THE BINDING OF THE PEROXIDASE OXIDATION PRODUCTS OF INDOLE-3-ACETIC ACID TO HISTONE

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Summary: Oxidation of indoleacetic acid-2-^{14}C by horseradish peroxidase caused a 2 to 14 fold increase in the binding of this hormone to calf thymus histone. Gel electrophoresis revealed from two to ten times more binding to the monomer and dimer of the arginine-rich histone fraction than to other fractions. Inhibition of this binding by glutathione and the U.V. spectra of the oxidation products indicated that 3-methyleneoxindole was the oxidation product that was bound. The data suggest that 3-methyleneoxindole reacted with sulfhydryl residues of the arginine-rich histone.

INTRODUCTION

It had been reported that an IAA oxidase in plants, which appeared to be a peroxidase (1, 2), inactivated the auxin indole-3-acetic acid (IAA) and that the oxidase activity was inversely related to growth (3). But recent experiments support the alternate view that a peroxidase oxidation of IAA activated this plant hormone. It has been shown that gibberellic acid-stimulated growth of peas was associated with an increased IAA oxidation (4), that the cytokinin- and IAA-stimulated growth of tobacco callus culture correlated with the synthesis of peroxidase isoenzymes (5), and that IAA oxidase activity was related to growth and tumor formation in tobacco leaves (6).

Tuli and Moyed (7) demonstrated that 3-methyleneoxindole, a major peroxidase oxidation product of IAA (1), had greater auxin activity than IAA itself. Macko *et al.* (8) found that peroxidase stimulated the germination and growth of a fungus, *Puccinia graminis* f. sp. *triciti*. Stahmann and Demorest (9) reported that fungal infection in plants increased the number and activity of peroxidase isoenzymes before there was an increase in activity of other enzymes. A regulatory role for peroxidase was proposed. In this paper we report that

oxidation of IAA by horseradish peroxidase greatly increased its binding to calf-thymus histone fractions and that the nature of this binding suggests a regulatory role for the oxidation product 3-methyleneoxindole.

MATERIALS AND METHODS

Calf-thymus histone was purchased from Worthington. Horseradish peroxidase (RZ = 0.6) was obtained from Pentex. IAA-2-^{14}C was a preparation of Nuclear-Chicago and was used at a specific activity of 1.85×10^7 CPM/μmole.

All operations were carried out in 0.05 M sodium acetate buffer, pH 5.0. Histone was treated with 0.01 M dithiothreitol for 1 hour at 37° and then passed through Sephadex G-25 under nitrogen to recover reduced histone free of dithiothreitol. Aliquots of this reduced histone were then added to solutions of IAA-2-^{14}C and peroxidase or appropriate controls, which had been previously incubated for 1 hour at room temperature. After 30 minutes, the mixture was passed through Sephadex G-25 to recover histone free from unbound IAA-2-^{14}C. An aliquot of the histone fraction was subjected to gel electrophoresis for 3 hours in 6 M urea according to Panyim and Chalkley (10), except that 5 M urea was used in the sample solution in place of sucrose. The gels were stained overnight as above (10) and electrophoretically destained. The gels were scanned with a Joyce Chromoscan and histone was calculated from the areas of the scanned peaks. The stained bands were cut-out and counted for ^{14}C-label according to Fambrough and Bonner (11).

RESULTS

The results shown in Fig. 1 indicate that the specific activity in all histone fractions was greatly increased by peroxidase oxidation of the IAA-2-^{14}C When the IAA was oxidized with peroxidase the specific activity increased three to five times in different histone fractions except histone 2',2'',2''' where it increased fourteen fold (compare A and B, Fig. 1). When reduced glutathione was added to the IAA and peroxidase just before the histone, the specific activity was decreased by about one-half of that for IAA and peroxidase in three

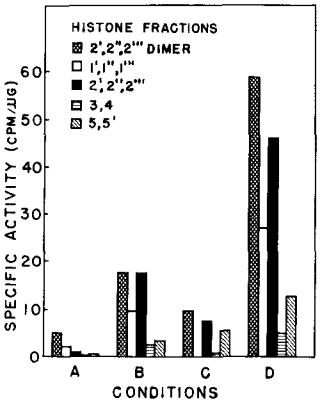

Fig. 1. The specific activity of ^{14}C-label from IAA in different gel electrophoretic fractions of calf-thymus histone under various conditions. The final incubation mixture contained IAA-2-^{14}C (1.18 x 10^{-4} M), peroxidase (10 µg/ml), and histone (1.0 mg/ml). The conditions were A.) IAA-2-^{14}C and histone, B.) IAA-2-^{14}C, peroxidase, and histone, C.) same as B. except 10^{-4} M reduced glutathione was included just before adding histone, and D.) same as B. except IAA-2-^{14}C and peroxidase was heated at 100° for 10 minutes and cooled to room temperature just before adding histone. In the absence of histone (not shown) the ^{14}C-labeling was only 3% of that found in B.. The histone nomenclature is that of Panyim and Chalkley (10).

histone fractions (compare B and C, Fig. 1). In histone 1',1'',1''' no binding was detected, but in histone 5,5' it increased. When the IAA-peroxidase reaction products were heated and cooled before adding the histone, the binding was increased two to three times (compare B and D, Fig. 1). The specific activity was greatest in histone 2',2'',2''' and its dimer. These fractions contained from two to nine times more specific activity than other histone fractions (see B, C and D, Fig. 1). No observable differences in the gel histone patterns of samples A through D of Fig. 1 could be found. Apparently, the bound IAA oxidation product did not significantly alter the mobility of the histone fractions.

