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STOKES' RADIUS CHANGES OF SOLUBILIZED GRAPE CATECHOL OXIDASE

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Key Word Index—*Vitis vinifera*; Vitaceae; grape; catechol oxidase; Stokes' radius; enzyme activation.

Abstract—The Stokes' radius of grape catechol oxidase was determined at pH 7·0 and during its reversible and irreversible activation at pH 5·0. The results are consistent with the view that the activation is due to a conformational change in the enzyme.

INTRODUCTION

Since it was first reported by Mallete and Dawson [1], multiple forms of catechol oxidases have often been described in the literature. Harel and Mayer [2] reported eight forms of solubilized grape catechol oxidase. During acrylamide gel electrophoresis at pH 7·9 four of these forms migrated slowly, while the other four migrated rapidly.

In a previous paper [3], we have described the transformation of some of the fast migrating forms into other forms, some of which were transient while others seemed to be stable, slow-running forms. The irreversible transformation of the fast-migrating enzyme forms into the slow ones was caused by various treatments, such as prolonged storage at pH 5·0, short incubation in acidic conditions at high ionic strength or prolonged incubation in 4 M urea. A reversible enzyme transformation was also described. Short exposure of enzyme to pH 5·0 increased enzyme activity ten-fold at pH 7·0. The enzyme activity at pH 7·0 decreased, on standing, to the level prior to the pH 5·0 treatment. This transformation may be due to a reversible conformational change in the enzyme.

We now report further evidence for conformational changes in grape catechol oxidase, based on changes in its Stokes' radius during its reversible activation.

RESULTS AND DISCUSSION

The partition coefficient [4], K_{av}, of grape catechol oxidase was determined on Sephadex G 200 at pH 7·0 and 4·5 by the rapid flow method of Sachs and Painter [5]. For this purpose we used undenatured enzyme preparation, showing only rapidly migrating electrophoretic forms. At pH 7·0 the enzyme eluted sharply at K_{av} 0·48. At pH 4·5 the elution pattern showed a peak at K_{av} 0·76 followed by extensive tailing extending beyond the column total volume. The tailing suggested that the enzyme was absorbed on the Sephadex [6,7]. To compete with the polysaccharide, 0·5 M sucrose was added to the eluant. At pH 7·0 this did not influence the elution pattern, while at pH 4·5 sucrose changed the pattern, the enzyme eluting sharply at K_{av} 0 (Table 1, Fig. 1).

Above pH 6·5, K_{av} of undenatured enzyme was about 0·5 while at pH values below 5·5 the K_{av} was zero in the presence of sucrose when the enzyme was reversibly activated. In some experiments sucrose was not required for the enzyme to elute at zero K_{av} at pH values equal or below 5·5, suggesting that in these cases the enzyme had been irreversibly activated (denatured). Around pH 6·0 the enzyme elution pattern showed two peaks, one at zero K_{av} the other at about 0·5 K_{av}. It seemed as if the enzyme underwent a change in shape with an apparent *pKa* of about 6·0.

Table 1. Partition coefficient and Stokes' radius of solubilized grape catechol oxidase using gel filtration through Sephadex G200

Eluant	K_{av}	R_s of elution peak: Our calibration (Å)	R_s of elution peak: Laurent Killander calibration* (Å)
Phosphate buffer, 5 mM, pH 7·0	0·48	26	31
Acetate buffer, 5 mM, pH 4·5	0·76 with tailing	14	16
Phosphate buffer, 5 mM, pH 7·0 + 0·5 M sucrose	0·48	26	31
Acetate buffer, 5 mM, pH 4·5 + 0·5 M sucrose	0	>40	>80

* Our calibration reached only 36 Å, we therefore also used the calibration of Sephadex G 200 by Laurent and Killander [4].

These results indicate that undenatured solubilized grape catechol oxidase has a Stokes' radius, R_s, of about 30 Å at pH 7·0 and a Stokes' radius greater than 80 Å at pH 5·0.

