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EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID TREATMENT ON THE SOLUBLE AND CELL WALL BOUND PROTEIN IN *CICHORIUM INTYBUS* ROOT TISSUE

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Abstract—Measurements have been made of protein and activities of enzymes bound to the cell wall of chicory root tissue. It has been shown that promotion of cell enlargement brought about by treatment with the highly active growth regulator 2,4-dichlorophenoxyacetic acid (2,4-D) does not arise from bound enzyme released from the wall. Experiments with inhibitors of protein synthesis suggest that the enhanced invertase and hydrolase activity found in soluble protein extracts after 2,4-D treatment arises from the synthesis of small amounts of protein.

INTRODUCTION

TREATMENT of tissue disks cut from chicory root with 10^{-5} M solutions of the plant growth regulator 2,4-dichlorophenoxyacetic acid (2,4-D) results in a large uptake of water and a marked increase in invertase activity.[1] Similar results have been reported for tuber tissue from Jerusalem artichoke.[2] In both cases, no increase in enzyme activity was obtained when tissue was treated with the inactive analogue 3,5-dichlorophenoxyacetic acid (3,5-D).

In the work described above, enzyme activities were measured in a soluble protein extract and it was suggested[1,2] that the increased activity observed after 2,4-D treatment might be due to a loosening of bound enzyme from the cell wall. Some enzymes, and particularly some forms of invertase are known to be bound to the plant cell wall.[3] Moreover, the altered chromatographic behaviour of invertase extracted from tissue treated with growth regulator added some support to the concept of release of bound enzyme.[1] The present study describes analyses of both bound and soluble enzyme before and after growth regulator treatment. In addition, inhibitors of various aspects of protein synthesis were used in an attempt to relate the relatively small increase in protein which was found to the large increase in enzyme activity.[1,2]

RESULTS

The effect of different treatments on invertase and hydrolase activity is shown in Table 1. In untreated chicory root, invertase activity is present both in the bound and soluble forms, the total activity being nearly equally divided between both forms. Ageing the tissue disks

[1] FLOOD, A. E., RUTHERFORD, P. P. and WESTON, E. W. (1970) *Phytochemistry* **9**, 2431.
[2] RUTHERFORD, P. P., WESTON, E. W. and FLOOD, A. E. (1969) *Phytochemistry* **8**, 1859.
[3] EDELMAN, J. and HALL, M. A. (1965) *Biochem. J.* **95**, 403.

in water for 72 hr produced little change in activity due to soluble enzyme but to a considerable increase in the bound form. Treatment with 2,4-D however led to a large increase in the activity due to soluble enzyme accompanied also by further increase in bound enzyme activity.

TABLE 1. THE EFFECT OF 5-FLUOROURACIL ON THE INVERTASE AND HYDROLASE ACTIVITIES OF SOLUBLE AND INSOLUBLE PROTEIN PREPARATIONS

Treatment	Water uptake (%)	Protein*		Units of invertase activity $\times 10^6$		Units of hydrolase activity $\times 10^6$	
		Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble
Untreated	—	12·4	7·9	45	44	226	153
Water	32	12·7	16·1	469	43	494	264
10^{-5} M 2,4-D	182	18·6	24·4	1628	2442	1221	413
2·5 mM 5-Fluorouracil	33	10·3	14·4	436	42	497	266
10^{-5} M 2,4-D + 2·5 mM 5-Fluorouracil	89	10·8	14·6	441	662	615	402

All treatments were for 72 hr. * mg protein/g initial dry wt. One unit of invertase activity represents 2 μ-mol of hexose liberated/min/mg initial dry wt at 25°. One unit of hydrolase activity represents 1 μmol of hexose liberated/min/mg initial dry wt at 25°.

The large effect of growth regulator treatment on activity due to bound enzyme is underlined by the investigations on hydrolase activity, where the most striking increase due to treatment with 2,4-D was associated with the insoluble protein fraction.

The amount of soluble protein and that bound to insoluble cell wall material is shown for the various treatments in Table 1. Treatment of the tissue with water for 72 hr had no effect on the total amount of bound protein but treatment with 2,4-D led to approximately 50% increase. On the other hand increases in soluble protein occurred with water treatment and there was a further increase with the 2,4-D treatment.

Table 1 also illustrates the effect of 5-fluorouracil on the invertase and hydrolase activities of protein preparations from chicory root tissue. It is well known that ageing of chicory root disks in water leads to a marked increase in total soluble protein (e.g. Laties[4]) and to an increase in hydrolase activity but little or no change in invertase activity.[1] 5-Fluorouracil had no effect on the enzyme activities but it reduced the amount of soluble protein synthesized. However, in the presence of 2,4-D the effect of the inhibitor was more dramatic. The increase in water uptake and the associated large increase in invertase activity due to 2,4-D treatment was considerably reduced. On the other hand there was no effect on hydrolase activity though the total soluble protein was reduced to the level found when the tissue was incubated with a solution of 5-fluorouracil alone.

