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INHIBITION BY ACTINOMYCIN D OF WATER UPTAKE AND INVERTASE AND HYDROLASE ACTIVITIES INDUCED IN JERUSALEM ARTICHOKE TUBER TISSUE DISKS BY TREATMENT WITH 2,4-DICHLOROPHENOXYACETIC ACID

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Abstract—Actinomycin D was found to inhibit the water uptake and both the invertase and hydrolase activities induced by treatment of Jerusalem artichoke tissue disks with 10^{-5}M solutions of 2,4-dichlorophenoxyacetic acid. None of the treatments were found to produce any measurable change in the amounts of total soluble protein or invertase or hydrolase activity. These results provide further evidence that increases in the activities of both invertase and hydrolase induced by 2,4 D are dependent upon the continued synthesis of a messenger RNA.

INTRODUCTION

THERE IS considerable evidence that plant growth-regulators are involved in nucleic acid and protein synthesis, which in turn is related to plant growth.[1] However, there is comparatively little information on any specific effects of growth-regulators on the activation of enzymes and even less on actual enzyme synthesis.[2] Probably, the only well established example of specific auxin-induced enzyme synthesis in plants is that found in the aleurone cell of cereal seeds which synthesizes several hydrolases more rapidly in response to treatment with gibberellic acid.[3] There are, however, several examples of increases in enzyme activity produced by growth-regulator treatment which could well be due to induced enzyme synthesis.[2]

One of the most studied plant materials is Jerusalem artichoke tissue disks, in which it has been shown that the activities of both invertase and hydrolase are greatly increased by growth-regulator treatment.[4] By the use of specific protein synthesis inhibitors, radioactive labelling and protein separation and purification it has been shown that continued synthesis of RNA and of protein are required for the growth-substance induced expansion growth of the Jerusalem artichoke tissue disks.[5–9] Although suggestions have been made of the possible synthesis of one or more enzymes and their mode of action,[7] no definite evidence has been obtained.

[1] J. L. KEY, *Ann. Rev. Plant Physiol.* **20**, 449 (1969).
[2] P. FILNER, J. L. WRAY and J. E. VARNER, *Science* **165**, 358 (1969).
[3] J. E. VARNER and G. R. CHANDRA, *Proc. Natl. Acad. Sci. U.S.* **52**, 100 (1964).
[4] P. P. RUTHERFORD, E. W. WESTON and A. E. FLOOD, *Phytochem.* **8**, 1859 (1969).
[5] K. V. THIMANN and G. M. LOOS, *Plant Physiol.* **32**, 274 (1957).
[6] L. D. NOODEN and K. V. THIMANN, *Plant Physiol.* **40**, 193 (1965).
[7] L. D. NOODEN and K. V. THIMANN, *Plant Physiol.* **41**, 157 (1966).
[8] Y. MASUDA, *Plant Cell Physiol.* **7**, 75 (1966).
[9] Y. MASUDA, *Plant Cell Physiol.* **7**, 573 (1966).

In this work, actinomycin D (Act D), which has been shown to block specifically the DNA dependent RNA synthesis,[10] has been used to obtain further information on the mode of action of the synthetic growth-regulator, 2,4-dichlorophenoxyacetic acid (2,4 D) which greatly enhances the activities of both invertase and hydrolase present in Jerusalem artichoke tubers.

RESULTS

Table 1 shows the effects of Act D on the water uptake and the increases in both invertase and hydrolase activities induced in Jerusalem artichoke tuber tissue disks by treatment with 10^{-5} M solutions of 2,4 D. Also included are the amounts of total soluble protein and of protein possessing invertase or hydrolase activities. The results for both water treated and untreated disks are also presented.

TABLE 1. ENZYME ACTIVITY, WATER UPTAKE AND AMOUNTS OF PROTEIN IN EXTRACTS FROM JERUSALEM ARTICHOKE DISKS AFTER TREATMENT AT 25°

Treatment		Water* uptake (%)	Protein†			Enzyme activity	
Day 1	Day 2–4 inclusive		Total	Invertase	Hydrolase	Invertase‡	Hydrolase§
Untreated		—	10·6	3·5	5·0	10	204
Water	Water	14	9·0	2·3	4·2	19	201
Water	10^{-5} M 2,4 D	109	9·5	4·2	4·4	814	1229
1 mg/l. Act D	10^{-5} M 2,4 D	58	8·7	4·0	3·9	221	796
10 mg/l. Act D	10^{-5} M 2,4 D	48	10·2	4·5	5·0	108	309
10^{-5} M 2, 4 D	10^{-5} M 2,4 D	112	8·9	3·6	4·3	707	1863
1 mg/l. Act D + 10^{-5} M 2,4 D	1 mg/l. Act D + 10^{-5} M 2,4 D	89	9·4	3·9	4·8	480	1499
10 mg/l. Act D + 10^{-5} M 2,4 D	10 mg/l. Act D + 10^{-5} M 2,4 D	43	9·6	3·8	5·2	55	464

* Increase in weight compared with the initial weight.

