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Indoleacetaldehyde Reductase of *Cucumis sativus* L.

KINETIC PROPERTIES AND ROLE IN AUXIN BIOSYNTHESIS[1]

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ABSTRACT

Indoleacetaldehyde reductase catalyzes the conversion of indoleacetaldehyde to indole ethanol in extracts of *Cucumis sativus* L., with reduced pyridine nucleotide required as co-substrate. NADH and NADPH result in markedly different enzyme behavior, as reflected in reaction kinetics and in responses to inhibitors and activators. It is not yet clear whether there are two separate enzymes, one specific for NADH and the other for NADPH, or whether there is a single enzyme differentially influenced by the two co-substrates.

In the presence of NADH, the indoleacetaldehyde reductase activity was inhibited by NaCl and displayed hyperbolic kinetics under all conditions tested. However, in the presence of NADPH the enzyme was activated by NaCl at concentrations up to 0.1 molar. Under certain conditions with NADPH as co-substrate, the enzyme showed kinetics sigmoidal with respect to indoleacetaldehyde concentration and was strongly inhibited by high concentrations of NADPH. It is possible that this substrate inhibition of the NADPH-linked indoleacetaldehyde reductase activity by NADPH, as well as the sigmoidicity with respect to indoleacetaldehyde concentration, may function in the regulation of auxin biosynthesis.

Several lines of evidence support the view that the rate of auxin biosynthesis is subject to regulation. Since the demonstration of IEt[4] as a naturally occurring constituent of some higher plants (12, 13) it has become apparent that the metabolism of this compound plays a central role in the regulation of auxin synthesis where it occurs. The enzyme IEt oxidase (16) is subject *in vitro* to feedback regulation by IAA (10). In tomato, the application of an excess of [3-^{14}C]tryptophan leads to little increase in the pool size of free IAA; however, there is a marked accumulation of label in the IEt pool and a 2- to 3-fold increase in that pool size (7), suggesting that IEt is acting as a flexible storage depot for auxin precursor as first proposed by Rayle and Purves (14).

We previously described the isolation and characterization of indole-3-acetaldehyde reductases from cucumber seedlings (3) and suggested that they play an important role in auxin synthesis. IAAld occurs naturally in cucumber, and exogenously supplied IAAld is readily converted to IEt *in vivo* (11). *In vitro*, IAAld reductase catalyzes the reduction of IAAld to IEt at the expense of reducing equivalents from NADH or NADPH. We suggested the occurrence of multiple IAAld reductases, each specific for a particular pyridine nucleotide, distinguishable with respect to several properties (3). Our first report dealt with enzyme purification, product proof, substrate specificity, and pH behavior (3). We have also reported the association of NADPH-specific activity with microsomal components in seedling homogenates; the NADH-specific IAAld reductase activity appeared only in the cytosol (1).

We have subsequently reexamined the evidence for multiple IAAld reductases. In addition, we have examined the possibility that IAAld reductase may have properties contributing to the regulation of the rate of auxin biosynthesis. The results and implications of these studies are presented below.

MATERIALS AND METHODS

Preparation of Reagents. Indoleacetaldehyde was purchased as the bisulfite adduct from Sigma Chemical Co. and stored desiccated at −20 C. IAAld was freed from the bisulfite as previously described (3) and used immediately to avoid decomposition. Final concentrations of free IAAld were determined spectrophotometrically by applying Beer's law with ϵ_M^{280} = 5,400 l/mol·cm (3). NADH and NADPH were obtained from Sigma and stored desiccated at −20 C. NAD(P)H solutions were prepared immediately before use, and concentrations were determined using the mol wt corrected for bound water as assayed by Sigma. Periodically, concentrations were confirmed spectrophotometrically. All other reagents were analytical grade from Mallinckrodt or Baker.

Preparation of Enzyme. Seeds of *Cucumis sativus* L. cv. National Pickling were sown in Vermiculite saturated with tap water. Seedlings were grown for 7 days under a 14-h light, 10-h dark cycle at 25 C, harvested, and extracted. Enzyme extraction and purification were as previously described (3). The procedures included pH and ammonium sulfate precipitations, removal of contaminating proteins by batch extraction with Bio-Rex 70, cellulose phosphate ion exchange chromatography and gel filtration (Sephadex G-150), yielding an enzyme preparation purified approximately 700-fold in IAAld reductase activity.

In the previous paper (3) we presented data for the elution of IAAld reductases from Sephadex G-150. Eluted fractions (6 ml) were assayed by a modified Salkowski reaction (16) after a 30-min incubation with IAA1D and NAD(P)H. In the present study Sephadex G-150 gel filtration, the final purification step, was performed as before with the exception that the enzyme assay reaction time was decreased from 30 min to 5 min when NADH was co-substrate and to 1 or 2 min when the co-substrate was NADPH. This modification in assay reaction time avoided the artifact described under "Results and Discussion," of the present report. Gel filtration of the ammonium sulfate-concentrated cel-

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[4] Abbreviations: IEt: indole-3-ethanol; IAAld: indole-3-acetaldehyde.

lulose phosphate eluate (see ref. 3) now yields a single peak of IAAld reductase activity which may represent either a single enzyme species or two or more enzymes with differing co-substrate specificity.

