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SOLUBILIZATION OF JERUSALEM ARTICHOKE TUBER CHROMATIN BY 2,4-DICHLOROPHENOXYACETIC ACID

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Key Word Index—*Helianthus tuberosus*; Compositae; Jerusalem artichoke tuber; chromatin; 2,4-dichlorophenoxyacetic acid; solubilization; histone; DNA.

Abstract—Chromatin from the tuber of the Jerusalem artichoke (*Helianthus tuberosus*) was solubilized in 2,4-dichlorophenoxyacetic acid (2,4-D) solution (100 mM) at pH 7·0. This solubilization was much affected by the pH; below 6·0 less chromatin was solubilized. The elution pattern of the products on gel filtration with Sepharose 4B showed that the solubilization was caused by the dissociation of the DNA and associated proteins. The pattern of polyacrylamide gel electrophoresis of histones extracted from the chromatin solubilized by 2,4-D was quite different from those of histones extracted from the original chromatin or from NaCl (2·0 M) solubilized chromatin. The F1 and F3 fractions seemed to be little affected by 2,4-D, but the F2a1, F2a2 and F2b fractions were greatly decreased. In addition, the ratios of histones and non-histone proteins to DNA changed considerably in 2,4-D solubilized chromatin in an inverse manner. None of these changes were observed with NaCl, which suggests that the behaviour of 2,4-D for the solubilization of chromatin differs substantially from that of NaCl.

INTRODUCTION

The DNA in the chromosomes of eukaryotes is known to be associated with other macromolecules, such as histones, non-histone proteins and RNA. This complex has been called chromatin and it is the biological activity of this whole fraction which apparently determines gene regulation, cell division and cell differentiation.

The solubilization of chromatin is necessary if one is to be able to study its structure, properties and function. Many workers have reported that chromatin can be solubilized by inorganic salt solutions [1–4], large volumes of water [5,6], salyrganic (mersalyl) acid [7] and other chemical reagents [8,9]. The solubilization of chromatin by salt solutions of high ionic strength (e.g. 2·0 M NaCl) was found to be due to the dissociation of the primary electrostatic binding between proteins and DNA [10,11]. On the other hand, chromatin has been shown to progressively swell on dilution with water, finally forming a gel [5,6,12], in which the DNA and proteins are not dissociated.

While studying the mode of action of the auxin, 2,4-dichlorophenoxyacetic acid (2,4-D) as a plant growth regulator in cell division, we found that the chromatin from the tuber of the Jerusalem artichoke was solubilized when treated with relatively high concentrations (100 mM) of 2,4-D solution at pH 7·0. In the work reported here, we have investigated the differences in the solubilization of chromatin by 2,4-D as compared with NaCl.

RESULTS

Solubilization of chromatin

Jerusalem artichoke tuber chromatin was completely solubilized at 4° with 2,4-D solutions of concentrations greater than 100 mM at pH 7·0 (Fig. 1). It should be noted that 2,4-D is not soluble above 500 mM at pH 7·0 and 4°. The resulting solution was very viscous and the addition

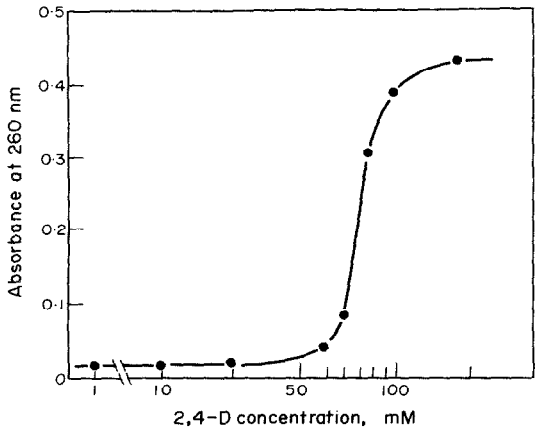

Fig. 1. Effects of 2,4-D concentration on the solubilization of chromatin. Chromatin was treated with 2,4-D solns at various concentrations. Absorptivity, after treatment of the solubilized chromatin with 5% perchloric acid at 90° for 15 min, was measured at 260 nm.

