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Regulation of β-Glucan Synthetase Activity by Auxin in Pea Stem Tissue

II. METABOLIC REQUIREMENTS[1]

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ABSTRACT

The 2- to 4-fold rise in particle-bound β-glucan synthetase (uridine diphosphate-glucose:β-1,4-glucan glucosyltransferase) activity that can be induced by indoleacetic acid in pea stem tissue is not prevented by concentrations of actinomycin D or cycloheximide that inhibit growth and macromolecule synthesis. The rise is concluded to be a hormonally induced activation of previously existing, reversibly deactivated enzyme. The activation is not a direct allosteric effect of indoleacetic acid or sugars. It is blocked by inhibitors of energy metabolism, by 2-deoxyglucose, and by high osmolarity, but not by Ca^{2+} at concentrations that inhibit auxin-induced elongation and prevent promotion of sugar uptake by indoleacetic acid, and not by α,α′-dipyridyl at concentrations that inhibit formation of hydroxyproline. Regulation of the system could be due either to an ATP-dependent activating reaction affecting this enzyme, or to changes in levels of a primer or a lipid cofactor.

Under conditions previously defined (37), treatment of pea stem tissue with IAA or other auxins causes a rapid, temperature-dependent rise in the level of particle-bound β-glucan synthetase activity (UDP-glucose:β-1,4-glucan glucosyltransferase) obtainable from the tissue. This report considers how this hormonal effect depends upon cellular metabolism. A somewhat similar investigation of the effect of IAA, applied in lanolin paste, on GDP-glucose-dependent glucan synthetase activity in stem tissue of whole decapitated pea seedlings was published recently (48).

METHODS

Plant material and glucan synthetase preparation and assay were as described previously (37). Briefly, pea stem segments 8 mm long cut from the region of maximal elongation rate were kept in a moist atmosphere at 35 C for 2.5 to 3 hr to reduce glucan synthetase activity substantially below the initial level; then a medium containing usually 30 mM sucrose was added and incubation at 35 C was continued for 1 hr. Then the tissue was treated with plus- or minus-IAA media at a specified temperature. In the case of inhibitor experiments the media also contained 16 mM K phosphate buffer, pH 6.1, and when necessary to ensure establishment of the appropriate inhibition by the time of addition of IAA the sucrose pretreatment media also contained the inhibitor, as specified for the experiments reported. At the end of the treatment period the tissue was chilled on ice, washed with ice water, and used for preparation and assay of glucan synthetase (37).

***In Vivo* Isotope Incorporation Experiments.** Samples of 50 segments were incubated in 3.0 ml of labeled substrate in 16 mM K phosphate buffer, pH 6.1, on a reciprocating shaker (120 oscillations/min) at 25 C. At the conclusion of incubation the segments were washed thoroughly with ice water. For assay of incorporation from sugars into particles and cell wall the segments were ground in the same manner (37) as for glucan synthetase assay; the cell wall residue (1,000*g*) and particle pellet (40,000*g*) from the procedure were suspended in scintillation fluid, in the case of the particle pellet only after it had been extracted overnight with 2:1 chloroform-methanol to remove lipids. For assay of incorporation from leucine into protein the segments were similarly ground in ice water, the cold 1,000*g* supernatant was made 9.0% in trichloroacetic acid, and precipitated protein was recovered by centrifugation and washed once with trichloroacetic acid and twice with 95% ethanol before being suspended in scintillation fluid for determination of radioactivity. In all cases the total absorbed radioactivity was estimated by adding the radioactivity found in the cell wall debris to the total radioactivity of the 1,000*g* supernatant as determined by scintillation counting of an aliquot thereof. Per cent incorporation means incorporated radioactivity as per cent of total absorbed radioactivity estimated as just stated.

