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Inactivation by Phenylglyoxal of the Specific Binding of 1-Naphthyl Acetic Acid with Membrane-Bound Auxin Binding Sites from Maize Coleoptiles

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ABSTRACT

The specific binding of 1-[^{3}H]naphthyl acetic acid (NAA) to membrane-bound binding sites from maize (*Zea mays* cv INRA 258) coleoptiles is inactivated by phenylglyoxal. The inactivation obeys pseudo first-order kinetics. The rate of inactivation is proportional to phenylglyoxal concentration. Under conditions at which significant binding occurs, NAA, *R* and *S*-1-naphthyl 2-propionic acids protect the auxin binding site against inactivation by phenylglyoxal. Scatchard analysis shows that the inhibition of binding corresponds to a decrease in the concentration of sites but not in the affinity. The results of the present chemical modification study indicate that at least one arginyl residue is involved in the positively charged recognition site of the carboxylate anion of NAA.

Binding sites for the plant hormone IAA and for its synthetic analog NAA[1], first detected in membrane particle preparations from maize coleoptiles by Hertel *et al.* (12), have been further characterized chemically (19) and shown to be associated with ER (7, 9, 15, 18). The molecular specificity of the sites has been investigated by measuring the ability of a series of analogs to compete for NAA binding (20). Since the specificity of the sites is in rather good agreement with the specificity of the coleoptile straight growth test, it has been suggested that these binding sites might represent receptors involved in the *in vivo* auxin action (20). The proteic nature of the sites has been demonstrated (6, 19) and previous attempts have been made to determine some of the amino acid residues, essential for auxin binding (6, 19, 29). It has been reported that at least one cysteinyl residue is likely to play a prominent role in the binding site (6, 29). Other data (10, 19) suggest the presence of an essential reducible group, possibly a disulfide. It has also been proposed by Venis (29) that a histidyl residue might be involved in the binding of the carboxylic function of the auxin molecule. The auxins IAA and NAA (pK_a 4.7 and 4.3, respectively) are mainly unprotonated at the physiological pH values and their binding, in part, probably results from an electrostatic interaction between a positively charged area of the binding site and their carboxylic moiety. Competition studies have shown that neutral derivatives such as methyl esters or amides of IAA and NAA, or the analogous nitriles, are bound with low affinity in comparison with the parent acetic acids (20). Therefore, the carboxylic anion of auxin appear to be of importance for *in vitro* binding.

Since the introduction of arginyl-specific reagents of the dicarbonyl type (27, 30) an increasing number of reports have shown that arginyl residues play an important and general role in enzymes acting on anionic substrates and cofactors (22). The presence of one or more arginyl residues in the auxin binding site may thus be a possibility although lysine and histidine are other potential candidates. In this work, the former possibility was investigated, taking advantage of phenylglyoxal, a highly selective reagent for the modification of arginyl residues (3, 27, 28).

MATERIALS AND METHODS

Chemicals. 1-[4-^{3}H] Naphthyl acetic acid (5.5 Ci/mmol) was purchased from the C.E.A. (France). Unlabeled NAA was obtained from Sigma. Phenylglyoxal monohydrate was a product from Aldrich. The *R* and *S* isomers of 1-naphthyl 2-propionic acid were a generous gift from Dr. A. Fredga (Uppsala, Sweden). All other chemicals were of the highest quality available.

Plant Material. Maize seeds (*Zea mays* cv INRA 258) were allowed to germinate on damp filter paper in the dark at 25°C. After 2 d, embryos were exposed for 2 h to red light (2300 ergs cm^{-2} s^{-1}; Mazda fluorescent tubes with a Rohm filter ROT 501) and then returned to the dark. After 5 d, coleoptiles were separated from primary leaves and kept chilled on ice. Harvesting and all subsequent procedures were performed in day light.

Isolation of Membranes. The membrane preparation was performed at 0 to 4°C. Coleoptiles (100 g, fresh weight) were homogenized in a mortar in 100 ml of medium containing 0.5 M D-mannitol, 1 mM EDTA, 5 mM 2-mercaptoethanol, 0.5% BSA, and 0.1 M Tris-HCl (pH 7.6). The homogenate was filtered through a nylon cloth having a pore diameter of 50 μm. During grinding, the medium was maintained at pH 7.6 with 1 M Tris. The filtrate was first centrifuged at 12,000*g* for 15 min to eliminate unbroken cells, cell wall fragments, starch, nuclei, and most of the mitochondria. The supernatant of this step was then centrifuged at 100,000*g* for 60 min. The resulting membrane pellet was resuspended in 100 ml of 0.1 M Tris-HCl (pH 7.6) and centrifuged again at 100,000*g* for 60 min. This final membrane preparation was then used in the chemical modification experiments.

