are dependent on temperature. In the 100–120° range there was no noticeable temperature dependence, so it may be that in the lower temperature range (65–100°) equilibrium was not reached. Such a situation might lead to curvature of the ln t_{net} or ln t_{corr} vs. $1/T$ plots. Further studies should be made to determine the exact nature of the temperature dependence.

Because in this study determinations of thermodynamic quantities depend on retention time and retention volume, and because retention data depend directly on the flow rate of the carrier gas, it is important to ascertain that the thermodynamic quantities as determined here are not functions of flow rate. In the general experiments a flow rate of 25 ml/min was used. For three compounds experiments were made in which each compound was studied at flow rates of 20, 25, and 30 ml/min. Though retention times vary widely with flow rate, as expected, the corrected retention volumes were remarkably constant for a given temperature, so that Gibbs energies of adsorption as derived from corrected retention volumes will not be dependent on flow rate. Heats of adsorption were observed to vary only slightly within experimental error, for different flow rates.

LITERATURE CITED

Berlin, E., U.S. Department of Agriculture, Washington, D.C., private communication, 1973.
Berlin, E., Anderson, B. A., Pallansch, M. J., *J. Dairy Sci.* **55**, 1396 (1972).
Breschia, F., Arents, J., Meirlich, H., Turk, A., "Fundamentals of Chemistry; A Modern Introduction", Academic Press, New York and London, 1966, p 297.
Gale, R. L., Beebe, R. A., *J. Phys. Chem.* **68**, 555 (1964).
Greene, S. A., Pust, H., *J. Phys. Chem.* **66**, 55 (1958).
Gregg, S. J., "The Surface Chemistry of Solids", Reinhold, New York and London, 1951, pp 17–44.
Habgood, H. W., in "The Solid-Gas Interface", Flood, E. A., Ed., Marcel Dekker, New York, N.Y., 1967, p 635.
Hinsch, D. M., Micromeritics Instrument Corp., Norcross, Ga. 30071, private communication, 1973.
Hoare, M. R., Purnell, J. H., *Trans. Farad. Soc.* **52**, 22 (1956).
Kiselev, A. V., Yashin, Y. I., "Gas-Adsorption Chromatography", translated by J. E. S. Bradley, Plenum Press, New York and London, 1969, pp 11–141.
Lee, I., Nickerson, T. A., Bernhard, R. A., *J. Dairy Sci.*, in press (1975).
Lim, S. G., Nickerson, T. A., *J. Dairy Sci.* **56**, 843 (1973).
Moore, W. J., "Physical Chemistry", 3rd ed, Prentice-Hall, Englewood Cliffs, N.J., 1962, pp 744–745.
Nickerson, T. A., Dolby, R. M., *J. Dairy Sci.* **54**, 1212 (1971).
Noller, C. R., "Chemistry of Organic Compounds", W. B. Saunders, Philadelphia, Pa., 1957, p 829.
Okamura, J. P., Sawyer, D. T., *Sep. Purif. Methods* **1**(2), 409 (1972).
Yabumoto, K., Jennings, W. G., and Pangborn, R. M., *J. Food Sci.*, in press (1975).

Received for review September 16, 1974. Accepted January 2, 1975.

Characterization of Phosphatase of Intact Maize Roots

Ralph B. Clark

Conditions for optimum assay of intact maize (*Zea mays* L.) root phosphatase are described. *p*-Nitrophenyl phosphate was the most effective of all substrates tried. Phosphatase activity was linear up to 1.5 hr at 0.25 to 1.0 m*M*. At *p*-nitrophenyl phosphate concentrations above or below these linearity decreased sooner. Phosphatase activity was inhibited by *p*-nitrophenol above 0.36 m*M*, phosphorus above 0.26 m*M*, molybdenum above 0.02 m*M*, and aluminum above 0.37 m*M*. Calcium, magnesium, iron, and zinc at concentrations up to tenfold that of the original solution were not inhibitory. Optimum activity was obtained between pH 3 and 7 and at 35–50°. Fibrous roots had higher phosphatase activity than prop roots and 21-day-old roots had higher activity than younger or older roots.