Density Gradient Fractionation of *in Vivo*-labeled Particles. Resuspended particles prepared as described for glucan synthetase (37), but using ^{3}H-glucose-fed tissue, were layered over a 20 to 50% linear sucrose gradient containing 50 mM tris, pH 8.0; 1 mM EDTA; and 1 mM dithiothreitol, in a 2.5- × 7.5-cm tube. This was centrifuged for 3 hr at 25,000 rpm (65,000*g*) at about 4 C in a SW-25 rotor in the Spinco model L ultracentrifuge. The gradient was fractionated into 1.5-ml fractions, and duplicate aliquots of each were assayed for glucan synthetase as described above, ^{14}C in the product being determined by a scintillation spectrometer. The remainder of each fraction was diluted with an equal volume of homogenization medium and centrifuged at 48,000*g* for 30 min. The resultant pellets were suspended in scintillation fluid for determination of ^{3}H.

RESULTS

Effect of Inhibitors of RNA and Protein Synthesis. Experiment 1 of Table I shows the effect of pretreatment with actino-

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Table I. *Effect of Actinomycin D and Cycloheximide on Response of Glucan Synthetase to IAA*

After 2.5 hr in moist air at 35 C, tissue was pretreated for 1 hr at 35 C with 50 mM sucrose-16 mM K phosphate, pH 6.1, without or with antibiotic as indicated. Samples were assayed at the end of this pretreatment, and after an additional 1 hr at 25 C (35 C in experiment 3) in the same medium without or with addition of 17 μM IAA. Each figure is the mean of assays on duplicate tissue samples. Elongation was measured on parallel samples of segments that were incubated for 2 hr in plus-IAA medium after pretreatment.

Treatment	Glucan Synthetase Assay			Segment Elongation in the Presence of IAA
	End of Pretreatment	Minus IAA	Plus IAA	
	dpm			%
Experiment 1				
Control	3010	2730	6210	14
Actinomycin D, 25 μg/ml	3080	3610	6310	8
Cycloheximide, 10 μg/ml	3400	3720	6340	5
Experiment 2				
Control	2110	2820	5330	11
Cycloheximide, 60 μg/ml	2560	3260	4530	4.5
Experiment 3				
Control (35 C)	980	720	3590	15
Cycloheximide, 20 μg/ml (35 C)	1150	1630	3030	7

mycin D or cycloheximide at concentrations that are known to be strongly inhibitory to RNA or protein synthesis, respectively, and to growth. The inductive effect of IAA treatment on synthetase activity was not suppressed. Table III gives data on incorporation of leucine into protein showing that under the conditions used cycloheximide very strongly inhibits protein synthesis.

Because the failure of cycloheximide to prevent IAA-induced rise in synthetase activity was contrary to general experience with hormonal effects on enzyme activities, an extensive series of experiments with cycloheximide was run, including treatment at both 25 and 35 C, and with cycloheximide concentrations up to 60 μg/ml. The strongest effects obtained are shown as experiments 2 and 3, Table I. Inhibition was only partial even at 60 μg/ml, and under these conditions the possibility exists of an effect of cycloheximide on energy metabolism (12) which would be inhibitory to the synthetase response as detailed below. To complicate the interpretation, cycloheximide invariably caused some *increase* in synthetase activity in the minus-IAA control samples, which reduced (but did not eliminate) the difference between them and the IAA-treated samples.

Dependence on Energy Metabolism. The top section of Table II shows the effect, on the synthetase response to IAA, of some inhibitors and uncouplers of respiratory metabolism. Table III gives the effects of these agents under the same conditions on leucine incorporation into protein as a measure of the extent of their influence on energy metabolism. Each of these agents largely or entirely suppressed the promotive effect of IAA on synthetase activity. Furthermore, these agents caused a marked decline in the level of synthetase activity in the minus-IAA controls.

Effect of Nonmetabolic Inhibitors. Mannitol at 0.25 M and sucrose at 0.3 M largely prevented the increase in synthetase activity in response to IAA, as illustrated by data given in Table II. At this osmolarity, elongation of the tissue in response to IAA is largely suppressed. On the other hand, inhibition of elongation by Ca^{2+} did not prevent the response of synthetase activity to IAA, as shown by data given in Table IV.