Binding Assay. The assay is based on the centrifugation method for particle-associated ligand-binding sites, generally used for membrane-bound hormone receptors (8). Experimental conditions for the assay were mainly derived from the work of Ray *et al.* (19) with some minor modifications. Binding experi-

[1] Abbreviations: NAA, 1-naphthyl acetic acid; PGO, phenylglyoxal; R-NP and S-NP, *R* and *S*-1-naphthyl 2-propionic acids, respectively; pm/gfw, picomole per gram of tissue fresh weight; k_d, dissociation constant.

ments were performed at 0 to 4°C in 1 ml polycarbonate tubes. [^{3}H]NAA (55 nCi) in 20 μl ethanol was added to a 1-ml sample of the membrane suspension in binding medium (the composition of which will be described for each type of experiment) to give a final concentration of 10^{-8} M. To an identical membrane sample, the same amount of labeled NAA was added but in the presence of a high amount of unlabeled NAA (final concentration: 10^{-3} M). After 30 min, the tubes were centrifuged for 30 min at 35,000*g*. The supernatants were discarded and the tube walls carefully wiped with paper towels. Membrane pellets were allowed to dissolve overnight in 300 μl of 10 mM Tris-HCl (pH 8) containing 4% Triton X-100 (w/v). The tube contents were transferred to scintillation vials and the tubes rinsed with 200 μl Triton X-100 solution. Ethanol (500 μl) and Bray's solution (10 ml) were then added and the radioactivity was measured. Specific and nonspecific binding defined as in Ray *et al.* (19) and Cuatrecasas (8) were determined by this method.

Modification of the NAA Binding Sites. Membranes were suspended at 0 to 4°C in 80 mM sodium bicarbonate buffer (pH 8.3) containing various PGO concentrations (0–15 mM). The protein concentration was 0.7 mg/ml. For each PGO concentration tested, a kinetic study of binding site inactivation was made. Incubation was performed at 30°C and the reaction stopped by cooling the samples in ice. For each PGO concentration, time zero of the kinetic was a sample kept in ice. After cooling, the pH of each sample was shifted to 7.0 by addition of an equal volume of 80 mM phosphate buffer (pH 5.9) containing 0.5 M sucrose and 10 mM $MgSO_4$. Specific NAA binding of each sample was then directly measured.

Protection against PGO Inactivation. The protective effect of NAA and related compounds against PGO inactivation of the auxin binding sites was studied. In these experiments, membranes were suspended at 0 to 4°C usually to 0.7 mg protein/ml in a buffer containing 40 mM sodium bicarbonate and 40 mM sodium phosphate (pH 7.0) with or without 15 mM PGO. The protective ligands were added to the incubation medium in ethanolic solutions to a final concentration of 10^{-3} M (if not otherwise indicated). The final concentration of ethanol never exceeded 1%. About 15 min after the ligand addition, the incubation was performed at 30°C for 60 min. Reaction was stopped as previously described. The samples were then pelleted at 100,000*g* for 45 min. The membrane pellets were subsequently washed twice in the bicarbonate-phosphate buffer (pH 7.0) in order to eliminate the ligand in the further binding test. For each wash, 3 ml of buffer were used per mg of protein. Finally, the membranes were resuspended in the binding medium containing 20 mM phosphate buffer, 0.5 M sucrose, and 5 mM $MgSO_4$ at pH 7.0 and assayed for specific NAA binding determination. In some cases, the specific binding of NAA on each sample was analyzed using a concentration series of NAA (technic of isotopic dilution) and the number of binding sites and their affinity determined by means of Scatchard plots.

Assay of Stability of PGO-Binding Site Complexes at Acidic pH. Membranes (0.7 mg protein/ml) were treated with or without 10 mM PGO (pH = 8.3) at 30°C for various times (0–50 min). After centrifugation, the membranes were resuspended in citrate buffer at pH = 5.5 for binding assay.

Competition of Specific NAA Binding by PGO. Membranes were resuspended at 0 to 4°C to 0.7 mg protein/ml in the binding medium at pH 7.0 (described in the preceding paragraph) in the presence of increasing PGO concentrations from 10^{-5} to 10^{-2} M. A control sample was prepared without PGO. [^{3}H]NAA binding (specific plus nonspecific) was determined for each sample according to the method previously described. The value of nonspecific binding was also determined using a control sample in the presence of 10^{-4} M unlabeled NAA.