Changes in the electrophoretic behaviour and Sephadex elution pattern of grape catechol oxidase have been described by Harel *et al.* [8]. The enzyme preparation used in the latter paper, was solubilized using Triton X-100. As judged from Sephadex elution it had a R_s of about 30 Å. The R_s decreased markedly on denaturing by urea. In the present paper, treatment of enzyme with acid pH also resulted in a shift of the R_s from about 30 to 14 Å. However, in the presence of sucrose the shift due to acid pH is towards a much higher R_s value. In all our experiments we have never succeeded in converting small aggregate of the enzyme, e.g. monomer, to larger aggregates such as the tetramer. It seems unlikely that sucrose alone would induce aggregation.

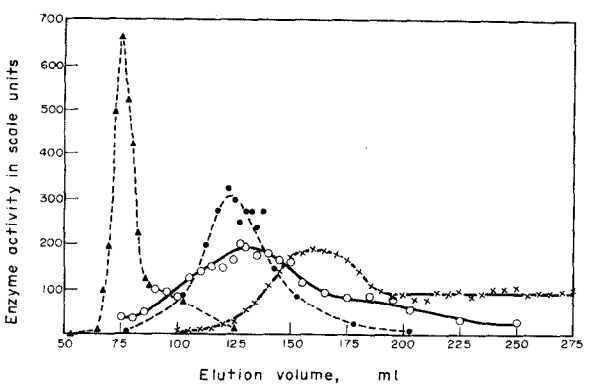

Fig. 1. Gel filtration of solubilised grape catechol oxidase on Sephadex G 200. Freshly prepared enzyme was eluted from the column with 5 mM Pi buffer pH 7·0 ○, 5 mM NaOAc buffer pH 4·5 ×, 5 mM Pi buffer containing 0·5 M sucrose pH 7·0 ●, or 5 mM NaOAc buffer containing 0·5 sucrose pH 4·5 ▲.

We therefore interpret the increase in Stokes' radius as representing an unfolding of the enzyme molecule rather than an association of several enzyme molecules, because it has been possible to induce the same enzyme transformation by different treatments some of which were unlikely to induce association. Such treatments were prolonged incubation at pH 5·0 or in urea 4 M and digestion with trypsin [3,8]. All these treatments irreversibly increased enzyme activity at pH 7·0 and changed its electrophoretic mobility from fast moving forms to slow moving ones.

Although our conclusion that acid pH induces an increased Stokes' radius of the enzyme by unfolding is consistent with all the data, this conclusion requires further experimental confirmation. One way of confirming this would be by determining the sedimentation constant of the activated enzymes.

EXPERIMENTAL

Lyophilized chloroplasts were prepared as previously described [3] from grapes, *Vitis vinifera*. Chloroplasts, 4 g, were rinsed 2× each time with 150 ml dist H_2O. Next chloroplasts were washed with cold 80% Me_2CO (150 ml × 3) to remove lipids. Resultant residue was extracted with 1 mM Pi buffer,

pH 7·0 and extract dialysed against the same buffer. Insoluble material was removed by centrifugation. Resultant supernatant was used as enzyme preparation. This preparation showed only 3 bands on electrophoresis and had a sp. act. 3× that of enzyme solubilized using extraction with Triton X-100.

Gel filtration was carried out according to Sachs and Painter [5]. The column was 35 mm in dia. and 850 mm high. Void volume, 75 ml, was determined with dextran blue. Total vol, 190 ml, was determined with DNP-L-alanine. R_s calibration was carried out using cytochrome *c*, soyabean trypsin inhibitor, ovalbumin, haemoglobin and bovine serum albumin. The K_{av} values of the standards were similar to the ones observed by previous authors [4,5] and the plot of K_{av} vs R_s was linear. Data for the Stokes' radius were taken from Laurent and Killander [4]. Flow rate was maintained at 1·5 ml/min with a peristaltic pump, 2·5 ml fractions were collected. This allowed us to determine the enzymes K_{av} within about 2 hr. Enzyme activity was determined by measuring oxygen consumption using a Clark polarographic electrode according to Mayer *et al.* [9].

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