The width of cellulose microfibrils in the pit membrane of softwood tracheid*

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針葉樹仮道管の壁孔膜のセルロースミクロフィブリル幅

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Abstract

The width of cellulose microfibrils in the margo of pit membrane of softwood tracheids, where microfibrils exist individually and can be observed without any chemical or mechanical treatments, was electron-microscopically examined with the negative staining technique. The microfibrils in the margo were found to be 40-50 Å in width.

要　　旨

針葉樹材仮道管の壁孔膜のマルゴのミクロフィブリルは，化学的，機械的な処理を行わなくても孤立した状態で観察できる。このミクロフィブリルを負染色法により電子顕微鏡で観察すると，その幅は 40-50Å であった。

Introduction

Many investigations on the structure of cellulose microfibrils in wood cell wall have been carried out since the advent of electron microscope. However, the width of microfibrils has been generally investigated for those isolated by mechanical disintegration, such as an homogenizer or ultrasonics, or by chemical treatments such as delignification, and was not for those which exist individually even under native conditions as seen in the margo of pit membrane of softwood tracheids.

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In this paper, the cellulose microfibrils of the margo of bordered pits in tracheids of a few softwood species are examined by the method of ultrathin sectioning with negative staining and shadow-casting techniques in order to elucidate the fine structure. Particularly the interests of the study have been concentrated into the width of cellulose microfibrils.

Materials and methods

The wood species examined were Akamatsu [*Pinus densiflora* SIEB. et ZUCC.] and spruce [*Picea abies* (L.) KARST] as Pinus type bordered pit memebrane,[1] and Sugi [*Cryptomeria japonica* D. DON] as Taxus type membrane.[1]

Fresh never-dried samples were dehydrated in a graduated ethanol series and embedded in a mixture of n-butyl methacrylate and methyl methacrylate (1:3) following routine procedures. Since the bordered pits in the radial wall of earlywood tracheid are more numerous than in the tangential wall,[2] the radial sections in earlywood were chosen for observation. Sections were cut on a Porter-Blum MT-1 ultramicrotome with a diamond knife, and picked up on copper grids coated with Formvar films reinforced with carbon. After removal of the methacrylate resin with toluene, some sections were negatively stained with a 2% aqueous solution of uranyl acetate for 1–2 days, and examined with an electron microscope (JEM-7). Other sections were shadow-cast with platinum-palladium and also examined.

In a preliminary experiment, it was noted that the printing paper expanded 1.0 to 1.5% in one direction and contracted 0.1% in the other during processing, but these values are not large enough to have any substantial impact on the measurement. No expansion was detected for the negatives.

Results

The margo microfibrils were observed using the negative staining technique (Figs. 1 and 2). For the width measurement, microfibrils individually observed were chosen from the slightly stained area (Fig. 3-B). Two hundred measurements were made on microfibrils from each species. Figure 4 shows the resulting frequency curves. The mode was found to be 40–50 Å in width for the species examined.

In order to make a comparison with the method of negative staining, a metal shadow-casting technique was also used (Fig. 5). Ohad's method[3] for measuring microfibril width was utilized. The width is obtained by plotting the image width (which is measured directly on the photographs) versus sin β (β is the angle between the microfibril axis and the direction of shadow-casting, see also Fig. 5), and by extrapolating to $\beta=0$ by the least square method (Fig. 6). The microfibril widths of the three species determined by the shadow-casting technique were around 50 Å.