RESULTS AND DISCUSSION

Since optimal conditions for the selective modification of the guanidinium group of arginyl residue by PGO have been defined recently (3, 28) these were used to study the possible PGO modification of auxin binding sites. Figure 1 shows a time-dependent decrease of the specific NAA binding, following membranes incubation with 15 mM PGO at 30°C. After 50 min, 90% of the specific binding measured at pH = 7.0 has been inactivated. On the opposite, the binding sites appear to be quite stable in bicarbonate buffer alone (control) since no decrease but slight activation of specific binding is observed after incubation. Another important point is that the values for the nonspecific binding (the nonsaturable component of ligand binding to the membrane) remained constant with time of incubation and were similar for PGO-treated and untreated membrane samples (data not shown). After modification, the stability of the putative PGO-auxin binding site adduct was checked at slightly alkaline pH (*i.e.* in the conditions of the modification). It was shown that a 17-h dialysis of the PGO-modified binding sites against bicarbonate buffer leads to a very poor recovery of the binding activity (Fig. 1). When remaining activity after modification is 10% of the control, it can be seen that dialysis yields an activity which is 17% of the dialyzed control, indicating that the presumed PGO-binding site adduct is essentially nonreversible at slightly alkaline pH, a result consistent with the formation of a covalent linkage. Moreover, it was checked that the specific binding inactivation is a temperature-dependent phenomenon. It was shown that in bicarbonate (pH = 8.3), the membranes suspended

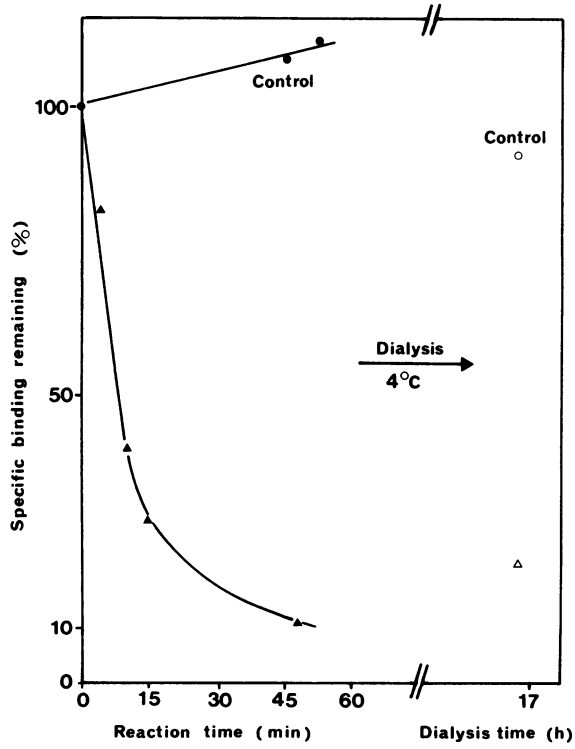

FIG. 1. Left side, Time course of inactivation of the auxin binding site (protein concentration of the membranes: 0.7 mg/ml) by 15 mM PGO in 80 mM bicarbonate buffer (pH 8.3) ▲——▲). Corresponding control incubated without PGO (●——●). The reaction is performed at 30°C. Right side, Per cent specific binding remaining of dialyzed membrane samples previously reacted 50 min with (Δ) or without (O) 15 mM PGO. The samples were dialyzed against 80 mM bicarbonate (pH 8.3). Note: Each point is the mean of duplicates. The range between the two values never exceeded 8% of the mean value.

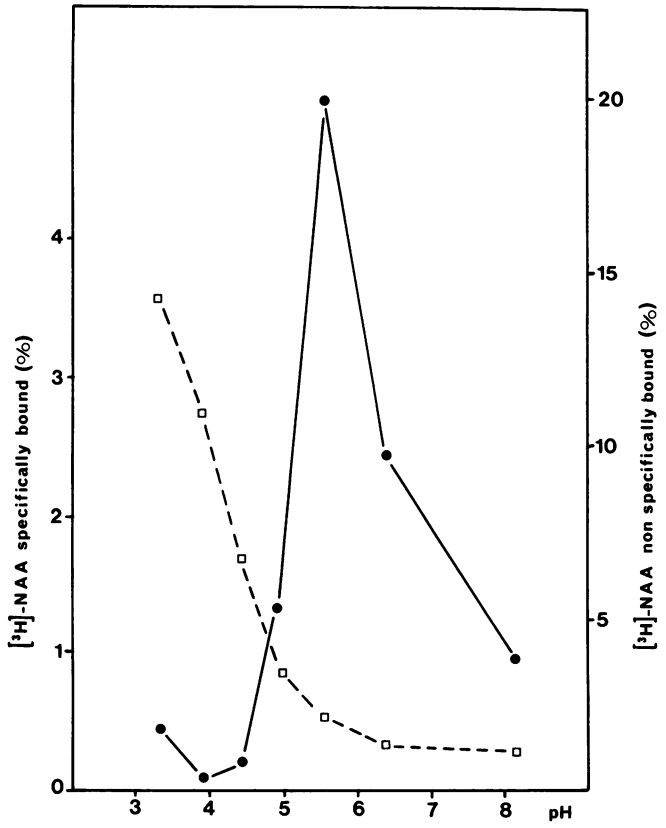

FIG. 2. Effect of pH upon specific (●——●) and nonspecific (□——□) binding of [³H]NAA on the ER-enriched membrane fraction prepared according to Hartmann-Bouillon and Benveniste (11). After the centrifugation step on sucrose gradients, the ER-rich fraction was collected, washed with Tris (pH 7.6), and tested for NAA binding at various pH values fixed by 20 mM citrate buffer. The binding medium also contains 5 mM $MgSO_4$ and 0.5 M sucrose. Note: Each point is the mean of duplicates. The range between the two values never exceeded 8% of the mean value.

