

Studies on Violet-colored Acid Phosphatase: Inactivation of Soybean Enzyme by Cysteine¹⁾

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The properties of soybean (*Glycine max*) acid phosphatase, violet-colored and manganese-containing, were further studied. The enzyme was significantly inactivated by the treatment with cysteine in alkaline solution. The enzyme was more inactivated under an aerobic condition than under an anaerobic condition. The inactivation was accompanied by the reduction of the absorbance of the enzyme at 540 nm. The mechanisms of the inactivation were also discussed.

Keywords—acid phosphatase; cysteine; soybean; *Glycine max*; inactivation by cysteine; manganese; metalloenzyme

There have been numerous reports dealing with the properties of acid phosphatase from many different living organisms.³⁾ However, inactivation of acid phosphatase by sulfhydryl compounds including cysteine is not reported.

We reported previously the purification and some properties of acid phosphatase from soybean (*Glycine max*).^{4,5)} The enzyme contains manganese and characterized by violet color (absorption maximum is at around 540 nm).

In our present study on the properties of soybean acid phosphatase, it was found that the enzyme was inactivated by cysteine. This paper describes inactivation of the soybean enzyme by cysteine.

Materials and Methods

Materials—The following substances were purchased: L-cysteine, dithiothreitol, DL-penicillamine, N-acetyl-L-cysteine, L-cysteinemethylester and β -mercaptoethanol from Nakarai Chemicals Co.; *p*-nitrophenyl phosphate, L-cysteic acid and DL-homocysteine from Wako Pure Chemical Co.; cysteamine from Sigma Chemical Co.; reduced glutathione from Kojin Co.; S-carboxymethyl-L-cysteine from Proteine Research Foundation in Japan. All other reagents used were analytical grade.

Preparation and Assay of the Acid Phosphatase—Purified acid phosphatase was prepared as described in the previous paper.⁴⁾ Protein concentration was determined spectrophotometrically at 280 nm in 0.01 M phosphate buffer, pH 6.0, using the factor $E_{1\%}^{1\text{cm}} = 21.4$.⁴⁾ The acid phosphatase activity was determined by measuring the rate of liberation of *p*-nitrophenol from *p*-nitrophenyl phosphate at 35°, pH 5.5 as described in the previous paper.⁴⁾

Results

Inactivation of the Acid Phosphatase by Cysteine

When the acid phosphatase was treated with cysteine at pH 8.5, the enzyme was inactivated in course of time as shown in Fig. 1. After 60 min treatment with 1 mM cysteine the enzyme lost 55% of the original activity. The other sulfhydryl compounds such as reduced

- 1) A part of this study was presented at the 96th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1976.
- 2) Location: 5-Nakauchi-cho Misasagi, Yamashina-ku, Kyoto, 607, Japan.
- 3) V.P. Hollander, "The Enzymes," Vol. IV, ed. by P.D. Boyer, Academic Press Inc., New York, 1971, p. 449.
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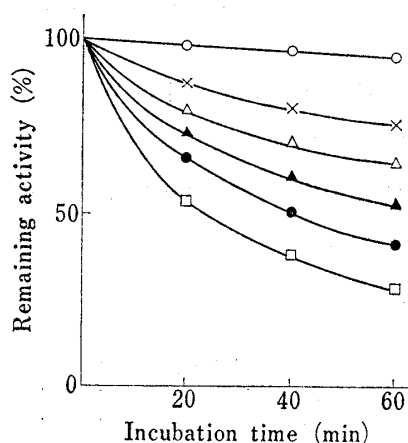


Fig. 1. Inactivation of the Acid Phosphatase by the Treatment with Sulphydryl Compounds

The acid phosphatase ($2 \mu\text{g}$) was treated with 1 mM sulphydryl compounds at 20° in 0.75 ml of Tris-HCl, pH 8.5. After various time, aliquots were withdrawn and then assayed for the enzyme activity. ○, control sample; ×, reduced glutathione; △, β -mercaptoethanol; ▲, penicillamine; ●, cysteine; □, dithiothreitol.