† Mg protein $\times 10^3$ per mg initial dry wt.

‡ 1 unit of invertase activity represents the liberation at 25° of 2 μmoles of hexose per min per mg initial dry wt. $\times 10^6$.

§ 1 unit of hydrolase activity represents the liberation at 25° of 1 μmole of hexose per min per mg initial dry wt. $\times 10^6$.

Act D always reduced the 2,4 D induced water uptake, being most effective at the higher concentration of 10 mg/l. At the lower concentration of 1 mg/l., initial treatment of the disks with Act D was considerably more effective in inhibiting water uptake than when solutions containing both Act D and 2,4 D were used. Very considerable reductions in the 2,4 D induced invertase and hydrolase activities occurred in all treatments which included Act D. The higher concentration, 10 mg/l., caused a considerably larger decrease in the activities of both enzymes, than the lower concentration of 1 mg/l. Initial treatment of the

[10] E. REICH and I. H. GOLDBERG, *Prog. Nucl. Acid Res. Mol. Biol.* **3**, 183, (1964).

disks with Act D alone rather than mixtures of 2,4 D and Act D was usually more effective in reducing enzyme activity. Act D had no measurable effect on the amounts of either total soluble protein or of protein possessing invertase or hydrolase activity.

DISCUSSION

It has already been shown that, although Jerusalem artichoke tissue disks behave in the same general qualitative way, the responses, and in particular the growth-substance induced water uptake, increase considerably throughout the storage period reaching a maximum and reasonably constant value after between 4 and 6 months.[11,12] Therefore, the Jerusalem artichokes used for this work were stored for 6 months, by which time the tubers showed signs of sprouting. Also, the experiments were carried out over a short time interval of 3 weeks. Choice of duration of treatment totalling 4 days, and of a growth-regulator and its concentration, 10^{-5} M 2,4 D, provided a large uptake of water. This growth-substance induced water uptake—over 100 per cent—was between two and three times larger than that obtained by either Thimann[7] or Masuda[9] and their co-workers, and so considerably facilitated the assessment of the effects of Act D.

Although the results presented in this paper are not strictly comparable with those obtained by other workers[7,9] it is possible to calculate from all the data the percentage inhibition of the growth-substance induced water uptake caused by Act D. Table 2 shows that treatment of the Jerusalem artichoke tissue disks with Act D, either prior to or together with growth-regulator always caused considerable inhibition of the induced water uptake. The close agreement between the results obtained when 2,4 D was used and those for 3-indole acetic acid[7] (IAA) provide further evidence for a similar mode of action for both the naturally occurring auxin, IAA and the synthetic growth-regulator, 2,4 D.[13]

TABLE 2. INHIBITION BY ACT D OF THE UPTAKE OF WATER INDUCED IN JERUSALEM ARTICHOKE TISSUE DISKS AT 25° BY GROWTH-SUBSTANCE TREATMENT

First treatment	Duration of treatment (hr)	Second treatment	Duration of treatment (hr)	Inhibition* (%)	Ref.
1 mg/l. Act D	24	2·21 mg/l. 2,4 D	72	54	—
10 mg/l. Act D	24	2·21 mg/l. 2,4 D	72	64	—
30 mg/l. Act D	20	10 mg/l. IAA	70	43	7
20 mg/l. Act D	16	1 mg/l. 2,4 D	72	0	9
1 mg/l. Act D + 2·21 mg/l. 2,4 D	24	1 mg/l. Act D + 2·21 mg/l. 2,4 D	72	23	—
10 mg/l. Act D + 2·21 mg/l. 2,4 D	24	10 mg/l. Act D + 2·21 mg/l. 2,4 D	72	70	—
30 mg/l. Act D + 10 mg/l. IAA	20	30 mg/l. Act D + 10 mg/l. IAA	70	67	7

* $\%\ \text{Inhibition} = \dfrac{\text{growth substance uptake} - (\text{growth substance} + \text{act D}) \text{ uptake}}{\text{growth substance uptake} - \text{water control uptake}} \times 100.$

[11] D. ADAMSON, *Can. J. Botany* **40**, 719 (1962).
[12] P. P. RUTHERFORD, C. M. GRIFFITHS and R. L. WAIN, *Ann. Appl. Biol.* **58**, 467 (1966).
[13] P. P. RUTHERFORD and D. R. BARD, *Phytochem.* (in press).