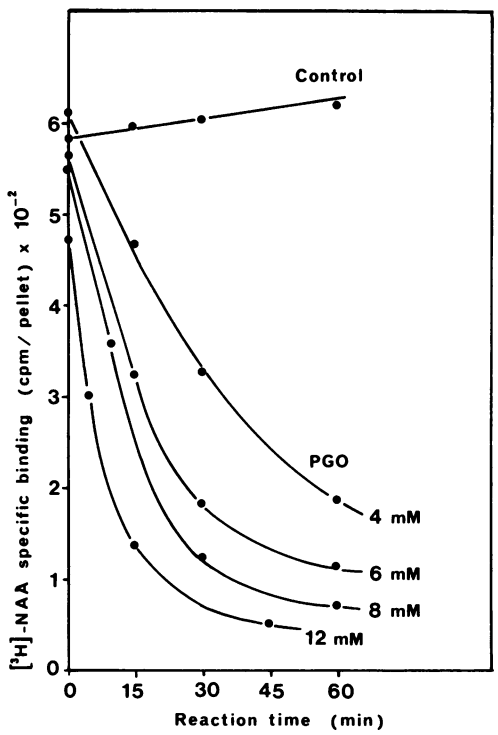

FIG. 3. Time course of inactivation of the auxin binding site (protein concentration of the membranes: 0.7 mg/ml) by PGO at various concentrations. The reaction conditions are the same as in Figure 1. [³H]NAA specific binding is measured at pH 7.0 as described in the experimental section. Note: Each point is the mean of duplicates. The range between the two values never exceeded 8% of the mean value.

in 15 mM PGO solutions for 1 h at 0°C and subsequently washed by sedimentation and homogenization in fresh buffer, fully kept their initial ability to bind auxin (data not shown), indicating that PGO does not chemically react with the NAA binding site at low temperature. In the conditions we have used for the modification, it has been demonstrated (3, 27, 28) that PGO reacts quickly with arginyl residues and extremely slowly with lysyl and cysteinyl residues. In addition, the Schiff bases and thiohemiacetals (25) which could form with these two later residues are unstable. In this respect, the observed irreversible inactivation of specific binding is in agreement with the formation of a PGO-arginyl adduct.

Conditions Used for the Binding Assay. It has been shown in previous studies (19) that specific binding of NAA is optimal at pH 5.5 and auxin binding studies are generally performed at this pH. However, we noticed that, contrary to the results of Ray *et al.* (19), at pH 7.0, a significant binding occurs (Fig. 2). Since the orientation of NAA binding sites in the transverse plane of the various membrane vesicles types remains unknown, the possibility that a part of the binding sites is oriented on the inner surface of sealed membrane vesicles has to be considered. The NAA molecules which mainly occur as anions at pH 7.0 should hardly penetrate the charged membrane of sealed vesicles. If, however, some diffusion takes place, some time is necessary to reach equilibrium between the inside and the outside of the vesicles. After addition of the ligand, we always used to wait for a 30-min period before centrifugation. This might explain the fact that we observe a significant specific binding at pH 7.0 in our experiments.

In this work, all the binding experiments were performed at pH 7.0. The reason is that, at this pH, in a mixture of bicarbonate and phosphate buffer, conditions convenient for both modification and protection studies could be met. Another major reason is that, when the binding experiments were performed in citrate buffer at pH = 5.5, no differences could be detected between the binding values measured for the control and for the PGO-treated membranes, suggesting unstability of the PGO-auxin binding site complex under these conditions.

Kinetics of the Binding Sites Inactivation by PGO. The kinetic mechanism of the reaction of membrane-bound auxin binding sites with PGO was investigated, studying the time course of inactivation at various PGO concentrations. The chemical modification was performed in bicarbonate buffer at 30°C, stopped at 0°C and, after a shift of pH to 7.0 by phosphate buffer addition, binding was measured. Results, plotted as specifically bound [³H] NAA per membrane pellet against time of the reaction (Fig. 3), show that the speed of binding inactivation depends on PGO concentration. It should be pointed out that binding values of the nonincubated samples (time 0) decrease with increasing concentration of PGO. This effect on binding resulted from the competition of PGO (remaining in the medium) with [³H]NAA in the course of the binding assay as further explained. Therefore, for each PGO concentration tested, the inactivation of binding was calculated with respect to the nonincubated sample containing the same PGO concentration. When the results of Figure 3 are plotted as semilogarithmic plots, pseudo-first order kinetics (5) are obtained at any concentration of PGO tested, suggesting that inactivation of the auxin binding site is due to the selective reaction of PGO with an essential arginyl residue (or several essential arginyl residues of similar reactivities). From the slopes

