Electrophoretic Analysis of Histones from Gibberellic Acid-treated Dwarf Peas

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ABSTRACT

Histones from the epicotyls of light-grown dwarf peas (Pisum sativum L. cv. Little Marvel) which had been treated with gibberellic acid were compared to histones from control dwarf peas by the method of polyacrylamide gel electrophoresis. The histone complements were found to be unaltered in the electrophoretic mobility and relative quantity of the individual fractions. The ratio of histone to DNA was also unaffected by treatment with gibberellic acid. The investigation confirmed earlier reports that over 95% of the histone of peas is contained in seven molecular species and that one of these can exist both as an oxidized disulfide dimer and as a reduced monomer. Evidence is presented which indicates that only the monomer form exists in vivo in the pea epicotyl tissue and that the oxidized dimer is an artifact of extraction. The implications of the data concerning the mechanism of action of gibberellic acid are discussed.

There is abundant evidence that gibberellic acid exerts its influence on plant growth and development by affecting nucleic acid metabolism and enzyme synthesis. Treatment of barley endosperm with the hormone results in the de novo synthesis of α-amylase (15) and the GA-induced formation of the enzyme is inhibited by actinomycin D (10). Chandra and Duynstee (8) have shown that GA causes the formation of a rapidly labeled, high molecular weight fraction of RNA in barley endosperm which they proposed contains the messenger RNA that codes for α-amylase. The effects of GA on protein synthesis and RNA metabolism have also been demonstrated in other systems (9, 16, 19–21). This work has led to the hypothesis that GA causes derepression of specific portions of the genome permitting the synthesis of specific messenger RNAs.

Histones have long been suspected of being directly involved in gene control (2). Isolated pea bud chromatin (primarily a complex of DNA and histone) has a much reduced capacity to direct DNA-dependent RNA synthesis in vitro relative to that of free DNA (4). Chromatin can be separated into two fractions of differing histone-DNA ratios; the higher the ratio, the lower the template activity (4). Furthermore, the template activity of chromatin increases as histones are removed by salt (23). Chromatin extracted from dormant potato buds which had been treated with ethylene chlorohydrin (a compound which mimics the effect of GA in breaking the dormancy of buds) has been shown to support a rate of DNA-dependent RNA synthesis 10 times that of chromatin from control buds (31).

In view of these ideas on both the mechanism of action of GA and the biological role of histones, this study was undertaken to determine whether the histones from the epicotyls of light-grown dwarf peas were altered either qualitatively or quantitatively by treatment with GA.

MATERIALS AND METHODS

Seeds of Little Marvel peas (Pisum sativum L.) were soaked in tap water for 24 hr with aeration, planted in sterilized sand, and grown under normal greenhouse conditions. When the plants were 8 days old and had developed four internodes, half the plants were treated with a drop of 1 × 10−4 M GA in 0.05% Tween 20 applied to each apex. After 48 hr the treated seedlings were taller than the control seedlings and were slightly chlorotic. At this time the epicotyls were removed and frozen on Dry Ice.

Histones were extracted from treated and control plants essentially by the method of Bonner et al. (3). The method was modified by including 0.025 M sodium bisulfite to inhibit proteolysis (28) and by treating the tissue with 0.01 M MgCl₂ for 30 min prior to disruption. This procedure yielded relatively pure nuclei which were ruptured by homogenization in 0.02 M EDTA. When the ionic strength of the suspension of ruptured nuclei is reduced, a highly viscous, clear gel of chromatin is formed (3). Histones were extracted from the chromatin with 0.4 M H₂SO₄.

Histones were dissolved in 0.9 N acetic acid, 15% sucrose (w/v). They were applied to gels containing 15% acrylamide (w/v), 0.9 N acetic acid, and 2.5 M urea as described by Panijm and Chalkley (25). Electrophoresis was performed at 130 V for 3½ hr on the short (10 cm) gels and at 190 V for 16 hr on the long (24 cm) gels. After electrophoresis the gels were stained with amido black and scanned in a Gilford model 2000 densitometer. The amount of protein in each electrophoretic band was determined by analyzing the densitometer traces with the DuPont 310 curve resolver. Data presented are based on eight extractions of dwarf pea histones.

Chromatin from GA-treated and control pea epicotyls was fractionated into DNA, histone, and non-histone protein (3). The amount of DNA was determined by the Burton modification of the diphenylamine method (6) and protein by the method of Lowry et al. (22).

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RESULTS

The timing of GA treatment and harvest of the tissue was chosen so that if the hormone did cause a change in the histone complement, the change would most likely be in effect at the time of sampling. Under the same conditions for growth of peas used in this investigation, Nordahl (24) has shown that the most dramatic change in the rate of elongation occurs 48 hr after treatment with GA. After this time the internodes of the treated plants elongate rapidly while the length of the internodes of the control plants increases only slightly. Also, Broughton (5) has shown that the amount of RNA per internode of light-grown peas which have been treated with GA is twice that of control peas 48 hr after treatment. Thus, 48 hr after treatment was considered to be the time when any GA-induced change in the histone complement should be detected if such a change occurred.