Enzyme Assays. Enzyme reaction rates were determined by recording the continuous decrease in A at 340 nm with a Beckman Acta V spectrophotometer using the double beam mode and cuvettes of 10-mm path length. Some experiments were performed with cuvettes of 2-mm path length, and these are identified in the appropriate figure legends. All assays were started by the addition of enzyme to a quantity of premixed substrates [IAAld and NAD(P)H] and effectors. Actual volumes and concentrations varied with each type of experiment and are described in the figure legends.

RESULTS AND DISCUSSION

How Many Enzymes Are Present? We reported previously that homogenates of cucumber seedlings contain one NADH-specific and two NADPH-specific IAAld reductases (3). We later discovered that we erred in this assessment, and our error points out a danger in using the Salkowski reagent for monitoring the interconversion of IAAld and IEt. This reagent yields a bright pink color in the presence of a mixture of IAAld and IEt (16). The color reaction reaches a maximum at a certain ratio of IEt to IAAld and falls off rapidly as this ratio is exceeded. We now realize that we underestimated the amount of NADPH-linked IAAld reductase activity in the Sephadex G-150 gel filtration fractions and that the true peak of activity had achieved a nearly complete conversion of IAAld to IEt in the assay tubes. Thus, this peak of activity yielded no pink color whereas the less active shoulders appeared as peaks of activity since enough IAAld remained to react with the IEt and Salkowski reagent (Fig. 2 of ref. 3). When this experiment is repeated under the conditions described under "Materials and Methods," quite different results are obtained. We now report that IAAld reductase activity elutes as a single peak from Sephadex G-150 (Fig. 1). The position of the peak is the same whether NADH or NADPH is used as co-

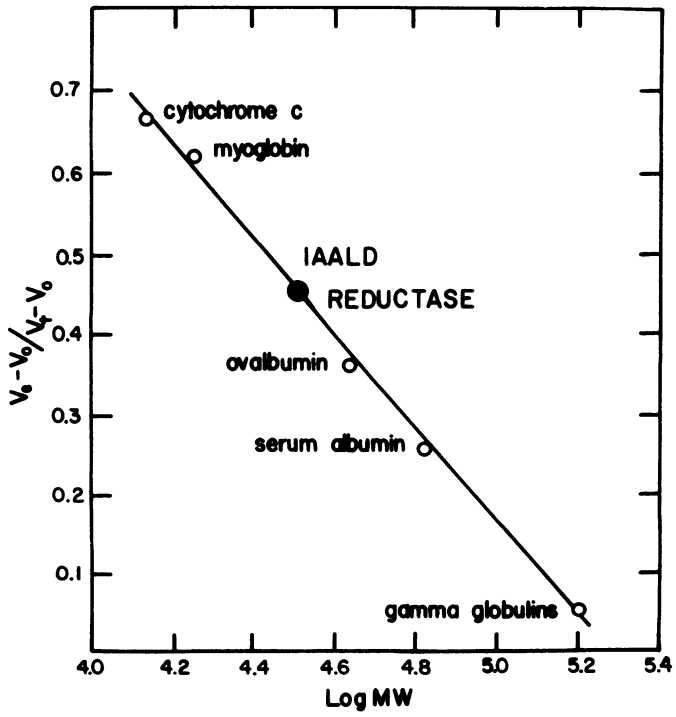

FIG. 1. Estimation of mol wt of indoleacetaldehyde reductase by calibrated Sephadex G-150 gel filtration.

substrate. Our preparations consistently demonstrate from 3 to 20 times as much activity with NADPH as with NADH, depending upon the purification step and assay conditions.

Since the NADPH- and NADH-linked IAAld reductase activities now appear to co-chromatograph on Sephadex G-150, with

Table I. *Effects of Inhibitors on Indoleacetaldehyde Reductase*

Enzyme preparation (0.2 ml) was added to a reaction mixture consisting of 0.5 ml of 0.8 mM IAAld in water, 0.2 ml of 0.66 mM NAD(P)H in 0.1 M Na-phosphate (pH 7.2), and 0.2 ml of inhibitor in water; and the assay was started without preincubation. The initial velocity was taken as the continuous decrease in A_{340} versus time, and the results are expressed as a percentage of the control rate. The (100%) control rate for the NADPH-linked activity was 82 nmol NADPH oxidized/min·ml of enzyme; for the NADH-linked activity it was 17 nmol NADH oxidied/min·ml enzyme. Each datum in the table is the average of two or more assays.

