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HYDRODYNAMIC PROPERTIES OF MUSHROOM TYROSINASE

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Key Word Index—*Agaricus bisporus*; Agaricaceae; tyrosinase; mushroom; hydrodynamic properties; enzyme.

Abstract—The hydrodynamic properties of mushroom tyrosinase were determined at pH 6.5 using a Sephadex G-200 column. From the comparison of its gel-filtration behaviour with those of standard proteins, the following parameters were calculated: MW (122 500 ± 1%), Stokes' radius (42.75×10^{-8} cm^2/sec), diffusion coefficient (5.048×10^{-7} cm^2/sec) and frictional ratio (1.26). These values suggest a globular conformation of this enzyme.

INTRODUCTION

Tyrosinase (monophenol, dihydroxyphenylalanine: oxygen oxidoreductase, EC 1.14.18.1) is a copper-containing bifunctional oxidase catalysing the *o*-hydroxylation of monophenols to diphenols and the oxidation of *o*-diphenols to corresponding quinones [1]. The enzyme is found in various plants, micro-organisms and animals, and has been purified from a number of sources [2–5]. The enzyme from mushrooms has been studied extensively with respect to substrate specificity [6], state of copper [7], effect of inhibitors [8] and the mechanism of action [9]. Recently, Lerch [10] reported the amino acid sequence of the thermolabile form of *Neurospora* tyrosinase and suggested a globular conformation of this enzyme. Despite the wealth of information available, the native conformation of tyrosinase is largely unknown. The present study characterizes the enzyme molecule on the basis of MW, Stokes' radius, diffusion coefficient and frictional ratio.

RESULTS AND DISCUSSION

The MW and other hydrodynamic parameters were determined at pH 6.5. The elution volumes, V_e, for different proteins were analysed in terms of K_d, the distribution coefficient [11] and K_{av}, the available distribution coefficient [12]. The column was equilibrated with phosphate buffer and various marker proteins and tyrosinase eluted under the identical experimental conditions. The V_e was measured from the elution profile of the proteins by extrapolating both sides of the peak to an apex wherever needed. The V_e for tyrosinase was 113 ml. The void volume, V_o, was measured by passing Blue Dextran 2000 and found to be 72 ml. The V_o was checked frequently during the course of these experiments to measure the change, if any, but no change in V_o was observed.

The major proteins with their molecular parameters and distribution coefficient are listed in Table 1. The gel filtration data were analysed employing the theoretical

Table 1. Molecular parameters and distribution coefficients of marker proteins used in gel filtration experiments

Protein	MW* ($\times 10^{-4}$)	Stokes' radius† ($\times 10^8$ cm)	V_e/V_o	K_d	$K_d^{1/3}$	K_{av}	$(-\log K_{av})^{1/2}$
1. α-Chymotrypsinogen	2.57	22.4	2.24	0.677	0.878	0.777	0.331
2. Ovalbumin	4.30	27.3	2.01	0.555	0.822	0.637	0.443
3. Bovine serum albumin	6.90†	35.5	1.76	0.418	0.747	0.480	0.564
4. Bovine serum albumin (dimer)	13.80†	43.0	1.53	0.289	0.661	0.331	0.693
5. Immunoglobulin	16.00	48.4	1.47	0.258	0.636	0.296	0.727

* Values taken from ref. [19].
† Values taken from ref. [20].

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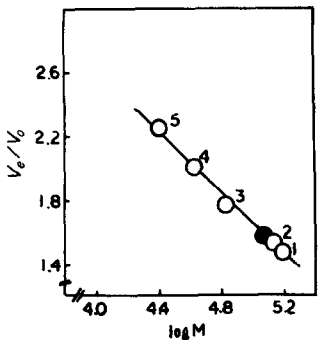

Fig. 1. Plot of V_e/V_o versus log M utilizing gel filtration data of proteins on Sephadex G-200 column. Immunoglobulin (1), bovine serum albumin (dimer) (2), bovine serum albumin (monomer) (3), ovalbumin (4), α-chymotrypsinogen A (5) and tyrosinase (●).

treatments of Porath [11], Laurent and Killander [12] and Ackers [13]. The linear relationship between log M and V_e/V_o is shown in Fig. 1 for the range of MW investigated and fitted the following equation developed by least squares.

