CHANGES IN THE BASICITY OF HISTONE FRACTIONS DURING CALLUS INDUCTION

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Summary

The ratio of the histone to the non-histone protein fraction increases as callus induction (dedifferentiation by 2,4-dichlorophenoxyacetic acid (2,4-D)) progresses. Formation of a complex(es) between 2,4-D and the histone fraction causes a decrease in the level of histone and an increase in the level of non-histone protein. The changing basicity of histones, which form complexes with 2,4-D, seems to cause the apparent increases in non-histone fraction levels. Amino acid analysis showed that the lysine composition of the non-histone fraction, in 2,4-D treated tissue, increased over that of the control.

2,4-Dichlorophenoxyacetic acid (2,4-D) formed most interesting complexes with lysine-rich histones during an early stage of callus induction with 2,4-D (Yasuda and Yamada 1970). When 2,4-D lysine-rich histone complexes are formed the basicity of the lysine-rich histones seems to change. This change most probably is the cause of decreases in levels of lysine-rich histones in the histone fractions sampled during callus induction. Gurley et al (1964) reported that lysine rich histones inhibited DNA polymerase more than arginine-rich histones did. This suggests that decreases in lysine-rich histone from the histone fractions are involved in derepression of DNA polymerase and that increases in cell division, due to uncontrolled DNA replication, cause callus induction (dedifferentiation of tissue). We investigated ; 1) the formation of complexes by 2,4-D with histones, and 2) changes in histone and non-histone fractions extracted from chromatin, using amino acid analysis and other techniques.

Material and Methods

Segments of pea epicotyl (Pisum sativum var. Alaska), about two

centimeters long, from young pea plants grown in the dark under sterile conditions were inoculated on a 1/10 concentration of synthetic medium (Linsmaier and Skoog 1965) with and without 10^{-5} M 2,4-D-2-C^{14} (29 mCi/mM, the Radiochemical Center, England). About 100 g of pea epicotyl from an inoculation on a 1/10 concentration of synthetic agar medium with 10^{-5} M 2,4-D-2-C^{14} was sampled at 0, 20 and 48 hours after inoculation. These samples were homogenized with 0.05 M Tris buffer at pH 8.0 containing 0.01 M β-mercaptoethanol and 0.001 M $MgCl_2$ (grinding medium), for 1.5 minutes.

Preparation of chromatin

Chromatin was prepared by the methods of Huang and Bonner (1962) and Srivastava (1968). The homogenate was filtered through 6 layers of cheesecloth then was centrifuged at 4,000 g for 30 minutes. The pellets obtained were resuspended in the grinding medium and were repelleted at 10,000 g for 15 minutes. The products were, in turn, twice pelleted from 0.25 M sucrose containing 0.001 M β-mercaptoethanol at 10,000 g for 15 minutes. The resulting pellets were suspended in 0.05 M Tris buffer (pH 8.0) containing 0.001 M β-mercaptoethanol, then were centrifuged at 10,000 g for 20 minutes. This treatment was repeated twice. Pellets of crude chromatin were suspended in 0.05 M Tris buffer (pH 8.0), then were layered on 1.7 M sucrose in 0.01 M Tris buffer (pH 8.0) and 0.001 M β-mercaptoethanol in cellulose nitrate tubes. The tubes were centrifuged at 22,000 rpm for 3 hours in an RPS 25-2 Hitachi rotor. Purified chromatin pellets were suspended in 0.01 M Tris buffer and dialyzed against the same buffer overnight.

Preparation of histones and non-histone proteins

Dialyzed chromatin was suspended in 0.2 N HCl solution by hand in a Ten-Broeck homogenizer, then it was centrifuged at 10,000 g for 20 minutes. Supernatants constituted the histone portion of the chromatin. Pellets were shaken by hand in 0.2 N NaOH in a Ten-Broeck homogenizer then were centrifuged at 10,000 g for 20 minutes. The supernatants formed the non-histone protein fraction. 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 volumn of 20 % trichloroacetic acid (TCA) was added to the fractionated solution and the whole was transferred to glass fiber paper (Whatman GF/C). This was washed with 10 % TCA, ethanol and ether. Radioactivity was counted with a Beckman LS-100 Scintillation Counter.

Amino acid analyses

Histone and non-histone samples (2-3 mg) were hydrolyzed in 2 ml of constantly boiling 6 N HCl under N_2-gas in a sealed Thunberg tube for 24 hours at 105 °C. Amino acid analyses were performed using an automatic amino acid analyzer (Yanagimoto LC 5-S).