Inhibitor	Relative Enzyme Activity			
	NADPH-linked inhibitor concn.		NADH-linked inhibitor concn.	
	1 mM	10 mM	1 mM	10 mM
	% of control			
Water control	100	100	100	100
Sodium azide	80	81	103	84
EDTA	60	2	90	0
Sodium fluoride	65	37	100	106
Potassium cyanide	27	1	0	0
Iodoacetic acid	78	51	100	38
N-Ethylmaleimide	54	0	17	0

Table II. *Effects of Metal Salts on the Activity of Dialyzed NADPH-linked Indoleacetaldehyde Reductase*

The enzyme preparation was dialyzed 24 h against 60 volumes of 10 mM NaCl in 0.5 mM Na-phosphate (pH 7.5) and then diluted 5-fold with the same buffer. The reaction mixture consisted of 0.4 ml of 0.9 mM indoleacetaldehyde in water, 0.2 ml of 0.75 mM NADPH in water, 0.2 ml of 10 mM metal salt, and 0.2 ml of dialyzed, diluted enzyme preparation; and the initial velocity was measured as the continuous decrease in A_{340} versus time. The activity in the presence of each metal salt is expressed as the per cent of the NaCl rate, and each datum is the average of two or more assays. The (100%) control rate was 39 nmol NADPH oxidized per min per ml enzyme. Controls have shown that the difference in ionic strength between, for example, 2 mM NaCl and 2 mM $MgSO_4$ leads to an insignificant effect on the initial velocity.

Metal Salt	Activity
	% NaCl rate
NaCl	100
$MnCl_2$	139
$MgSO_4$	129
$MgCl_2$	128
$CaSO_4$	129
$Ca(NO_3)_2$	128
$ZnCl_2$	52
$ZnSO_4$	48
$CdCl_2$	0
$HgCl_2$	0
$CuSO_4$	0
KNO_3	105
KCl	85
$Co(NO_3)_2$	94
$CoCl_2$	94
NH_4Cl	89
NH_4NO_3	105
$NaNO_3$	96

