Pergamon

PII: S0031-9422(96)00055-1

Phytochemistry, Vol. 45, No. 5, pp. 879–880, 1997

0031-9422/97 $17.00+0.00

ISOLATION, PURIFICATION AND PARTIAL CHARACTERIZATION OF SULPHITE OXIDASE FROM *MALVA SYLVESTRIS*

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(*Received in revised form* 27 *November* 1996)

Key Word Index—*Malva sylvestris*; Malvaceae; sulphite oxidase; sulphite.

Abstract—Sulphite oxidase (EC 1.8.3.1) was isolated from *Malva sylvestris* leaves and partially characterized. The enzyme was homogeneous with respect to its size and charge, as judged by gel filtration and PAGE. The M_r of the enzyme as estimated by gel filtration was 27000. The values of Stokes' radius and frictional ratio were 2.29 nm and 1.16, respectively, and the K_m was 5.33×10^{-3} M.

INTRODUCTION

Sulphite oxidase catalyses the final reaction in oxidative degradation of sulphur-containing amino acids: $SO_3^{2-} + A + H_2O \rightarrow SO_4^{2-} + AH_2$. The enzyme is physiologically important, its absence may even lead to death [1–3]. The enzyme has been purified from a variety of animal and bacterial sources [4–5]. Although activity of sulphite oxidase has been observed in some plants [6], so far, purification of the enzyme to homogeneity has not been reported from any plant source. The present work describes purification and some properties of sulphite oxidase from *Malva sylvestris* leaves.

RESULTS AND DISCUSSION

On screening various plants for the presence of sulphite oxidase, *Malva sylvestris* showed considerable activity. The results of the purification process are summarized in Table 1. Heat treatment of the crude extract resulted in loss of about 25% of original protein, but loss of enzyme activity was only 18%. On a DEAE-cellulose column, this enzyme preparation was resolved into two fractions: a bound and an unbound fraction. The elution profile of the enzyme from Sephadex G-200 column showed a major peak and a small shoulder. A purification of 8.3-fold was achieved after the final step. The enzyme preparation appeared to be 95% homogeneous, as judged by gel filtration and PAGE. Elution of the enzyme as a single peak from Sephadex G-200 column and a single band on SDS-PAGE indicated the size homogeneity of the protein.

The Sephadex G-200 column was calibrated by marker proteins of known M_r Stokes' radii. A plot of V_e/V_o against log M_r for marker proteins yielded a straight line [7], and analysis by the method of least squares gave the equation: $V_e/V_o = -1.54 \log M_r + 9.48$. The value of V_e/V_o for the enzyme was 2.54 giving a M_r about 27000. For the determination of Stokes' radius of the enzyme, the gel filtration data was also treated according to ref. [8]. Least square analysis of the data yielded a straight line (Fig. 1) which obeyed the equation: $(-\log K_{av})^{1/2} = 0.1987a - 0.0594$. The value obtained for the Stoke's radius 'a' was 2.29 nm. The values of M_r and Stoke's radius as determined were used to calculate the fric-

Table 1. Summary of purification of sulphite oxidase from *Malva sylvestris*

Purification step	Total protein (mg)	Total activity (nKat)	(nKat mg^{-1})	Purification (fold)	Yield (%)
Crude extract	855	2070	2.42	1	100
Heat treatment	643	1690	2.62	1.1	82
Ion exchange chromatography	101	1150	11.4	4.7	55
Sephadex gel chromatography	29	583	20.1	8.3	28

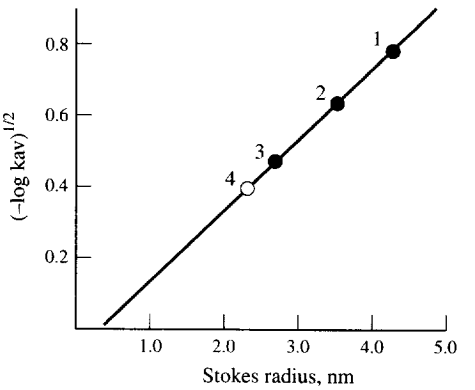

Fig. 1. Plot of (-log K_{av})$^{1/2}$ against Stokes' radius for gel filtration data of marker proteins: (1) BSA dimer; (2) BSA monomer; (3) ovalbumin and (4) isolated sulfite oxidase, on Sephadex G-200 column.

tional ratio which was 1.16. The M_r was considerably lower than the value (110 000) reported for chicken liver and bovine liver sulphite oxidase. The data showing the relationship between substrate concentration and enzyme activity were analysed according to ref. [9]. The K_m of the enzyme was 5.33×10^{-3} m. The K_m value as obtained was considerably higher than that reported for the beef and dog liver and spinach sulphite oxidase [10, 11], indicating that the enzyme from *Malva sylvestris* has lesser affinity for sulphite as substrate than the enzyme isolated from other plant and animal tissues. The absorption maxima of the enzyme were at 220 and 277 nm. A small peak was also found at 570 nm that was 5% the height of the peak at 277 nm. This could be due to a heme prosthetic group.

EXPERIMENTAL

Preparation of Me_2CO *powder.* Fresh young *Malva sylvestris* leaves grown in local fields (100 g) were washed, deribbed, patted dry and homogenized for 3 min in 200 ml of 0.05 M KPi buffer, pH 7.8. The homogenate was poured in 1.2 l chilled Me_2CO, stirred for 3 min and washed with 1 l of chilled Me_2CO. The filter cake was dried by a mixt. of Me_2CO and Et_2O (1:1) under vacuum and then sifted in air for a few min.

Extraction with buffer. 17 g of Me_2CO powder was suspended in 300 ml of 0.05 M KPi buffer, pH 7.8, and stirred for 45 min at 4°.

Heat treatment. The crude extract obtained after extraction with buffer was heated at 56° for 3 min, chilled and centrifuged at 10 000 rpm in a fixed angle rotor for 10 min, at 4°.

Column chromatography. The sample obtained after heat treatment was fractionated on a DEAE-cellulose column. The adsorbed protein was eluted with 0.05 M KPi buffer, pH 7.8 containing 0.95 M NaCl. The active frs were pooled, dialysed and concd by lyophilization. The concd sample was applied to Sephadex G-200 column and eluted with 0.05 M KPi buffer, pH 7.8. The active fractions were pooled and concd. Enzyme purity was ascertained by disc PAGE [12] using 7.5% gel. SDS-PAGE was also performed on 7.5% acrylamide gel in 0.1 M NaPi buffer, pH 7.0, containing 0.2% SDS by the method of ref. [13].

Assay of sulphite oxidase. Sulphite oxidase activity was assayed at 25° as described in ref. [14]. The reaction mixt. (2.5 ml) contained 0.5 ml each of 1×10^{-3} M EDTA, 4×10^{-3} M K ferricyanide, 2×10^{-2} M Na_2SO_3 and 5×0.05 M KPi buffer, pH 7.8. The final concn of Na_2SO_3 was 4 mM, i.e. less than the K_m. The reaction was initiated by 0.5 ml of the enzyme and the change in A at 420 nm was monitored.

Determination of protein. Protein was determined by the method of ref. [15]. The protein profiles in column chromatography were followed by measuring the A of the eluate at 278 nm.

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