Planta (1981) 152:365–368

Planta

Activities of some enzymes of lignin formation in reaction wood of *Thuja orientalis*, *Metasequoia glyptostroboides* and *Robinia pseudoacacia*

H. Kutsuki and T. Higuchi
Research Section of Lignin Chemistry, Wood Reseach Institute, Kyoto University, Gokasho Uji-shi, Kyoto-fu 611, Japan

Abstract. The activities of the following five enzymes which are involved in the formation of lignin have been compared in reaction wood and in opposite wood: phenylalanine ammonia lyase (EC 4.3.1.5), caffeate 3-*O*-methyltransferase (EC 2.1.1.–), *p*-hydroxycinnamate:CoA ligase (EC 6.2.1.12), cinnamyl alcohol dehydrogenase (EC 1.1.1.–) and peroxidase (EC 1.11.1.7). The activities of the four first-named enzymes in the compression wood of *Thuja orientalis* L. and *Metasequoia glyptostroboides* Hu et Cheng were 2.8 ± 1.4-fold and 2.6 ± 1.5-fold higher than those in opposite wood, respectively, whereas peroxidase had the same level of activity in either type of wood. On the other hand, no differences were observed in the activities of the five enzymes between tension and opposite woods of *Robinia pseudoacacia* L. These findings are well in accord with the chemical structure of lignin in the compression and tension woods of the three species studied: high content of lignin rich in condensed units in compression wood, and little difference in lignin between tension and opposite woods.

Key words: Lignin formation, enzymes – Peroxidase – Phenylalanine ammonia lyase – Reaction wood – Wood, reaction and opposite.

Introduction

Reaction wood, which is commonly formed in non-vertical stems and in branches, is divided into two groups: compression wood and tension wood. The former is formed in the lower part of the stem of gymnosperms; the latter, in the upper part of the stem of angiosperms. The wood opposite to the compression or the tension wood is called opposite wood, and seems to have the normal wood characteristics (Sarkanen and Hergert 1971).

The lignin in compression wood has been intensively studied (Bland 1958; Côté et al. 1967; Erickson et al. 1973; Sakakibara 1977; Tomimura et al. 1980) and has been found to differ from that of normal wood in three respects: (1) high lignin content (in *Larix*, 39% in compression wood and 28% in normal wood), (2) high degree of condensed units (in *Abies*, 79% in compression-wood lignin and 48% in normal-wood lignin), (3) high content of *p*-hydroxyphenyl units which results in low methoxyl content (in *Larix*, 12.64% of methoxyl in milled-wood lignin of compression wood and 15.19% in that of normal wood).

In contrast, only limited information is available on the lignin of tension wood (Bland 1958; Morohoshi and Sakakibara 1971a, b; Tomimura et al. 1980), but it has been suggested that the chemical properties of the lignin in tension wood are almost the same as of that in normal wood. However, it has been reported that the lignin content is slightly less in tension wood than in opposite wood (in *Fraxinus*, 21% in tension wood and 24% in opposite wood).

We have extracted five enzymes known to be involved in lignin biosynthesis from the xylem of reaction wood and opposite wood of two gymnosperms; *Metasequoia glyptostroboides* and *Thuja orientalis*, and one angiosperm, *Robinia pseudoacacia*, and compared their activities with those from opposite wood, in order to contribute to the characterization of lignin in reaction wood. The enzymes studied were phenylalanine ammonia lyase (PAL), caffeate 3-*O*-methyltransferase (OMT), *p*-hydroxycinnamate:CoA ligase (PCL), cinnamyl alcohol dehydrogenase (CAD) and peroxidase (PO).

Abbreviations: CAD = cinnamyl alcohol dehydrogenase (EC 1.1.1.–); OMT = caffeate *O*-methyltransferase (EC 2.1.1.–); PAL = phenylalanine ammonia lyase (EC 4.3.1.5); PCL = *p*-hydroxycinnamate: CoA ligase (EC 6.2.1.12); PO = peroxidase (EC 1.11.1.7)

0032-0935/81/0152/0365/$01.00

Materials and methods

Seven-year-old branches of *Thuja orientalis* L. and *Metasequoia glyptostroboides* Hu et Cheng, and one-year-old ones of *Robinia pseudoacacia* L. were collected in our campus, Uji, Kyoto as sources of the enzymes. After removing the bark, the xylem adjacent to the cambial zone of the reaction and the opposite wood was scraped off separately (Fig. 1). The location of compression wood in a branch was identified by its dark-brown color and that of tension wood was determined with the $ZnCl_2$-I_2 reagent (Konoshima 1954) which stains the gelatinous layers of tension wood purple. Moreover the nature of the tension wood was confirmed morphologically with a light microscope in cross sections stained with $ZnCl_2$-I_2.