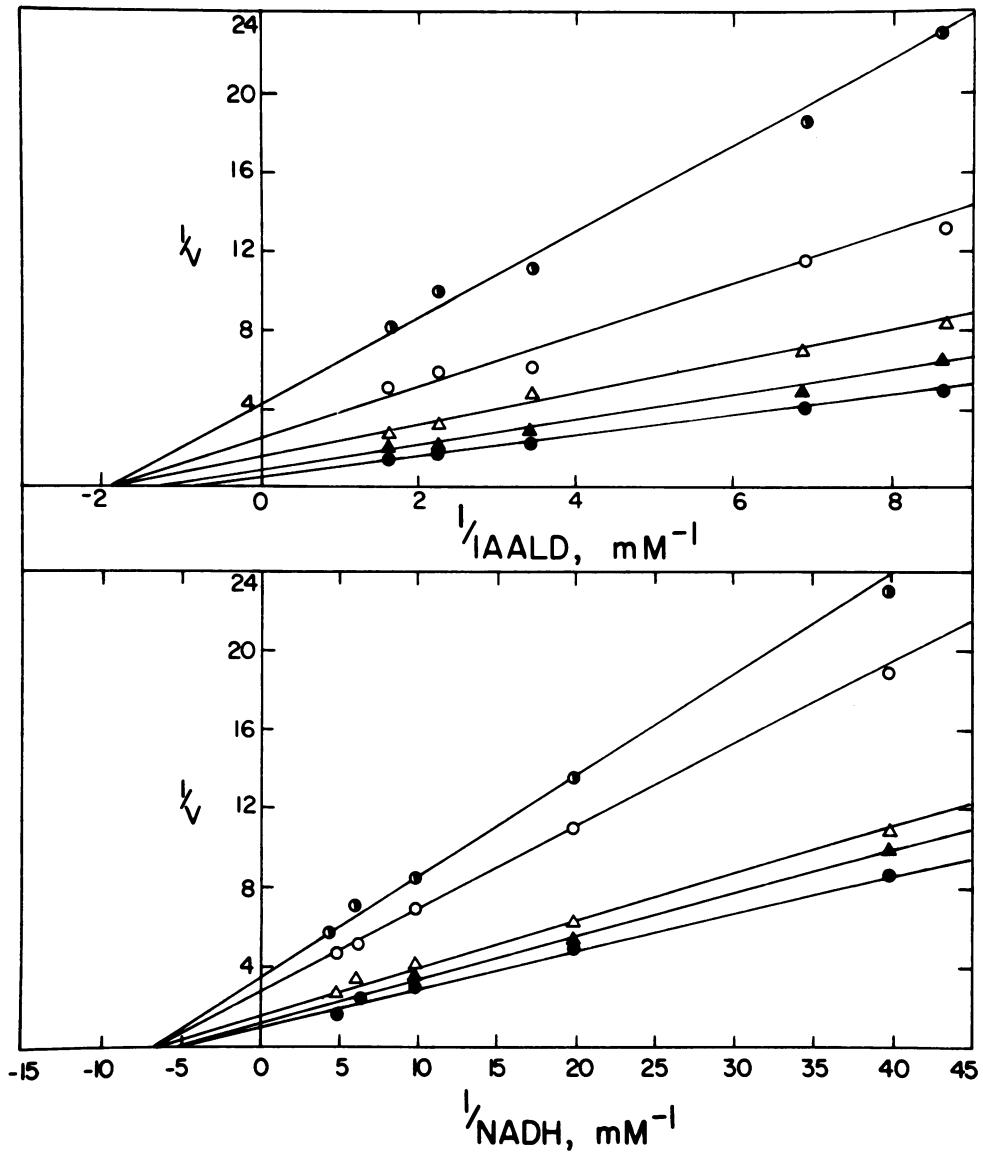

FIG. 2. Reaction kinetics of NADH-linked indoleacetaldehyde reductase. Reactions were started by the addition of 0.1 ml enzyme preparation in 50 mM Na-phosphate (pH 7.5), to bring the total reaction volume to 0.8 ml. Substrates were without buffer, and the final ionic strength was 17 meq/l (based on phosphate ion $pK_a = 6.8$). Initial velocity expressed as change in A_{340} per 100 s. Top: reciprocal initial velocity *versus* reciprocal IAAld concentration at five different NADH concentrations. (●): 0.2 mM NADH; (▲): 0.15 mM; (△): 0.10 mM; (○): 0.05 mM; (◑): 0.025 mM. Bottom: reciprocal initial velocity *versus* reciprocal NADH concentration at five different IAAld concentrations. (●): 0.58 mM IAAld; (▲): 0.43 mM; (△): 0.29 mM; (○): 0.14 mM; (◑): 0.11 mM.

an apparent mol wt of approximately 32,000, we must consider whether these activities are the result of two or more distinct enzymes or are actually attributable to a single enzyme which cross-reacts with the pyridine nucleotides. Unfortunately, a definitive answer is not yet at hand. It has not been possible to resolve the two activities convincingly by DEAE-cellulose or cellulose-phosphate chromatography or by ammonium sulfate fractionation. We have reported a partial separation of two activities by centrifugal methods during subcellular localization studies (1). However, the low activities observed in those experiments keep them from being absolutely conclusive. NADH- and NADPH-linked IAAld reductase activities do differ markedly in such properties as substrate specificity and response to pH and NaCl concentration (3) as well as in their kinetic properties and responses to chemical inhibitors and metal salts (see below). This does not prove that there are separate NADH- and NADPH-specific enzymes, but it does keep the question alive. The interpretation of our other results does not depend crucially upon the resolution of this question. In what follows, we shall refer to NADPH- and NADH-linked "activities" without claiming that they either are or are not separate enzyme species. In any given experiment, only one reduced pyridine nucleotide was employed.

Effects of Chemical Inhibitors. The effects of several known enzyme inhibitors on the IAAld reductase activities are shown in Table I. This study was carried out without preincubation of enzyme with inhibitor. Thus, observed inhibitions were due to enzyme-inhibitor reactions which occurred during mixing or during the enzyme assay (several minutes duration). Quantitative differences between the NADH- and NADPH-linked IAAld reductase activities were observed with respect to metal-reactive inhibitors. While both activities were inhibited very slightly by sodium azide and strongly by EDTA, only the NADPH-linked activity was inhibited by sodium fluoride at concentrations up to 10 mM. The NADH-linked activity, on the other hand, appeared to be more sensitive to inhibition by cyanide.

Both iodoacetate and *N*-ethylmaleimide strongly inhibited these

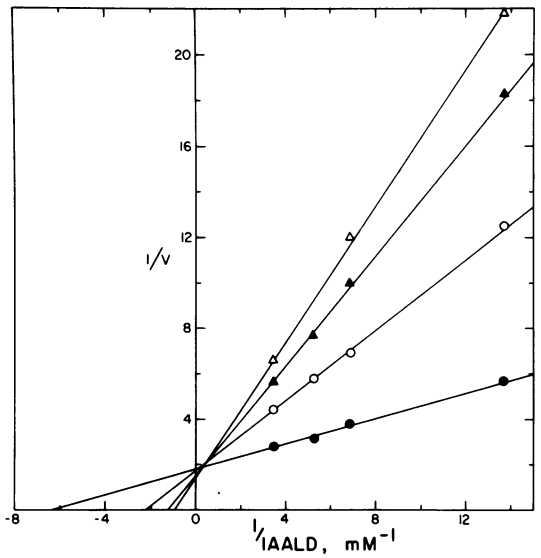

FIG. 3. Inhibition of the NADH-linked IAAld reductase by NaCl, with respect to IAAld. Reciprocal initial velocity *versus* reciprocal IAAld concentration is shown for four different concentrations of NaCl. The reaction mixture contained 0.15 mM NADH; and the reaction was started by the addition of 0.2 ml of enzyme preparation in 50 mM Na-phosphate (pH 7.5) to bring the total reaction volume to 1.0 ml. Velocity is normalized to the change in A_{340} per 1,000 s. Reaction concentrations of NaCl were: (●): 0.0 mM NaCl; (○): 50 mM; (▲): 100 mM; (△): 200 mM.

