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KINETICS OF SULFITE OXIDASE PURIFIED FROM *MALVA SYLVESTRIS*

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ABSTRACT

Sulfite oxidase was purified from *Malva sylvestris* (Sunchul) leaves by acetone fractionation, heat treatment , ion - exchange chromatography and gel permeation chromatography methods. The activity of the enzyme was determined using a coupled assay with sodium sulfite as substrate and potassium ferricyanide as co - substrate. Decrease in absorbance at 420nm was monitored.The pH and temperature optima of the enzyme were found to be 7.8 and 30°C , respectively. Km and Vmax as determined by using different methods were 3.34mM and 1.13mM/min respectively. The activation energy of the enzyme was 71.3kj/mole.

INTRODUCTION

Sulfite oxidase an enzyme that catalyses the oxidation of sulfite to sulfate , the terminalreaction in the oxidative degradation of the sulfur containing amino acids. It has also beenassociated in the detoxification of sulfur dioxide/sulfite. Plants with higher level of sulfite oxidase have been shown to be less susceptible to sulfur dioxide insult (Javeed , 1998). The enzyme has been purified and characterized extensively in animals (Macleod *et al*. 1961, Astashkin et al., 2002 andFeng et al. 2003) but there are only few reports on its existence inplants (Jager *et al*. 1986; Ganai *et al*. 1997; Eilersetal, 2001 and Hille *et al*. 2003). We report here the Kinetics of the enzyme isolated from *Malva sylvestris*.

MATERIALS AND METHODS

Fresh sunchul leaves were used as study material and Sunchul was obtained from local fields.All chemicals used in this study were of highest purity com-

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mercially available.Protein concentrations were determined by Bradford (1976) using Bovine serum albumin as the standard. Absorbance was measured by Spectronic-20 spectrophotometer.

Enzyme assay

The assay of sulfite oxidase involved the use of sodium sulfite solution (2mM), potassium ferricyanide (2mM), EDTA (1mM), 0.25 M potassium phosphate buffer, pH 7: 8 and sulfite oxidase solution.

Sulfite oxidase was isolated from sunchul leaves by acetone precipitation, heat treatment, ion exchange chromatogramy and Sephadex gel filtration method as described by Ganai *et al*. 1997. The purified solution of sulfite oxidase was used as the enzyme source.

Sulfite oxidase was assayed at pH 7.8 and temperature of 30°C by a little modification of the method described by *Cohen and Frictovich* (1971). The activity of the enzyme was indicated by decrease in absorbance at420nm of potassium ferricyanide used as an indicator substrate in the coupled assay.

The assay mixture (2.5mL) contained 0.5mL of 0.25M potassium phosphate buffer pH of 7.8 ,0.5mL of 2mM potassium ferricyanide , 0.5ml of 1mM ETDA and 0.5mL of enzyme solution. The reaction rates were corrected from the non enzymatic oxidation of sulfite by running enzyme and substrate blanks. The enzyme activity was expressed in terms μ mole of substrate converted into product /min./mL.

Effect of time

The main objective of this experiment was to familiarize one with the selection of incubation time for measurement of enzyme activity. Reaction mixtures were recorded at 420nm in the time interval up to 7min. and data ptoted as activity versus time.

Effect of substrate concentration

This experiment demonstrates how substrate concentration affects the activity of sulfite oxidase and this in turn helps in the determination of kinetic parameters like Km and Vmax. The effect of sulfite (substrate) on the activity of sulfite oxidase was studied by varying the concentration of sodium sulfitefrom O.8mM to5.6mM while keeping other conditions constant. The data was analyzed in the form of *Lineweaver - Burk* (1934), Hanes - *Woolf, Woolf Aungistinson - Hofstee and Ediae - Sctcharrd plots* (Segel, 1976) for the calculation of Km and Vmax.

Effect of pH

The effect of pH on the sulfite oxidase activity was investigated using citrate - phosphate buffer from pH range 5 - 7 , phosphate buffer of pH range 7-8 and tris - HCI pH range 8-10 . The main objective of this experiment was to familiarize one with the selection of optimum pH of the reaction mixture.

Effect of temperature

The effect of temperature on sulfite oxidase activity was investigated at pH 7.8 in 0.05M potassium phosphate buffer.The reaction mixtures were incubated at different temperature (ranging 15° C-50° C). The main objective of this experiment was to choose optimum temperature of the reaction mixture.