Studies have shown phosphatase activities of intact roots may play a significant role in making nonavailable forms of phosphorus more available for plant use (Bieleski, 1973; Ridge and Rovira, 1971; Woolhouse, 1969). Considerable phosphatase activity in intact roots is located near root surfaces (Hall and Butt, 1968; Ridge and Rovira, 1969), especially under phosphorus deficiency conditions (Bieleski and Johnson, 1972; Reid and Bieleski, 1970). Phosphorus-deficient *Spirodela* hydrolyzed more glucose 1-phosphate in the growth medium than in the plant tissue (Bieleski and Johnson, 1972) and it was suggested that, under these conditions, the function of root phosphatase was to utilize P-esters in the growth medium. Thus, P-esters appear to be hydrolyzed by making contact with plant roots without being absorbed inside the root.

Phosphatase activity has generally been determined on tissue extracts or on purified preparations (Hollander, 1971). Information on phosphatase activities of intact roots is limited and many of the conditions for optimum activity have not been given (Bieleski and Johnson, 1972; Hall and Butt, 1968; Reid and Bieleski, 1970; Ridge and Rovira, 1971; Spencer, 1954; Woolhouse, 1969). The purpose of this study was to determine optimum conditions for phosphatase assay of intact maize roots.

Department of Agronomy, Agricultural Research Service, U.S. Department of Agriculture, Ohio Agricultural Research and Development Center and the North Central Region, Wooster, Ohio 44691.

EXPERIMENTAL SECTION

Growth of Plants. The maize (*Zea mays* L.) inbred Pa36 was chosen for this study because of its use in other phosphorus nutrition studies (Clark and Brown, 1974). Plants were grown in full-strength nutrient solutions containing (m*M*) 2.6 Ca, 1.8 K, 0.6 Mg, 0.9 NH_4N, 6.9 NO_3N, 0.5 S, 0.5 Cl, 0.069 P, 0.007 Mn, 0.019 B, 0.002 Zn, 0.0006 Mo, 0.0005 Cu, and 0.038 Fe as Fe-hydroxyethylenediaminetriacetate (FeHEDTA). These nutrient levels were adequate to sustain optimum growth for 15 to 17 days. The plants were grown in a glasshouse with added light (19 klx, 30 cm from the source) in 18-l. plastic containers (4 plants/container).

Phosphatase Assay Solutions. Phosphatase assay solutions were full-strength nutrient solutions with added substrate (*p*-nitrophenyl phosphate) and treatment salts where necessary. Solutions were adjusted to pH 4.0 and

Table I. Phosphatase Activity of Intact Maize Roots with Various P-Esters

Substrate	μmol of inorganic P hr^{-1} g^{-1} dry wt
p-Nitrophenyl phosphate	52.6 ± 2.3
β-Glycerophosphate	16.0 ± 0.9
Glucose 1-phosphate	5.6 ± 0.8
Phenyl phosphate	5.4 ± 0.9
α-Naphthyl phosphate	2.8 ± 0.8

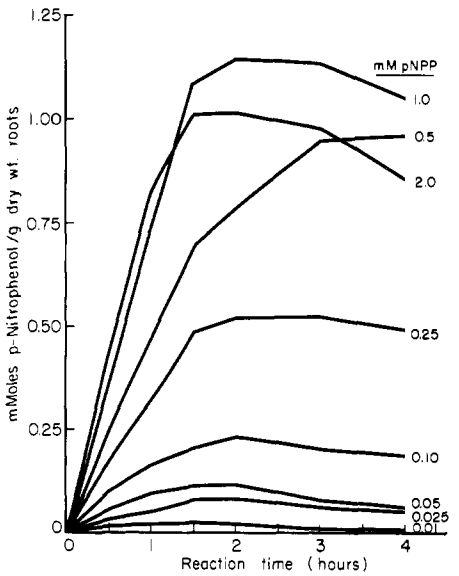

Figure 1. Phosphatase activity of intact maize roots with *p*-nitrophenyl phosphate concentration (pNPP).

put in assay beakers at 200 ml/beaker. Each assay beaker was enveloped with black plastic and covered by a dark plexiglass lid containing a slit for the plant and a small hole for entry of an aeration tube.