The results of the first experiment comparing histones from GA-treated and control dwarf peas indicated that GA caused the appearance of no new histones and did not alter the electrophoretic mobilities of existing histones (Fig. 1). However, it was noted that Histone 2 (nomenclature of Fig. 1) appeared to be present in greater quantity in the control tissue. This histone is known to exist in two forms: a reduced form (band 2) and an oxidized form (a disulfide dimer) which, because of its higher molecular weight, migrates just slower than the bands designated Histone 1 (27). (See Fig. 3). Treatment of an aliquot of the whole histone with 0.5 M 2-mercaptoethanol and 8 M urea before electrophoresis causes the slower moving band to disappear completely and results in a concomitant increase in the amount of protein in the band designated Histone 2. In the initial preparations the relative amounts of Histone 2 present in the monomer and dimer forms varied considerably and independently of GA treatment. Therefore, it was suspected that at least some of the dimer was formed by oxidation during extraction and subsequent handling prior to electrophoresis and thus was not a true indication of the state of the molecule in vivo. However, if the histone samples are obtained by a rapid extraction and dissolved immediately before electrophoresis, the slower moving, oxidized band is totally absent from both the control and GA-treated tissue, as shown in Figure 1. Thus Histone 2 exists as a monomer in vivo in pea epicotyl tissues.

The histone bands have been numbered to correspond to the nomenclature of Panyim and Chalkley (26) for histones from vertebrate animals. This nomenclature has been adopted in order to facilitate comparisons of histones extracted from diverse sources and analyzed by polyacrylamide gel electrophoresis. The banding pattern shown in Figure 1 is very similar to that of Fambrough and Bonner (12, 13) with the exception of the mobility of the oxidized form of Histone 2 (Histone III in their nomenclature). The histones of Little Marvel and Alaska peas (those used by Fambrough and Bonner) have been examined in this laboratory and found to be identical (29). Thus, the difference in mobility is due to the different conditions imposed during electrophoresis. The three bands of Histone 1 probably correspond in order of increasing electrophoretic mobility to Histones I, I, and I. Histone 3 corresponds to II. Histone 4 is not named in the system of Fambrough and Bonner, but is clearly present as indicated by the shoulder on the peak of Histone II, in densitometer tracings. Histone 5 corresponds to Histone IV.

Of the five major electrophoretic groups of histone from peas, two have electrophoretic mobilities identical to histone groups of calf thymus. One of these, Histone 5, has been shown by DeLange et al. (11) to have identical amino acid sequence in peas and calf thymus with the exception of two conservative amino acid replacements. The only other histone which has identical mobility in mammalian and pea tissue is the cysteine-containing Histone 2 (27). The monomers from both sources have identical mobilities, as do the dimers.

Table I gives the average values from eight preparations for the proportion of the total histone in each of the histone bands from GA-treated and control dwarf pea epicotyls. The amount of protein applied to the gels was kept in the range in which the absorbance due to the bound amido black is linearly proportional to the amount of protein in each band (14). Thus, the area under each peak of a densitometer tracing is proportional to the amount of protein in the corresponding band. These data along with those of Figure 1 showing the mobilities of histone from both tissues indicate that GA treatment has not affected the histone complement either qualitatively or quantitatively.

Whole pea histone is composed of seven major bands as shown in Figure 1. However, in Figure 2 it can be seen that in order to make the complex curve from the curve resolver superimposable on the densitometer tracing, it is necessary to add two small curves on either side of the curve corresponding to the band of Histone 2 (monomer form). That these small curves correspond to visible bands present in low quantity is shown in Figure 3 which compares the region of Histones 2,

![Electrophoretic patterns of histones from GA-treated and control peas. The bands of histone increase in electrophoretic mobility from left to right. Minor bands not visible in the photographic representation of the gels are present and identical in histones from both sources.](image-url)
3, and 4 as resolved by a short (10 cm) gel and as they are more highly resolved by a long (24 cm) gel. In the calculations of the amount of histone in each group, these two low intensity bands have been included with Histone 2. GA does not change the mobilities or proportion of histone in these two low intensity bands.

There remained the possibility that GA causes a change in the amount of total histone relative to the amount of DNA without changing the relative amounts of the various histone fractions. Therefore, samples of chromatin from control peas and from peas treated with GA were analyzed. Table II shows that the ratios of histone to DNA and of nonhistone protein to DNA in chromatin are not altered by treatment of the plants with GA.

**DISCUSSION**

It has been proposed that histones act as gene repressors (2, 4). Although the mechanism of such repression has not been elucidated, it has been argued on the basis of experiments in vitro on the template activity of DNA-histone complexes that portions of DNA which are complexed with histone cannot be transcribed (4, 18, 23). Other workers have suggested that a portion of DNA which is fully complexed with histones may be "activated" for transcription by acetylation in situ (1) or phosphorylation (17) of the histone molecules.

These ideas led to studies which indicated changes in the proportion of the lysine-rich fraction of histone during development of pea cotyledons (14), differences in the proportions of the histone fractions in various organs of the pea plant (14), differences in the histone-DNA ratios in various organs of the pea plant (4), and differences in the histones of vernalized and control winter wheat (30). Implicit in these investigations is the idea that during development some genes become activated and others repressed by addition or removal of histone molecules, and that the extent of change is great enough to be detected by variation in either the proportions of the individual histone fractions or in the ratio of total histone to DNA.

This study revealed no difference in the histone complements of control peas and peas which had been treated with GA. Two explanations are possible: first, that GA does not act by modifying the histone complement, and second, that GA does cause changes in histones, but that the changes are too subtle to be detected by the methods employed. We do not favor the second possibility because of the large and dramatic effects of GA on RNA metabolism, seen as a doubling in the rate of RNA synthesis in pea stem segments in vivo (5), and as a doubling in the rate of RNA synthesis in vitro by pea nuclei isolated in the presence of GA (19), which would presumably be due to more than the very subtlest changes in gene expression.

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**LITERATURE CITED**