of EtOH caused the precipitation of proteins and DNA in the form of long white fibers. The behaviour of the solubilized chromatin in the 2,4-D solution was very similar to that in an NaCl solution, though the concentration of 2,4-D for complete solubilization was only one tenth that for NaCl (2·0 M). However, the solubilization of chromatin by 2,4-D, unlike that of NaCl, was very dependent on the pH (Fig. 2). At pH levels below 6·0, less chromatin was solubilized. (Since at pH 3·5, 2,4-D itself was precipitated from the solution, the solubilization of chromatin could not be tested below this point.) Above pH 6·0, the solubilization of chromatin by 2,4-D was unaffected by the pH.

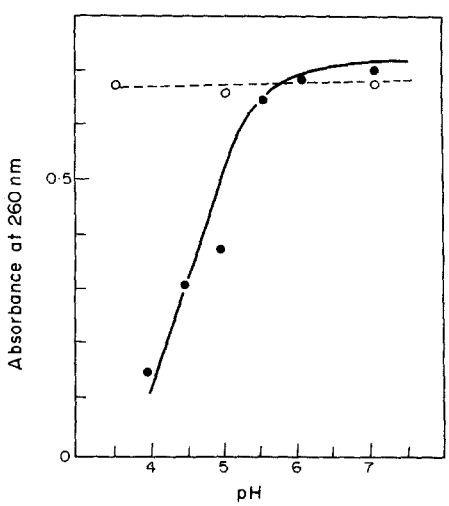

Fig. 2. Effects of pH on the solubilization of chromatin. Chromatin was treated with 2,4-D soln (100 mM) or NaCl soln (2·0 M) at different pH values (see Fig. 1 for analysis).

Table 1. The solubilization of chromatin by various 2,4-D analogues

2,4-D Analogues	Amounts of solubilized chromatin (*A* values at 260 nm)
2,4-Dichloro-phenoxyacetic acid	1·800
o-Chloro-	0·446
m-Chloro-	0·510
m-Bromo-	0·495
p-Chloro-	0·730
p-Nitro-	0·575
p-Methoxy-	0·328

Chromatin was treated with various 2,4-D analogues (100 mM) at pH 7·0. Absorptivity, after treatment of the solubilized chromatin with 5% perchloric acid at 90° for 15 min, was measured at 260 nm.

Chromatin was also treated with various 2,4-D analogues at a concentration of 100 mM at pH 7·0. As shown in Table 1, chromatin was partially solubilized by all the 2,4-D analogues used and the amounts of chromatin solubilized increased in the order of *p*-methoxy < *o*-chloro < *m*-bromo < *m*-chloro < *p*-nitro < *p*-chloro ≪ 2,4-dichlorophenoxyacetic acid. We also tested the effect of indole-3-acetic acid (IAA), a natural auxin, on the solubilization of chromatin. However, no solubilization was observed by the IAA–potassium salt at either 100 or 200 mM at pH 7·0 after 1 hr stirring.

Polyacrylamide gel electrophoresis of histones

The patterns of histones obtained by polyacrylamide gel electrophoresis of the extracts from the original chromatin, from NaCl (2·0 M) and from 2,4-D (200 mM) solubilized chromatin are shown in Fig. 3. The pattern of histones from NaCl solubilized chromatin (Fig. 3b) was very similar to that of the original chromatin (Fig. 3a), indicating that all the histone fractions could be extracted with NaCl from Jerusalem artichoke tuber chromatin, as has been reported by many investigators [4]. However, the pattern of histones extracted from the chromatin solubilized by 2,4-D was quite different. The F1 and F3 fractions in the pattern seemed to be little affected by 2,4-D,