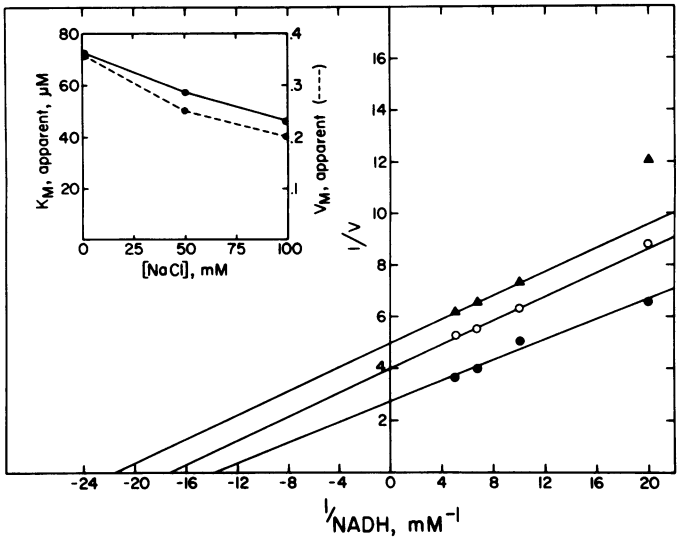

FIG. 4. Inhibition of NADH-linked IAAld reductase by NaCl, with respect to NADH. Reciprocal initial velocity *versus* reciprocal NADH concentration for three different NaCl concentrations. The reaction mixture contained 0.4 mM IAAld, and the reaction was started and the velocity expressed as in Figure 3. The ionic strength was 27.5 meq/l (based on phosphate ion $pK_a = 6.8$) before the addition of NaCl. Reaction concentrations of NaCl: (●): 0 mM; (○): 50 mM; (▲): 100 mM.

activities, with the latter being more effective. This provides partial but not conclusive evidence for the involvement of sulfhydryl groups in the action of IAAld reductase. Many NAD(P)H-linked enzymes have participatory sulfhydryls at their active sites (9).

Effects of Metal Salts. IAAld reductase, as isolated from the final gel filtration step, was not activated by the addition of various divalent metal salts. However, following exhaustive dialysis the NADPH-linked activity (but not the NADH-linked activity) became responsive to certain metal ions (Table II). At 2 mM, Mn^{2+}, Mg^{2+}, and Ca^{2+} activated the dialyzed enzyme between 20 and 40% over the 2 mM NaCl rate. These ions are also those which are most reactive with the inhibitors sodium fluoride, sodium azide, and EDTA (17). The results of Tables I and II thus suggest that IAAld reductase may depend upon one or more of these divalent metal cations.

Kinetics of IAAld Reductase with NADH as Co-substrate. Figure 2 shows the reaction kinetics of the NADH-linked IAAld reductase activity. The Lineweaver-Burk plots indicate a sequential mechanism (both substrates add to the enzyme before any product dissociates) and are consistent with either random or ordered addition of the substrates. The apparent K_m values from the data of Figure 2 are 500 μM and 140 μM for IAAld and NADH, respectively. We have noted some variability in the K_m values obtained from individual enzyme preparations; and, further, it will be shown that the apparent K_m values for both substrates are marked functions of the NaCl concentration. The discrepancy in K_m values for NADH between Figures 2 and 4 is attributable to the fact that different enzyme preparations were employed in the experiments and that the very low ionic strength (17 meq/l) in Figure 2 favors a high K_m value. For these reasons, we cannot yet determine the *in vivo* K_m values, if indeed the enzyme operates under steady-state kinetic conditions *in vivo*.

We have reported that NaCl concentration is a strong effector of IAAld reductase (3), the NADH-linked activity being inhibited by NaCl. Figures 3 and 4 show in more detail the nature of the inhibition by NaCl. It is uncompetitive with respect to NADH and competitive with respect to IAAld. Given that the addition of substrates is random, the simplest explanation for this kinetic pattern is that NaCl inhibits the binding of IAAld to both free enzyme and the NADH-enzyme complex. If the reaction mechanism is ordered, with NADH binding before IAAld, the action of NaCl is restricted to the inhibition of IAAld binding to the NADH-enzyme complex (15).