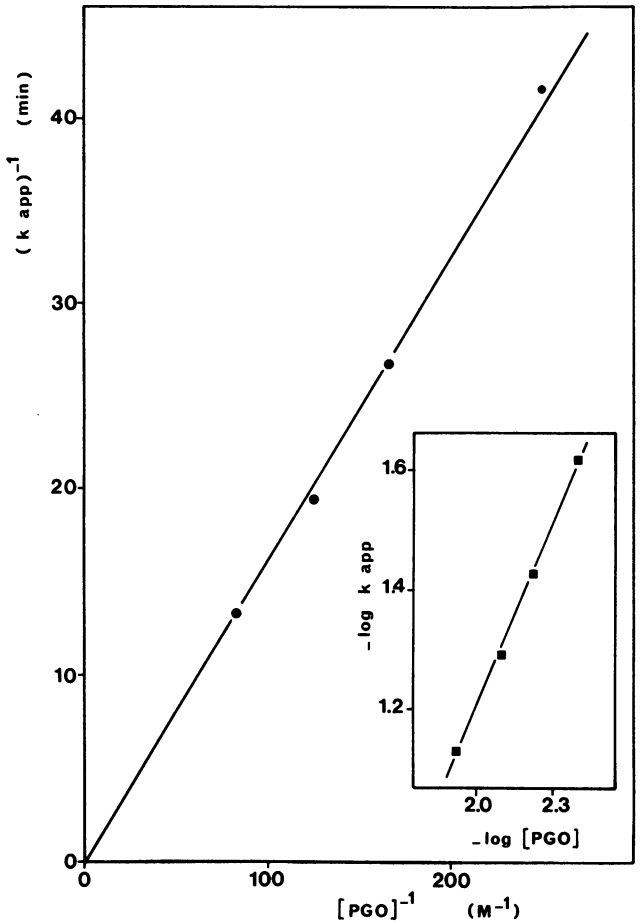

FIG. 4. Dependence of k_{app} upon the concentration of PGO, plotted as reciprocals. Inset: Plot of log k_{app} *versus* log [PGO].

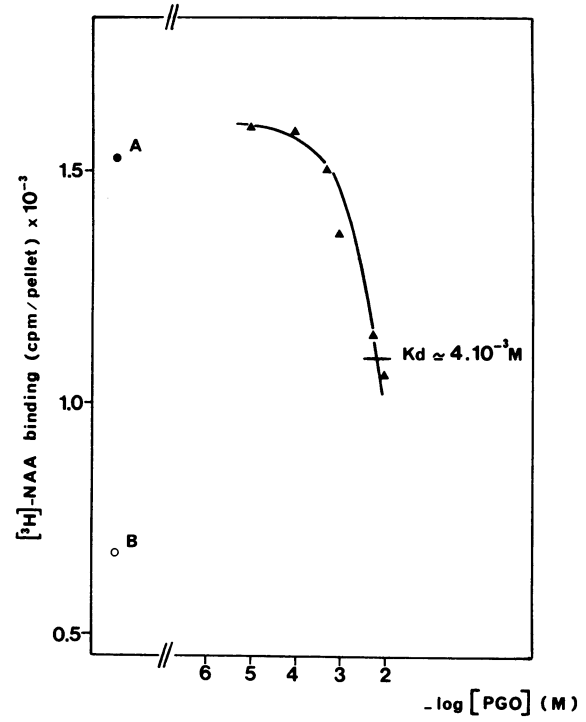

FIG. 5. Displacement curve of [^{3}H]NAA from its binding sites by increasing concentrations of PGO (▲——▲). Points A and B correspond to [^{3}H]NAA binding in the presence (○) or not (●) of 10^{-4} M unlabeled NAA. A − B = specific binding. Note: Each point is the mean of triplicates. The range between the three values never exceeded 8% of the mean value.

of the semilogarithmic plots, the values of the pseudo-first order rate constants for inactivation (k_{app}) were calculated. The dependence of k_{app} upon the concentration of PGO studied using a double reciprocal plot (13) yields a straight line crossing the origin (Fig. 4) indicating that, in the course of the modification of the sites, (*i.e.* in bicarbonate buffer at pH 8.3), no reversible PGO-auxin binding site complex seems to be formed prior to inactivation. The reciprocal of the slope of such a plot is then equal to the rate constant k for inactivation (k = 5.9 M^{-1} min^{-1} at pH 8.3 and 30°C: mean of three experiments). The order (n) of inactivation with respect to PGO concentration was determined according to Levy *et al.* (14). Since $k_{app} = k$ [PGO]^n, a plot of log k_{app} *versus* log [PGO] allows the determination of n with accuracy (Fig. 4, inset), which was found equal to 1.00; 1.02; 1.02 in three separate experiments. The simplest interpretation of the above results is that the modification of the auxin binding sites by PGO, leading to inactivation of the specific auxin binding proceeds according to a bimolecular process. This is fully consistent with the data of Takahashi (27) who demonstrated that the slow kinetic step leading to formation of the PGO-arginine adduct involves one molecule of arginine and one molecule of PGO.