Phosphatase Assay. Plant roots were gently rinsed with deionized water and individual plants (uniform as possible) were introduced into each beaker to start the reaction. Control reactions were aerated solutions without plants. At timed intervals, 3.0-ml samples were drawn from each beaker and added to test tubes containing 1.0 ml of 2 *N* NaOH. The tubes were shaken and centrifuged at 3000*g* for 2 min and the absorbance at 410 nm determined (Bessey et al., 1946) using a Gilford Model 240 spectrophotometer. Concentrations of *p*-nitrophenol formed by the phosphatase mediated hydrolysis of *p*-nitrophenyl phosphate were determined by reference to standard curves of *p*-nitrophenol. In tests using P-esters other than *p*-nitrophenyl phosphate, phosphatase activity was assayed by determining the amount of inorganic phosphate formed, using the Fiske–SubbaRow (Fiske and SubbaRow, 1925) method for P.

Plants were not assayed until after they had been illuminated for a minimum of 6 hr during the day. Plants were also illuminated during the assay. A minimum of three plants was assayed per treatment level. The pH and temperature of the assay solutions were recorded at the time of sampling. Except for experiments in which pH, temperature, and substrate concentration were treatment variables, final pH values were 4.5 ± 0.3, temperatures were 30 ± 2°, and substrate concentrations were 0.1 m*M*.

Although samples were taken from each reaction mixture at several timed intervals, the results are reported using the 0.5-hr reaction time. At the conclusion of experiments, roots were thoroughly water rinsed, blotted dry, weighed for fresh weight, dried at 70° for a minimum of 4 days, and weighed for dry weight. Results were comparable on a fresh or dry weight basis; consequently, phosphatase activity is reported on a dry weight basis.

Table II. Effect of *p*-Nitrophenol, Inorganic Phosphorus, Molybdenum, and Aluminum on Phosphatase Activity of Intact Maize Roots

Added, m*M*	μmol of *p*-nitrophenol hr^{-1} g^{-1} dry wt
p-Nitrophenol	
0	64.8 ± 5.5
0.18	63.3 ± 4.4
0.36	63.2 ± 8.2
0.72	44.8 ± 2.1
1.44	2.2 ± 0.1
2.88	1.9 ± 0.2
P	
0	46.8 ± 5.6
0.065	50.4 ± 6.6
0.13	42.3 ± 3.5
0.26	28.9 ± 4.1
0.52	28.6 ± 0.3
1.03	18.5 ± 0.9
Mo	
0	40.4 ± 1.7
0.0006	38.3 ± 0.8
0.006	37.3 ± 0.3
0.02	19.5 ± 1.5
0.06	12.9 ± 1.0
Al	
0	50.4 ± 6.6
0.09	49.3 ± 6.8
0.19	52.5 ± 4.0
0.37	27.0 ± 1.5
0.74	22.0 ± 1.4

RESULTS AND DISCUSSION

Phosphatase activity was higher with *p*-nitrophenyl phosphate as substrate than with β-glycerophosphate, glucose 1-phosphate, phenyl phosphate, and α-naphthyl phosphate (Table I). Phosphatase activities with β-glycerophosphate, glucose 1-phosphate, phenyl phosphate, and α-naphthyl phosphate were 30, 11, 10, and 5%, respectively, that of *p*-nitrophenyl phosphate. When inorganic P is the product measured in the assay, reactions allowed to continue 1 hr and beyond decrease from curves noted when the hydrolyzed organic molecule is measured. This is because inorganic P is absorbed by the roots and is no longer available for assay.

