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# ACID PHOSPHATASE FROM MAIZE SCUTELLUM: NEGATIVE CO-OPERATIVITY SUPPRESSION BY GLUCOSE

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**Key Word Index**—*Zea mays*; Gramineae; maize; scutellum; germination; acid phosphatase; negative co-operativity.

**Abstract**—Acid phosphatase purified from maize scutellum, upon acylation with succinic anhydride, still shows negative co-operativity for the hydrolysis of glucose-6-phosphate at pH 5.4. This phenomenon is abolished by glucose, for both native and succinylated enzymes, through stimulation of the initial velocities at sub-optimal substrate concentrations. However, negative co-operativity for the enzymatic hydrolysis of *p*-nitrophenylphosphate at pH 5.4 is suppressed only at high concentrations of glucose. Furthermore, the hydrolysis of *p*-nitrophenylphosphate is non-competitively inhibited (low affinity form of the enzyme molecule) by glucose, which suggests the existence of different substrate binding sites.

## INTRODUCTION

We have recently shown that, at pH 5.4, the kinetics of an acid phosphatase (EC 3.1.3.2) purified from maize scutellum, deviates significantly from Michaelis–Menten behavior [1]. It has also been shown that a kinetic transition from negative co-operativity (at pH 5.4) to Michaelian behavior (at pH 6.7) is associated with a pH dependence on the $K_m$ values for the hydrolysis of substrates [2]. Because this monomeric enzyme [3] probably has a single catalytic site [1], and negative co-operativity (at pH 5.4) for the hydrolysis of *p*-nitrophenylphosphate is suppressed by succinylation, we have supposed that the source of this phenomenon could be the ionization of the enzyme molecule [1]. In order to further study the relationship between kinetic and structural properties of this acid phosphatase, we investigated the kinetic properties of the enzyme interaction with glucose, which suppresses the negative co-operativity phenomenon for the hydrolysis of both substrates. Furthermore, our results provide some evidence for the existence of different substrate binding sites on the enzyme molecule.

## RESULTS AND DISCUSSION

Figures 1 and 2 show, respectively, the double-reciprocal plots of initial velocities for native and succinylated enzymes, at pH 5.4, as a function of the glucose-6-phosphate concentration. It can be observed that these plots did not give a straight line for native and succinylated enzymes and are characterized by a Hill coefficient [4] lower than one (Table 1), which is compatible with a negative co-operativity phenomenon [5]. Thus, extensive succinylation of the enzyme molecule did not abolish this phenomenon for the hydrolysis of glucose-6-phosphate at pH 5.4, as has been shown in the hydrolysis

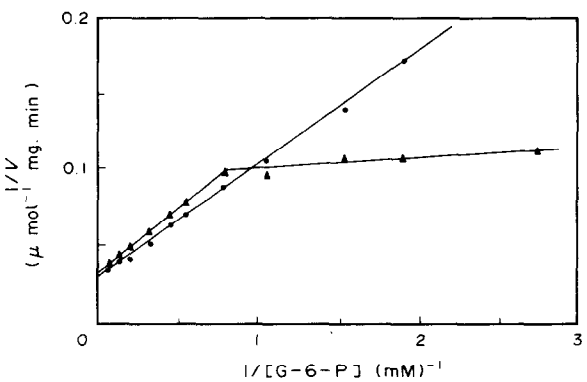


Fig. 1. Double-reciprocal plots of velocity of maize acid phosphatase as a function of glucose-6-phosphate concentration at pH 5.4. Each tube contained 430 ng of native enzyme. (▲), (●) Acid phosphatase velocity without and with 0.1 M glucose, respectively.

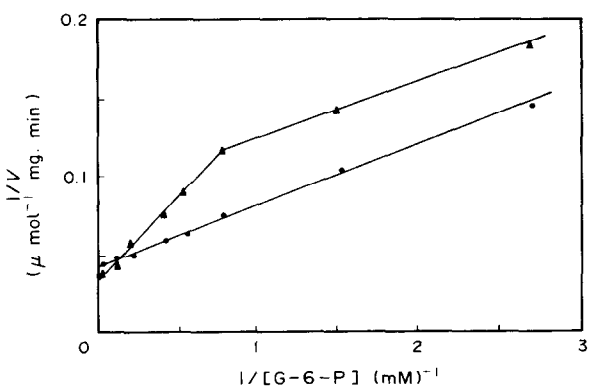


Fig. 2. Double-reciprocal plots of velocity of maize acid phosphatase as a function of glucose-6-phosphate concentration at pH 5.4. Each tube contained 430 ng of succinylated enzyme. (▲), (●) Acid phosphatase velocity without and with 0.1 M glucose, respectively.