Reversal of Auxin Effect by Inhibitors. The right-hand column in Table V shows the result of adding 1 mM KCN or 0.2 M mannitol to segments 1 hr after addition of IAA, a time at which a large increase in synthetase activity had been induced

Table II. *Effects of Metabolic and Osmotic Inhibitors on Glucan Synthetase Response*

After 2.5 hr in moist air at 35 C, tissue was pretreated with 50 mM sucrose in 16 mM K phosphate buffer, pH 6.1, plus inhibitor where so indicated, for 1 hr at 35 C, then incubated in sucrose-phosphate plus inhibitor with or without 17 μM IAA at 25 C for 1 hr (2 hr in the last two experiments).

Inhibitor	Conc.	Glucan Synthetase Assay	
		Minus IAA	Plus IAA
		dpm	
None (control)	...	1700	4430
KCN	1 mM	680	790
NaN_3	2 mM[1]	890	1200
2-4-Dinitrophenol	0.3 mM[1]	1030	1780
CCCP	0.15 mM[1]	950	1980
None (control)	...	2975	4690
Mannitol	0.25 M	2430	2510
Sucrose	0.30 M	1970	2300
None (control)	...	2200	5730
2-Deoxyglucose	50 mM	750	1210
2-Deoxyglucose	50 mM[1]	450	880
None (control)	...	3450	6200
2,2'-Dipyridyl	0.2 mM[1]	...	6960

[1] Pretreated with inhibitor for 1 hr prior to addition of IAA to the plus-IAA samples.

Table III. *Effects of Inhibitors on Protein and Cell Wall Polysaccharide Synthesis and on Incorporation into Particles*

Tissue was pretreated as in comparable experiments in Tables I and II, 0.5 μc of ^{14}C-leucine or ^{14}C-sucrose (uniformly labeled) was then added, and incubation continued for 1 hr at 25 C. Each figure is the mean of duplicate samples.

Inhibitor	Concn.	^{14}C-Leucine		^{14}C-Sucrose		
		Uptake relative to control	Incorporation[1] into Protein	Uptake relative to control	Incorporation[1] into Cell wall	Incorporation[1] into Cell particles
	mM	%	%	%	%	%
None (control)	...	100	24.5	100	14.4	4.6
Cycloheximide	0.035	54.6	2.9	64.3	13.0	5.2
Cycloheximide	0.21	40.6	2.3	61.0	14.6	5.7
KCN	1.0	44.9	2.1	72.3	1.4	1.0
NaN_3	2.0	32.3	2.3	...	...	...
2,4-Dinitrophenol	0.3	36.8	5.3	...	...	...
CCCP	0.15	34.7	4.6	32.1	1.9	1.5
2-Deoxyglucose	50.0	44.4	26.0	48.3	0.8	1.2
Mannitol	250.0	...	...	86.9	11.9	3.9

[1] Incorporation as percentage of uptake.

as shown by the control (IAA without inhibitor) data in the left-hand column. One hour later the synthetase activity had been reduced to the control (minus-IAA) level by mannitol and below the control level by cyanide. These agents therefore cause a rapid and complete reversal of the IAA effect on synthetase activity.

Effect of 2,2'-Dipyridyl. To test whether formation of hydroxyproline-containing protein might be involved in the auxin regulation of synthetase activity, the effect of 2,2'-dipyridyl was determined. At 0.2 mM this agent had no effect on the increase in synthetase in response to IAA (Table II), even though concentrations lower than 0.2 mM are known to inhibit hydroxylation of proline completely (2, 9).