The wood tissue (10 g) was cut into small pieces and homogenized in a mortar at 4° C with 100 mM tris(hydroxymethyl)aminomethane(Tris)-HCl buffer, pH 7.2 (30 ml), containing 10% glycerol and 10 mM mercaptoethanol, Polyclar AT (polyvinylpyrrolidone, cross-linked; GAF Corp., New York, N.Y., USA; 1 g) and sea sand (10 g). Glycerol and mercaptoethanol were omitted from the buffer when PO was extracted because of their inhibitory effects on this enzyme. The homogenate was squeezed through cheesecloth, centrifuged at 10,000 *g* for 15 min at 4° C, and the supernatant used as enzyme source.

Enzyme assays were performed by the methods listed in Table 1 (Zucker 1975; Kutsuki et al. 1981 a, b, c). Each assay was repeated three times and the mean value was calculated. Protein in the enzyme solution was determined by the method of Lowry et al. (1951) with bovine serum albumin as standard.

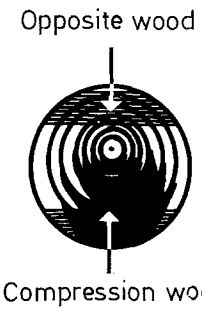

Fig. 1. Location of compression and opposite woods in a branch of *Thuja orientalis*. Compression wood and the parts used as enzyme source are shown in black and hatching, respectively

Results and discussion

Compression wood. The results with compression and opposite woods of *Thuja orientalis* and *Metasequoia glyptostroboides* are shown in Figs. 2 and 3. The activities of PAL, OMT, PCL and CAD, which are responsible for the formation of guaiacyl lignin, were markedly higher in the compression wood in comparison with those in the opposite wood, namely, 2.8 ± 1.4-fold in *Thuja orientalis* and 2.6 ± 1.5-fold in *Metasequoia glyptostroboides,* although no significant differences were observed in the protein concentrations in compression and opposite woods. The same tendency was found for the activities of OMT and CAD, enzymes active in biosynthesis of syringyl lignin, although these enzymes seem to contribute only little to the formation of lignin in these two gymnosperms since gymnospermous lignin mainly consists of guaiacyl units (Creighton et al. 1944).

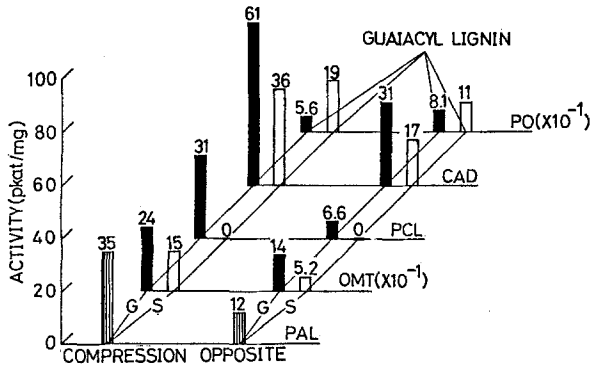

Fig. 2. Enzyme activities of compression and opposite woods in *Thuja orientalis*. PO activity was defined as ΔA_{470} min^{-1} mg^{-1} and the other activities were expressed as pkat/mg. PAL = phenylalanine ammonia lyase; OMT = caffeate *O*-methyltransferase; PCL = *p*-hydroxycinnamate:CoA ligase; CAD-cinnamyl alcohol dehydrogenase; PO = peroxidase; G = guaiacyl-lignin pathway; S = syringyl-lignin pathway

Table 1. Enzyme assays used

Enzyme	Phenolic substrates		Method[a]	Reference
	Guaiacyl-lignin pathway	Syringyl-lignin pathway		
PAL	Phenylalanine	Phenylalanine	290 nm	Zucker (1975)
OMT	Caffeic acid	5-Hydroxyferulic acid	^{14}C	Kutsuki (1981 a)
PCL	Ferulic acid	Sinapic acid	346 nm[b]	Kutsuki (1981 c)
CAD	Coniferaldehyde	Sinapaldehyde	340 nm	Kutsuki (1981 b)
PO	Guaiacol	2,6-Dimethoxyphenol	470 nm	[c]