Further experiments with 5-fluorouracil revealed a similar effect on the insoluble protein, both in the presence of water and 2,4-D.

In comparable experiments, puromycin, actinomycin-D and ethidium bromide had no effect on the water uptake, soluble or insoluble protein or any of the bound or soluble enzyme activities. For this reason the detailed results of these experiments are not given.

DISCUSSION

The suggestion[1,2] that the increased activity induced in both chicory and artichoke tissue by 2,4-D treatment might arise from a loosening of enzyme bound to the cell wall now

[4] LATIES, G. G. (1959) *Arch. Biochem. Biophys.* **79**, 364.

seems unlikely. Large increases in enzyme activity bound to insoluble cell wall material always accompany the increases in activity found in soluble protein preparations. Although the activity associated with the insoluble cell wall material cannot be described as due to cell wall bound enzyme *in vivo*,[5,6] this level of activity would not be expected if all the increased soluble activity arose solely from enzymes loosened from the cell wall.

The simplest explanation of increased enzyme activity would be in terms of increased protein synthesis. The figures for soluble protein given in Table 1 are in agreement with results of other workers. The increase in protein associated with ageing the tissue in water is well known[4] and the additional increase in soluble protein due to 2,4-D treatment has been described previously.[1] In an attempt to see whether the increased water uptake and increased enzyme activity were related to the measured increases in protein, we studied the effect on the system of some inhibitors of protein synthesis.

5-Fluorouracil, which inhibits ribosome synthesis and has been used in Jerusalem artichoke studies, had marked inhibitory effects on water uptake, total protein synthesis and invertase activity both bound and soluble. It inhibited the synthesis of protein when disks were aged in water but had no effect on the initial enzyme activities (which are not increased by water treatment). Inhibition of enzyme activity was confined to increments of enzyme activity associated with protein increases. There was no significant effect on hydrolase activity after 2,4-D treatment. However, the effect of the auxin on the hydrolase activity is small compared with that on invertase activity and this may explain the absence of any significant inhibition. Although the increase in protein after 2,4-D treatment is small compared with the increase in enzyme activity, the fact that both parameters are reduced by the inhibitor treatment indicates that they are related.

Rutherford[7] has reported that actinomycin-D reduced the water uptake and invertase and hydrolase activity in Jerusalem artichoke tissue disks following 2,4-D treatment. With chicory root disks neither actinomycin-D nor ethidium bromide, which behaves like actinomycin-D but is a smaller molecule, showed any inhibitory effect. Puromycin, which inhibits the binding of amino acids to tRNA also had no inhibitory effect. It is likely that the difference shown by artichoke tuber and chicory root tissue towards inhibitors of protein synthesis is related to differences in cell permeability.

The present study provides evidence that the effect of 2,4-D on chicory root tissue is not to loosen enzyme protein bound to the cell wall. The enhanced extractable enzyme activity observed is more likely due to the synthesis of small amounts of protein of high specific activity. Analytical studies recently carried out in this laboratory have indicated that proteins of high specific activity may be present in the tissues following treatment with 2,4-D.

EXPERIMENTAL

Chicory roots, variety Magdeburg, were obtained from F. G. Harrison, Bury St. Edmunds, Suffolk and stored and sampled as already described.[1] Tissue disks were prepared from the roots and incubations carried out as described by Rutherford *et al.*[8]

Protein preparations from the disks. Soluble protein preparations were obtained and their invertase and hydrolase activity determined by methods already described.[1] The residue from the extraction of soluble

[5] HAWKER, J. S. (1969) *Phytochemistry* **8**, 337.
[6] RICARDO, C. P. P. and AP REES, T. (1970) *Phytochemistry* **9**, 239.
[7] RUTHERFORD, P. P. (1971) *Phytochemistry* **10**, 1469.
[8] RUTHERFORD, P. P., GRIFFITHS, C. M. and WAIN, R. L. (1966) *Ann. Appl. Biol.* **58**, 467.
[9] EDELMAN, J. and JEFFORD, T. G. (1964) *Biochem. J.* **93**, 148.

protein was dialysed against 5 mM phosphate buffer, pH 7·5 (4 changes) to remove the cysteine and the diethyldithiocarbamate used in the initial extraction. Aliquots (about 1 g) of the dialysed material were suspended in 25 ml of a solution that was 0·1 M with respect to acetate pH 5 buffer and either 0·3 M with respect to sucrose (for invertase assay) or contained sufficient substrate M[9] equivalent to 1 g fructose (for hydrolase assay). The suspension was agitated gently for 4 hr at 25° and then filtered through a Buchner funnel. Suitable aliquots of the filtrate were taken for analysis of reducing sugars by the salicylate method.[10]

Protein determinations. Soluble protein was determined by the method of Lowry[11] and bound protein by micro Kjeldahl.[12]

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[11] LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDLE, R. J. (1951) *J. Biol. Chem.* **193**, 265.
[12] CHIBNALL, A. C., REES, M. W. and WILLIAMS, E. F. (1943) *Biochem. J.* **37**, 354.