

Thermal stability of catalases active in dormant saffron (*Crocus sativus L.*) corms

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Summary

Catalase activity was detected in crude extract prepared from dormant saffron (*Crocus sativus L.*) corms. The activity was independent of pH in the range 6.0 - 11.0. Thermostability studies suggested the presence of three isoenzymes with transition temperatures of 30°C, 45°C and 60°C, respectively, as given by Arrhenius plots. When stained for catalase activity gel electropherograms of extract revealed 3 distinct bands with apparent molecular weight of 323,000, 295,000 and 268,000, respectively. Thus it appeared that at least three isoenzymes of catalase were present in dormant saffron corms.

Introduction

Crocus sativus L. has been cultivated since ancient times as the source of saffron, a substance renowned for its medicinal and flavoring properties. The plant is carefully described in botanical books, but there is no available information on the basic aspects of its physiology and biochemistry. Crocus species are perennial plants adapted to overcome a dry dormant period in the form of an underground corm [1]. Although enzymatic activities are necessarily limited during a dormant period some are essential for survival. For example, even an underground corm is exposed to diverse kinds of stress, such as heat, chilling, drought and others that can all lead to oxidative stress. Thus enzymes involved in antioxidant defenses are likely to be active even during dormancy. Among the numerous enzymes that plants possess for their defense against oxidative damage are catalases [2,3] and peroxidases [4-6]. We reported previously the presence of peroxidative activity in dormant saffron corms [7]. Catalase (E.C.1.11.1.6) has the advantage to catalyze the dismutation of H₂O₂ into H₂O and O₂ without requiring an additional source of reducing power as all peroxidases do. The purpose of this research was to investigate and characterize catalase activity in dormant saffron corms.

Materials and Methods

Extracts from dormant saffron (*Crocus sativus L.*) corms were prepared in phosphate buffer 0.1 M, pH 7.0, as previously described [7]. Protein concentration was determined by the Lowry method. Catalase activity was measured by following the dismutation of H₂O₂ spectrophotometrically [8] using an extinction coefficient for H₂O₂ at 240 nm of 27 M⁻¹.cm⁻¹. All assays were done at room temperature (~ 22-25°C) with an Aminco DW-2 spectrophotometer. 30 μ l extract were added to 0.01 M citrate-phosphate-borate buffer, at given pH, containing H₂O₂ in final concentration of 2 to 40 mM (for K_M, V_{MAX} determinations), or 10 mM (for all other assays); the reaction mixture total volume was 3 ml. One unit of catalase was defined as the amount of enzyme decomposing 1 μ mol H₂O₂/min. Thermal stability was studied by incubating aliquots of extract at various temperatures for 10

min (unless otherwise specified) and measuring their activity at room temperature after brief cooling in ice. Electrophoretic separation of proteins in corm extract and activity staining for catalase were performed as described in reference [9].

Results and Discussion

H₂O₂ dismutation was observed upon addition of extract to the reaction mixture as described in materials and methods, indicating the presence of catalase in dormant saffron corm extract. The enzymatic activity was detectable over a wide pH range (5.0 - 11.5) with maximum activity (21 μ mol H₂O₂ removed per min per mg protein) found at pH 6.0 to 11.0 (Fig. 1). However, while K_M and V_{MAX} values at pH 6.0, 7.0, 9.0 and 11.0 were similar, as

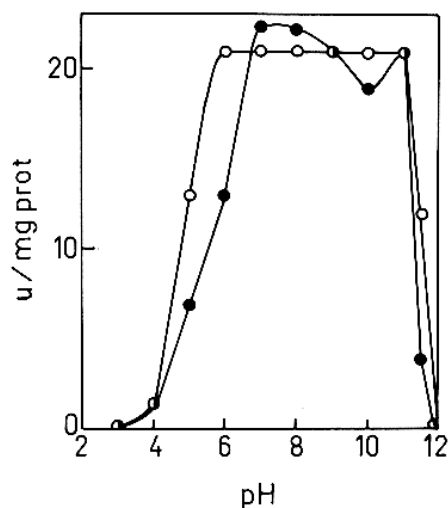


Figure 1: pH dependency of catalase activity in dormant saffron corm. The enzymatic activity, expressed in units per mg protein, was measured in corm extract either directly (open circles) or after incubation of the extract for 10 min at 50°C (closed circles).

shown in Table 1, the catalytic efficiency (calculated per mg extract protein) at pH 6.0 was half that at pH 7.0 and 9.0 and was 2/3 the value found at pH 11.0

The thermostability of the catalase activity in dormant corm extract was also studied. When the extract was incubated at 50°C for various lengths of time and then tested at

Table 1
Kinetic parameters at various pHs

pH	K _M (mM)	V _{MAX} ^(*) (mM.min ⁻¹)	V _{MAX} /K _M ^(*) (min ⁻¹)
6.0	8	6.4	0.8
7.0	6	9.0	1.5
9.0	5	8.0	1.6
11.0	7	7.8	1.1