Reversible Inhibition of NAA Binding by PGO at 0°C. As previously mentioned, the binding values of the time zero samples of the kinetic experiments (Fig. 3) decrease with the increasing concentrations of PGO. Since in these experiments, the binding assay is performed after a shift of pH to 7.0 by phosphate buffer addition, PGO is then present at a concentration half of that in the modification reaction. As we have checked that PGO cannot irreversibly inactivate NAA binding at 0°C, the remaining possibility is that PGO reversibly alters NAA binding. The inhibiting effect was studied at various PGO concentrations in phosphate buffer (pH 7.0). In the presence of 4 × 10^{-3} M PGO, we found that half of the specifically bound [^{3}H]NAA was displaced from the sites (Fig. 5) confirming our previous observations (Fig. 3). Assuming that PGO competes for the NAA binding site, this value can be regarded as the dissociation

Table I. *Protection Study of the Auxin Binding Site against Inactivation by PGO in the mixture of Bicarbonate and Phosphate Buffers (40/40 mM) at pH 7.0*

Membrane samples (0.4 mg protein/ml) were incubated for 60 min at 30°C with the indicated substances. Washing of the membranes and binding were performed as described in the experimental section. The dissociation constants of the auxin binding sites for S-NP and R-NP, determined according to the method of Ray *et al.* (20), were found to be 1.2 and 40 μM, respectively, at pH 5.5 in 20 mM citrate buffer, 0.5 M sucrose, and 5 mM $MgSO_4$ (unpublished data).

Treatment	Specific Binding[a]	
	cpm/mg protein	*% of control*
Control	1006	100
+ PGO (15 mM)	550	55
+ PGO (15 mM) + S-NP (1 mM)	1303	130
+ PGO (15 mM) + R-NP (1 mM)	1200	119

[a] Data represent means of duplicates. The error on the mean is evaluated to ± 10%.

Table II. *Values of K_d and Sites Concentration Obtained from Scatchard Plots*

Treatment	K_d	Sites Concn.	
	μmol/l	pm/gfw	% of control
Control	1.0	13.8	100
+ PGO (15 mM)	1.0	8.7	63
+ PGO (15 mM) + NAA (10^{-3} M)	1.1	14.6	106

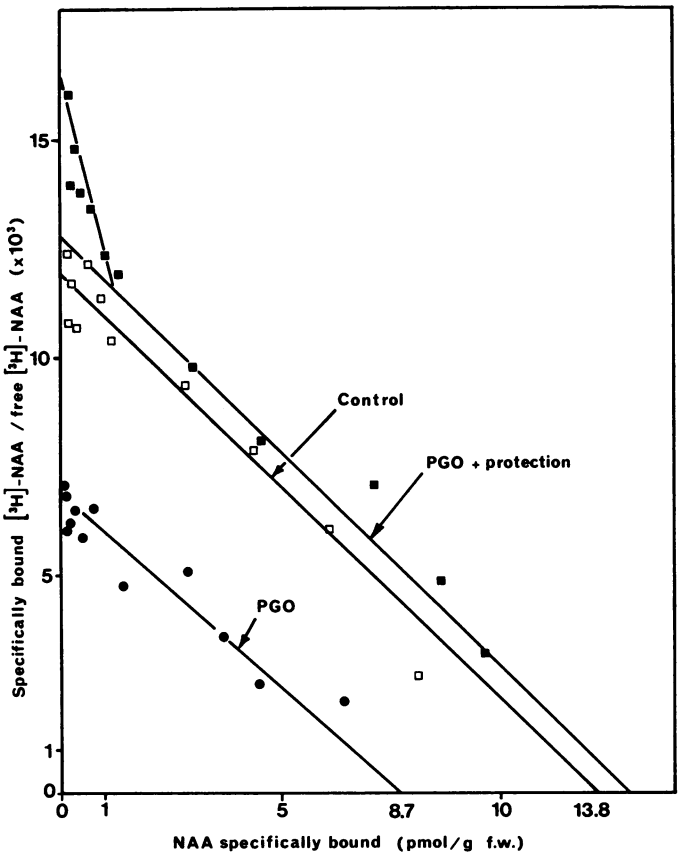

FIG. 6. Effect of PGO inactivation with (■——■) or without protection (●——●) on the two characteristic parameters of binding: the dissociation constant and the sites concentration. Membrane samples (0.7 mg protein/ml) were incubated for 60 min at 30°C as detailed in the experimental section. In this case, the protection was 10^{-3} M NAA. Note: Each point is the mean of duplicates. The range between the two values never exceeded 8% of the mean value.

constant of the auxin binding sites towards PGO under the experimental conditions fixed for the binding assay (2, 19).

