THE EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE BASICITY OF PROTEINS DURING CALLUS INDUCTION

Takeshi YASUDA and Yasuyuki YAMADA
Department of Agricultural Chemistry, Kyoto University, Kyoto 606, Japan

Received 7 August 1971

1. Introduction

2,4-Dichlorophenoxyacetic acid (2,4-D) is effective in inducing calluses from differentiated tissues and organs of plants (dedifferentiation) [1–3]. We studied the mechanism of callus induction and found that during this induction, 2,4-D formed complexes with proteins, and interestingly with histones [4, 5]. At the same time, we observed that the amounts of relatively basic protein fractions from DEAE cellulose column chromatography decreased, while amounts of more acidic protein fractions increased. These results seem to be caused by: (1) qualitative changes in protein synthesis activity, or (2) complex formation by 2,4-D with proteins. This study investigates the complex formation of 2,4-D-2-^{14}C with proteins, and confirms that changes in the basicity of proteins are due to complex formation by 2,4-D with proteins, and not by qualitative changes in protein synthesis.

2. Materials and methods

Segments of pea epicotyl (*Pisum sativum* var. Alaska) from etiolated young pea plants grown under sterile conditions were inoculated on a synthetic agar medium [6] with 10^{-5} M 2,4-D or 2,4-D-2-^{14}C (29 mCi/mmole, Radiochemical Centre, England). Conditions of incubation were darkness at 25°.

2.1. *Leucine-^{14}C incorporation*

Pea epicotyl segments were incubated on a synthetic agar medium with or without 2,4-D for 48 hr. Three gram samples of segments were incubated for 6 hr with 3 μCi of leucine-^{14}C (U) (270 mCi/mmole, Daiichi Pure Chemicals, Japan) in 3 ml of a liquid medium.

2.2. *2,4-D-2-^{14}C complex formation*

Pea epicotyl segments were incubated on a synthetic agar medium with 2,4-D-2-^{14}C for 24 hr, then were transplanted to fresh medium with cold 2,4-D for 30 hr.

2,3, *Extraction and DEAE cellulose column fractionation*

Each sample was homogenized with 0.1 M sodium phosphate buffer (pH 7.5), then filtered through cheese cloth. The filtrate was centrifuged for 20 min at 10,000 *g* and dialyzed against 0.01 M sodium phosphate buffer (pH 7.5) overnight. After dialysis, the crude extracts were applied to a DEAE cellulose column. Protein fractions were prepared by stepwise elution with these solutions: (F1) 0.01 M Na-phosphate buffer (pH 7.5), (F2) 0.10 M NaCl in 0.01 M Na-phosphate buffer (pH 7.5), (F3) 0.30 M NaCl in 0.01 M Na-phosphate buffer (pH 7.5), (F4) 0.60 M NaCl in 0.01 M Na-phosphate buffer (pH 7.5) and (F5) 0.20 N NaOH. Protein concentrations were determined by the method of Lowry et al. [7]. ^{14}C radioactivity was determined as follows. An equal volume of 20% trichloroacetic acid (TCA) was added to the fractionated solution and the whole was transferred to a glass fiber paper (Whatman GF/C). This was washed with 10% TCA, 80% ethanol and ether.

		cpm	protein (γ)	Specific activity (cpm/γ)
control	F1	347	170	204
	2	2485	480	418
	3	3590	720	498
	4	815	150	543
	5	1960	360	545
2,4-D treated	F1	582	132	340
	2	1770	379	462
	3	2880	610	470
	4	1108	210	502
	5	1700	450	377

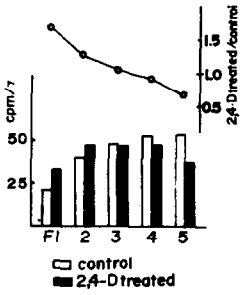

Fig. 1. The incorporation of leucine-^{14}C into protein fractions of pea epicotyl segments treated with and without 2,4-D. Proteins were extracted with 0.1 M Na-phosphate buffer (pH 7.5) and were applied to a DEAE cellulose column. Protein fractions were prepared by stepwise elution with solutions F1 to F5 (see section 2.3). The bar graph at the right is based on data of specific activity (2,4-D-^{14}C; cpm/μg protein) of 2,4-D treated tissue to that of the control.

Radioactivity was counted with a Beckman LS-100 scintillation counter.