^(*)Calculated per mg extract protein

room temperature, at pH 7.0, the catalase activity was preserved even after 3 h incubation at 50°C. It even slightly increased when the exposure at 50°C lasted for 10 to 30 min. 10 min was chosen as the length of incubation at various temperatures. The pH activity profile

obtained after preincubation at 50°C for 10 min and shown in Fig. 1, suggested the possible presence of isoenzymes. Compared to the control, the activity at pH 6.0 was reduced by 40% while that at pH 7.0 was slightly increased (Fig. 1). The extract was then incubated at different temperatures for 10 min and tested at various pHs (Fig. 2). When tested at pH 6.0, the catalase activity was maximum after preincubation at 25°C; it dropped to 60% of that value when the extract was preincubated at various temperatures from 30°C to 50°C and it then dropped abruptly as the preincubation temperature increased. When tested at pH 11.0,

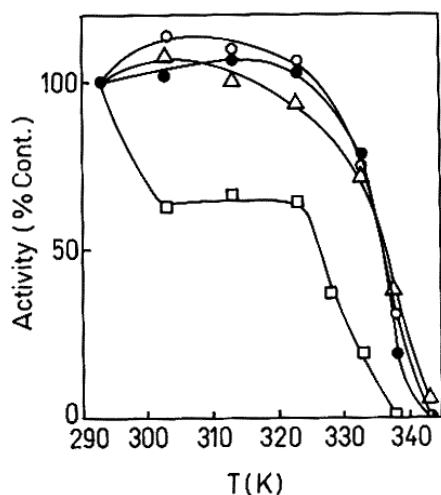


Figure 2: Effect of temperature on the enzymatic activity at various pHs. Extract was incubated at the indicated temperature for 10 min and then tested at room temperature, at given pH, after brief cooling in ice. pH 6.0 (squares); pH 7.0 (closed circles); pH 9.0 (open circles); pH 11.0 (open triangles).

the catalase activity was maximum after preincubation at 30°C, and when tested at pH 7.0 or 9.0, it was maximum after preincubation at 50°C (Fig. 2). In agreement with these results, Arrhenius plots of $\log k$ vs $1/T$ (Fig. 3) showed transition temperatures of 30°C and 50°C when the enzymatic activity was tested at pH 6.0, 45°C when tested at pH 11.0, and 60°C when tested at pH 7.0 and 9.0.

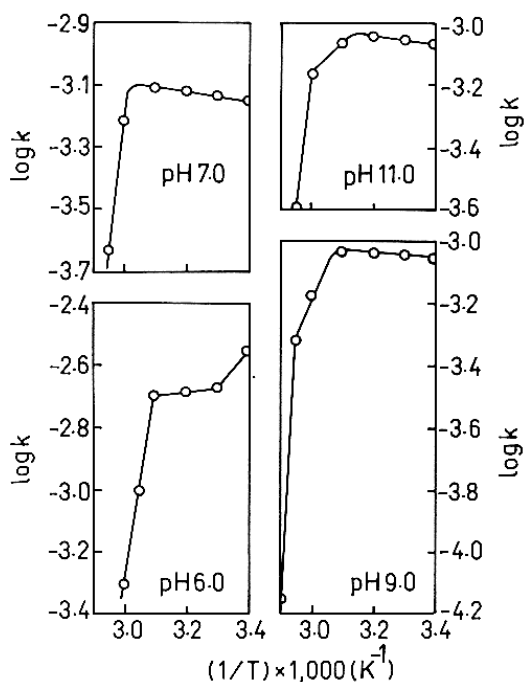


Figure 3: Arrhenius plots of enzyme denaturation.

The presence of three isoenzymes was confirmed when the extract was submitted to non-denaturing polyacrylamide gel electrophoresis. Three distinct bands corresponding to apparent molecular weight of 323,000, 295,000 and 268,000, were revealed after staining for catalase activity (Fig. 4).



Figure 4: Non-denaturing polyacrylamide gel electrophoresis of extract stained for catalase activity. Bands 1, 2 and 3 corresponded to apparent molecular weights of 323,000, 295,000, and 268,000, respectively. Left lane was loaded with 10 μ l extract (130 μ g protein) and right lane was loaded with 5 μ l extract (65 μ g protein).

In conclusion, three catalase isoenzymes of apparent molecular weight 323,000, 295,000 and 268,000, were detected in dormant saffron corms. Quantitatively, the same number of units of enzyme per mg extract protein was detectable at pH 6.0 through 11.0. However, one of the enzyme, active at pH 6.0, was very sensitive to temperature (inactivated by 40% after 10 min at 30°C). The other two were more resistant to temperature; one of them, active at pH 7.0 - 9.0, still showed 110% activity (compared to room temperature) after 10 min at 50°C, while the other, active at pH 11.0, showed 90% activity after 10 min at 50°C.

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