Protection against PGO Inactivation. When the modification of the auxin binding sites was performed in 80 mM bicarbonate buffer (pH 8.3), we found that neither 0.1 mM *R*- nor 0.1 mM *S*-1-naphthyl 2-propionic acids were able to protect the binding against inactivation by 15 mM PGO. This result is not surprising since in bicarbonate buffer (pH 8.3), no binding of auxin to the site is likely to occur (20). In contrast, under conditions where auxin does interact with the site, full protection is obtained. This is evidenced by performing the inactivation reaction at pH 7.0 in the mixture of phosphate and bicarbonate buffers (40/40 mM). This mixture of buffers, previously used in the kinetic experiments has been shown to be a quite convenient medium for the binding assay. In these new conditions for the modification, the inactivation rate is lowered as expected due to the lower pH and lower bicarbonate concentration (3, 4). However, it remains sufficiently high to allow a significant inactivation of the binding sites in a 1-h period (Table I). Both 1 mM R-NP and 1 mM S-NP completely prevent the inactivation of specific binding by 15 mM PGO at 30°C, while specific binding of a nonprotected sample is 55% of a control without PGO, after a 1-h incubation. It is interesting to notice a 30% and 19% activation, respectively, for S and R-NP with respect to the control. This effect can be explained assuming that, in the course of the incubation at 30°C, the sites saturated with protective ligands have an increased stability compared to the unprotected ones. Convenient conditions for both modification and protection of the binding sites being established, it became particularly interesting to analyze the effects of the PGO inactivation with or without protection on the two characteristic parameters of specific NAA binding: the concentration of the sites on the membrane and their dissociation constant (K_d). These two parameters were determined by the use of Scatchard plots (24). In Figure 6, it can be seen that the K_d of the NAA binding sites remains unchanged after PGO inactivation. The observed inhibition of specific binding is clearly due to a 37% decrease of the concentration of the sites (Table II; Fig. 6). In the presence of 1 mM NAA as protection, PGO does not affect the binding site affinity nor does it produce any decrease of the concentration of binding sites. In this case, it is interesting to note a slight increase in the concentration of the sites (Table II; Fig. 6). Again, this effect might be explained by an improved stability of the sites when they are saturated with NAA. But, it can also be argued that this phenomenon results from the unmasking of cryptic binding sites, due to the incubation of the membranes with NAA. This hypothesis would be consistent with the appearance of a high affinity binding as evidenced in Figure 6. The above protection study clearly shows that PGO acts at the auxin binding site or in its close environment. These results also suggest that inactivation of binding is probably not due to a modification of the three-dimensional structure of the protein.

We have demonstrated that the incubation of membrane-bound auxin binding sites with PGO leads to the irreversible inactivation of the specific binding of NAA, when incubation is performed at 30°C and binding measured at pH 7.0. Such an inactivation does not occur at 0°C. Inactivation is rapid and follows pseudo-first order kinetics until approximately 90% of the specific binding is lost. Kinetic data and protection studies suggest that the chemical reaction is bimolecular and specifically takes place near or at the binding site. Since PGO is known to be a highly selective reagent for arginyl residues (3, 27, 28) these data strongly suggest that at least one arginyl residue is involved in the binding of the auxin carboxyl group. It has been proposed (29) that a histidyl residue might occur in the auxin binding site and be responsible for the binding of the auxin carboxyl group. This assumption was made in order to explain the decrease of NAA binding activity with increasing pH in the range 6 to 7. It was also derived from binding site inactivation results obtained with the diazonium salt of 2,5-dichloro 3-amino benzoic acid at pH 6. However, this reagent as well as other diazonium salts (23), has not been shown to be highly selective for histidine (29) and it might react for instance with the presumed arginine(s) of the anion auxin binding site. Various results from literature show that the carboxyl anions of enzymes substrates generally anchor to guanidinium ions rather than to imidazolium ions (1, 17, 21, 26). Our results are consistent with the presence of at least one arginine in the active site of the receptor but do not exclude the possible presence of another charged residue (*e.g.* histidine) (16).