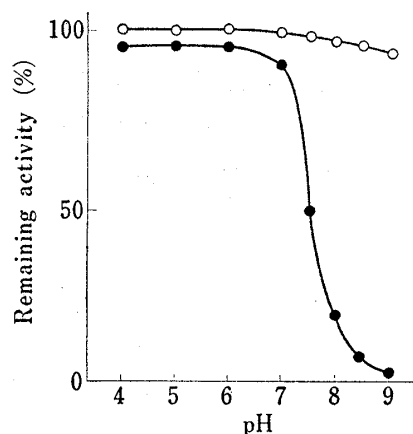


Fig. 2. Effect of pH on the Inactivation of the Acid Phosphatase by Cysteine

The acid phosphatase ($2 \mu\text{g}$) was treated with 100 mM cysteine (●) or without cysteine (○) at 20° in 0.75 ml of various pH values of 0.05 M Tris-maleic acid. After 60 min, aliquots were withdrawn and then assayed for the enzyme activity.

TABLE I. Effect of Analogues of Cysteine on the Acid Phosphatase

Substances	Remaining activity (%)
None	100
Cysteine	43
Cysteamine	35
Homocysteine	57
Cysteinemethylester	42
N-Acetylcysteine	64
Cysteic acid	96
S-Carboxymethylcysteine	98

The acid phosphatase ($2 \mu\text{g}$) was treated with 1 mM analogues of cysteine at 20° in 0.75 ml of 0.05 M Tris-HCl, pH 8.5. After 60 min, aliquots were withdrawn and then assayed for the enzyme activity.

glutathione, β -mercaptoethanol, penicillamine and dithiothreitol were also able to inactivate the enzyme (Fig. 1). Dithiothreitol was the most potent inhibitor in the sulphydryl compounds examined.

The enzyme was inactivated by cysteine significantly in alkaline solution (Fig. 2).

Effect of Cysteine Derivatives

Some kinds of cysteine derivatives were examined their ability to inactivate the acid phosphatase at pH 8.5. The results are shown in Table I. The inactivation activity of the derivatives without substituent on the sulphydryl group, *e.g.*, cysteamine and cysteinemethylester, was also observed. However, the derivatives with substituent on the sulphydryl group, *e.g.*, cysteic acid and S-carboxymethylcysteine, did not develop the activity. It seems likely that sulphydryl group is essential to develop the inactivation activity.

Effect of Oxygen on the Cysteine Inactivation

To determine whether or not oxygen is essential for the acid phosphatase inactivation by cysteine, the inactivation study was made under an anaerobic condition (Fig. 3).

The inactivation activity of cysteine was depressed to some extent under an anaerobic condition.

Correlation between the Activity and the Absorbance at 540 nm of the Enzyme by the Treatment with Cysteine

The manganese in soybean acid phosphatase contributes not only to the enzyme activity but also to violet color of the enzyme.⁵⁾ The correlation between the enzyme activity and the absorbance at 540 nm of the enzyme was determined under the treatment with cysteine (Fig. 4). The absorbance at 540 nm reduced with a loss of the enzyme activity.

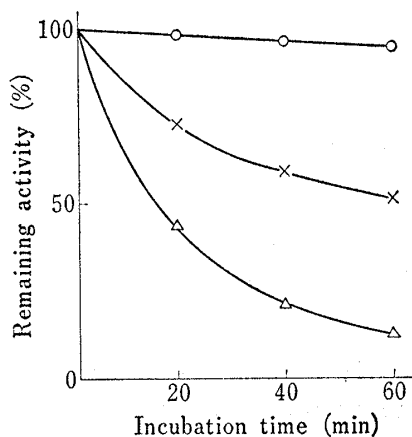


Fig. 3. Inactivation of the Acid Phosphatase by the Treatment with Cysteine Under Aerobic and Anaerobic Conditions

The acid phosphatase (2 μ g) was treated with 100 mM cysteine at 20° in 0.75 ml of 0.05 M Tris-HCl, pH 8.5, under aerobic and anaerobic conditions. For the experiment under anaerobic condition, oxygen in the reaction mixture was replaced with nitrogen gas. After various time, aliquots were withdrawn and then assayed for the enzyme activity. ○, control sample (in air and N₂ gas); ×, cysteine (in N₂ gas); △, cysteine (in air).

