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HYDROLYSIS OF α-D-GLUCURONATE-1-PHOSPHATE BY EXTRACTS FROM *LEMNA MINOR*

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INTRODUCTION

Although the enzymic conversion of glucuronate-1-phosphate to glucuronate has been observed in preparations obtained from rat kidney [1], it does not appear to have been studied in plants. The existence of a similar enzymic conversion in plants is reported in this paper. The enzyme has been prepared from duckweed (*Lemna minor*) growing in axenic culture. The hydrolysis of glucuronate-1-phosphate provides one route for the formation of glucuronic acid in plants. Glucuronic acid is also known to be formed from *myo*-inositol [2] by the activity of *myo*-inositol oxygenase (EC 1.13.99.1). When D-glucuronate-[^{14}C] is fed to plants, label can be detected in L-gulonate [3], D-xylose and sucrose [4].

RESULTS AND DISCUSSION

The existence of glucuronate-1-phosphate activity was established in preparations obtained by grinding in buffered medium, $(NH_4)_2SO_4$ precipitation of the protein and subsequent dialysis. Controls in which either glucuronate-1-phosphate or duckweed extract was omitted from the assay were suitably low in inorganic P.

Table 1. Effect of culture age on ammonium sulphate fractionation of glucuronate-1-phosphatase activity

$(NH_4)_2SO_4$ fraction as % saturation	% of total glucuronate-1-phosphatase activity	
	Sensecent cultures	Young cultures no sensescent fronds
0–30	8.2	11.5
30–50	10.4	72.1
50–100	81.4	16.4

Table 2. The pH response of glucuronate-1-phosphatase activity

pH	Activity expressed as % of pH 5 activity*
3	42
4	79
5	100
6	87
8	31
9	23

* Using 50–100% $(NH_4)_2SO_4$ fraction from senescent cultures.

$(NH_4)_2SO_4$ fractionation was carried out on crude extracts and typical results are shown in Table 1. The age of the culture influenced the fraction in which the maximum enzyme activity was detected. The response to pH, determined using a preparation obtained by dialysis of the 50–100% $(NH_4)_2SO_4$ fraction from senescent cultures is shown in Table 2. Similar results were obtained using a preparation including all protein precipitated in saturated $(NH_4)_2SO_4$. These results suggest that the pH optimum for glucuronate-1-phosphatase is between 4 and 6. The acid phosphatases of plants are characterized by pH optima within this range [5].

EXPERIMENTAL

Lemna minor collected from a local swamp was obtained in axenic culture using the method of ref. [6]. The growth medium used was the M medium described in ref. [7] which contained in addition 20 μm tartaric acid, 30 μM EDTA and 1% (w/v) sucrose. Medium was adjusted to pH 4.6 with KOH before autoclaving and inoculation.

Preparation of enzyme. This was carried out at 0–2%. About 25 g (fr. wt) was ground in 2 vol. 0.2 M NaOAc buffer (pH 5) and centrifuged at 1500 *g* for 5 min. The supernatant was filtered through cheesecloth to remove floating particles and centrifuged at 16000 *g* for 15 min. The supernatant was used for $(NH_4)_2SO_4$ fractionation. Precipitated fractions were collected by centrifugation at 16000 *g* for 15 min, taken up in H_2O and dialysed against 5 changes of H_2O.

Enzyme assay. The reaction mixture contained 4 μmol of the K salt of α-D-glucuronate-1-phosphate, 100 μmol of NaOAc buffer (pH 4 or 5) or glycine–HCl (pH 3) or phthalate–NaOH (pH 6) or Tris-HCl (pH 8 or 9), 8 μmol $MgSO_4$ and *Lemna* extract in a total vol. of 1 ml. After 15 min incubation at 30°, the reaction was terminated by adding 1 ml of 10%w/v TCA. Centrifuging to remove precipitated protein was only necessary for unfractionated prepns. Inorganic P was determined in suitable samples by the method of ref. [8], as modified in ref. [9].

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