Kinetics of IAAld Reductase with NADPH as Co-substrate. In contrast with the NADH-linked activity, the NADPH-linked IAAld reductase activity is strongly increased by NaCl up to concentrations of 0.1 M (3). Figures 5 and 6 describe the kinetics of this activation. Lineweaver-Burk plots with respect to either

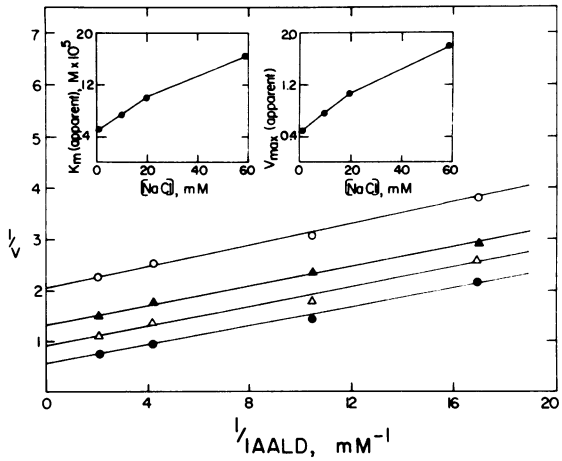

FIG. 5. Activation of NADPH-linked IAAld reductase by NaCl, with respect to IAAld. Reciprocal initial velocity *versus* reciprocal IAAld concentration for four different NaCl concentrations. The reaction mixture contained 80 μM NADPH; the reaction was started by the addition of 0.2 ml of enzyme preparation in 20 mM Na-phosphate (pH 7.5), to bring the total reaction volume to 1.1 ml. Velocity normalized to change in A_{340} per 100 s. Reaction concentrations of NaCl: (○): 0 mM; (▲): 10 mM; (△): 20 mM; (●): 60 mM.

substrate gave parallel lines for different concentrations of NaCl. (This is analogous to the situation observed with an uncompetitive inhibitor, but in this case NaCl is an activator rather than an inhibitor.) These results are consistent with the hypothesis that NaCl acts to accelerate a rate-limiting step past ternary complex (IAAld-enzyme-NADPH) formation. This hypothesis is valid whether the reaction mechanism involves either ordered addition or random addition of substrates, and whether the reaction is in steady-state or rapid equilibrium.

Figure 7 shows the reaction kinetics of the NADPH-linked IAAld reductase activity under conditions of low NADPH concentration (<40 μM) and low ionic strength (30 mM). Typical Lineweaver-Burk kinetics were observed. Under these conditions the apparent K_m values were 125 μM and 11 μM for IAAld and NADPH, respectively. It is important to note that the kinetic parameters (K_m and V_{max}) are strong functions of pH and ionic strength or NaCl concentration (Figs. 5 and 6, see also ref. 3), and it is impossible to make physiological inferences as to the *in vivo* enzymic affinity for the substrates in the absence of information concerning intracellular (or compartment) pH and ionic strength.

Marked deviations from the simple kinetics of Figure 7 were observed when the initial rate was examined over a much wider range of NADPH concentrations. Substrate (NADPH) inhibition occurred at high NADPH concentrations, and this inhibition was a strong function of the IAAld concentration (Fig. 8). The concentration of NADPH giving half-maximal inhibition increased from 0.2 to 0.8 mM as the IAAld concentration increased from 68 to 400 μM. The data of Figure 7 were obtained with concentrations of NADPH and IAAld within the steeply rising (noninhibited) range of Figure 8 and at low ionic strength. Note that the substrate inhibition shown in Figure 8 was observed at the relatively high ionic strength of 98 meq/l. In contrast, the NADH-linked activity showed no substrate inhibition under the same conditions of substrate concentration range and ionic strength.

A potentially artifactual situation is encountered when observing initial rates by the change with time in A_{340} at high NADPH concentrations (4, 5). At high concentrations of NADPH, the apparent molar extinction coefficient may deviate from its normal, low concentration value because of NAD(P)H fluorescence or

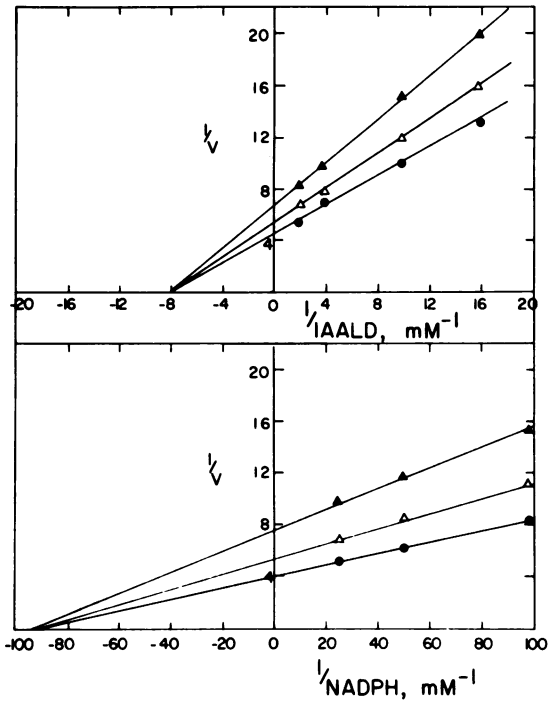

FIG. 7. Reaction kinetics of NADPH-linked IAAld reductase. Reactions started by addition of 0.2 ml of enzyme preparation in 50 mM Na-phosphate (pH 7.5) to bring total reaction volume to 0.9 ml. Substrates were without buffer, and the final ionic strength was 29 mM. Initial velocity normalized to change in A_{340} per 100 s. Top: reciprocal initial velocity versus reciprocal IAAld concentration at three different NADPH concentrations. (●): 40 μM NADPH; (△): 20 μM; (▲): 10 μM. Bottom: reciprocal initial velocity *versus* reciprocal NADPH concentration at three different concentrations of IAAld. (●): 0.51 mM IAAld; (△): 0.25 mM; (▲): 0.10 mM.

