Oxidases participate in the formation of wood

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The process of wood formation Wood is the major renewable resource of timber for building, pulp for papermaking and is the main source of fuel in developing countries[1]. Trees also fix millions of tonnes of carbon dioxide into wood, which locks up this 'greenhouse' gas and influences global temperature control.

New wood is formed by an extraordinary differentiation process. Trees increase in girth by depositing annual rings of wood during spring and summer (Fig. 1). A transverse section from a branch of Sitka spruce in May (Fig. 1, insert), illustrates the various stages of the differentiation process. New wood cells arise from the division of meristematic cells in the vascular cambium (VC), which lies between the old wood and the bark or phloem tissues. The undifferentiated new wood cells are initially spherical and thin-walled, and appear to be near-identical to the cambial cells. After expansion and elongation (E), the new wood cells deposit a thick cellulose-rich secondary wall inside their original walls that becomes encrusted and waterproofed with the plastic-like polymer, lignin (SCL). The secondary cell wall is the main component of fixed carbon in wood and its formation represents a huge investment of photosynthetic products. During maturation (M), the cells lose their cellular contents and die. The fully differentiated wood cells are hollow, interconnected tubes with reinforced and waterproofed walls that function to transport water from the roots to the leaves.

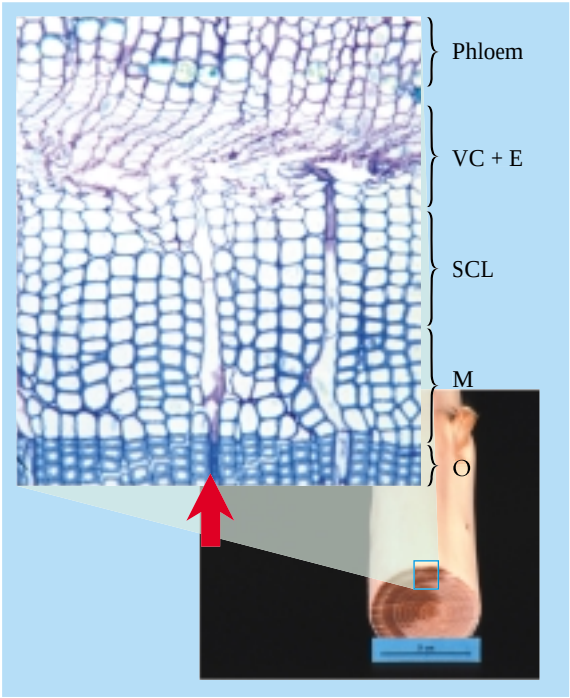

Figure 1 New wood formation in Sitka spruce. A branch of Sitka spruce showing the old wood, new wood and phloem and bark. The expanded insert shows the phloem, vascular cambium (VC) and enlarging wood cells, developing wood cells with lignified secondary cell walls (SCL) and mature wood cells (M). The arrow denotes the position of the vascular cambium.

Oxidase activity co-localises with lignifying wood cells Oxidase activity can be detected in the differentiating wood cells of the gymnosperm, Leyland cypress (Fig. 2a), by specific staining with a colour-generating reagent. Oxidase activity is localised in the differentiating wood cells that have begun to deposit their secondary cell walls but it is absent from the old wood, the vascular cambium and the phloem cells. On closer inspection, it can be seen that the pattern of oxidase activity effectively co-localises with the presence of lignin in differentiating wood cells (compare Figs 2a & 2b). Oxidase

