PLANT TISSUE PHOSPHODIESTERASE ACTIVITIES

W. E. Razzell*

Faculty of Agriculture, University of British Columbia

Vancouver 8, Canada

Received December 17, 1965

Enzyme preparations purified from snake venom, hog kidney and hog liver possess the common property of hydrolyzing p-nitrophenyl thymidine 5'-phosphate and eliminating nucleoside 5'-phosphates from a variety of nucleoside 5'-diesters and pyrophosphates (Razzell, 1963). Data on all such reactions reinforce the conclusion of Razzell and Khorana (1959a) that the type reaction

$$R - O - P - O - R' + H_2O \rightleftarrows R - O - P - OH + H - O - R'$$

(R = nucleoside or deoxynucleoside; R' = any suitable residue) proceeds at rates governed by the leaving groups (R' - O - H). It has become clear that the best leaving groups observed to date are p-nitrophenol and α-naphthol (Sierakowska, Szemplinska and Shugar, 1963), and that the ability of such enzymes to catalyze a stepwise degradation of ribo- or deoxyribo-oligonucleotides (Razzell & Khorana, 1959b) is dependent on the high pH of the reaction (optimum at 9.1 - 9.3) and on divalent ion (Mg^{++}). In contrast, potato nucleotide pyrophosphatase (Kornberg and Pricer, 1950) which eliminates nucleoside 5'-phosphate from NAD^+ and FAD and deoxynucleoside 5'-phosphate from p-nitrophenyl thymidine 5'-phosphate at pH values between 4.5 and 7.5 in the presence of divalent ion chelators (EDTA), does not catalyze the stepwise degradation of

*Supported in part by a grant from the Jane Coffin Childs Memorial Fund.

polynucleotides (Razzell, 1965). However, NAD^+ is a good competitive inhibitor of the hydrolysis of p-nitrophenyl thymidine 5'-phosphate by the potato enzyme and thus both substrates appear to be hydrolyzed by the same enzyme (Razzell, 1965).

Experiments are reported below which show that plant tissues possess two distinct enzymes, one of which appears to correspond to the animal tissue phosphodiesterase (phosphodiesterase I) and the other to the nucleotide pyrophosphatase isolated from potato.

EXPERIMENTAL

Seedlings were obtained from peas and corn following germination in tap water at 22° in the dark until the growth had reached a height of 3-4 cm. The cotyledon(s) and plumule were removed together and the remaining seed (without the coat) was saved separately. Seed and seedlings were each homogenized in a blendor micro assembly in 0.05 M Tris-acetate, pH 7.5 and the suspensions centrifuged at 15,000 x g for 20 min. at 4°. The supernatant solutions were stored at 4°.

Crude enzyme preparation from peeled potatoes was obtained by homogenization in 0.4 saturated ammonium-sulfate according to Kornberg & Pricer (1950); the filtered, centrifuged solution was employed without further precipitation of the soluble proteins.

Enzyme assays were performed as previously described (Razzell, 1963, 1965) with variations in Mg^{++} or chelators as indicated subsequently.

RESULTS

Samples of potato homogenate, adjusted to the pH of the test at 4°, were examined for activity against p-nitrophenyl thymidine 5'-phosphate at various pH values, with and without Mg^{++} or EDTA. From Fig. 1 it is apparent that the greater activity is exhibited at pH values below 8, where the reaction is independent of Mg^{++}, although appreciable Mg^{++}-dependent activity remains at pH 9.3. Thus, there are two activities in potato; the nucleotide pyrophosphatase of Kornberg and Pricer and phosphodiesterase

I. Attempts to purify the latter from potato extracts have so far been unsuccessful.*

The extracts of corn (monocotyledenous) and peas (dicotyledenous) were assayed for activity at pH 9.3 and 7.2 with Mg^{++} or EDTA, as shown in Table I.

TABLE I. Hydrolysis of p-nitrophenyl thymidine 5'-phosphate in extracts of plant tissues.

Tissue	pH 9.3 Phosphodiesterase I +Mg^{++}	pH 9.3 Phosphodiesterase I + EDTA	pH 7.2 Phosphodiesterase III +Mg^{++}	pH 7.2 Phosphodiesterase III + EDTA
Peas - seedling	15.7	1.8	3.9	3.9
- seed residue	14.0	1.1	10.7	10.6
Corn - seedling	36.0	3.1	11.5	11.7
Potato	4.4	0.5	7.6	7.6

Activities are expressed as μmoles p-nitrophenol liberated per hr per gram wet tissue. EDTA, when present, 10^{-2} M; Mg^{++}, 10^{-2} M.

