible within the limits of this experiments. This means that sodium hydroxide solution had enough activity to dissolve tori even at low concentration such as 0.125 % of it,

and that this solution was one of strong reagent as a solvent of pectic substance. But its lowest limitation of enough concentration to dissolve pectic substance in pit membranes including tori is to be inquired furthermore.

7. On the whole, the effect of drying methods for dissolving tori with sodium hydroxide solution showed no remarkable difference between two drying treatments, that is drying at 105° C and air drying at room temperature. But as a general tendency, the wood dried at 105° C effected slightly better for dissolving tori than the air dried one.

8. As to the dissolution of pit membranes or tori, the difference of wood species, that is sugi and KUROMATSU, was also not remarkable, but daringly speaking, a tendency was recognized that tori in KUROMATSU was slightly more soluble than that of sugi.

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