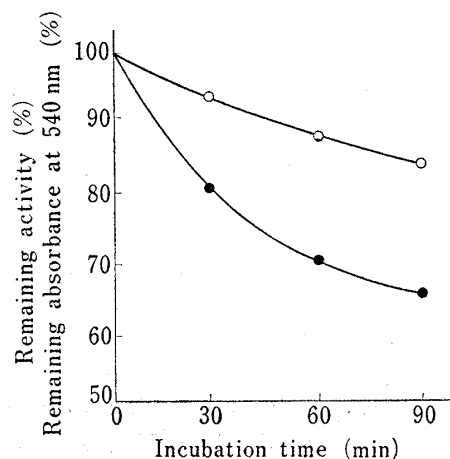


Fig. 4. Correlation between the Activity and the Absorbance at 540 nm of the Acid Phosphatase by the Treatment with Cysteine

The acid phosphatase (1 mg) was treated with 10 mM cysteine at 20° in 2.0 ml of 0.05 M Tris-HCl, pH 8.5. After various time absorbance at 540 nm was measured and aliquots were withdrawn to assay the enzyme activity. ●, acid phosphatase activity; ○, absorbance of the enzyme at 540 nm.

Discussion

Cysteine is one of chelating agents and it is well known that cysteine inactivates effectively alkaline phosphatase in alkaline solution by the chelation with zinc at the active site of the enzyme.⁶⁾

In the previous paper, we reported that ethylenediaminetetraacetic acid (EDTA) inactivated soybean acid phosphatase accompanying with the disappearance of the absorbance of the enzyme at 540 nm. We concluded that the phenomenon was caused by the chelation with manganese of the enzyme.⁵⁾

Cysteine and some of the derivatives also inactivated significantly soybean acid phosphatase in alkaline solution (Fig. 1, Fig. 2, Table I). Figure 4 shows that the absorbance of the enzyme at 540 nm was reduced with a loss of the enzyme activity by cysteine, though the reduction was not completely proportional to the inactivation; in the case of EDTA the reduction of the absorbance was completely agreed with the inactivation of the enzyme.⁵⁾ The facts suggest that cysteine inactivates the enzyme by the chelation with manganese in the enzyme molecule.

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However, cysteine inactivated the enzyme more potently under an aerobic condition than under an anaerobic condition (Fig. 3). If the inactivation was caused only by the chelation, the enzyme had to be inactivated to the same extent under both conditions. Additional factor(s) must be considered. As one of the factor(s) we presume a contribution of certain reactive species which are produced from cysteine under an aerobic condition. In fact, Misra reported that superoxide radical, hydroxyl radical and thiyl radical, which were very reactive species, were generated during autoxidation of sulfhydryl compounds in alkaline solution.⁷⁾

The inhibitory effect of cysteine to metalloenzyme is generally recognized as chelation with essential metal of enzyme. However, the results obtained in this study may suggest that the inhibitory effect of cysteine must be also discussed by considering additional factor(s) other than the chelating effect. Further studies to elucidate the additional factor(s) is necessary.

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Conformational Analysis of Prostaglandins. IV.¹⁾ Relationship between Melting Point and Calculated Conformational Energy of Prostaglandins²⁾

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A conformational energy calculation was carried out on nine prostaglandins; PGE₁, 11-epi PGE₁, 15-epi PGE₁, 11,15-epi PGE₁, PGA₁, 15-epi PGA₁, PGF_{1α}, PGF_{1β}, and PGB₁. The number of sterically allowed backbone conformations obtained by the computer experiment is related to the state of prostaglandins. The prostaglandin having a large number of sterically allowed backbone conformations (more than 70) was in an oily state, and the prostaglandin which had a small number of sterically allowed backbone conformations was in a crystalline state. Melting point of prostaglandin is related to conformational energy difference between the mean conformational energy and the lowest conformational energy of all sterically allowed conformations calculated by the computer experiment.

Keywords—computer experiment; melting point; prostaglandins; PGA; PGB; PGE; PGF

Introduction

A relationship between melting point and conformational energies of prostaglandins calculated by the computer experiment was studied, and relationship between states of prostaglandins and number of sterically allowed conformations was also examined. Crystalline state is the state in which exists only one conformer possessing the lowest conformational energy. Oily state is the state including not only one but many conformers. Populations of the conformers are given by statistical mechanics.

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2) A part of this work was presented at the 96th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1976.

3) Location: 2-1, Oshika 2-chome, Shizuoka 422, Japan.