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COPPER CONTENT AND AMINO ACID COMPOSITION OF CATECHOL OXIDASE FROM CLAIRETTE GRAPES

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Key Word Index—*Vitis vinifera*; Vitaceae; grape, cv. Clairette; catechol oxidase; copper content; amino acid composition.

Abstract—Catechol oxidase was purified 330 × from chloroplasts of Clairette grapes. The MW of the enzyme is 80 000 and its Cu content is half an atom per molecule. The amino acid composition of the enzyme is also described.

INTRODUCTION

Catechol oxidases (*o*-diphenol: O_2 oxidoreductase, E.C. 1.14.18.1) from higher plants are difficult to purify and there are relatively few reports on their MW and other properties. The published data on purified catechol oxidase from potato [1], spinach beet [2] and fungi [3–6] show that there is great similarity between the enzymes. Grape catechol oxidase has been described in some detail [7–10]; here we report the MW, Cu content and amino acid composition of the purified enzyme.

RESULTS AND DISCUSSION

The purification was done in two steps: extraction of the enzyme from freeze-dried chloroplasts after removal of lipids with 80% acetone; and chromatography on a column of hydroxyl-apatite. The enzyme was purified 334-fold with an overall yield of 30% (Table 1). The active fractions from several successive runs were pooled and the MW, Cu content and amino acid composition were determined.

The enzyme sedimented as a single, apparently homogeneous peak. Its MW is 80000, based on sedimentation velocity ($S_{20,w} = 5.0$ S) and an estimated diffusion coefficient ($D_{20,w} = 5.58\ cm^2/sec \times 10^7$), assuming a partial specific volume of 0.727. The Cu content, based on the amino acid composition, is 0.04%. This corresponds to half an atom of Cu per molecule, suggesting that some Cu might have been lost during the purification, as has been observed by other workers [5,6,11]. The minimum Cu content of the enzyme is therefore one atom per molecule, assuming that the enzyme is composed only of protein. No evidence was found for the presence of a sugar moiety in the enzyme. The amino acid composition of the purified enzyme is shown in Table 2. Aspartic acid is present in relatively large amounts, followed by glutamic acid, proline and alanine. The content of cystine and histidine is relatively low. Methionine is apparently absent.

The MW of the subunit (monomer) of catechol oxidase from higher plants has frequently been reported to be about 30–40 000 [1–3,12,13], and that from mushroom 30–32 000 [4–6]. Where determined, the Cu content appears to be one atom per monomer molecule [4–6]. The isolation of a catechol oxidase with a MW of 80 000

Table 1. Purification of grape catechol oxidase

	Specific activity (μlO_2/mg protein/min)	Purification
Freeze-dried chloroplasts	122	1
Extract of chloroplasts with Pi buffer after pre-extraction of lipids with 80% Me_2CO	19726	162
Enzyme eluted with 30 mM Pi buffer pH 7.0 from a column of hydroxyl-apatite	40726	334

Table 2. Amino acid composition of grape catechol oxidase

Amino acid	Amount (g/100 g protein)	Residues per 80000 mol. wt. (nearest integer)
Lysine	6.86	36
Histidine	2.57	12
Arginine	4.89	22
Aspartic Acid	12.97	78
Threonine	5.61	36
Serine	6.42	48
Glutamic acid	10.18	56
Proline	7.67	52
Glycine	5.23	44
Alanine	5.70	50
Half-cystine	present*	1–2†
Valine	4.95	36
Methionine	—	—
Isoleucine	5.33	34
Leucine	7.95	42
Tyrosine	8.13	36
Phenylalanine	5.07	24

* Only small amounts of half-cystine were found. † Minimal estimate. Tryptophane was not determined.

may indicate the presence of a stable dimer. However, the low content of Cu does not support such a view. It was not possible to dissociate the enzyme (e.g. by gel electrophoresis in sodium dedecylsulphate) while retaining its activity. Potato catechol oxidase was reported to have a MW of 72800 with 50% content of an RNA-like component and about one atom of Cu per molecule. We found no indication of a non-protein constituent in the enzyme from grapes, though this possibility cannot be ruled out.

We have previously reported the partial purification and some of the properties of catechol oxidase from table grapes [7,9]. The MW estimated by gel filtration through Sephadex was 55–58000. Partial degradation of the enzyme [9] and underestimation by the gel filtration method may account for the discrepancy between the two values. It is unlikely that the difference is due to the difference in grape varieties used.

The amino acid composition of grape catechol oxidase shows considerable similarities with that reported for the enzymes from potato [1] and spinach beet [2]. The grape enzyme is richer in proline and tyrosine but has relatively less leucine than the potato enzyme. Compared with the spinach beet enzyme, the catechol oxidase from grapes has relatively more glutamate and tyrosine and less valine and leucine. The differences between the grape enzyme and fungal tyrosinases [3–6] are somewhat greater.

EXPERIMENTAL

Chloroplasts were prepared from grapes, *Vitis vinifera*, cv. Clairette (Bourbaulenc) as previously described [7,8]. Isolated, freeze-dried chloroplasts were pre-extracted with cold 80% Me_2CO as described by Lerner *et al.* [8]. The extract in 1 mM Pi buffer pH was applied to a hydroxyl-apatite column (Serva) equilibrated with the same buffer. 'Inactive' protein was washed-off the column with 50 ml mM Pi buffer pH 7 and the enzyme was collected by eluting with 75 ml 30 mM buffer. The purified enzyme was stabilized by the addition of 0.05 ml Ampholine pH 3.5–10.0 and kept in the cold. Determination of Cu content, ultracentrifugation and amino acid analysis were performed as previously described [14]. The estimate of the partial specific volume of the enzyme was calculated from the partial specific volumes of the constituent amino acids. Catechol oxidase activity was determined by the use of a polarographic oxygen electrode, in 0.1 M citrate buffer pH 4.8 with 5 mM 4-methylcatechol as substrate. Protein was determined according to Lowry *et al.* [15].

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AMINO ACID COMPOSITION AND MOLECULAR WEIGHT OF *BOTRYTIS CINEREA* LACCASE

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Key Word Index—*Botrytis cinerea*; laccase; glycoprotein; amino acid composition; copper content.

Abstract—The extracellular laccase from *Botrytis cinerea* is shown to be a glycoprotein, of MW 56000, containing at least one Cu atom/molecule. Its amino acid conposition shows an exceptionally low content of basic amino acid and very high content of threonine and serine.

INTRODUCTION

In a previous paper [1] we reported on some properties of laccase partially purified from the fungus *Botrytis cinerea*. An unusual feature of the enzyme was its very low isoelectric point, pH 2.5. We therefore decided to purify and characterize the enzyme further.

RESULTS AND DISCUSSION

The extracellular laccase from *Botrytis* was precipitated from its culture medium with acetone and redissolved in buffer. Following ultrafiltration it was applied to a DEAE cellulose column and eluted with 0.04M phosphate citrate buffer, pH 6. The final purification was 30 × com-