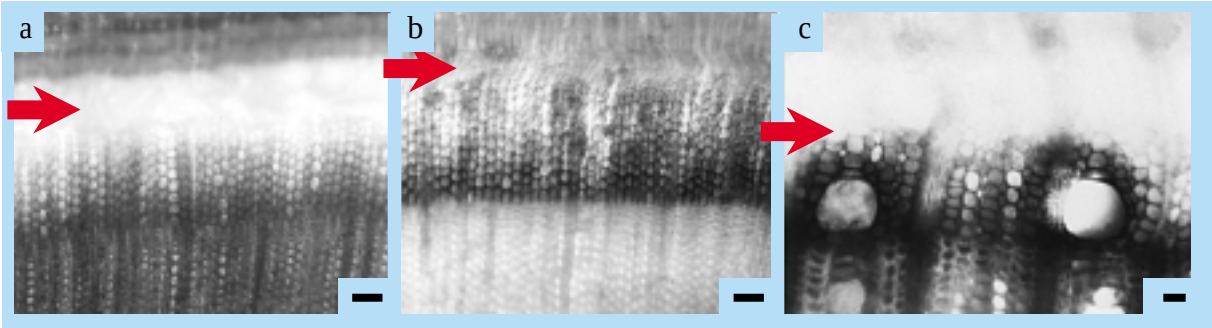

Figure 2 Oxidase activity and lignin deposition in developing wood cells. Figure 2a shows the oxidase activity present in developing wood cells of Leyland cypress. Figure 2b shows the extent of lignification of these wood cells. Figure 2c shows the presence of oxidase activity in the lignifying developing wood cells of sycamore. Arrows denote the position of the vascular cambium. All bars represent 25 µm.

activity is also specifically expressed in the lignifying cells of the differentiating wood of the angiosperm, sycamore (Fig. 2c). Indeed, oxidase activity showed the same pattern of expression in the lignifying, differentiating wood of a taxonomically diverse range of tree species. It has been found in the most ancient species of the gymnosperms, *Ginkgo biloba*, and the oldest family of the angiosperms, the Magnolias, as well as other representative species from all the main gymnosperm and angiosperm families. The antiquity and ubiquity of oxidase activity in tree species suggests that it may have a conserved role in wood formation in trees.

The role of oxidase activity in wood formation Oxidase activity has been postulated to be involved in the final step of the biosynthesis of lignin[2], catalysing the oxidation and polymerisation of lignin monomers, monolignols, to lignin in the walls of the wood cells (Fig. 3). Peroxidases acting with hydrogen peroxide can also catalyse this reaction and there has been some discussion about the relative roles of the two enzymes. Two observations strongly support a role for oxidases in lignin polymerisation. Activity-staining studies suggested that oxidase activity was localised in the walls of lignifying wood cells (Fig. 2) and biochemical studies[3,4] confirmed that oxidase activity was closely associated with the cell walls of the differentiating wood cells, the site of lignin formation. Secondly, oxidases extracted from the differentiating wood of Sitka spruce (and other species) oxidise monolignols *in vitro*[5] to form lignin-like polymers. In addition, oxidases produced the same three dimeric intermediates as peroxidases and hydrogen peroxide from the monolignol, coniferyl alcohol (Fig. 4). Both enzymatic systems produced the dimers in the same proportions, which indicated that the reactions proceeded through the same intermediates and produced similar products. Therefore, oxidases are equally able to carry out this biologically important reaction as peroxidases.

Purification and identification of an oxidase from a conifer Oxidase activity was extracted from the cell walls of the differentiating wood of Sitka spruce and purified by sequential lectin affinity, immobilised metal affinity and gel permeation chromatography steps (Fig. 4, lanes A-D). The purification was characterised by an increase in the specific oxidase activity

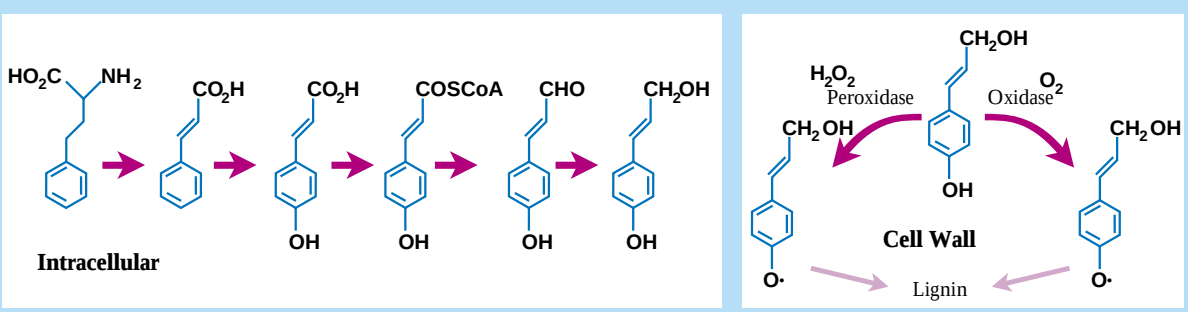

Figure 3 Oxidases in lignin biosynthesis. Oxidases and peroxidases have both been postulated to be involved in the final step of lignin biosynthesis, the oxidation and polymerisation of monolignols into lignin.