It is clear that the results with potato are not representative of actively growing tissue of other dicotyledons, since the extracts of pea seedlings and residual pea seed storage material show greater activities of phosphodiesterase I than of the enzyme active at low pH in the absence of Mg^{++} (which shall be referred to as phosphodiesterase III until its relationship to nucleotide pyrophosphatase is clarified). Further, the corn seedling also shows the approximate 3:1 ratio of phosphodiesterase I:III. The activities of plant extracts are a small fraction of the phosphodiesterase I activities of animal tissues, which are 300-500 μmoles/h/gm. wet wt.

It has been possible to confirm the observations of

*A black precipitate of polyphenols appears to adsorb the phosphodiesterase I, but does not greatly affect the nucleotide pyrophosphatase; 2-mercaptoethanol at 10^{-2} M prevents blackening but promotes rapid inactivation of phosphodiesterase I preferentially.

Kornberg and Pricer (1950), that the nucleotide pyrophosphatase from potato which is active in liberating AMP and NMN from NAD^+ is soluble in contrast to the NAD^+-splitting enzyme from kidney (Kornberg and Lindberg, 1948), (i.e., phosphodiesterase I). For example, homogenates of pea and corn seedlings (approximately 15% w/w) were centrifuged at 110,000 x g for 60 min. at 6°. The concentrations of the activities of both phosphodiesterases I and III in the fraction of the supernatant solution at the upper half of the centrifuge tubes were greater than 75% of the original concentrations. The solubility of phosphodiesterase I from the plants is in marked contrast to the behaviour of the enzyme in kidney and liver (Razzell 1961b, 1965) and in animal tumors (V. R. Potter and D. H. Ives, personal communication).*

This multiplicity of phosphodiesterases in plants and the general ubiquity in nature of polynucleotidases begs for integration

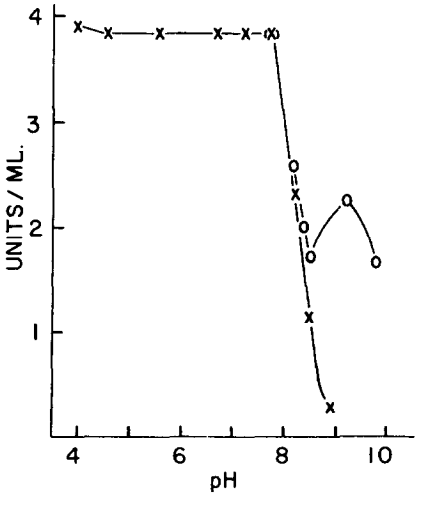

Fig. 1. Rates of liberation of p-nitrophenyl thymidine 5'-phosphate by 0.4 sat. ammonium sulfate supernatant fraction from potato. Included in the assay mixtures were EDTA, 10^{-2} M (crosses) or $MgCl_2$, 10^{-2} M (circles).

*The high pH optimum and EDTA sensitivity which characterize the phosphodiesterase I in diverse animal tissues (Razzell, 1961a, 1963) are also properties of purified preparations with polynucleotidase activity. Because of the prevalence of various polynucleotidases in plant extracts (Khorana, 1961) it is impossible to assess the polynucleotidase activity of plant phosphodiesterase I from diverse sources without extensive purificatio

into the catabolic and anabolic pathways of metabolism. That such enzymes are not required for cell replication under all conditions, however, is apparent from the fact the mammalian cells in culture lack several polynucleotidases found in the organized tissues of origin (Razzell, 1965).

REFERENCES

Khorana, H. G., (1961) in P. D. Boyer, H. Lardy and K. Myrbäck (Editors), The Enzymes, Academic Press, New York, Vol. 5, p. 79.
Kornberg, A., and Lindberg, O., (1948), Federation Proc., 7, 165.
Kornberg, A., and Pricer, W. E. Jr., (1950), J. Biol. Chem., 182, 763.
Razzell, W. E., and Khorana, H. G., (1959a), J. Biol. Chem., 234, 2105.
Razzell, W. E., and Khorana, H. G., (1959b), J. Biol. Chem., 234, 2114.
Razzell, W. E., (1961a), J. Biol. Chem., 236, 3028.
Razzell, W. E., (1961b), J. Biol. Chem., 236, 3031.
Razzell, W. E., (1963) in S. P. Colowick and N. O. Kaplan (Editors), Methods in Enzymology, Academic Press, New York, Vol. VI, p. 236.
Razzell, W. E., (1965) , Can. J. Biochem. Physiol., in press.
Sierakowska, H., Szemplinska, H., and Shugar, D., (1963), Acta Biochimica Polonica, X, 399.