Figure 1 shows phosphatase activities of maize roots with increasing concentrations of *p*-nitrophenyl phosphate over a period of 4 hr. At substrate concentrations between 0.25 and 1.0 m*M*, the reaction was linear for 1.5 hr before leveling off. At 2 m*M* substrate concentration, the reaction was linear for 1 hr and at substrate concentrations below 0.1 m*M* the reaction was linear for only 0.5 hr.

When reactions were allowed to continue 4 hr, the absorbance reached a maximum and then decreased. These decreases were attributed to *p*-nitrophenol absorption by the roots at low *p*-nitrophenyl phosphate concentrations and to both root absorption and inhibition of phosphatase by *p*-nitrophenol at high *p*-nitrophenyl phosphate concentrations. *Agrostis tenuis* Sibth. roots absorbed more *p*-nitrophenol after 2 hr than before (Woolhouse, 1969). *p*-Nitrophenol was also inhibitory to phosphatase at concentrations of 0.72 m*M* and above (Table II).

Table III. Effect of pH and Temperature on Phosphatase Activity of Intact Maize Roots

pH		
Initial	Final	μmol of *p*-nitrophenol hr^{-1} g^{-1} dry wt
3	3.1	69.2 ± 3.4
4	4.1	68.9 ± 3.5
5	5.1	60.1 ± 7.1
6	6.0	60.8 ± 7.0
7	6.8	63.7 ± 3.2
8	7.6	52.2 ± 2.9
9	8.5	16.7 ± 1.6
10	9.6	2.5 ± 0.3
Temp, °C		
10		16.2 ± 1.2
20		24.0 ± 1.7
25		38.2 ± 1.4
30		63.1 ± 2.3
35		75.6 ± 3.6
40		73.0 ± 5.0
50		86.7 ± 0.8

Phosphatase activity with 2 m*M* *p*-nitrophenyl phosphate exceeded the activity at 1 m*M* within the first hour only (Figure 1). Purified extracts do not require such high concentrations of substrate (Hollander, 1971). Intact roots may require higher substrate concentrations because roots are spread heterogeneously throughout the solution.

Relatively small amounts of *p*-nitrophenol yielded relatively high absorbance values. Absorbance was over 0.300 for 0.072 μ*M* *p*-nitrophenol. Readings as high as 1.00 were commonly attained within 0.5 hr for 3.0-ml aliquot samples (from a total of 200 ml) with 0.25 m*M* *p*-nitrophenol phosphate and roots from single plants of 0.3–0.5 g dry weight.

In this study, best results of maize root phosphatase activities were obtained when analyses were made within 1 hr after the roots were introduced into the reaction mixture. Substrate concentration and amount of root material determined the best time to take samples for analysis.

The effects of *p*-nitrophenol and several inorganic elements on phosphatase activity were studied. Phosphatase was inhibited 31% by 0.72 m*M* *p*-nitrophenol, 38% by 0.26 m*M* P, 49% by 0.02 m*M* Mo, and 46% by 0.37 m*M* Al (Table II). Phosphatase activity was inhibited 97% at 1.44 m*M* *p*-nitrophenol. Higher concentrations of the above compounds were even more inhibitory. Calcium, Mg, Fe, and Zn were not inhibitory up to 26 m*M* Ca, 6 m*M* Mg, 0.38 m*M* Fe, and 20 μ*M* Zn, tenfold higher concentrations than original full-strength nutrient solutions. The plants had no prior equilibration with *p*-nitrophenol or the inorganic salts. Longer exposure of roots to these compounds or growth of plants at various P, Mo, and Al concentrations might cause an even greater change in phosphatase (Bieleski and Johnson, 1972; Clark and Brown, 1974; Reid and Bieleski, 1970; Spencer, 1954). The plant genotype might also affect the response of phosphatase to the mineral elements (Clark and Brown, 1974).

