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EVIDENCE FOR THE FORMATION OF METHOXYL GROUPS OF FERULIC AND SINAPIC ACIDS IN *BAMBUSA* BY THE SAME *O*-METHYLTRANSFERASE*

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Key Word Index—*Bambusa* Graminae; bamboo; *O*-methyltransferase; lignin biosynthesis; angiosperm plant; methylation; feedback control; role of 5-hydroxyferulic acid.

Abstract—The two methylation reactions, i.e. caffeate to ferulate (FA) and 5-hydroxyferulate to sinapate (SA), in the biosynthesis of guaiacyl and syringyl lignins in angiosperms were demonstrated to be catalyzed by the same enzyme in bamboo. This follows from the facts that: the ratio (SA/FA) obtained for *O*-methyltransferase remains constant during purification of the enzyme; chromatography on DEAE-cellulose, Sephadex G100 and G200, and analyses by polyacrylamide gel electrophoresis and isoelectric focusing in pH gradients showed that the two methylating activities belonged to a single enzyme protein; caffeate and 5-hydroxyferulate compete each other in the formation of the enzyme-substrate complex, the latter substrate showing greater affinity for the enzyme. Thus, feedback control may operate at the methylation step, caffeate to FA, in biosynthesis of angiosperm lignin.

INTRODUCTION

SINCE *O*-methyltransferase (OMT) was extracted from higher plants by Finkle *et al.*,[1,2] the role of this enzyme in ligninification has been well studied[3–5] and have shown that the methoxyl groups in both guaiacyl and syringyl lignins are formed at the hydroxycinnamic acid stage by mediation of OMT with *S*-adenosylmethionine. It was previously proposed[6] that the gymnosperm and angiosperm methylating enzymes were denoted as 'mono-' and 'di-function OMT', respectively, on the grounds that the former usually catalyzes only one methylation (FA formation),[5–7] whereas the latter catalyzes two methylations (FA and SA formation).

The present investigation describes evidence for the formation of the methoxyl groups of ferulic (FA) and sinapic (SA) acids in bamboo shoots by the same 'di-function OMT'. In addition, evidence suggests the possibility that feedback control of this enzyme may operate in the biosynthesis of angiosperm lignin.

RESULTS AND DISCUSSION

Table 1 shows that bamboo OMT is purified about 100-fold finally by ammonium sulfate fractionation, DEAE-cellulose ion exchange chromatography, and gel filtration through

* The present investigation was presented at the 9th *Phytochemistry Symposium* (Tokyo) and the *Annual Meeting of Agricultural and Chemical Society of Japan* (Tokyo) in 1973.

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[4] SHIMADA, M., OHASHI, H. and HIGUCHI, T. (1970) *Phytochemistry* **9**, 2463.
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[6] SHIMADA, M., FUSHIKI, H. and HIGUCHI, T. (1973) *Mokuzai Gakkaishi* **19**, 13.
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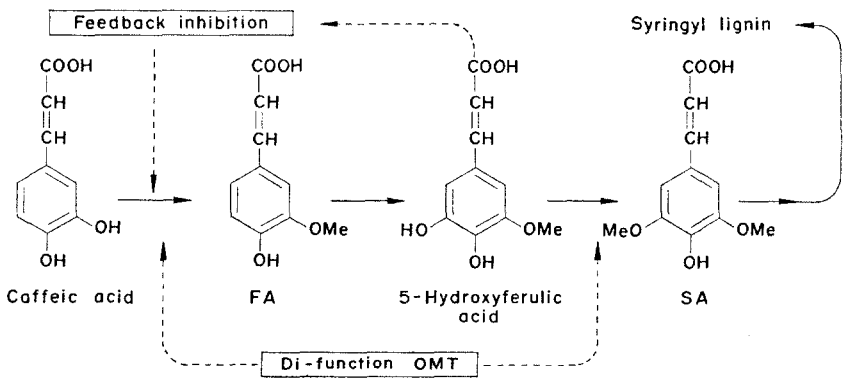
TABLE 1. PURIFICATION OF *O*-METHYLTRANSFERASE FROM *Bambusa*