A slow rate of penetration of Act D together with a comparatively short storage period for the tubers could account for Masuda's inability to obtain any inhibition.[9]

The large increases found in both the invertase and hydrolase activities of the soluble protein extracted from Jerusalem artichoke disks after 4 days treatment with 10^{-5} M solutions of 2,4 D are similar to those already reported for disks treated for 3 days.[4] From the data presented in Table 3 it can be seen that Act D whether used prior to or together with 2,4 D was almost equally effective in reducing the growth-substance induced increase

TABLE 3. INHIBITION BY ACT D OF THE INCREASES IN BOTH INVERTASE AND HYDROLASE ACTIVITIES INDUCED IN JERUSALEM ARTICHOKE TISSUE DISKS AT 25° BY GROWTH-SUBSTANCE TREATMENT

First treatment (24 hr)	Second treatment (72 hr)	Inhibition* of increase in invertase activity (%)	Inhibition* of increase of hydrolase activity (%)
1 mg/l. Act D	2·21 mg/l. 2,4 D	75	45
10 mg/l. Act D	2·21 mg/l. 2,4 D	89	90
1 mg/l. Act D + 2·21 mg/l. 2,4 D	1 mg/l. Act D + 2·21 mg/l. 2,4 D	33	22
10 mg/l. Act D + 2·21 mg/l. 2,4 D	10 mg/l. Act D + 2·21 mg/l. 2,4 D	95	84

* % Inhibition =

$$\frac{\text{growth substance activity} - (\text{growth substance} + \text{act D}) \text{ activity}}{\text{growth substance activity} - \text{water control activity}} \times 100.$$

in both the invertase and hydrolase activities of the soluble extractable protein. Generally, the higher concentration of Act D, 10 mg/l., was about twice as effective as the lower concentration of 1 mg/l. and caused about 90 per cent inhibition. These inhibitory effects of Act D indicate that the large growth-substance induced increases in both the invertase and hydrolase activities of the soluble protein extracted from the Jerusalem artichoke disks are dependent on the continued synthesis of a messenger RNA just as is the growth-substance induced water uptake.

Although other workers[5,8] have found that growth-substance treatment produced some increase in the amounts of protein, in this work neither Act D nor 2,4 D was found to have any measurable effect on the amounts of total soluble protein which could be extracted from the Jerusalem artichoke tissue disks (Table 1). Moreover, although not 100 per cent efficient, it was possible to separate the soluble protein into fractions possessing almost entirely either invertase or hydrolase activity, and even then there was no increase in the amounts of protein in the active fractions obtained from 2,4 D treated samples (Table 1). Since the increases found by other workers[5,8] in the amounts of protein in growth-substance treated disks are considerably smaller than the increases in either invertase or hydrolase activities, and since only total and not specific protein was measured, it seems likely that 2,4 D treatment of the Jerusalem artichoke tissue disks does increase the specific activity of both invertase and hydrolase. Furthermore, the evidence presented

indicates that the increases in the specific activities of the two enzymes are both dependent upon the continued synthesis of a messenger RNA.

EXPERIMENTAL

Biological Material and Treatment

The Jerusalem artichoke tubers were obtained from plants grown from a single original tuber. The tubers were lifted in November and stored in moist peat at 4 ± 2° until use the following April and May. The preparation and treatment of the tissue disks followed methods already described by Rutherford *et al.*[12] Six Petri dishes each containing 6 disks were used for each treatment. After treatment for 24 hr at 25° the disks were taken from the dishes, any solution was removed using sterilized paper tissue, and then the disks were transferred to a new solution for incubation for a further 72 hr at 25°. Each disk was then dried, reweighed and the water uptake was determined.

Protein Extraction, Fractionation, Determination and Assay for Invertase and Hydrolase Activity

The disks for each treatment were bulked and the soluble protein was extracted and fractionated by methods described previously.[4,14]

A sample of 36 untreated disks was similarly treated. The invertase and hydrolase activities of both the unfractionated and fractionated protein were measured.[4] The amounts of total soluble protein and of the fractions possessing either invertase or hydrolase activities were measured by the micro Kjeldahl estimation of N_2.

The whole experiment was repeated twice more and the averaged results are presented in the Tables.

[14] J. EDELMAN and T. G. JEFFORD, *Biochem. J.* **93**, 148 (1964).