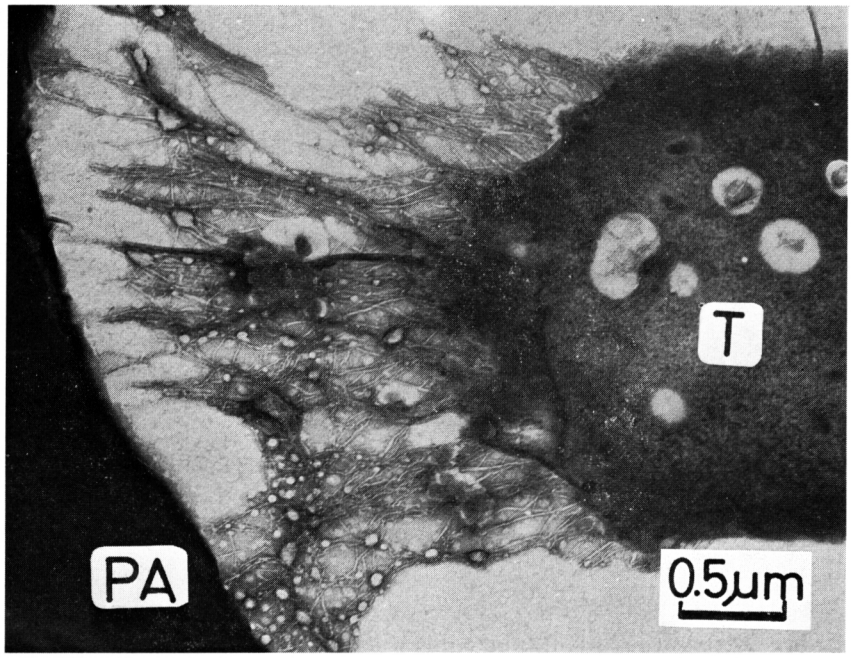

Fig. 1. Electron micrograph of microfibrils in the margo connecting between the pit annulus (PA) and the torus (T) in Sugi (*Cryptomeria japonica*). Negatively stained with uranyl acetate.

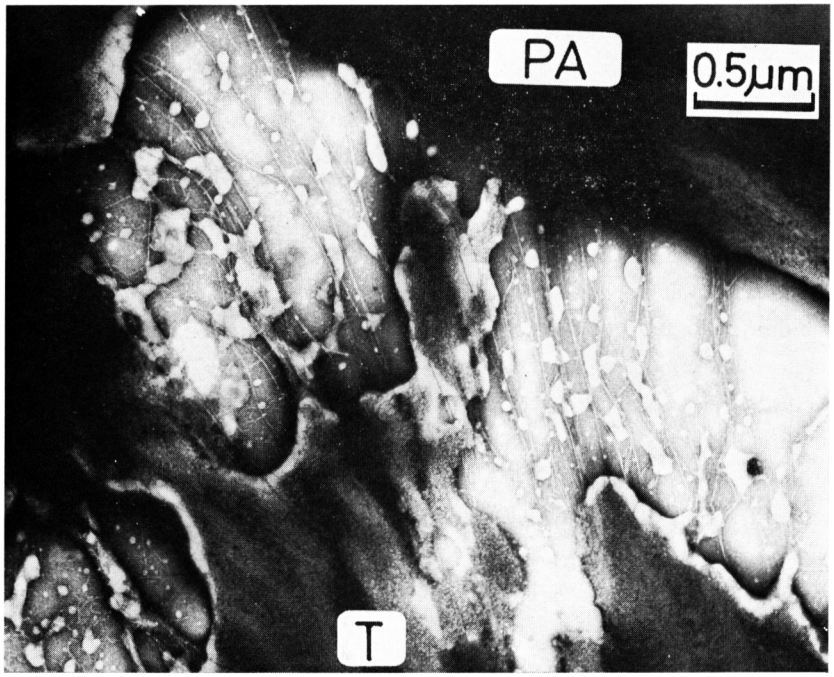

Fig. 2. Simlar to Fig. 1 but in spruce (*Picea abies*).