$$V_e/V_o = 6.470 - 0.9636 \log M \quad (1)$$

According to Porath [11], K_d of a protein molecule can be related to its Stokes' radius (r) which is directly proportional to $M^{1/3}$

$$M^{1/3} = 119.47 - 103.07 K_d^{1/3} \quad (2)$$

Laurent and Killander [12] obtained an expression which shows the relationship between Stokes' radius and K_{av} and predicts a linear relationship between $(-\log K_{av})^{1/2}$ and r.

$$(-\log K_{av})^{1/2} = 0.154 r + 0.008 \quad (3)$$

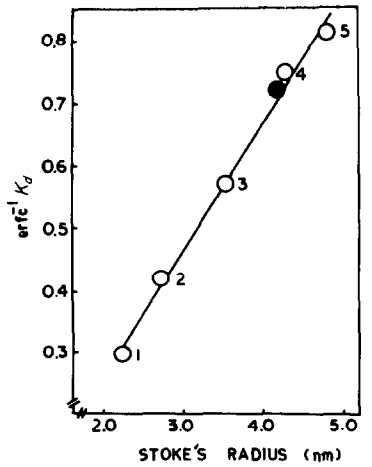

Fig. 2. Plot of $erfc^{-1} K_d$ versus Stokes' radius. The straight line was drawn by the method of least squares. α-Chymotrypsinogen A (1), ovalbumin (2), bovine serum albumin (monomer) (3), bovine serum albumin (dimer) (4), immunoglobulin (5) and tyrosinase (●).

According to Ackers [13], K_d is given by the error function complement (erfc) of Gaussian distribution.

$$K_d = \text{erfc}\,(r - r_o)/b_o$$

and

$$r = r_o + b_o \text{erfc}^{-1} K_d$$

r_o and b_o are the calibration constants for the gel. The above equation shows a linear relationship between r and $erfc^{-1} K_d$ (Fig. 2) which fits the following equation of a straight line.

$$\text{erfc}^{-1} K_d = 0.20 r - 0.14 \quad (4)$$

The values of V_e/V_o, K_d and K_{av} for tyrosinase were found to be 1.57, 0.312 and 0.358, respectively. Using equations (1) and (2), the MW was calculated to be 121 600 and 123 500, respectively. The average value, 122 500, is in good agreement with the reported MW (128 000 ± 5%) for mushroom enzyme [7]. The Stokes' radii from equations (3) and (4) were calculated to be 42.8×10^{-8} and 42.7×10^{-8} cm, respectively (average value—42.75×10^{-8} cm). The Stokes' radius value of 30×10^{-8} cm was reported for the rapidly migrating electrophoretic form of grape catechol oxidase [14] which is somewhat lower than the value obtained in the present study. Grape enzyme exists in multiple forms [15, 16] and this difference in Stokes' radius may be due to the conformational differences among the multiple forms.

The diffusion coefficient, D, of the enzyme corresponding to 42.75×10^{-8} cm value of Stokes' radius was calculated using the following equation and found to be 5.048×10^{-7} cm²/sec.

$$D = KT/6\pi\eta r,$$

where K is the Boltzman constant, η is the viscosity of the solvent in poise and T is the absolute temperature.

A value of 1.26 of frictional ratio, f/f_o, of the enzyme was obtained using the equation

$$f/f_o = r/(3\bar{V}_2 M/4\pi N)^{1/3},$$

where $\bar{V}_2$ is the partial specific volume of the tyrosinase and N is the Avogadro's number. A value of 0.758 ml/g of $\bar{V}_2$ was used [17]. These results, when compared to the values obtained for standard proteins, suggest a globular conformation of this enzyme.

EXPERIMENTAL

Mushroom tyrosinase (lyophilized, salt free, grade III) was purchased from Sigma Chemical Company. The enzyme was purified according to ref. [18] and the prepn was homogeneous by the criteria of column chromatography and polyacrylamide gel electrophoresis.

The analytical column of Sephadex G-200 was prepared according to the procedure of Pharmacia Fine Chemicals, Sweden. Columns with the min dead space at the outlet were used for chromatography. The column (2.1 × 54 cm) was packed with previously swollen Sephadex G-200 and *ca* 3 vol. of eluent was passed in order to stabilize and equilibrate the gel bed. The homogeneity of the bed was checked by passing Blue Dextran 2000 soln at a concn of 2 mg/ml. Flow rate was maintained at 20 ml/hr and 2 ml fractions were collected. The sample in 0.1 M Pi buffer, pH 6.5, containing 4–8 mg protein was applied to the column and was eluted with the same buffer.

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