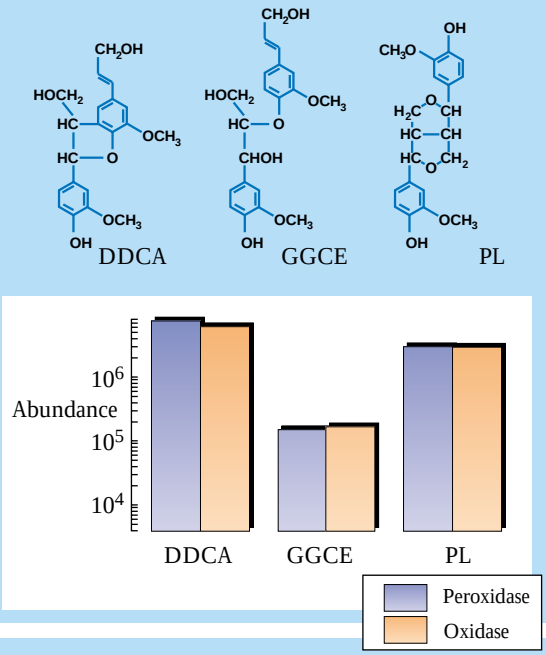

Figure 4 Products of coniferyl alcohol oxidation by oxidases and peroxidases
The oxidation of coniferyl alcohol (a) by oxidases or peroxidases produced the same three dimeric intermediates I, II and III.

and a decrease in the number of polypeptides in the samples after each step. One polypeptide with a relative molecular mass of 80 kDa became enriched as the specific oxidase activity increased (see Fig. 4, arrow) and was the main candidate for the oxidase activity. This polypeptide was selected for protein sequencing and it yielded an amino-terminal protein sequence that had homology to plant laccase-type oxidases. This was the first protein sequence of a laccase-type oxidase from a conifer.

Conclusions Oxidase activity is specifically expressed in the differentiating wood of a taxonomically diverse range of tree species and may be involved ubiquitously in wood formation. These enzymes can oxidise and polymerise monolignols and are expressed in the correct subcellular locale of lignifying cells to be involved in monolignol oxidation. Our studies strongly suggest

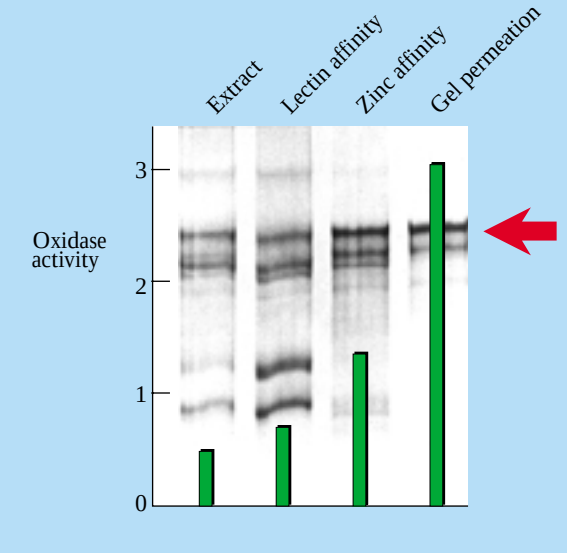

Figure 5 Purification of an oxidase from the developing wood of Sitka spruce. The oxidase activity was enriched by these sequential purification procedures. The polypeptide at 80 kDa (denoted by arrow) was selected for protein sequencing.

that the oxidase activity associated with the lignifying wood of conifers is mainly due to the expression of laccase-type enzymes. The apparent duplication of roles between oxidases and the peroxidases in lignin polymerisation may be a consequence of the importance of lignin biosynthesis in wood formation and tree growth but it is possible that the two enzymes have separate roles in lignin deposition.

References

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