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LITERATURE CITED

1. ADAMS MJ, M BUEHNER, K CHANDRASEKHAR, GC FORD, ML HACKERT, A LILJAS, MG ROSSMANN, IE SMILEY, WS ALLISON, J EVERSE, NO KAPLAN, SS TAYLOR 1973 Structure-function relationships in lactate deshydrogenase. Proc Natl Acad Sci USA 70: 1968–1972
2. CHENG Y, WH PRUSOFF 1973 Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. Biochem Pharmacol 22: 3099–3108
3. CHEUNG ST, ML FONDA 1979 Reaction of phenylglyoxal with arginine. The effect of buffers and pH. Biochem Biophys Res Commun 90: 940–947
4. CHEUNG ST, ML FONDA 1979 Kinetics of the inactivation of *Escherichia coli* glutamate apodecarboxylase by phenylglyoxal. Arch Biochem Biophys 198: 541–547
5. CORNISH-BOWDEN A 1976 Basic principles of chemical kinetics. *In* Principles of Enzyme Kinetics. Butterworths, London, pp 1–13
6. CROSS JW, WR BRIGGS 1978 Properties of a solubilized microsomal auxin-binding protein from coleoptiles and primarily leaves of *Zea mays*. Plant Physiol 62: 152–157
7. CROSS JW, WR BRIGGS 1979 Solubilized auxin binding protein. Planta 146: 263–270
8. CUATRECASAS P 1974 Membrane receptors. Annu Rev Biochem 43: 169–213
9. DOHRMANN U 1975 Charakterisierung der *In vitro*-bindung des Pflanzenhormons Auxin bei Koleoptilen von *Zea mays* L. PhD thesis. Freiburg-im-Breisgau, Federal Republic of Germany
10. DOHRMANN U, PM RAY 1976 Measurements of auxin binding to solubilized receptor sites of corn seedling tissue. Carnegie Inst Wash Yearbook 75: 395–399
11. HARTMANN-BOUILLON MA, P BENVENISTE 1978 Sterol biosynthetic capability of purified membrane fractions from maize coleoptiles. Phytochemistry 17: 1037–1042
12. HERTEL R, KST THOMSON, VEA RUSSO 1972 In vitro auxin binding to particulate cell fractions from corn coleoptiles. Planta 107: 325–340
13. KITZ R, IB WILSON 1962 Esters of methane sulfonic acid as irreversible inhibitors of acetylcholinesterase. J Biol Chem 237: 3245–3249
14. LEVY HM, PD LEBER, EM RYAN 1963 Inactivation of myosin by 2,4-dinitrophenol and protection by adenosine triphosphate and other phosphate compounds. J Biol Chem 238: 3654–3659
15. NORMAND G, MA HARTMANN, F SCHUBER, P BENVENISTE 1975 Caractérisation de membranes de coléoptiles de Maïs fixant l'auxine et l'acide N-naphtyl phtalamique. Physiol Vég 13: 743–761
16. PATTHY L, J THESZ 1980 Origin of the selectivity of α-dicarbonyl reagents for arginyl residues of anion-binding sites. Eur J Biochem 105: 387–393
17. PHELPS DC, Y HATEFI 1981 Inhibition of D(−)-β-hydroxybutyrate dehydrogenase by butanedione, phenylglyoxal, and diethyl pyrocarbonate. Biochemistry 20: 459–463
18. RAY PM 1977 Auxin-binding sites of maize coleoptiles are localized on membranes of the endoplasmic reticulum. Plant Physiol 59: 594–599
19. RAY PM, U DOHRMANN, R HERTEL 1977 Characterization of naphthaleneacetic acid binding to receptor sites on cellular membranes of maize coleoptile tissue. Plant Physiol 59: 357–364
20. RAY PM, U DOHRMANN, R HERTEL 1977 Specificity of auxin-binding sites on maize coleoptiles membranes as possible receptor sites for auxin action. Plant Physiol 60: 585–591
21. RIORDAN JF 1973 Functional arginyl residues in carboxypeptidase A. Modification with butanedione. Biochemistry 12: 3915–3923
22. RIORDAN JF, KD MC ELVANY, CL BORDERS JR 1977 Arginyl residues: Anion recognition sites in enzymes. Science 195: 884–886
23. RIORDAN JF, BL VALLE 1972 Diazonium salts as specific reagents and probes of protein conformation. Methods Enzymol 25: 521–531
24. SCATCHARD G 1949 The attractions of proteins for small molecules and ions. Ann New York Acad Sci 51: 660–672
25. SCHUBERT MP 1936 Compounds of thiol acids with aldehydes. J Biol Chem 114: 341–350
26. SHOUN H, T BEPPU, K ARIMA 1980 An essential arginyl residue at the substrate-binding site of p-hydroxybenzoate hydroxylase. J Biol Chem 255: 9319–9324
27. TAKAHASHI K 1968 The reaction of phenylglyoxal with arginine residues in proteins. J Biol Chem 243: 6171–6179
28. TAKAHASHI K 1977 The reactions of phenylglyoxal and related reagents with amino acids. J Biochem 81: 395–402
29. VENIS MA 1977 Affinity labels for auxin-binding sites in corn coleoptile membranes. Planta 134: 145–149
30. YANKEELOV JA JR 1972 Modification of arginine by diketones. Methods Enzymol 25: 566–579