Effects of Different Carbohydrates. Table VI summarizes results of a number of experiments in which the effectiveness of various sugars and related compounds was compared with that of sucrose in supporting the induction of synthetase activity by IAA. The naturally occurring hexoses were all effective, but somewhat less so than sucrose at equal concentrations; the same was true of the disaccharide maltose, whereas cellobiose was somewhat less effective. The natural pentoses D-ribose and L-arabinose were only moderately effective while D-arabinose, the methyl pentose L-fucose, and the sugar alcohol mannitol had virtually no effect, as did acetate. Results with various other compounds, omitted from Table VI because they were not used also in incorporation experiments, are as follows (all tested at 30 mM). Equimolar mixtures of glucose and fructose had the same effect as glucose or fructose separately and did not equal the effect of sucrose. The trisaccharide raffinose was as effective as maltose; the trisaccharide melezitose, disaccharides melibiose, trehalose, and lactose, hexoses D-sorbose and L-rhamnose, and glycerol, had effects comparable with natural pentoses (25–40% of the effect of sucrose). The disaccharide gentiobiose, the glycoside β-methylthiogalactoside, and the hexose relatives 3-*O*-methylglucose, myoinositol, and L-ascorbic acid had no effect.

The impression created by this survey is that carbohydrates that might plausibly be converted metabolically to glucose and glucan products are active in the response, those for which conversion is probably marginal are marginally active, while compounds that cannot be converted to glucose are inactive, even if (like acetate) they are metabolizable in respiration. Incorporation tests with labeled sugars described below substantially verified this supposition.

Table IV. *Effect of IAA on Glucan Synthetase Activity and on Uptake and Incorporation from Labeled Sugars in Presence of Ca^{2+}*

After 2.5 hr in moist air at 35 C plus 1 hr pretreatment at 35 C with 20 mM $CaCl_2$ containing sugar (no IAA) at indicated concentrations, samples of 50 segments were incubated 2 hr at 25 C in 20 mM $CaCl_2$, plus ^{14}C-sugar (0.10 μc/μatom C) and 17 μM IAA where indicated. Ca^{2+} plus IAA-treated segments elongated 4%, whereas comparable IAA controls without Ca^{2+} elongated 12%.

Treatment, 20 mM $CaCl_2$ plus	Glucan Synthetase Assay	Uptake and Incorporation of ^{14}C-Sugar		
		Cell wall	Particles	Total uptake
	dpm	*dpm*		
30 mM sucrose	1,720	14,300	2,690	75,000
IAA + 30 mM sucrose	5,920	18,360	3,510	77,100
60 mM sucrose	1,920	23,400	4,500	131,500
50 mM glucose	1,820	8,200	1,840	46,800
IAA + 50 mM glucose	4,600	11,600	2,320	48,100

Table V. *Effect of Inhibitors When Added after IAA*

After 2.5 hr in moist air at 35 C and incubation in 50 mM sucrose for 1 hr at 35 C, tissue was placed in sucrose without or with 17 μM IAA at 25 C (zero time). Samples were transferred from sucrose-IAA to the same medium plus KCN or mannitol, or chilled to 2 C, at 10 min and at 60 min, the former set being worked up at 60 min for synthetase assay along with plus- and minus-IAA controls, and the latter set being assayed along with further controls at 120 min.

Treatment	Glucan Synthetase Activity at	
	60 min	120 min
	dpm	
No IAA	2050	2730
IAA	5375	6525
IAA → (KCN [1 mM] + IAA)	1125[1]	1790[2]
IAA → (mannitol [0.2 M] + IAA)	2340[1]	2780[2]

[1] Transferred to inhibitor media at 10 min.
[2] Transferred to inhibitor media at 60 min.

Table VI. *Effects of Carbohydrates on Glucan Synthetase Activity and on in vivo Incorporation*

After 2.5 hr in moist air at 35 C tissue was treated for 2 hr at 25 C with 17 μM IAA alone or plus one of the substrates (30 mM). Synthetase data express the increase in synthetase activity above that in IAA alone, as a per cent of the increase caused by sucrose. Incorporation was measured using similarly treated tissue incubated 1 hr at 25 C in 30 mM ^{14}C-substrate containing 0.33 μc/ml (sample size, 50 segments).

