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# PROPERTIES OF LACCASE IN *SCHINUS MOLLE*

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**Key Word Index**—*Schinus molle*; Anacardiaceae; laccase; MW; amino acid composition; carbohydrate composition; glycoprotein.

**Abstract**—The properties of laccase isolated from *Schinus molle*, including its MW, amino acid and carbohydrate composition, are described. The enzyme is distinct from *Rhus* laccase both in $K_m$ and in carbohydrate composition.

## INTRODUCTION

Laccases are an unusual group of glycoproteins which catalyse the oxidation of *o*- and *p*-diphenols [1]. They have been studied quite extensively and it is clear today that fungal and higher plant laccases show significant differences in their properties. The higher plant laccase studied in greatest detail is that of *Rhus* [2, 3], but surprisingly few details are available on other laccases. It has been shown that all members of the Anacardiaceae, including *Schinus molle*, contain extracellular laccase [4]. We therefore undertook to purify and characterize this enzyme.

## RESULTS AND DISCUSSION

After preparation of an acetone powder, the enzyme was purified by a three-step procedure—extraction with buffer, ammonium sulphate fractionation, collecting the fraction precipitating between 70 and 100% saturation, followed by passage through a Biogel P150 column. The degree of purification was 18-fold. The sp. act. of the purified enzyme was 23000 $\mu$l $O_2$/min/mg protein. The amounts isolated were inadequate to express the results in terms of the dry wt. On electrophoresis on cellulose acetate the purified enzyme moved as a single band which stained both for enzyme activity, protein and carbohydrate. This was evidence for its homogeneity. The enzyme moved as a single sharp peak in ultracentrifugation with $s_{20,w}$ of 6.2 S and $D_{20,w}$ of 4.8 ($10^7$ cm$^2$/sec). The MW, assuming $V = 0.70$, was 105000. A value of $V = 0.70$ was determined for *Rhus* laccase [5]. SDS gel electrophoresis gave an apparent MW of 96000, close to that obtained by ultracentrifugation, suggesting that the protein is made up of a single subunit. It must be remembered that SDS electrophoresis of glycoproteins often gives anomalous results for various reasons [6]. These values are close to those reported for *Rhus* [5, 7, 8]; (Pecht, personal communication).

The enzyme had a pH optimum of pH 6.2, with quinol as the substrate, with apparent $K_m$ of $1.25 \times 10^{-3}$ M for quinol and 9.4% inambient atmosphere for $O_2$. The pH optimum is similar to that observed for some *Rhus* species, but the $K_m$ is appreciably lower than that previously reported for higher plant laccases, which was of the order of $10^{-2}$ M [5, 9]. No $K_m$ for $O_2$ of *Rhus* laccase appears to have been reported.

The purified enzyme contained 3–4 atoms Cu/mol enzyme, again similar to other reports for laccase assuming a MW of 105000. We determined the sugar content of the enzyme using the anthrone reagent to give a rough idea of total sugar content using glucose as a standard. Detailed analysis of the constituent sugars, using GC, was also carried out. The two methods gave sugar contents of 62 and 47% respectively, again based on protein determination as described. Only a few precise determinations of the sugar content of *Rhus* laccase exist and these are of the same order of magnitude [1]. Sugar analysis showed the presence of arabinose, xylose, mannose, galactose, glucose and glucosamine, the relative amounts being 11% arabinose, 1.7% xylose, 19% mannose, 55% galactose, 4.6% glucose and 8.4% glucosamine. This shows at least two significant differences from the *Rhus* laccase (Pecht, personal communication)—the absence of fucose and the preponderance of galactose. Glucose is present in much greater amounts in the *Schinus* laccase than that of *Rhus*, and mannose is lower. The sugar composition is suggestive of an N-glycosidic carbohydrate–peptide linkage as commonly found in other plant glycoproteins [10], but this requires further study.

We also determined the amino acid composition of the enzyme (Table 1). The amino acid composition shows a rather low content of basic amino acids, but no special other features. It is not very significantly different from the laccase of *Rhus* and peach [7, 11]. Apparently cysteine and cystine were destroyed during hydrolysis or reacted with sugars.