Purification procedure	Total protein (mg)*	Total activity (units)† FA	SA	Specific activity (units/mg) FA	SA	Recovery (%) FA	SA	SA/FA ratio	Purification fold
1 0–70% $(NH_4)SO_4$	2900	5980	7300	2·06	2·52	100	100	1·22	1
2 20–55% $(NH_4)SO_4$	1300	6890	8640	5·3	6·8	118	118	1·26	3
3 DEAE-cellulose	118	5480	6870	46·5	58·3	92	93	1·25	23
4 Sephadex G200	65	3720	4180	57·5	64·0	63	57	1·11	28
5 DEAE-cellulose	1·5	300	368	200·0	244·0	5	5	1·22	97

* Protein contents were determined by the method of Lowry *et al.*[10] with Folin reagent.

† One unit of the enzyme activity is defined as the amount of OMT which catalyzes the formation FA or SA with 10^4 cpm for 1 hr at 30°. The reaction mixture contained 0·1 ml each of the enzyme solution, 0·1 M cysteine, mercaptoethanol, $MgCl_2$, and iso-ascorbate, and *S*-adenosylmethionine-$^{14}CH_3$ (0·25μ mol, 0·025 μCi), and 0·2 ml each of caffeate or 5-hydroxyferulate (0·5 μmol) and 0·5 M phosphate buffer (pH 8·0).

Sephadex G200. During purification the SA/FA ratio remains constant within the limits of experimental error (Table 1). In a second purification, the same constant ratio was also obtained with 70-fold purified enzyme. During these purifications, it was observed that the peaks of FA- and SA-activities were not separated by chromatography on DEAE-cellulose (0–0·25 M KCl linear gradient elution), Sephadex G200, and Sephadex G100. However, more definite evidence that the two methylating activities for FA and SA formation belong to a single enzyme protein, was provided by polyacrylamide gel electrophoresis and isoelectric focusing in pH gradients. In both cases the FA- and SA-activities were detected in the same protein fraction. These analyses showed that isoelectric point of bamboo OMT is pH 4·61 at 4°.



SCHEME 1. THE POSSIBLE FUNCTION OF FEEDBACK CONTROL IN BIOSYNTHESIS OF ANGIOSPERMOUS LIGNIN.

Further demonstration that the formation of FA and SA is catalyzed by the same enzyme was provided by kinetic studies with both crude and purified OMT. The Lineweaver–Burk plots for the methylation of caffeate to FA in the presence and absence of 5-hydroxyferulic acid (10^{-4} M) gave a typical competitive inhibition pattern, showing that the FA formation was greatly inhibited by 5-hydroxyferulic acid. The finding that the two substrates compete with each other is consistent with the presence of a single methylating enzyme.

Alternatively, however, such competitive inhibition suggests a regulatory role of 'di-function OMT' in the metabolism of hydroxy-cinnamic acids involved in lignin biosynthesis. Since K_m values for caffeate and 5-hydroxyferulate were found to be 5×10^{-5} and

[10] LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J. (1951) *J. Biol. Chem.* **193**, 265.

1×10^{-5} M, respectively, in excess *S*-adenosylmethionine, the preference of the latter in the formation of an enzyme substrate-complex shows that feedback control of FA production is possibly controlled by varying pool sizes of 5-hydroxyferulic acid (Scheme 1). However, more detailed investigations are in progress in order to report in the full paper.

EXPERIMENTAL

Preparation and assay of bamboo OMT were the same as described previously[5] except that bamboo shoots (1·5 kg) were homogenized in the phosphate buffer (pH 7·5) without Polyclar AT. Polyacrylamide gel electrophoresis was carried out according to Davis.[8] Isoelectric focusing in pH gradients was performed in LKB-ampholine column by use of carrier ampholite of pH 3–6 range at 7°.[9]

[8] DAVIS, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121,** 788.
[9] VESTERBERG, O. and SVENSON, H. (1966) *Acta Chem. Scand.* **20,** 820.