COMPLEX FORMATION BY 2,4-DICHLOROPHENOXYACETIC ACID WITH HISTONES DURING CALLUS INDUCTION

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Summary

During callus induction (dedifferentiation by 2,4-dichlorophenoxyacetic acid (2,4-D)) the relatively basic protein fractions from DEAE cellulose column chromatography decreased, while the more acidic protein fractions increased. There was also an increase in complex formation by 2,4-D. 2,4-D formed particularly notable complexes with lysine-rich histones at an early stage of callus induction. This complex formation by 2,4-D with lysine-rich histones seems to be the cause of the decrease in the lysine -rich portions of histone fractions during callus formation.

Auxins, plant growth regulators, are essential for inducing callus from plant tissues and organs. This process has been termed dedifferentiation by Yamada (1966). The concentration of auxins required to induce callus varies from plant to plant. 2,4-Dichlorophenoxyacetic acid (2,4-D), a synthetic auxin, is generally the most effective. In Alaska pea epicotyl, 2,4-D induces callus at concentrations higher than 10^{-6} M, while indole-3-acetic acid (IAA), a natural auxin, does not induce callus at any concentration.

Though the role of auxins in triggering Dedifferentiation is not clear, previous work from our laboratory, based on Sephadex column chromatography and Pronase or alkali digestion (Yamada et al 1969), showed that 2,4-D combines with protein during callus induction.

This study investigates the changes in protein patterns and the relationship of 2,4-D to histones during callus induction.

Material and Methods

Two centimeter long segments of pea epicotyl (Pisum sativum var. Alaska) from young pea plants grown under sterile conditions were inoculated on a

synthetic medium (Linsmaier and Skoog 1965) with and without 10^{-5}M 2,4-D-2-C^{14} (29 mCi/mM, the Radiochemical Centre, England) or with 10^{-5}M IAA. About 3 g of epicotyl or pea callus (from inoculation of epicotyl tissue on a synthetic agar medium with 10^{-5}M 2,4-D for 3 weeks) was homogenated with 0.1M sodium phosphate buffer at pH 7.5. The homogenate was filtered through cheesecloth, then its filtrate was centrifuged for 20 minutes at 10,000g and dialyzed against 0.01M sodium phosphate buffer (pH 7.5) overnight. After dialysis, the crude extracts were applied to a DEAE cellulose column. Elution was performed by a stepwise increase of the NaCl concentration in the same buffer.

Chromatin was prepared by the method of Fambrough and Bonner (1966). Purified chromatin was twice extracted with 0.2 N HCl to give the histone fractions. The residue was extracted with 0.2 N NaOH to give the non-histone fraction. Histone extracts were neutralized with NaOH and dialyzed against 0.1 M acetic acid-NaOH buffer (pH 4.2). Using the modified Johns method (Johns et al 1960), they were separated on a CM cellulose column (0.8 x 5 cm).

Protein concentration was determined using the procedure of Lowry et al (1951). Radioactivity of 2,4-D-2-C^{14} was determined as follows. An equal volume of 20% trichloroacetic acid (TCA) was added to the fractionated solution and the whole was transferred to glass fibre paper (Whatman GF/C) and washed with 10% TCA, ethanol and ether. Radioactivity was counted with a Beckman Scintillation Counter.

Results and Discussion

Figure 1 shows DEAE cellulose chromatograms of phosphate buffer extracts (crude soluble protein). The control showed a high protein F_2 fraction peak and a lower protein F_5 peak (Fig 1a). In callus inducing tissue (Fig 1b) the F_2 peak decreased, while the F_5 fraction increased; as compared to the control (Fig 1a). This pattern was similar to that of callus (Fig 1d) alone. IAA, which does not induce callus from pea epicotyl, had no effect on peak patterns.

In Fig 1b, 2,4-D-2-C^{14} was incorporated in all the protein fractions

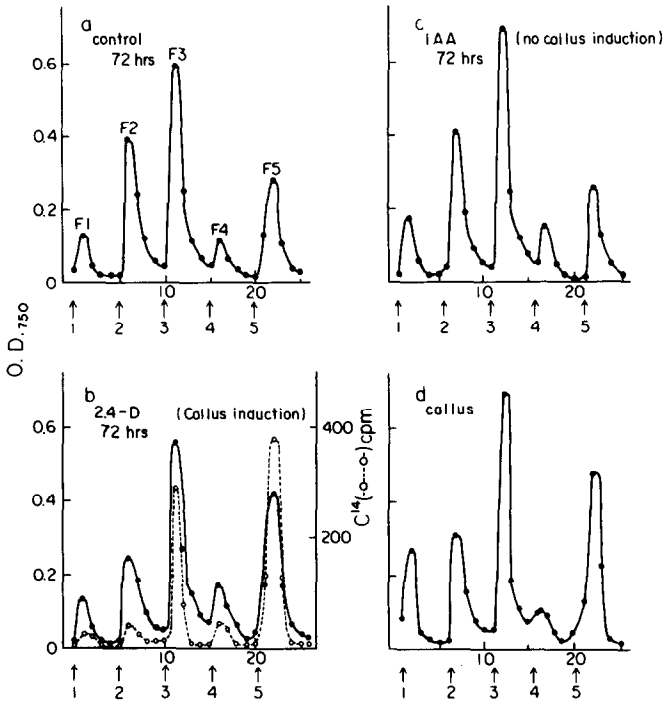

Fig 1 Elution patterns of phosphate buffer extracts on DEAE cellulose columns (0.8 x 5 cm.). Extracts were applied to the column in 0.01 M Na-phosphate buffer (pH 7.5) and were eluted stepwise with these solutions:

1. 0.01 M Na-phosphate buffer (pH 7.5)
2. 0.1 M NaCl in 0.01 M Na-phosphate buffer (pH7.5)
3. 0.3 M NaCl in 0.01 M Na-phosphate buffer (pH7.5)
4. 0.6 M NaCl in 0.01 M Na-phosphate buffer (pH7.5)
5. 0.2 N NaOH

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but formed complexes more readily with the F_3 and F_5 fractions. When 2,4-D-2-C^{14} was mixed with the isolated proteins from the control, in vitro, no incorporation of 2,4-D-2-C^{14} was observed in the protein fractions. Addition of 2,4-D (10^{-5} M) caused the levels of relatively acidic proteins (F_5 in Figs 1b,1d) to increase during callus induction or in callus, itself. The decrease in protein in the F_2 fraction and the increase in the F_5 fraction (more acidic than F_2 and with a higher incorporation of 2,4-D-2-C^{14}) suggests that 2,4-D forms a complex with proteins by combining with the basic moiety ($-NH_2$) and that some relatively basic proteins (in F_2) undergo changes to become more acidic (in F_5). Only one radioactive fraction was obtained from

hydrolysis of the radioactive protein fractions, which was identified as radioactive 2,4-D, by paper chromatography.

Radioactive 2,4-D was found in histone fractions from the CM cellulose column (Fig 2). Histone patterns at 20 hours showed no differences from the control at 0 hour. 2,4-D-2-C^{14} activity was observed in each histone

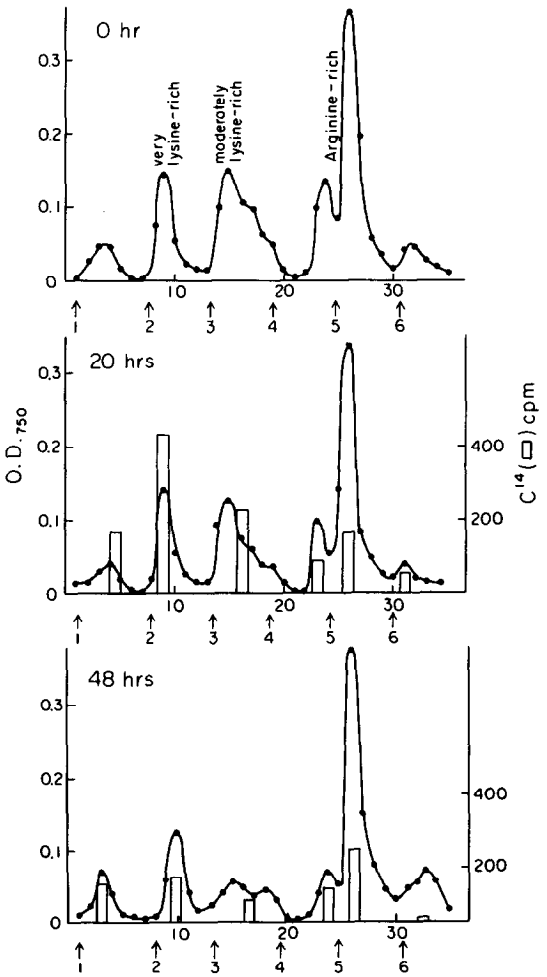

Fig 2 CM cellulose column chromatograms of histones. Histones were eluted stepwise with these solutions:

1. 0.1 M CH_3COOH-NaOH (pH 4.2)
2. 0.17 M CH_3COOH-NaOH (pH 4.2), 0.3 M NaCl
3. 0.17 M CH_3COOH-NaOH (pH 4.2), 0.42 M NaCl
4. 0.01 N HCl
5. 0.02 N HCl
6. 0.1 N HCl

fraction; the very lysine-rich, the moderately lysine-rich and the arginine -rich fractions, but incorporation of 2,4-D was particularly high in the very lysine-rich and moderately lysine-rich fractions. This high incorporation of radioactivity is indicative of an early stage of callus induction. After 48 hours, the levels of the lysine-rich histone fractions and the 2,4-D-2-C^{14} activity observed in those fractions decreased (Fig 2, bottom). These changes are the second stage for callus induction. The non-histone fraction (after 48 hours) showed, by comparison, a much higer radioactivity. Formation of complexes between 2,4-D and the lysine-rich histone fractions seems to be the cause of the decrease in the levels of lysine-rich portions of the histone fractions during callus induction. Gurley et al (1964) reported that lysine-rich fractions inhibited DNA polymerase more than arginine-rich fractions did. This also suggests that decreases in lysine -rich fraction levels are due to formation of complexes by lysine-rich histones and 2,4-D, and that this is related to callus induction (dedifferentiation of tissue).

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