RESULTS AND DISCUSSION

Sulfite oxidase was chosen for understanding enzyme kinetics, the reason being obvious that it follows a simple assay procedure and also it has not been developed full by other researchers . The effect of time on the rate of sulfite oxidase catalyzed oxidation of sulfite to sulfate is shown in Fig. (1). There was a linear increase in velocity up to 5 minutes but then it fell possibly due to fall in substrate concentration as the reaction proceeded. Thus the time of *incubation* may be chosen up to 5 min.

The curve of activity versus substrate concentration was a hyperbola Fig. (2). From the figure It can be seen that the velocity increases up to 3.5mM and then became constant. It is difficult to determine the exact value of Vmax from this plot and therefore the data were transformed into linear plots.

Double reciprocal plots as shown in Fig. 3 (a, b, c, d). The value of Km and Vmax obtained through least square analysis are listed in Table (1).

One can see that *Lineweaver -Burk* plot s not only linear transformation of velocity equation and one of the other linear plots described above may be more suitable for determination of kinetic constants.

The effect of pH on sulfite oxidase activity was investigated and the results obtained are presented in Fig.(4) and the data is the average of three readings. The pH optimum was found to be 7.8 and provides inferences about the pK values of this enzyme and enzyme substrate complex and also the groups participating in the catalytic action.

Table 1

Kinetic data for the enzyme sulfite oxidase catalyzed oxidation of sulfite.

Treatment of data	Km (M)	V max. (M/min)
Lineweaver- Burk plot	4.16×10^{-3}	1.3×10^{-3}
Hanes- Woolf plot	2.8×10^{-3}	1.05×10^{-3}
Woolf- Augustinsson-Hofstee plot	3.34×10^{-3}	1.14×10^{-3}
Eadie-Scatchard plot	3.05×10^{-3}	1.04×10^{-3}

*Km = 3.34×10^{-3} M

*Vmax = 1.13×10^{-3} (M/min.)

* Km and * Vmax value for the sulfite oxidase are taken as average of the four values.

The effect of temperature on sulfite oxidase activity was ivestigated at pH 7.8 in 0.05 M potassium phosphate buffer and the results are presented in (Fig. 5). The optimum temperature was found to be 30°C. The same data was used to determine the activation energy of the enzyme . The data on the effect of

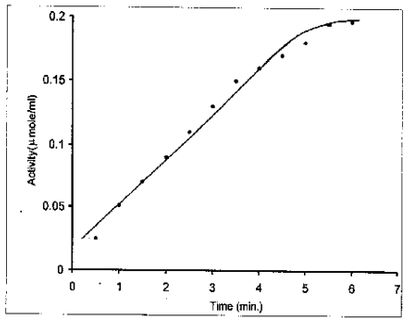

Fig. 1 Effect of time on sulfite oxidase activity.

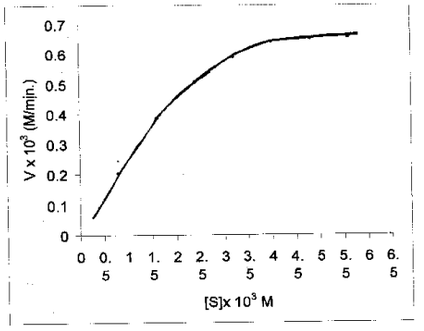

Fig. 2 Effect of substrate concentration on the activity of sulfite oxidase.

