Phytochemistry, Vol. 20, No. 11, pp. 2481–2482, 1981.
Printed in Great Britain.

0031-9422/81/112481-02 $02.00/0

ACID PHOSPHATASE FROM MAIZE SCUTELLUM: SUCCINYLATION AND SOME KINETIC PROPERTIES OF THE ACTIVE ENZYME

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(*Received* 24 *March* 1981)

Key Word Index—*Zea mays*; Gramineae; maize; scutellum; germination; acid phosphatase; succinylation; thermal inactivation.

Abstract—Acid phosphatase purified from maize scutellum did not dissociate into subunits upon acylation with succinic anhydride. The enzyme maintained its catalytic activity after succinylation of 52 free amino groups per molecule. The results also showed that free amino groups may play an important role in the maintenance of enzyme stability at pH values greater than 5.4.

INTRODUCTION

We have recently shown that acid phosphatase (EC 3.1.3.2) purified from germinating maize scutellum is probably a monomeric enzyme (MW 65000) and that SH groups might not be involved in the catalytic mechanism [1]. Studies with human prostatic acid phosphatase have also eliminated serine [2], tyrosine [3], tryptophan [3], cysteine [4] and lysine [4] from involvement in either catalysis or substrate binding.

For comparative purposes and in order to check our previous report on the aggregation state of the enzyme [1], we have extensively succinylated maize scutellum acid phosphatase. Our results provided no evidence for active site lysyl residues and confirmed the monomeric state of the maize scutellum acid phosphatase.

RESULTS AND DISCUSSION

The acid phosphatase purified from germinating maize scutellum [1] was found by titration with TNBS to contain 52 free amino groups per molecule. After succinylation (see Experimental), no free amino groups were detectable and the enzyme activity at pH 5.4 (*p*-nitrophenylphosphate and glucose-6-phosphate as substrates) was enhanced about 30%. These results indicated that the free amino groups of the protein may be unimportant for the maintenance of enzyme activity. No further reactivation of the modified enzyme was observed after treatment of the succinylated enzyme with 0.5 M hydroxylamine for 1 hr, at pH 7.0, at room temperature. These results indicated that succinylation did not affect groups other than amino groups, these groups probably being those of the tyrosyl, seryl or threonyl residues. The acylation of these groups was completely reversible after treatment with a nucleophile [5].

The MW of the succinylated enzyme was 75000 ± 3000 as determined by molecular exclusion chromatography. The increment on the apparent MW (65000) could be due to the incorporation of succinic acid into the protein molecule with an additional increase in the Stokes radius [6]. When the MW of the modified enzyme was estimated by disc electrophoresis, in the presence of SDS (see ref. [1]), only one protein band (MW *ca* 65000) was observed. These results confirm our previous results which suggested that the enzyme was in the monomeric form.

Inactivation behaviour at pH 5.4 and 6.7 (at 65°) is shown in Fig. 1. It can be seen that the inactivation velocities found were quite similar for the native enzyme at both pHs. Although the residual activity of the modified enzyme decreased more rapidly than for the native

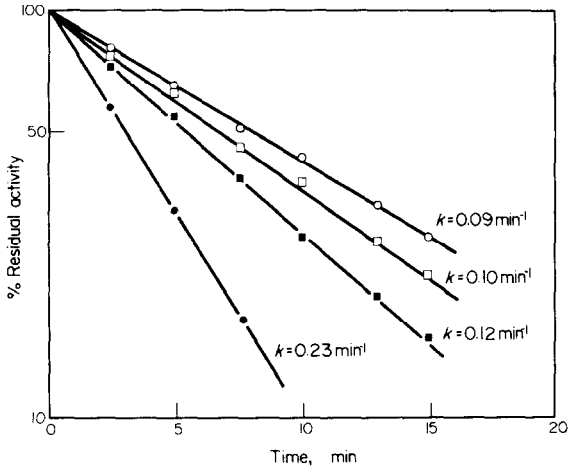

Fig. 1. Thermal inactivation of purified maize scutellum acid phosphatase. □-□, Native enzyme assayed at pH 5.4; ■-■, succinylated enzyme assayed at pH 5.4; ○-○, native enzyme assayed at pH 6.7; ●-●, succinylated enzyme assayed at pH 6.7.

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enzyme, we concluded that succinylation had not changed the enzyme stability at pH 5.4. However, at pH 6.7 the decrease in the residual activity was more marked, suggesting that the free amino groups were important for the maintenance of enzyme stability at this pH.