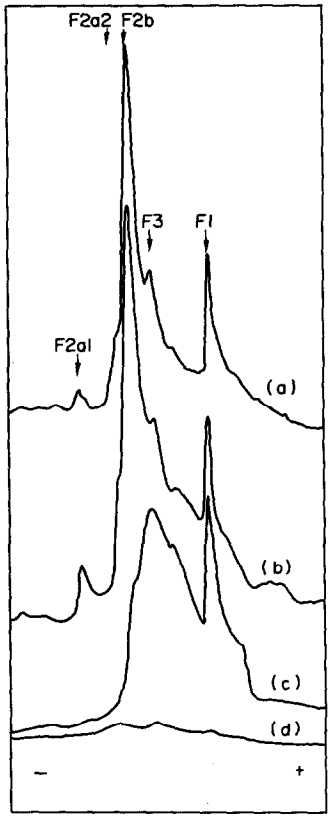

Fig. 3. Polyacrylamide gel electrophoresis of histones. (a) histones from original chromatin; (b) histones from NaCl (2·0 M) solubilized chromatin; (c) histones from 2,4-D (200 mM) solubilized chromatin; (d) histones from the residual chromatin after solubilization by 2,4-D (200 mM).

but the F2a2 and F2b fractions were greatly decreased and no F2a1 fraction was found (Fig. 3c). The chromatin residues after solubilization by 2,4-D were, therefore, treated with cold 0·2 N H_2SO_4 and the residual histones were again analysed by polyacrylamide gel electrophoresis. However, as shown in Fig. 3d, no histones were found showing that they were all solubilized by the 2,4-D treatment, although some may have been changed into non-extractable proteins with 0·2 N H_2SO_4.

Table 2. Chemical analysis of chromatin

Chromatin	Component			
	DNA	RNA	Histone	Non-histone protein
Original	1·0	0·57	1·10	2·86
NaCl solubilized	1·0	0·54	1·05	2·83
2,4-D solubilized	1·0	0·51	0·53	3·32

Values expressed as mg based on 1·0 mg DNA.

Chemical analysis of chromatin

The compositions of the chromatin solubilized by 2,4-D (200 mM) and NaCl (2·0 M), and those of original chromatin as the control are shown in Table 2. It should be noted that the chromatin obtained from Jerusalem artichoke tuber has a high content of non-histone protein in comparison with those reported from pea or barley [13,14]. The ratios of RNA, histones and non-histone proteins to DNA in the NaCl solubilized chromatin were almost identical with those in the original chromatin showing that none of the chromatin components solubilized by NaCl were seriously affected by the treatment. Interestingly, the composition of the non-histone proteins and histones of the 2,4-D solubilized chromatin was quite different; the histone level decreased to half that of the original chromatin while the non-histone proteins increased by almost the same value as that by which the histones decreased. This indicates that the solubilization of chromatin by 2,4-D changed some histones into acidic moieties which were then recovered in the non-histone protein fraction.

Gel filtration of chromatin on agarose

Separation by gel filtration on agarose between DNA and proteins in samples of chromatin solubilized by NaCl (2·0 M) or 2,4-D (150 mM) was

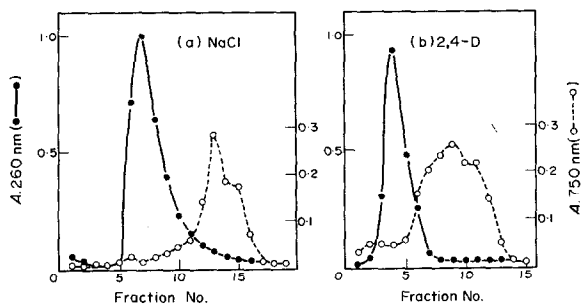

Fig. 4. Gel filtration on Sepharose 4B of chromatins solubilized by 2·0 M NaCl(a) and by 150 mM 2,4-D(b). One ml of each chromatin soln was applied to a column of Sepharose 4B equilibrated with 2·0 M NaCl or 150 mM 2,4-D. Bed dimensions: 30 × 1·5 cm. Flow rate: 2 ml/hr. Nucleic acids were determined by the absorptivity at 260 nm, proteins by the method of Lowry *et al.* [25].