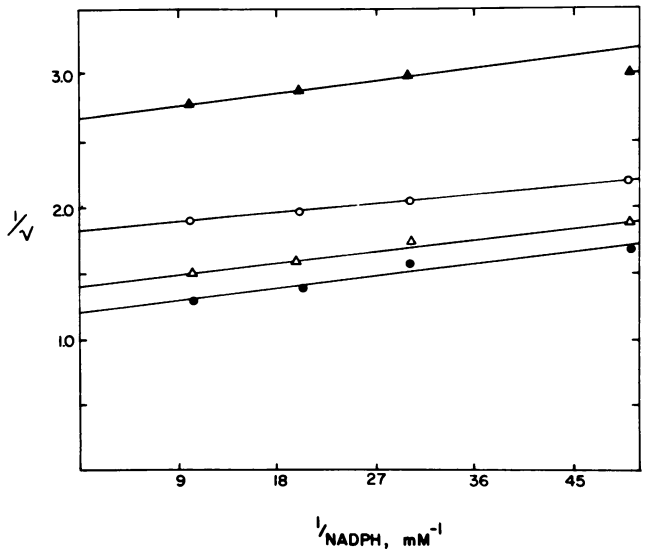

FIG. 6. Activation of NADPH-linked IAAld reductase by NaCl, with respect to NADPH. Reciprocal initial velocity *versus* reciprocal NADPH concentration for four different NaCl concentrations. The reaction mixture contained 0.2 mM IAAld, and the reaction was started and the velocity expressed as in Figure 5. Reaction concentrations of NaCl: (▲): 0 mM; (○): 10 mM; (△): 20 mM; (●): 30 mM.

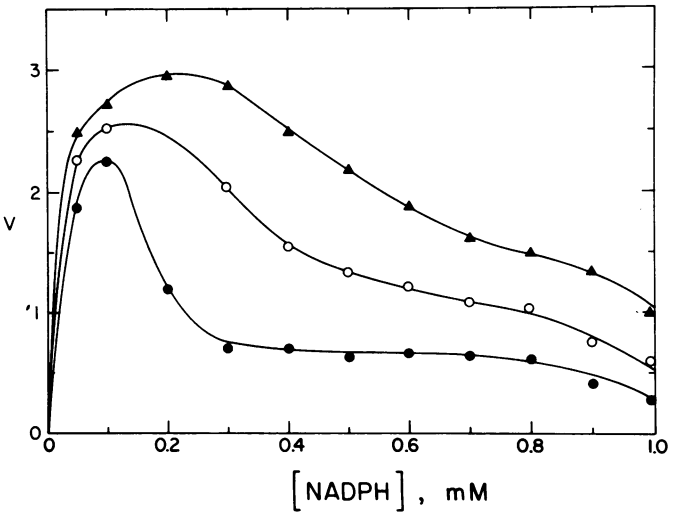

FIG. 8. Substrate inhibition of IAAld reductase by NADPH. Initial velocity *versus* NADPH concentration at three different concentrations of IAAld. Reaction mixtures consisted of 0.4 ml of IAAld in water, 0.2 ml of NADPH in 0.1 M Na-phosphate (pH 7.5), and 0.2 ml of enzyme preparation in 50 mM Na-phosphate (pH 7.5). Velocity expressed as change in A_{340} per 10^4 s as observed in a cuvette with a 2-mm optical path length. Ionic strength in this experiment was 97.5 meq/l. IAAld concentrations were: (▲): 0.4 mM; (○): 0.10 mM; (●): 0.06 mM.

inherent instrument stray light. Such effects lead to artifactually decreased apparent reaction velocities. This artifact was avoided by performing these experiments with a 2-mm path length cuvette yielding a constant extinction coefficient over an NADPH concentration range of 0–1 mM (0–1.25 *A*).

The substrate inhibition of the NADPH-linked activity by NADPH leads to other interesting kinetic properties. While the data of Figure 7 indicated hyperbolic kinetics with respect to IAAld at low, noninhibitory concentrations of NADPH, supraoptimal (inhibitory) concentrations led to sigmoidal kinetics with respect to IAAld (Fig. 9). The data are plotted by the Lineweaver-Burk method; nonlinear plots are indicative of nonhyperbolic kinetics, and concave plots (as obtained) are diagnostic of positively cooperative kinetics. While the double reciprocal plots for IAAld were linear at 0.2 mM NADPH, the plots became increasingly concave at 0.4, 0.6, and 0.8 mM NADPH.