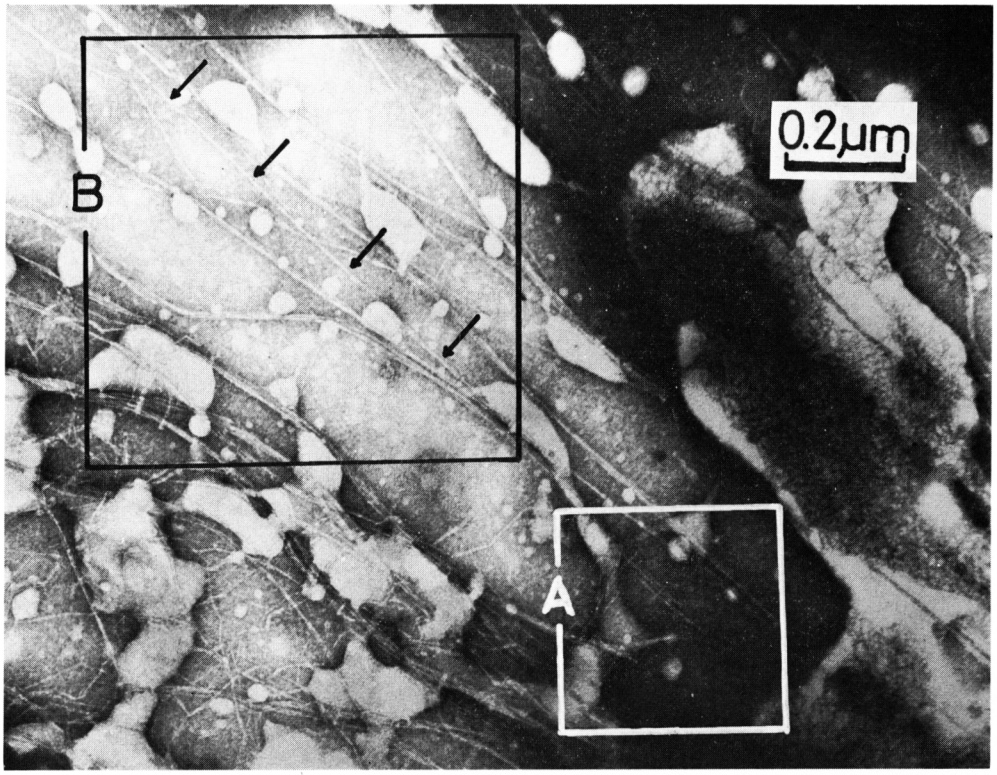

Fig. 3. Magnified micrograph of Fig. 2. Note the isolated microfibrils running from the pit annulus to the torus (arrows). The rectangular area (B), where microfibrils are lightly negatively stained, shows the part chosen for the measurement.

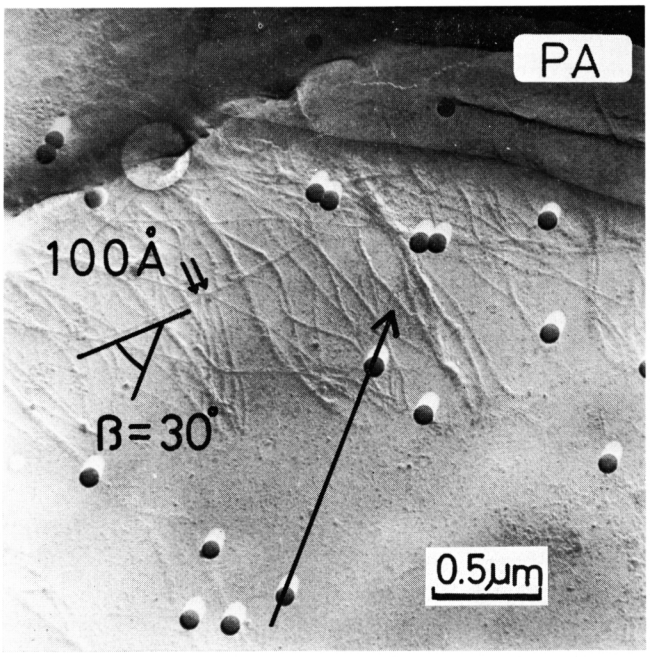

Fig. 5. Pt–Pd shadow-cast microfibrils in the margo of spruce (*Picea abies*). Polystylene latex spheres are sprayed to indicate the direction of the shadow-casting (single arrow). Note the isolated microfibril (double arrows) showing the image width 100 Å when the angle β between the long axis of the microfibril and the direction of the shadow-casting is 30°.