[a] Enzyme assays were performed at 30° C and activities were measured spectrophotometrically at the wave length shown. For the OMT assay, radioactivity incorporated into substrate from S-[$^{14}CH_3$]adenosylmethionine was measured

[b] 349 nm was used for sinapic acid

[c] PO was assayed with 5 mM substrate and 5 mM H_2O_2 in 100 mM potassium-phosphate buffer, pH 6.5

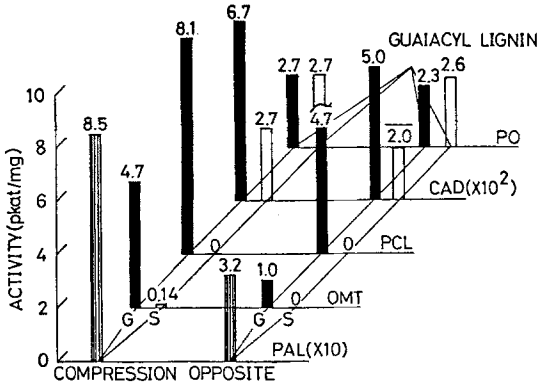

Fig. 3. Enzyme activities of compression and opposite woods in *Metasequoia glyptostroboides*. Activities and abbreviations as in Fig. 2

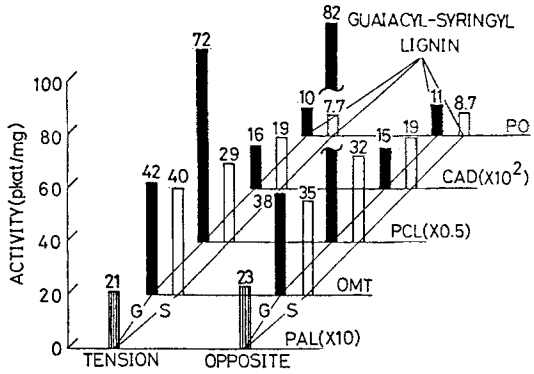

Fig. 4. Enzyme activities of tension and opposite woods in *Robinia pseudoacacia*. Activities and abbreviations as in Figs. 2 and 3

The increased activities of PAL, OMT, PCL and CAD in compression wood should result in larger amounts of lignin precursors, as compared to opposite wood, and this would at least partly explain why compression wood contains more lignin than opposite wood (Bland 1958; Côté et al. 1967; Sakakibara 1977). On the other hand, an increased supply of lignin precursors in compression wood could result in lignin biosynthesis by dehydropolymerization of coniferyl alcohol, analogous to the "Zulaufverfahren", i.e. one-time addition of coniferyl alcohol into PO solution containing H_2O_2 (Freudenberg 1956). The "Zulaufverfahren" gives a so-called "bulk polymer" which contains large amounts of condensed units (Sarkanen 1971) as found in the lignin in compression wood (Sakakibara 1977). Thus, this property of compression-wood lignin may also be ascribed to the increased activities of PAL, OMT, PCL and CAD in compression wood, as compared to that in opposite wood.

Peroxidase activity in both compression and opposite woods was found to be extremely high to compare with other enzymes examined, although an accurate quantitative evaluation was difficult because conversion of the unit of activity of PO into pkat/mg is difficult. Peroxidase activity in compression wood of *Metasequoia glyptostroboides* was slightly higher, while in *Thuja orientalis* it was in far lower than in opposite wood when guaiacol was used as substrate. Therefore, it seems that PO is not involved in the differential regulation of lignin biosynthesis between compression and opposite woods. However only soluble PO was studied and lignin biosynthesis could be madiated by cell-wall-bound POs.

We did not conduct experiments aimed at elucidating the reasons for the third characteristic of compression wood, namely, the high content of *p*-hydroxyphenyl units in its lignin (Erickson et al. 1973; Sakakibara 1977). This characteristic may be the result of the observed increase in the activities of PCL and CAD as these enzymes are involved in the biosynthesis of *p*-hydroxycinnamyl alcohol. However, it could also be the result of increased activity of cinnamate-4-hydroxylase (EC 1.14.13.11) and – or depression of *p*-hydroxycinnamate-3-hydroxylase (EC 1.14.17.2). Further studies are needed to elucidate this problem.