Results and discussion

The ratio of the non-histone protein fractions to the histone fraction increases as callus induction progresses (Table 1). Forty eight hours after 2,4-D treatment, the ratio of non-histone proteins to histones had increased to twice the value seen at zero hours. In absolute amounts at 48 hours non-histone protein also showed an increase over the value at zero hours. Radioactivities in the histone fraction, which were derived from 2,4-D-2-C^{14}, were detected from 20 hours after 2,4-D-2-C^{14} treatment and decreased (rather than increased) at 48 hours (Table 1). Radioactivity, however, was present in large amounts in the non-histone fraction at 48 hours. These radioactivities

Table 1 Ratios of non-histone proteins to histones and 2,4-D-2-C^{14} activity in each fraction during callus induction

	hrs after 2,4-D treatment		
	0	20	48
$\frac{\text{non-histone proteins}}{\text{histones}}$	0.23	0.28	0.46
	cpm/mg proteins		
Histones	--	354	321
non-histone protein	--	--	971

were not absolutely identifiable as the radioactivity of 2,4-D-2-C^{14} ; but, only one radioactive fraction was obtained from hydrolysis of radioactive soluble protein fractions. This was identified as radioactive 2,4-D by paper chromatography. Previous results (Yasuda and Yamada 1970) showed that at 20 hours, 2,4-D-2-C^{14} activity was found in histone fractions, particularly those with high levels of very lysine-rich and moderately lysine-rich components. After 48 hours, the levels of the lysine-rich histone fractions and the activities of 2,4-D-2-C^{14} in these fractions decreased.

Results of these experiments indicate that the formation of complexes between 2,4-D and the histone fractions causes a decrease in the level of histone fractions and an increase in the level of non-histone protein fractions. These and previous results (Yasuda and Yamada 1970) show that especially lysine-rich histone forms a complex with 2,4-D and that the basicity of lysine-rich histone becomes acidic. Changing basicity causes the increases in the apparent levels of the non-histone fraction. Thus, amino acid composition of non-histone fractions in the control and in 2,4-D treated pea epicotyl may reflect this changing basicity.

Non-histone fraction composition in the control and in tissue treated with 2,4-D for 72 hours is expressed as the number of moles of amino acid per 100 moles of the total amino acids recovered. To compare them with the non-histone fractions from pea epicotyl, the amino acid compositions of lysine-rich histone fractions from pea epicotyl are given in table 2. After 72 hours of treatment with 2,4-D (10^{-5}M), the lysine composition of the non-histone fraction in 2,4-D treated tissue increased over that of the control. Simultaneously, the serine content increased in 2,4-D treated tissue. This may be because lysine and serine are found together in large amounts, as reported previously (Iwai et al 1970).

Spelsberg and Sarkissian (1970) reported that acidic proteins associated with DNA were increased by indole-3-acetic acid (IAA), when bean hypocotyl was incubated with IAA. IAA also is an auxin but its ability to trigger callus induction is lower than that of 2,4-D.

Table 2 Amino acid composition of non-histone fractions and lysine-rich histone fractions in the control and in 2,4-D treated pea epicotyls

	non-histone fractions		lysine-rich histone fractions
	2,4-D	control	control
Lys	6.06 %	5.03 %	11.38 %
His	1.52	1.56	2.42
Arg	1.63	5.02	7.51
Asp	11.51	9.66	8.47
Thr	5.86	5.68	4.36
Ser	11.31	4.72	7.02
Glu	3.24	9.72	9.20
Pro	7.74	5.28	6.05
Gly	25.54	14.95	12.11
Ala	10.26	11.53	10.65
Val	3.55	7.42	5.08
Ileu	3.45	4.94	2.90
Leu	4.39	8.43	5.80
Tyr	1.77	1.98	2.42
Phe	1.09	4.09	3.15

Our studies indicate that 2,4-D forms a complex with histone ; especially with lysine-rich histones and that these lysine-rich histones are involved with DNA polymerase activity during callus induction.

The activity of histone appears to change as a result of 2,4-D histone complex formation. These histones are involved in the regulation of DNA synthesis and in the control of gene expression. Our results also show that the formation of a complex of 2,4-D with histones indicates a preference for binding the 2,4-D to the basic regions of the histones. When 2,4-D-2-C^{14} treatment, which made a complex with phosphate buffer soluble proteins, was followed by cold 2,4-D treatment for 30 hours, the radioactive complex composed of proteins with 2,4-D-2-C^{14} formed fractions which became more acidic as treatment progressed. This provides further evidence that formation of complexes by proteins with 2,4-D lessens protein basicity. Interaction of 2,4-D with histones in vitro will be presented in another communication.

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