Substrate (30 mM)	Effect on Synthetase Activity (+ IAA) Relative to Sucrose	Uptake Relative to Sucrose	Incorporation into	
			Cell wall	Particles
	%	%	*μatoms C/sample*	
Sucrose	100	100	69	17.1
Glucose	63	78	51	12.6
Mannose	68	74	34	6.9
Galactose	64	68	25	6.5
Maltose	67	46	40	9.1
Cellobiose	56	62	26	8.4
D-Ribose	35	74	19	4.4
L-Arabinose	41	97	4.3	1.6
D-Arabinose	4	79	0.8	0.6
L-Fucose	0	70	0.9	0.7
D-Mannitol	6	41	7.7	2.6
Acetate	0	156	1.2	0.9

2-Deoxyglucose strongly inhibits the synthetase response to IAA (Table II). Like inhibitors of energy metabolism, 2-deoxyglucose also depressed the minus-IAA synthetase level substantially below the control value. However, as shown by data in Table III, 2-deoxyglucose did not block energy metabolism as judged by the energy-dependent incorporation of leucine into protein. Incorporation of sugars into wall polymers was, however, strongly inhibited by 2-deoxyglucose, as considered in the next section.

Relation between Effects on Synthetase Activity and Incorporation into Particles and Cell Wall. Data in Table III show that 2-deoxyglucose, KCN, and CCCP,[2] at concentrations

[2] Abbreviation: CCCP: carbonyl cyanide *m*-chlorophenylhydrazone.

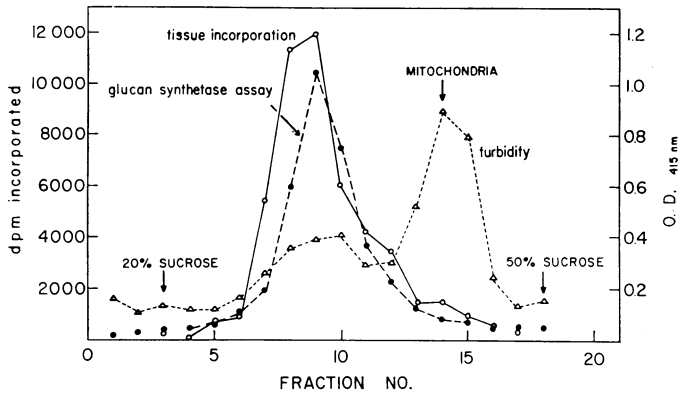

FIG. 1. Isopycnic linear 20 to 50% sucrose density gradient profile of ^{3}H-labeled particles from pea stem tissue incubated 15 min in ^{3}H-glucose (○). Aliquots of the gradient fractions were assayed for glucan synthetase activity using UDP-^{14}C-glucose (●). Turbidity (△) was assayed by measurement of absorbance at 415 nm. The major turbidity peak toward the bottom of the gradient is due to the mitochondria (38).

that suppress the response of synthetase activity to IAA and cause activity to decline below the control level, strongly inhibit incorporation from sucrose into the cell wall and into cytoplasmic particles of the standard synthetase preparation. Cycloheximide, on the other hand, did not inhibit these incorporation phenomena appreciably, and 0.25 M mannitol inhibited only moderately.

Figure 1 shows the isopycnic sucrose density gradient profile obtained by ultracentrifugation of cell particles prepared from pea tissue that had been incubated in labeled glucose. The incorporated radioactivity peaked sharply at the same density as that of the particles that bear glucan synthetase activity (38) and did not correspond with the distribution of cell particles in general, as shown by the turbidity profile. This is consistent with the assumption that incorporation into particles shown in Tables III, IV, and VI represents polysaccharide products associated with the glucan synthetase-bearing membrane elements.