The K_m values for native and succinylated enzyme (*p*-nitrophenylphosphate as substrate) were 0.3×10^{-3} M and 0.5×10^{-3} M at pH 5.4 and 1.6×10^{-3} M and 6.7×10^{-3} M at pH 6.7, respectively. The K_m values found at pH 5.4 were quite similar for native and succinylated enzymes. However, at pH 6.7, the K_m for the succinylated enzyme was 4 times higher than that of the native enzyme, indicating that the succinylated enzyme had a lower affinity for *p*-nitrophenylphosphate at this pH.

Thus, the K_m values and inactivation constants suggest that free amino groups may play an important role in the maintenance of enzyme conformation at a pH greater than 5.4.

EXPERIMENTAL

Assay procedures. The enzyme assays were carried out in either 0.1 M NaOAc buffer (pH 5.4) or 0.1 M Na maleate buffer (pH 6.7) using 2.0 ml of 6.0 mM *p*-nitrophenylphosphate as substrate at 37°. The reaction was stopped by the addition of 1.0 ml of 1.0 M NaOH, and the *p*-nitrophenol estimated by measurement of $E_{405\,nm}$ (ε 17800).

Glucose-6-phosphate hydrolysis was carried out in either 0.1 M NaOAc buffer (pH 5.4) or 0.1 M Na maleate (pH 6.7) using 2.0 ml of 30 mM glucose-6-phosphate as substrate and by measuring the Pi liberated by the method of ref. [7]. Incubations were carried out at 37° for 15–30 min and the reactions were terminated by addition of 1.0 ml cold 10% TCA.

All enzyme activities were measured in duplicate, for at least two time points. One unit of phosphatase activity is defined as 1.0 μmol substrate hydrolysed/min.

Protein was measured by the method of ref. [8] using BSA as a standard.

The number of free amino groups in the protein was determined by using TNBS [9].

The reaction of the enzyme with succinic anhydride was performed as in ref. [5]: 150 μl succinic anhydride in Me_2CO (200 mg/ml) was added to 1.0 mg of acid phosphatase in 3.0 ml 0.1 M NaPi buffer, pH 8.2, at 20°. The activity of the succinylated enzyme was measured before and after treatment with 0.5 M NH_2OH at pH 7.0 and at 20° for 1 hr. The NH_2OH treatment was used to deacylate any tyrosyl, seryl or threonyl residues which could have been modified in the course of the chemical reaction [5]. A control experiment established that in the absence of succinic anhydride the enzymic activity of native protein was unaffected. After succinylation, the modified enzyme was dialysed for 24 hr against 16 l. of 10 mM NaOAc buffer (pH 5.0) with four changes of the buffer and stored at 4°.

MW was measured by gel filtration, using a Sephadex G-200 column (2 × 120 cm) as described in ref. [10], equilibrated with 30 mM NaOAc buffer (pH 5.0) containing 10 mM EDTA, at a flow rate of 15 ml/hr (3 ml fractions). BSA, ovalbumin and trypsinogen were used as MW markers.

Enzyme purification. Germinating maize scutellum acid phosphatase was purified as described in ref. [1].

Thermal inactivation. The native or succinylated enzyme (4 μg/ml) was incubated at 65° in a final volume of 3.0 ml 0.1 M NaOAc buffer, pH 5.4, or 0.1 M maleate buffer, pH 6.7. At appropriate times aliquots (0.1 ml) were taken to measure, at pH 5.4, the residual *p*-nitrophenylphosphatase activity.

Acknowledgements—This work was supported by grants from FAPESP. S.A.R. was supported by a Research Fellowship from CAPES. We are grateful to Mrs Leda Verdiani-Tfouni for revision of the manuscript.

REFERENCES

1. Rossi, A., Palma, M. S., Leone, F. A. and Brigliador, M. A. (1981) *Phytochemistry* **20**, 1823.
2. Greemberg, H. and Nachmansohn, D. J. (1965) *J. Biol. Chem.* **240**, 1639.
3. Rybarska, J. and Ostrowski, W. (1975) *Acta Biochim. Pol.* **21**, 377.
4. McTigue, J. J. and Van Etten, R. L. (1978) *Biochim. Biophys. Acta* **523**, 407.
5. Means, G. and Feeney, R. E. (1971) in *Chemical Modification of Proteins*. Holden-Day, San Francisco.
6. Habeeb, A. F. S. A. (1967) *Arch. Biochem. Biophys.* **121**, 652.
7. Gomori, G. (1942) *J. Lab. Clin. Med.* **27**, 955.
8. Hartree, E. F. (1972) *Analyt. Biochem.* **48**, 422.
9. Habeeb, A. F. S. A. (1966) *Analyt. Biochem.* **14**, 328.
10. Andrews, P. (1964) *Biochem. J.* **91**, 22.