Phosphatase activity of intact maize roots was optimum between pH 3 and 7 (Table III). Above pH 7, activity decreased rapidly and continued to decrease above pH 9. The optimum pH suggests that phosphatase near the root surface is primarily acid phosphatase since alkaline phosphatase is active near pH 9. Optimum pH for purified acid phosphatase is 4 to 6 and activity declines rapidly below or above that range (Hollander, 1971). Apparently

Table IV. Effect of Root Type and Age on Phosphatase Activity of Intact Maize Roots

Root type[a]		μmol of *p*-nitrophenol hr^{-1} g^{-1} dry wt
All roots		43.9 ± 1.9
All fibrous roots		64.4 ± 1.7
Top-half fibrous roots		64.2 ± 2.1
Prop roots		24.7 ± 0.7
Root age,[b] days	Root wt, g dry wt/plant	
14	0.11	31.2 ± 1.2
17	0.17	44.6 ± 3.3
21	0.53	61.2 ± 3.4
28	1.49	36.7 ± 2.0

[a] All roots were 21 days old. [b] Days from germination. Plants were 7 days old when transferred to full-strength nutrient solutions.

the optimum pH range is narrower for extracts than for intact maize roots. This might be expected for intact root phosphatase, since it may be protected to some degree by adjacent or enclosing membranes near the root surface (Bieleski and Johnson, 1972).

Phosphatase activity increased with temperature to a plateau at 30–40° (Table III) and then increased further at 50°. At 50°, roots showed extensive injury and phosphatase might have been released from internal sources to the solution. Optimum phosphatase activity for purified extracts has been noted at 50° (Hollander, 1971); however, optimum growth conditions for corn are near 25–35°.

Phosphatase activity was determined on maize roots from different parts of the system and on roots of different ages (Table IV). Activity was similar for upper and lower fibrous roots. The newly developing prop roots had lower phosphatase activity. The prop roots are large and contribute greatly to total weight compared to fibrous roots, but little to the surface area. As secondary roots developed on the prop roots, phosphatase activity increased. Activity was higher for 21-day-old roots than for younger or older roots. Phosphatase activity may be more closely associated with surface area than with age of roots (Woolhouse, 1969) although root weight may serve as a useful guide under certain conditions (Ridge and Rovira, 1971).

ACKNOWLEDGMENT

The technical assistance of Georgianna Kirchhofer is greatly appreciated.

LITERATURE CITED

Bessey, O. A., Lowry, O. H., Brock, M. J., *J. Biol. Chem.* **164**, 321 (1946).
Bieleski, R. L., *Annu. Rev. Plant Physiol.* **24**, 225 (1973).
Bieleski, R. L., Johnson, P. N., *Aust. J. Biol. Sci.* **25**, 707 (1972).
Clark, R. B., Brown, J. C., *Crop Sci.* **14**, 505–508 (1974).
Fiske, C. H., SubbaRow, Y., *J. Biol. Chem.* **66**, 375 (1925).
Hall, J. L., Butt, V. S., *J. Exp. Bot.* **19**, 276 (1968).
Hollander, V. P., *Enzymes, 3rd Ed.*, **4**, 449 (1971).
Reid, M. S., Bieleski, R. L., *Planta* **94**, 273 (1970).
Ridge, E. H., Rovira, A. D., *New Phytol.* **70**, 1017 (1971).
Spencer, D., *Aust. J. Biol. Sci.* **7**, 151 (1954).
Woolhouse, H. W., in "Ecological Aspects of the Mineral Nutrition of Plants", Rorison, I. H., Ed., Blackwell Scientific Publications, Oxford, England, 1969, p 357.

Received for review September 26, 1974. Accepted January 9, 1975. Contribution from the U.S. Department of Agriculture, Journal Article No. 65-74. Mention of a company, trademark, or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may be suitable.