about complete and reproducible (Fig. 4). Many investigators have suggested that the bonds between DNA and proteins are principally ionic and that the great majority of proteins bound to DNA can hence be separated at a high ionic strength, such as with 2·0 M NaCl. However, since most of the proteins associated with the DNA in chromatin can be separated by treatment with 2,4-D, this cannot be the whole explanation. Nevertheless, the solubilization of chromatin by 2,4-D appears to be caused by the dissociation of DNA and proteins, and thus differs from the solubilization of chromatin on dilution in water where the DNA and proteins do not dissociate.

DISCUSSION

There are many reports [3,4] showing that increasing concentrations of inorganic salt solutions cause selective dissociation of histones in the form of deoxyribonucleo proteins (DNP). In our experiments, the amounts of chromatin solubilized by 2,4-D depended on the concentration (Fig. 1) suggesting that selective dissociation of the DNP might be also caused by 2,4-D. In addition, the results shown in Fig. 4b suggest that the solubilization of chromatin by 2,4-D might be caused by the dissociation of DNA and histones. This is well supported by the similarity of the elution patterns with those of chromatin dissociated by NaCl (Fig. 4a). But, it is doubtful whether 2,4-D acts in the same way as NaCl [7,8], because the concentration required is very low and the ionic strength of the carboxylic group of 2,4-D is smaller than Na^+Cl^-. So the solubilization by 2,4-D may involve the phenoxy group of the latter. The results shown in Table 1 suggest that the kinds and locations of substituents in 2,4-D may play an important role in solubilizing chromatin. However, the amounts of chromatin solubilized are affected by changes in pH; thus, the carboxylic group of 2,4-D must also be involved. In addition, results indicate that when chromatin is solubilized by 2,4-D (200 mM), all histones could be extracted with the DNA and other chromatin components (Fig. 3d, Table 2), but the pattern of histones from 2,4-D solubilized chromatin on polyacrylamide gel electrophoresis (Fig. 3c) differs considerably from that solubilizated by NaCl (Fig. 3b). Some changes in the histones were found to be due to changes in some histones extractable with 0·2 N H_2SO_4 (Table 2). No such observations have been made in the solubilization of chromatin by NaCl and by other salt solutions.

The question arises as to how the solubilization of chromatin, involving changes in histones, is caused by the action of 2,4-D. Several investigators have suggested that the interaction between histone and DNA is affected by modifications in histones, i.e. by acetylation [15], phosphorylation [16], methylation [17] or binding with various animal hormones [18]. Yasuda and Yamada [19] reported that when etiolated pea stem segments were explanted on a medium containing 2,4-D-[2-^{14}C] (10^{-5} M) and cell divisions were induced, radioactivity was incorporated into the tissue and was conjugated with histones in the chromatin. Furthermore, Yamada and Yasuda [20] reported that formation of complexes between 2,4-D and histone *in vivo* causes a decrease in the level of histone and an increase in the level of non-histone protein. Unpublished experiments show that when chromatin was solubilized by 2,4-D (200 mM) containing 2,4-D-[2-^{14}C], radioactivity was found in both the histone and non-histone protein fraction. We suggest, therefore, that the solubilization of chromatin by 2,4-D may be caused by a decrease in the interaction between DNA and histone due to modification of the histones by 2,4-D which results in the dissociation of DNA and proteins. 2,4-D has many actions with plant tissues such as the induction of cell division, cell enlargement, root formation, shoot formation and herbicidic effects. We are currently investigating the induction of DNA synthesis and cell division by 2,4-D in plant tissues during callus induction [21–23]. It should be noted that the most suitable concentration of 2,4-D for callus induction from Jerusalem artichoke tuber tissue is 10^{-5} M [23], while the concentration of 2,4-D for the solubilization of chromatin is several orders of magnitude higher (100 mM). However, the concentration of 2,4-D in the nucleus might be much higher than that in the medium. Further studies on the relationship between the physiological effects of 2,4-D *in vivo* and the solubilization of chromatin are obviously very important if we are to uncover the mechanism of action of this auxin.