3. Results and discussion

Fig. 1 shows the incorporation of leucine-^{14}C into protein fractions of pea epicotyl segments treated with and without 2,4-D. In callus inducing tissue (2,4-D treated) the amounts (μg) of relatively basic proteins (F2) decreased while those of relatively acidic proteins (F5) increased, as compared to the control. The radioactivity of leucine-^{14}C in the F2 fraction of 2,4-D treated tissue is lower than that in the control. The ratio of specific activity (radioactivity/proteins) of 2,4-D treated tissue to that of the control became lower for the acidic protein fractions (points on the curve in fig. 1). The fraction that increased during callus induction did not have a higher incorporation of ^{14}C-leucine. These results indicate that increases in relatively acidic proteins do not depend on *de novo* synthesis of proteins during callus induction.

Incorporation of 2,4-D into various fractions was investigated. After 24 hr, radioactive 2,4-D was found in every fraction, with the F2 fraction having the highest specific activity (radioactivity/proteins) (fig. 2A). To investigate the fate of 2,4-D-^{14}C, epicotyl segments cultured with 2,4-D-2-^{14}C were transferred

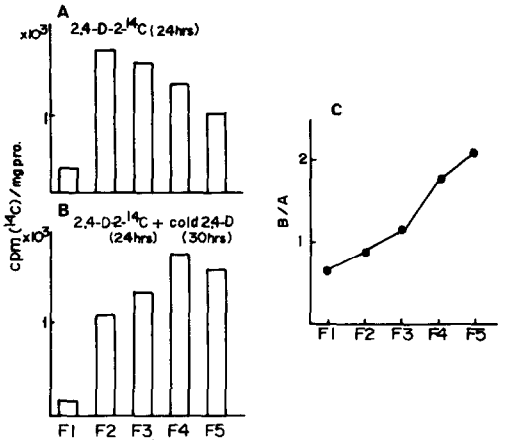

Fig. 2. 2,4-D-2-^{14}C in the protein fraction of 2,4-D-2-^{14}C treated pea epicotyl and the fate of 2,4-D-^{14}C after cold 2,4-D treatment. (A) DEAE cellulose column fractionation of proteins from pea epicotyl segments incubated with 2,4-D-2-^{14}C for 24 hr. Protein fractions were prepared by stepwise elution with solutions F1 to F5 (see section 2.3). (B) DEAE cellulose column fractionation of proteins from pea epicotyl segments incubated with 2,4-D-2-^{14}C for 24 hr, then incubated with cold 2,4-D for 30 hr. (C) The ratio of (B) to (A) in specific activities (2,4-D-^{14}C; cpm/mg protein).

to fresh media containing cold 2,4-D. Fig. 2B shows the specific activities (radioactivity/proteins) of the fractions (2,4-D-^{14}C treated for 24 hr, then chased with cold 2,4-D for 30 hr). Specific activity in F2 (the highest activity at transfer) became lower than that in the relatively acidic fractions (F3, F4 and F5). 2,4-D, which combined with the proteins, moved to the more acidic fractions. The ratio of the specific activity of each fraction in 2,4-D-2-^{14}C (24 hr) and in cold 2,4-D (30 hr) treated tissue to that in 2,4-D-2-^{14}C (24 hr) tissue is shown in fig. 2C. The more acidic protein fraction shows an increase in 2,4-D-^{14}C radioactivity. These results indicate that proteins which form complexes with 2,4-D become more acidic. The combination of 2,4-D with proteins, probably at the basic moiety ($-NH_2$), causes some relatively basic proteins to undergo changes, thus becoming more acidic. Previous results [8] showed that especially lysine-rich histones formed complexes with 2,4-D and that the basicity of lysine-rich histones became acidic. Change in the basicity of histones may occur in a way similar to that for proteins, and complex formation by 2,4-D with histones may

cause decreasing basicity in histones with a subsequent loosening of their attachment to DNA.

Acknowledgement

We thank Professor E. Takahashi for his encouragement during the course of this work.

References

[1] J.G. Torrey, J. Reinert and N. Merkel, Am. J. Bot. 49 (1962) 420.
[2] B. Kaul and E.J. Staba, Science 150 (1965) 1731.
[3] Y. Yamada, K. Tanaka and E. Takahashi, Proc. Japan Acad. 43 (1967) 156.
[4] Y. Yamada, T. Yasuda, M. Koge and J. Sekiya, Agric. Biol. Chem. 35 (1971) 99.
[5] T. Yasuda and Y. Yamada, Biochem. Biophys. Res. Commun. 40 (1971) 649.
[6] E.M. Linsmaier and F. Skoog, Physiol. Plant. 18 (1965) 100.
[7] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[8] Y. Yamada and T. Yasuda, Biochem. Biophys. Res. Commun. 43 (1971) 488.