The data of Figure 2 show that the greatest specific activity was bound to histone 2',2'',2''' and its dimer. Histone 1',1'',1''' showed high binding only at the highest peroxidase level. Histone 2',2'',2''' and its dimer had from two to ten times more specific activity bound to it than other histone fractions, with maximum labeling at 10 µg/ml of peroxidase.

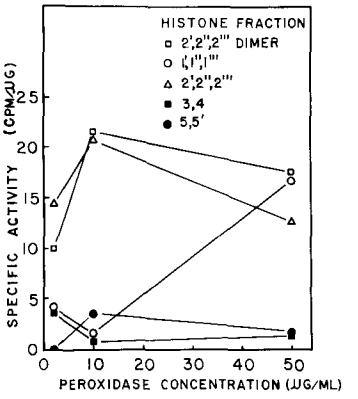

Fig. 2. The specific activity of ^{14}C-label from IAA in different gel electrophoretic fractions of calf-thymus histone as a function of peroxidase concentration. The final mixture contained IAA-2-^{14}C (5.85×10^{-5} M), histone (1.5 mg/ml) and peroxidase at 2, 10, or 50 µg/ml. In all samples the IAA and peroxidase was heated at 100° for 10 minutes and cooled to room temperature just before adding histone. The histone nomenclature is that of Panyim and Chalkley (10).

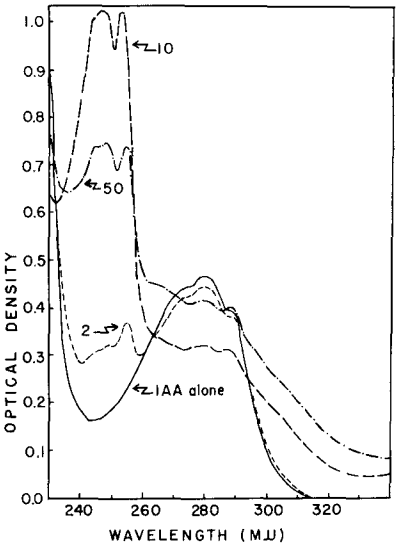

Fig. 3. The U.V. spectra of the peroxidase oxidation products of IAA as a function of peroxidase concentration. IAA (2.0×10^{-4} M) and peroxidase at 2, 10 or 50 µg/ml (shown as numbers next to tracings) were incubated 1 hour at room temperature. The solution was then heated for 10 minutes at 100° and cooled to room temperature before diluting 2-fold for U.V. measurements in a Cary 15 against blanks containing peroxidase plus buffer.

3-Methyleneoxindole has been shown to be a product of the oxidation of IAA by peroxidase (1). Its presence in our oxidation products is indicated by the

U.V. spectra shown in Figure 3. The doublet peak at 253 and 248 mμ is characteristic of 3-methyleneoxindole (1, 12). It increased as peroxidase was raised from 2 to 10 μg/ml but was decreased at 50 μg/ml. In a separate experiment, heating the reaction mixture also increased the quantity of 3-methyleneoxindole by two to three times as was indicated by the increased absorption of the doublet peak. This effect of heating was previously observed (1).

The reduction in the specific activity by added reduced glutathione (Fig. 1), which is known to conjugate with 3-methyleneoxindole (12), the agreement between maximum 3-methyleneoxindole formation and maximum binding (Figs. 2 and 3) and the increased specific activity after heating (Fig. 1) all indicate that the histones must have reacted with the 3-methyleneoxindole.

DISCUSSION

The often observed stimulation of RNA synthesis by IAA in plant tissue, cells, and nuclei (13) may be due to an IAA-induced increase in DNA template availability from plant chromosomes for DNA-dependent RNA synthesis. It has been found that chromatin from IAA treated plant tissue has a greater template activity for in vitro DNA-dependent RNA synthesis than chromatin from untreated plants (14, 15). Since histones are known to restrict the template activity of DNA (16), it would be expected that IAA or some derivative reduced the histone's restriction of template availability. The experiments reported in this paper showed that peroxidase oxidation of IAA-2-^{14}C greatly increased this hormone's binding to all calf-thymus histone fractions without significantly altering electrophoretic mobility. This result agrees with previous experiments showing that binding of IAA-2-^{14}C to histones in plant nuclei is not accompanied by changes in histone gel electrophoretic patterns (17).

The increased binding after heating and inhibition by glutathione suggest that 3-methyleneoxindole, a product of peroxidase oxidation of IAA (1), was the principal oxidation product which was bound to the histone fractions. This binding occurred most extensively with histone 2',2'',2''' and its dimer. Since

the arginine-rich (F3) (10, 18) calf-thymus histone 2',2'',2''' and its dimer contain free sulfhydryl groups while the other histone fractions do not (11, 18), the results support the suggestion that the 3-methyleneoxindole, which is known to conjugate readily with sulfhydryl groups in various compounds and some proteins (12), conjugated with the cysteine residues in the arginine-rich histone. Such a reaction may also occur with plant arginine-rich histone since the plant and animal cysteine-containing arginine-rich histones appear to be physically (18) and chemically (11) similar.

Finally, since the conjugation of 3-methyleneoxindole with the cysteine residues of arginine-rich histone would be expected to block disulfide formation, this conjugation could account for the IAA-induced increases in DNA template activity (14, 15). This might be expected since it has been found that metabolically inactive chromosomes have a higher disulfide content than active chromosomes (19). The disulfide form of the arginine-rich histone was more effective in restricting DNA template activity in DNA-dependent RNA syntheses than the sulfhydryl or sulfhydryl-blocked form (20). These experiments lend support to our view that some peroxidase isoenzymes may be involved in derepression of the plant genome during injury or disease (9) and suggest a possible regulatory role for peroxidase.

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