EXPERIMENTAL

Preparation of chromatin. Chromatin was prepared Jerusalem artichoke tuber [24] and after ultracentrifugation in 2·0 M sucrose, the chromatin pellet was well washed with a large vol. of NaCl (15 mM)–sodium citrate (1·5 mM) buffer (pH 7·0) to remove sucrose. The purified chromatin pellet was suspended in the same buffer to study solubilization of chromatin by 2,4-D, NaCl and various 2,4-D analogues.

Solubilization of chromatin. Effects of 2,4-D concentration: Chromatin (0·5 mg/ml) was suspended in 2,4-D solns of various concns at pH 7·0 (Fig. 1) and the suspension stirred slowly for 1 hr at 4°. Suspensions treated with 2,4-D at concns above 80 mM, became very viscous indicating that chromatin was being solubilized. Suspensions were then centrifuged at 8000 *g* for 15 min. Treatments were repeated several times and resulting supernatants were combined. All the macromolecules (DNA, RNA, histones and non-histone proteins, etc.) were precipitated by EtOH. After washing (EtOH), the ppts were treated with 5% perchloric acid (PCA) at 90° for 15 min to digest nucleic acids which were determined by measuring the absorbance at 260 nm.

Effects of pH. Chromatin was suspended in solns of 100 mM 2,4-D or 2·0 M NaCl at different pH values (pH was adjusted with dil. HCl or NaOH) and stirred slowly for about 1 hr. Subsequent procedures were as described above.

Solubilization by 2,4-D analogues: Chromatin (0·5 mg/ml) was suspended in various 2,4-D analogues (100 mM) at pH 7·0. Amounts of chromatin solubilized were determined as above.

Chemical analysis of chromatin. Chromatin suspensions were stirred slowly in 200 mM 2,4-D soln or in 2·0 M NaCl soln at pH 7·0 for 12 hr at 4° when the chromatin was solubilized completely. Then, EtOH (3-4 vol.) was added, and after centrifugation at 8000 *g* for 15 min, the ppt. was washed well with EtOH and dried *in vacuo*. From each ppt., histones were extracted with 0·2 N H_2SO_4, then non-histone proteins were extracted with 0·2 N NaOH. Proteins were determined by the method of Lowry *et al.* [25]. DNA and RNA were determined after treatment with 5% PCA at 90° for 15 min: DNA was then estimated by the diphenylamine reaction as modified by Burton [26], and RNA was estimated by the orcinol reaction [27]. As standards for DNA, RNA, histones and non-histone proteins, calf thymus DNA, yeast RNA, calf thymus histones and bovine serum albumin were used, respectively.

Polyacrylamide gel electrophoresis of histones. Histones extracted with 0·2 N H_2SO_4 from original chromatin or from NaCl (2·0 M) or 2,4-D (200 mM) solubilized chromatins, were pptd with EtOH. The ppts, after washing (EtOH), were dissolved in 0·9 N HOAc containing 15% sucrose, then layered on the top of a 15% polyacrylamide gel. Electrophoresis was performed according to the method of Panyim and Chalkley [28] and gels were stained with 1% amido black for 30 min at 70°. After destaining in 7% HOAc containing 20% EtOH the protein bands in the gels were scanned at 610 nm with a Gilford Gel Scanner.

Gel filtration of chromatin on agarose. Chromatin was suspended in 2·0 M NaCl or 150 mM 2,4-D soln and stirred slowly for 1 hr, then the soln was centrifuged for 15 min at 8000 *g* and chromatin solns obtained as the supernatants. Gel filtration of 1 ml was effected on a column of 4% agarose (Sepharose 4B) [11], 1·5 × 30 cm with a flow rate of 2 ml/hr. Nucleic acids eluted were determined by their absorptivities at 260 nm, after degradation by 5% PCA for 15 min at 90°, and proteins by the method of Lowry *et al.* [25].

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