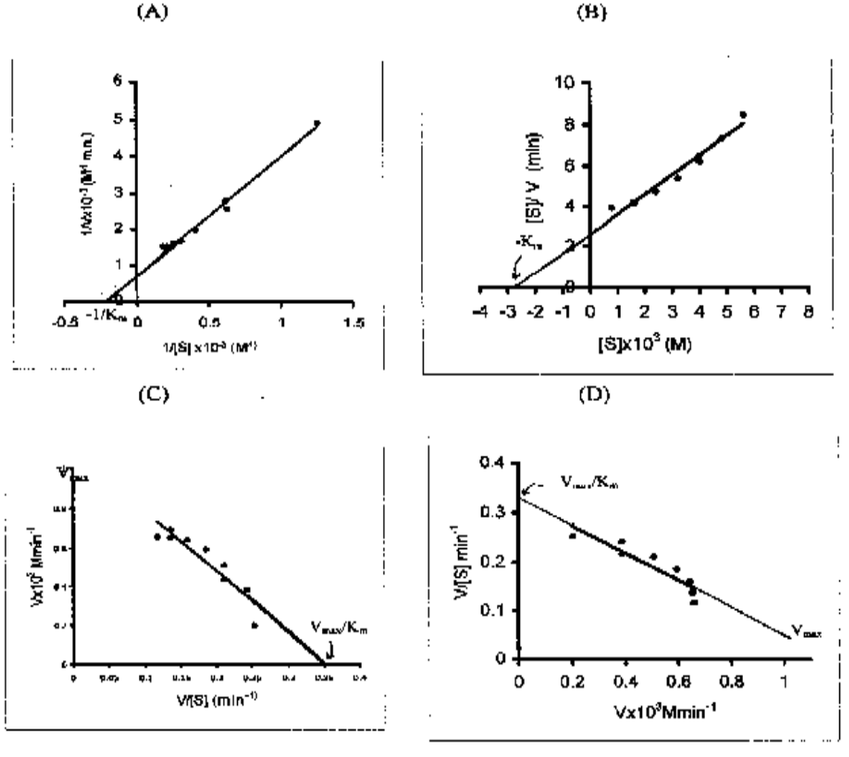

Fig. 3 Treatment of kinetic data by A) Line weaver -Burk plot, B) Hanes - Woolf plot,
C) Woolf Aungistinson - Hofstee polt,
D) Ediae - Scatchard plots for sunchul sulfite oxidase.

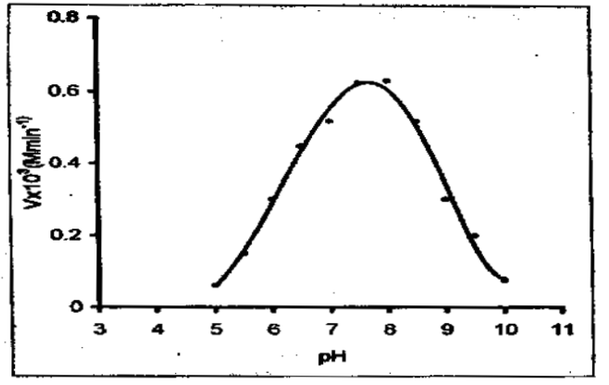

Fig. 4 pH dependence of sulfite oxidase activity. (Each value is the mean three replicates).

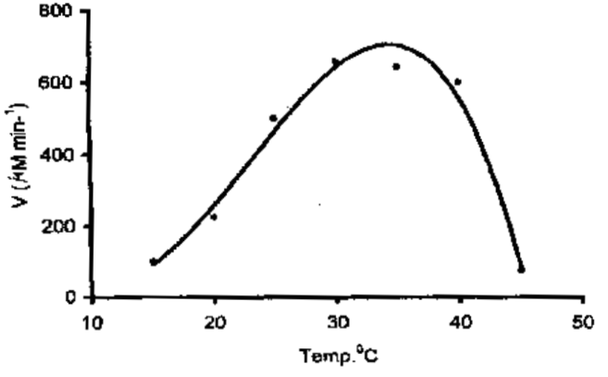

Fig. 5 Effect of temperature on sulfite oxidase activity. (Each value is the mean three replicates).

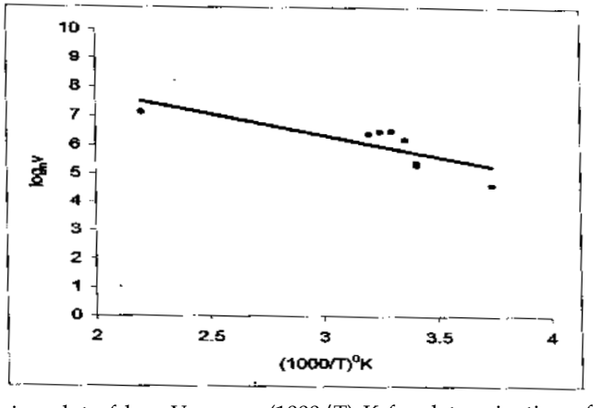

Fig. 6 Arrhenius plot of $\log_a$ V versus (1000/T) K for determination of activation energy of sulfite oxidase.

temperature on enzyme activity up to its optimum temperature was treated according to *Arrhenius* plot (Fig. 6) and the activation energy was found to be 71.3 kj/mole providing the information about the catalytic activity of the enzyme.

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