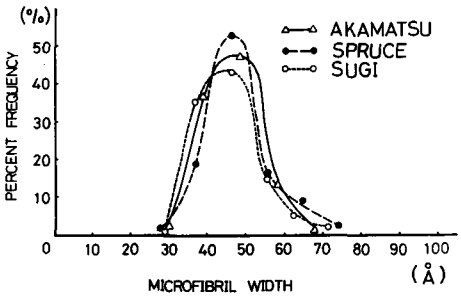

Fig. 4. Width distributions of microfibrils of Akamatsu (*Pinus densiflora*), spruce (*Picea abies*) and Sugi (*Cryptomeria japonica*).

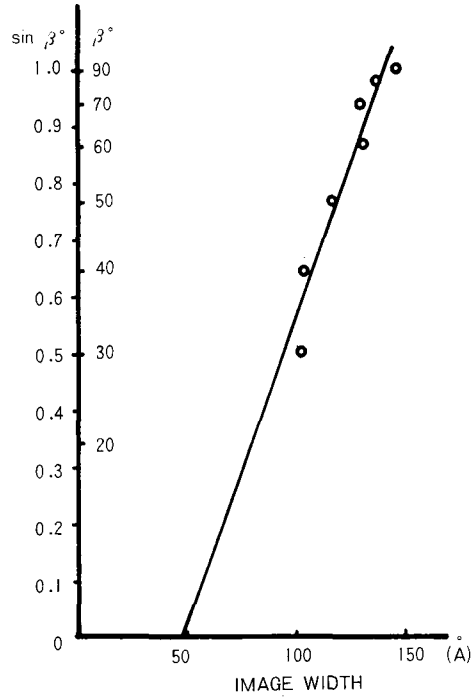

Fig. 6. Measurement of the microfibril width on micrographs of shadow-casting preparations. The image width is plotted against the sine of the angle β between the long axis of the microfibril and the direction of the shadow-casting. Extrapolation to $\beta=0°$ yields the real width of about 50Å.

Discussion

It has been difficult to obtain isolated microfibrils without any mechanical or chemical treatments. In order to overcome this difficulty, the isolated microfibrils in nature, i.e. microfibrils in the margo of the pit membrane, were selected for study. Never-dried samples were embedded in methacrylate resin in order to avoid pit aspiration. The negative staining technique used in this study is reported to have some difficulties. Preston[4] suggested that the application of the negative staining technique to bundles of microfibrils may lead to errors in the estimate of microfibril width because of an overlapping of images. Even with isolated microfibrils, measurement problems exist. For example, in a densely stained microfibril, the image has a tendency to be obscure and underestimated because the stain covers the upper surface of the microfibrils[5]; it is probably seen in Fig. 3–A. Therefore, microfibrils obviously isolated in a lightly stained part were chosen for measurement (Fig. 3–B). The microfibril widths obtained are shown in Fig. 4. This figure clearly shows no significant difference in microfibril width between the two bordered pit types studied.

Microfibril widths determined in this study are larger than those found in cell walls [ex. Goto et al. (6)]. This difference may be due to the fact that margo microfibril formation is not as restricted as cell wall microfibril formation, thus larger microfibrils may be formed in the margo, (which is suggested by HANNA and CÔTÉ[7]). The other possibility is that greater margo microfibril width permits greater resistance of the pit membrane to forces applied by liquid flow through the membrane. This problem of difference in microfibril width will be studied further together with consideration of the detailed mechanism of microfibril formation.

There is no tendency for the microfibril width to have any multiple of about 50 Å (Fig. 4), suggesting that so-called 35 Å elementary fibrils[8] are not a structural unit, at least in margo microfibrils.

The negative staining technique is thought to reveal the crystalline region as the translucent part on the photographic plate, i.e. the stain penetrates only the regions accesible to water.[9] On the other hand, the shadow-casting technique does not differentiate the crystalline and paracrystalline regions of the microfibril and therefore measurements included both regions. On the basis of the above assumptions, the comparison between the microfibril widths obtained by negative staining and by shadow-casting may indicate that there is a slight paracrystalline region around microfibril core.

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