Numerous models have been advanced to provide a theoretical basis for sigmoidal enzyme kinetics, but we have insufficient data to allow a definitive choice in the case of IAAld reductase. A model which appears to be particularly worthy of attention in this case is the purely kinetic model proposed by Ferdinand (6). This model postulates a two-substrate, random addition, nonprior equilibrium enzyme mechanism with a kinetically preferred pathway to the ternary complex. Subunit interactions are not necessary under these conditions to generate sigmoidal kinetics and substrate inhibition. IAAld reductase has a relatively low apparent mol wt; it shows substrate inhibition with respect to one substrate (NADPH) and sigmoidal kinetics with respect to the other (IAAld). These observations are all consistent with the Ferdinand scheme. Jensen and Trentini (8) have suggested that the D-*arabino*-heptulosonate 7-phosphate synthetase of *Rhodomicrobium vannielii* fits the Ferdinand model. They also based their arguments on the kinetic properties of the enzyme, which exhibits P-enolpyruvate-modulated substrate inhibition by erythrose-4-P and erythrose-4-P-modulated sigmoidal kinetics with respect to P-enolpyruvate.

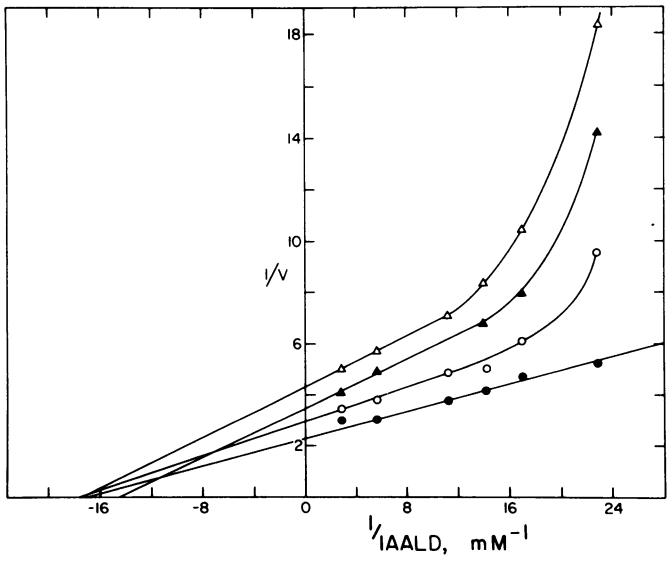

FIG. 9. NADPH-dependent cooperative kinetics with respect to IAAld. Reciprocal initial velocity *versus* reciprocal IAAld concentration for four different NADPH concentrations. Reaction mixture consisted of 0.4 ml of IAAld in water, 0.2 ml of NADPH in 0.1 M Na-phosphate (pH 7.5), and 0.2 ml of enzyme preparation, yielding a final ionic strength of 98 meq/l. Initial velocity is expressed as change in A_{340} per 1,000 s and was observed using a cuvette of 2-mm optical path length. NADPH concentrations: (●): 0.10 mM; (○): 0.30 mM; (▲): 0.50 mM; (△): 0.75 mM.

Possible Roles for IAAld Reductase in the Regulation of Auxin Biosynthesis. We have reported that IAA is a potent inhibitor of highly purified IEt oxidase (10), and experiments with a crude preparation of IAAld oxidase activity from cucumber seedlings have shown that this activity is inhibited by 2,4-D, a chemical analog of IAA (2). These results suggest that IAA regulates its own synthesis through feedback inhibition of the terminal enzyme of the pathway.

The enzyme kinetics described in this report suggest that IAAld reductase may also play a regulatory role in auxin biosynthesis. Increased activity of this enzyme would result in a diversion of flux away from IAA and toward a storage form, IEt, while a decrease in IAAld reductase activity would increase the rate of IAA production. The sigmoidal response of the IAAld reductase to IAAld may be significant in this regard. It could serve to buffer the rate of auxin synthesis against inordinate precursor flow. Higher concentrations of IAAld, by effectively activating the reductase, would divert the flux away from IAA and toward IEt.

A second, more speculative, suggestion arises from the observed substrate inhibition by NADPH. Its validity can be assessed only when methods are developed for the determination of NADPH concentration, ionic strength, and other relevant parameters within the cells and subcellular compartments in which IAAld reductase functions. If the usual concentration range of NADPH in the compartment in which IAA is produced should be supraoptimal with respect to the activity of IAAld reductase, the following picture would emerge. At higher concentrations of NADPH, the reductase would be substrate-inhibited, resulting in an increased flux to IAA. Thus, a high concentration of NADPH (presumably favorable to growth, which requires energy and reducing power) would be accompanied by a high level of available auxin. A drop in the NADPH concentration would alleviate the inhibition of the reductase, diverting the flux toward IEt accumulation; hence, the concentration of IAA would also drop. This would appear to be a useful mode of regulation—if it actually occurs.

We have now shown that three of the enzymes catalyzing the terminal reactions of auxin synthesis are subject to regulation *in vitro*. *In vivo* tracer kinetic studies have been initiated in order to test the relevance of these *in vitro* observations to the situation in the living plant.

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