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Table 1. Summary of some kinetic constants for the enzymatic hydrolysis of glucose-6-phosphate at pH 5.4 and 37°

| Enzyme | Addition | $K_m$(mM) | | $V_{max}$ (μmol · mgE min) | | $K_{cat}/K_m$ (mM · sec) | | $n$ |
|---|---|---|---|---|---|---|---|---|
| | | High | Low | High | Low | High | Low | |
| Native | — | 2.2 | 0.2 | 27.0 | 13.8 | 13.3 | 74.8 | 0.59 |
| | 0.1 M glucose | 2.1 | — | 29.0 | — | 15.0 | — | 0.97 |
| Succinylated | — | 2.3 | 0.3 | 29.7 | 11.4 | 14.0 | 41.2 | 0.72 |
| | 0.1 M glucose | 0.8 | — | 22.2 | — | 30.0 | — | 1.10 |

of *p*-nitrophenylphosphate [1]. From these results it seems clear that protonation–deprotonation of free amino groups of the enzyme molecule is not directly responsible for the appearance of negative co-operativity for the enzymatic hydrolysis of glucose-6-phosphate at pH 5.4. However, as can be seen in Table 1, enzyme succinylation leads to an increase in the Hill coefficient, which could indicate negative co-operativity suppression. Data from Figs. 1 and 2 also show that, for both enzymes, this phenomenon is similarly abolished by glucose, i.e. through stimulation of the initial velocities at sub-optimal substrate concentrations. Thus, negative co-operativity suppression by glucose seems not to be due to an inhibition of the low affinity form of enzyme molecule (Table 1) [1]. A conformational change, promoted by the binding of glucose on the enzyme molecule, may be responsible for suppression of this phenomenon. Furthermore, inspection of Fig. 2 and Table 1 shows a more marked effect of glucose on the succinylated enzyme which suggests that extensive acylation with succinic anhydride has affected the enzyme conformation at pH 5.4. From inactivation constants, we have concluded that free amino groups play a role in the maintenance of enzyme conformation only at a pH greater than 5.4 [3].

Although negative co-operativity is not observed at pHs greater than 6.7 [1], the initial velocities for the hydrolysis of glucose-6-phosphate by both native or succinylated enzymes is also stimulated by glucose at sub-optimal concentrations of substrate (not shown).

Figure 3 shows the double-reciprocal plots of initial velocities, at pH 5.4, as a function of *p*-nitrophenylphosphate concentration in the presence of glucose. It can be observed that the negative co-operativity phenomenon for the enzymatic hydrolysis of this substrate [1] is abolished by glucose at high concentrations only (0.5 M glucose). Furthermore, the enzymatic hydrolysis of *p*-nitrophenylphosphate is non-competitively inhibited by glucose (low affinity form of enzyme molecule).

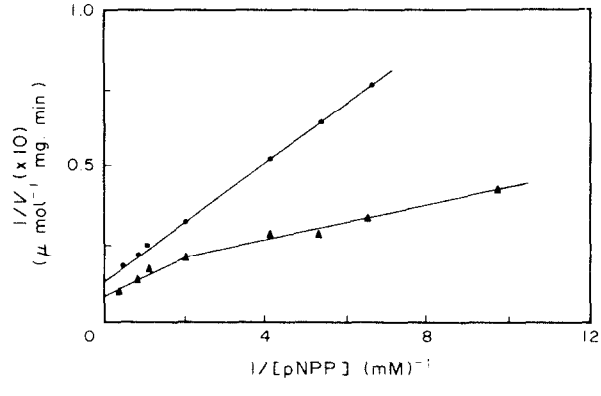


Fig. 3. Double-reciprocal plots of velocity of maize acid phosphatase as a function of *p*-nitrophenylphosphate concentration at pH 5.4. Each tube contained 390 ng of native enzyme. (▲), (●) Acid phosphatase velocity with 0.1 M and 0.5 M glucose, respectively.

Taken together, the results presented above suggest that the negative co-operativity phenomenon for the enzymatic hydrolysis of *p*-nitrophenylphosphate or glucose-6-phosphate, could not be explained by the same hyphothesis. Because this monomeric enzyme [3] probably has a single catalytic site [1], the existence of different substrate binding sites seems possible. If not, the same behaviour for the effect of glucose on hydrolysis of both substrates should be expected. Thus, the non-Michaelian behavior (at pH 5.4) after enzyme succinylation could be explained by the existence of two glucose-6-phosphate binding sites or, as proposed in the 'mnemonical' model, two conformational states of enzyme molecule [6–9].

## EXPERIMENTAL

Glucose-6-phosphate (monosodium salt) and *p*-nitrophenylphosphate were purchased from Sigma and Merck, respectively. All other chemicals were reagent grade.

*Enzyme preparation.* Acid phosphatase from germinating maize scutellum was purified as described in ref. [2].

*Assay procedures.* Glucose-6-phosphate hydrolysis was carried out in 0.1 M NaOAc buffer (pH 5.4) or 0.1 M Na maleate (pH 6.7) using 2 ml 30 mM glucose-6-phosphate as substrate and by measuring the Pi liberated by the method of ref. [10] with the exception that the solvent used was EtOAc. Incubation was carried out at 37° for 15–20 min and the reaction was terminated by adding 1 ml cold 10% TCA. *p*-Nitrophenylphosphate hydrolysis was carried out as described in ref. [2]. All enzyme activities were measured in duplicate, for at least two time points. 1 unit of phosphatase activity is defined as 1 μmol substrate hydrolysed per min. Succinylation of the enzyme molecule was performed as described in ref. [3].

*Kinetic studies.* Initial velocities were plotted according to the method of ref. [11]. Interaction constants ($n$) were determined by the method of ref. [4]. Kinetic constants given in this paper were obtained from linear-square analysis. When biphasic kinetic was observed, linear-square analysis was performed for each component of the curve (Table 1, low and high $K_m$ values).

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