

EFFECT OF DETERGENTS ON THE ALKALINE PHOSPHATASE OF *CUSCUTA REFLEXA*

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(Received 21 November 1973)

Key Word Index—*Cuscuta reflexa*; Cuscutaceae; alkaline phosphatase; detergents.

Abstract—The non-ionic detergents, in particular Tween 20, Tween 80 and Triton X100, stimulated the alkaline phosphatase activity of *Cuscuta reflexa* homogenates with fructose-1,6-diphosphate and β -glycerophosphate as substrates. The order of activation was usually less than 100%, suggesting that a true latency was not involved. A differential response was found towards the two substrates, indicating the existence of two enzyme activities.

INTRODUCTION

THE ADDITION of detergents to homogenates of a range of tissues is known to increase the activities of certain enzymes.¹ This paper reports the effects of non-ionic detergents on alkaline phosphatase activity towards fructose-1,6-diphosphate (FDP) and β -glycerophosphate (β -GP) in homogenates of *Cuscuta reflexa* Roxb.

RESULTS AND DISCUSSION

Tissues of *C. reflexa* possess powerful alkaline phosphatase activity with an optimum at pH 8 when tested towards FDP or β -GP.² Incorporation of Tween 20, Tween 40, Tween 60, Tween 80 and Triton X100, as buffered solutions immediately prior to assay, at a final concentration of 0.05% (v/v) (Tween 80 was also employed at 0.02 and 1% concentrations), did not effect any significant change in activity towards either FDP or β -GP. Experiments were therefore conducted to determine the effect on enzyme activity of preincubation with higher concentrations of detergent than those conventionally employed.^{3,4} A preincubation period of 90 min and a temperature of 0–2° were chosen arbitrarily and held constant, while the detergent concn was varied.

To 100 ml samples each of a stock homogenate 0.5, 1, 2 and 5 ml of the particular detergent (unbuffered) was added and the mixture re-homogenized in a Waring blender for 30 sec. Similarly, a sample of the control homogenate (100 ml) was also re-homogenized. These preparations were assayed after 90 min.

The changes induced by Tween 40 and Tween 60 were not significant, but Tween 80 led to a marked increase in the alkaline phosphatase activity for both substrates, and

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¹ KRISHNAN, P. S., VISWANATHAN, P. N. and MATTOO, R. L. (1969) *J. Sci. Ind. Res.* **28**, 181.

² MATTOO, R. L. (1973) *Biochem. Physiol. Pflanzen* **164**, 639.

³ NEWMARK, M. Z. and WENGER, B. S. (1960) *Arch. Biochem. Biophys.* **89**, 110.

⁴ ROUSSOS, G. G. (1962) *Biochem. Biophys. Acta* **64**, 187.

Tween 20 induced activation only towards β -GP. Triton X100 caused greater activation towards β -GP than FDP.

Not only the quantitative but even the qualitative reproducibility of the above results failed when the preincubation period was increased to 3 hr. The following experiments were therefore undertaken to elucidate the influence of the preincubation period with the detergent on the enzyme activity. The detergent concn at which either the degree of activation was highest or FDPase/ β -GPase ratio was lowest was selected. Of four 100 ml samples of stock homogenate, one was mixed with 1 ml Tween 20, the second with 5 ml Tween 80 and the third with 1 ml Triton X100, while the fourth served as control. All the samples were re-homogenized in a Waring blender for 30 sec and stored at 0°. The various homogenates were assayed 15 min after detergent treatment. The detergent-treated homogenates were assayed also at 60, 120 and 180 min, commencing from the time of detergent addition. The control homogenate (untreated) was assayed at the end of 180 min. There was 5–10% loss of activity towards both the substrates when the control homogenate was assayed after storage for 3 hr. The activation and/or deactivation of alkaline phosphatase was differential towards FDP and β -GP not only with different detergents but even under the influence of the same detergent over various lengths of time. On contact with Tween 80 and Triton X100 for 60 and 180 min respectively, the alkaline phosphatase for the first time showed a higher activity with FDP than with β -GP, a feature not encountered in any of the other analyses. Tween 80 apparently did not exert its maximal activating effect even at 5%. A concentration of Tween 20 and Triton X100 as high as five-fold the optimal concentration of 1% did not show any inhibition.

The general effect of the detergents was one of stimulation, but the extent of stimulation was less than 100%, suggesting that a true latency was not involved. Also, the degree of stimulation and the concentration of detergent for maximal stimulation differed with the detergent. The order of enzyme stimulation achieved with Tween 80 was significantly higher than observed with any detergent by Stetten and Burnett⁵ and Snoke and Nordlie⁶ for glucose-6-phosphatase activity.

The action of detergents was time-dependent. An activation occurring during a certain interval was followed in some cases by an inhibition or vice versa. The consistent occurrence of a change in the ratio of the alkaline phosphatase activity towards the two substrates indicates the possible existence of two separate enzymes or two distinct types of activity associated with a single protein in this parasite. *Cuscuta* has photosynthetic capability⁷ and alkaline FDPase is a key enzyme in the photosynthetic pathway.⁸

EXPERIMENTAL

Preparation of homogenates. All homogenates were 10% (w/v) and contained cysteine, added during cell-disruption, at a final concn of 17 mM. Homogenization of the tissue, or re-homogenization of a dispersion with detergent caused frothing at all detergent concn tested. This subsided in about 15 min after which the final vol. was adjusted.

Enzyme assay. The assay system for alkaline phosphatase was as reported earlier,⁹ using the Mg^{2+} -stimulated activity.² In the presence of detergents opalescence occurred during color development for orthophosphate. This was prevented by adding about 50 mg of activated Norit A following the trichloroacetic acid and prior to color

⁵ STETTEN, M. R. and BURNETT, F. F. (1967) *Biochim. Biophys. Acta* **139**, 138.

⁶ SNOKE, R. E. and NORDLIE, R. C. (1967) *Biochim. Biophys. Acta* **139**, 190.

⁷ MACLEOD, D. G. (1961) *Experientia* **17**, 542.

⁸ SMILLIE, R. M. (1960) *Nature* **187**, 1024.

⁹ MATTOO, R. L., VISWANATHAN, P. N. and KRISHNAN, P. S. (1969) *Physiol. Plantarum* **22**, 638.

development. Control experiments established that the addition of Norit did not alter the concn of orthophosphate in the TCA supernatants.

Acknowledgements—The authors are thankful to Prof. P. S. Krishnan for his interest in the study and to C.S.I.R., New-Delhi, for financial assistance.