Tension wood. The activities of the five enzymes examined were almost as high in the tension wood as in the opposite wood of *Robinia pseudoacacia*, namely 0.96 ± 0.11-fold (Fig. 4). These findings accord with the fact that the lignin in tension wood, in contrast to that in compression wood, is at most but little different in chemical structure as compared with the lignin in opposite wood (Morohoshi and Sakakibara 1971 a, b). The slightly lesser lignin content in tension wood than in opposite wood might be ascribed to the activation of polysaccharide biosynthesis, which causes the presence of gelatinous layers rich in cellulose in tension wood (Norberg and Meier 1966).

This work was partly supported by the Grant-in-Aid for Scientific Research (548047) from the Ministry of Education, Science and Culture of Japan. The authors are indebted to Dr. H. Kuroda of Research Section of Wood Biology in our Institute for help with the microscope observations.

References

Bland, D.E. (1958) The chemistry of reaction wood. Holzforschung **12**, 36–43

Côté, W.A., Kutscha, N.P., Simson, B.A., Timell, T.E. (1967) Studies on compression wood. Tappi **50**, 350–356

Creighton, R.H.J., Gibbs, R.D. and Hibbert, H. (1944) Studies on lignin and related compounds. LXXV. J. Am. Chem. Soc. **66**, 32–37

Erickson, M., Larsson, S., Miksche, G.E. (1973) Zur Struktur des Lignins des Druckholzes von *Pinus mugo*. Acta Chem. Scand. **27**, 1673–1678

Freudenberg, K. (1956) Beiträge zur Erforschung des Lignins. Angew. Chem. **68**, 508–512

Konoshima, M. (1954) Laboratory manual of botany. Hirokawa-shoten, Japan

Kutsuki, H., Shimada, M., Higuchi, T. (1981a) The role of *O*-methyltransferase in the formation of lignin and lignans in the genus *Thuja*. Mokuzai Gakkaishi (J. Japan Wood Res. Soc., Tokyo) **27**, 39–48

Kutsuki, H., Shimada, M., Higuchi, T. (1981b) A regulatory role of cinnamyl alcohol dehydrogenase in the formation of guaiacyl and syringyl lignins. Phytochemistry (in press)

Kutsuki, H., Shimada, M., Higuchi, T. (1981c) Distribution and roles of *p*-hydroxycinnamate: CoA ligase in lignin biosynthesis. Phytochemistry (in press)

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.L. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265–275

Morohoshi, N., Sakakibara, A. (1971a) The chemical composition of reaction wood. 1. Mokuzai Gakkaishi (J. Japan Wood Res. Soc., Tokyo) **17**, 393–399

Morohoshi, N., Sakakibara, A. (1971b) The chemical composition of reaction wood. II. Mokuzai Gakkaishi (J. Japan Wood Res. Soc., Tokyo) **17**, 400–404

Norberg, P.H., Meier, H. (1966) Physical and chemical properties of the gelatinous layer in tension wood fibers of aspen. Holzforschung **20**, 174–178

Sakakibara, A. (1977) Degradation products of protolignin and the structure of lignin. Rec. Adv. Phytochem. **11**, 117–139

Sarkanen, K.V. (1971) Precursors and their polymerization. In: Lignins, pp. 95–163, Sarkanen, K.V., Ludwig, C.H., eds. Wiley-Interscience, New York London Sydney Toronto

Sarkanen, K.V., Hergert, H.L. (1971) Classification and distribution. In: Lignins, pp. 43–94, Sarkanen, K.V., Ludwig, C.H., eds. Wiley-Interscience, New York London Sydney Toronto

Tomimura, Y., Yokoi, T., Terashima, N. (1980) Heterogeneity in formation of lignin. V. Mokuzai Gakkaishi (J. Japan Wood Res. Soc., Tokyo) **26**, 37–42

Zucker, M. (1965) Induction of phenylalanine deaminase by light and its relation to chlorogenic acid synthesis in potato tuber tissue. Plant Physiol. **40**, 779–784

Received 12 February; accepted 25 March 1981