Relatively few plant glycoproteins have been studied in any detail [10]. The most detailed reports are on a storage glycoprotein in seeds and on lectins. Knowledge of an additional extracellular glycoprotein with well-defined enzymic properties is therefore of some interest. Our results also show that considerable differences can exist in higher plant laccases from different species in the same genus.

Table 1. Relative amounts of amino acids in *Schinus molle* laccase

| | Relative amounts*† | % of total† |
|---|---|---|
| Lysine | 1.45 | 3.4 |
| Histidine | 2.2 | 3.2 |
| Arginine | 1.0 | 2.2 |
| Aspartic acid | 5.4 | 11.9 |
| Threonine | 3.7 | 8.1 |
| Serine | 3.7 | 8.1 |
| Glutamic acid | 3.6 | 7.9 |
| Proline | 3.4 | 7.7 |
| Glycine | 3.25 | 7.3 |
| Alanine | 3.45 | 7.7 |
| Valine | 3.0 | 6.6 |
| Methionine | 0.8 | 1.8 |
| Isoleucine | 2.6 | 5.7 |
| Leucine | 2.95 | 6.5 |
| Tyrosine | 2.4 | 5.2 |
| Phenylalanine | 2.8 | 6.0 |

* Results normalized, taking arginine as unity. Cysteine was not detected. Tryptophane was destroyed under the hydrolysis conditions.

† Means of two independent determinations.

## EXPERIMENTAL

*Collection of secretion.* The trunk of *Schinus molle* trees was cut and the secretion collected periodically and stored at 4–6°.

*Purification of enzyme.* The crude secretion (100 g) was homogenized with 1 l. of $Me_2CO$, allowed to stand at 2° and then centrifuged at 10000 *g* for 20 min. The ppt. was collected and air-dried at 2°. The $Me_2CO$ powder (3 g) was extracted with 10 mM Pi buffer, pH 6. The extract was centrifuged at 20000 *g* for 15 min. The supernatant was fractionated with $(NH_4)_2SO_4$ and the fraction precipitating between 70 and 100% satn collected. This fraction was redissolved in 10 mM Pi buffer, pH 6 and dialysed overnight with 2 changes of buffer. The extract was concd by ultrafiltration to 5 ml, using an Amicon PM 10 membrane. It was applied to a Biogel P 150 column 2.2 × 90 cm, equilibrated with 10 mM Pi buffer pH 6. The column was eluted with the same buffer and the fractions having highest laccase activity were collected, combined and concd by ultrafiltration. This extract was again applied to a Biogel column and eluted with 10 mM Pi buffer. The most active fractions were combined, concd and stored at 2°. The prepn was stable for several weeks.

*Enzyme activity* was determined with a polarographic $O_2$ electrode using 10 mM quinol as substrate [12]. $K_m$ values were determined from Lineweaver–Burk plots.

*Electrophoresis* was carried out in cellulose acetate using Tricine buffer 40 mM, at 25° for 2 hr at 200 V. Enzyme activity was determined by incubating the strips with 10 mM quinol containing 10 mM *p*-phenylenediamine in 10 mM Pi buffer [13]. Strips were stained for protein with 0.5% Amidoblack in MeOH–HOAc (9:1) and destained with MeOH–HOAc. SDS electrophoresis was carried out according to ref. [14]. The markers used were: phosphorylase 94000, albumen 67000, bovine albumen 43000, carbonic anhydrase 30000, trypsin inhibitor 20000 and α lactalbumen 14400.

Sugars on cellulose acetate strips were detected as described in refs. [15] and [16]. Total sugar in the enzyme was determined by the anthrone method [17] using glucose as standard. GC of the sugar was according to ref. [18]. Sedimentation measurements were made in a Spinco Model E analytical centrifuge equipped with Schlieren optics. Amino acid analyses were carried out on hydrolysed enzyme; hydrolysis was at 115° in 6 N HCl for 24 hr. Cu was determined in suitable samples of enzyme by atomic absorption spectroscopy, directly on the protein, without ashing. Protein was determined by the method of ref. [19] using BSA as standard.

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