Selected ^{14}C-labeled compounds from the list of those surveyed for activity in enhancing the synthetase response were tested for utilizability in cell wall synthesis and incorporation into particles as shown in Table VI. Sucrose gave the greatest incorporation while hexoses and maltose, which at the same concentration are somewhat less active in the synthetase response than sucrose, gave somewhat less incorporation. Cellobiose, D-ribose, and L-arabinose gave modest incorporation while that from D-arabinose, L-fucose, and acetate, which are inactive in the synthetase response, was negligible. Incorporation from mannitol was not negligible but was small compared to that from sugars that have a substantial effect on the synthetase response.

Data in Table VI indicate that the differences observed between sugars were due mainly to differences in metabolizability rather than in uptake.

Relation between IAA Effect and Sugar Uptake. In the absence of inhibitors IAA causes an increase in uptake of sugar from the medium (1). In view of the dependence of glucan synthetase activity upon exogenous sugar supply, it appeared possible that the increase in synthetase activity caused by IAA is actually due to promotion of sugar uptake by IAA. This was tested by treating with 20 mM $CaCl_2$, which is known to prevent promotion of sugar uptake by IAA (1). Results in Table IV show that a substantial effect of IAA on synthetase activity can be obtained without an appreciable increase in uptake, either of sucrose or of glucose. Incorporation into particles and cell wall is still increased by IAA under these conditions. Data in Table IV also show that only a minor increase in synthetase activity occurred when sucrose uptake was nearly doubled by raising the concentration of sucrose supplied to the tissue, in the absence of IAA.

These findings as well as uptake data given in Table III indicate that suppression of the glucan synthetase response to IAA by metabolic and osmotic inhibitors (Table II) is not due mainly to their effects on sucrose uptake. Cycloheximide does not suppress the synthetase response even though it reduces sucrose uptake (Table III). Furthermore, a substantial difference between minus- and plus-IAA treatments is observed in the complete absence of exogenous sugar (37), so even were the inhibitors to prevent sucrose uptake entirely (which they do not) this could not suppress the IAA effect as recorded in Table II.

DISCUSSION

The inductive effect of IAA treatment on β-glucan synthetase activity of pea tissue is not suppressed by concentrations of actinomycin D and cycloheximide that block RNA (54) and protein (Table III) synthesis in pea tissue, concentrations that completely prevent typical enzyme inductions by *de novo* protein synthesis in plant tissues (16, 18, 25, 28, 30), including pea (16, 23). I conclude, therefore, that the IAA effect is an activation of pre-existing enzyme rather than being due to new enzyme formation. This conforms with the fact that a substantial activation by IAA may be demonstrated only if the synthetase activity in freshly cut pea segments is allowed first to decline, and activation usually leads at most to a restoration of the initially existing level of activity (37). IAA appears to cause reversal of a temperature-dependent deactivation process that operates on the enzyme system in the absence of the hormone. The auxin-synthetase system is, therefore, of special interest as a type of hormonal regulation of enzyme activity different from that of *de novo* protein synthesis, upon which almost all emphasis has been placed in recent work with plant hormones and growth regulators (16, 18, 28, 30). It may perhaps be comparable with a variety of hormonal activation effects now known to influence enzyme activities in animal tissues—for example, those involving cyclic-AMP (39)—or with various nonhormonal enzyme activation effects known in plant systems (7, 19, 22, 31, 44, 45, 57).

Contrary to the results presented here, Spencer *et al.* (48) found that cycloheximide largely prevented protective and inductive effects of IAA on glucan synthetase activity (GDP-glucan as donor) of pea stem tissue as followed over periods of days in decapitated whole plants treated with IAA in lanolin paste. They concluded that regulation of activity probably involves turnover and synthesis of glucan synthetase protein. Hall and Ordin (20) also concluded from a cycloheximide experiment that the IAA effect on glucan synthetase observed in *Avena* coleoptiles depends upon protein synthesis. Induction of polysaccharide synthetase activity in certain other systems seems probably to be due to enzyme synthesis (11, 29, 36). The present experiments indicate by contrast an activation/deactivation mode of short term regulation of β-glucan synthetase activity in pea that may be analogous to known mechanisms of regulation of glycogen synthetase activity (6, 43, 47).

That the activation phenomenon is not due to a simple effector action of IAA or other auxins is indicated by the failure to obtain this effect by adding IAA over a wide concentration range *in vitro* to synthetase particles capable of activation *in vivo* (37). A small and variable activating effect of 2,4-D upon a glucan synthetase preparation from onion was reported

recently (52). However, no such effect could be obtained with the present system (37) despite its much larger capacity for activation by IAA *in vivo*.

From the results with inhibitors and uncouplers of respiration it is concluded that activation of glucan synthetase depends upon energy metabolism, presumably upon ATP. This is probably among the reasons why activation is not obtained *in vitro* by simply adding IAA or other auxins to isolated particles, nor *in vivo* by treatment with IAA in the cold (37).

Data given here and in Reference 37 show that activation depends upon availability of a metabolizable carbohydrate. This requirement cannot be satisfied by noncarbohydrate respiratory substrates such as acetate and appears, therefore, to be additional to the requirement for energy metabolism. Inhibition of activation by 2-deoxyglucose confirms this since, as in other systems (10, 40, 55, 56), 2-deoxyglucose does not suppress respiratory energy metabolism in peas (judged by incorporation of leucine into protein) but blocks severely the utilization of hexose for polysaccharide synthesis, again as in other systems (4, 14, 24, 58) in which this effect is due to formation of deoxyhexose nucleotides (13, 17, 27, 58).

Suppression of activation by mannitol and high concentrations of sucrose suggests that activation depends upon normal turgor pressure. Since this is a somewhat surprising requirement for a biochemical phenomenon, alternative interpretations have been considered. Osmotic inhibition of activation cannot be regarded as a consequence of inhibition of elongation, because IAA does activate the synthetase in nonelongating basal stem segments (37) and in segments whose elongation is inhibited by Ca^{2+} or cycloheximide. Since loss of turgor can cause a rise in the level of abscisic acid in pea tissue (46), I tested the effect of abscisic acid upon the activation response to IAA. No inhibition whatever was given by 10 μM and 0.1 mM abscisic acid, from two sources, in 2-hr experiments similar to those presented in "Results."

The requirement for turgor suggests that activation may depend upon some relatively subtle aspect of intact cell function such as occurrence of normal transport and secretory operation of the Golgi system.

In view of the effect of sugar supply on glucan synthetase activity, I considered whether the activating effect of IAA could be due simply to the fact that IAA promotes uptake of sugars by pea stem segments (1). This relatively trivial explanation is ruled out by the experiment of Table IV, which shows that, in presence of Ca^{2+}, IAA activates glucan synthetase without promoting sucrose or glucose uptake, and contrariwise an increase in sucrose uptake caused by raising the sucrose concentration of the medium does not lead to a substantial activation comparable to that caused by IAA.

Activation, presumably allosteric, of several polysaccharide synthetase systems by various sugars has been reported (15, 26, 34, 35, 42, 49, 51). However, under the conditions of the present glucan synthetase assay sucrose, fructose, or other carbohydrates that promote *in vivo* activation of pea glucan synthetase (Table VI) do not affect activity when added directly to the synthetase particles, even if these are prepared without the usual high concentration of sucrose in the homogenization and resuspension media. Therefore, it seems excluded that the activation of glucan synthetase by IAA is via effector action of sugars. Involvement of other effectors in the IAA response is not ruled out by data given here, but it is somewhat improbable because the assay is run with substantially washed particles.

Further alternatives, and their precedents in the literature on polysaccharide synthetase systems, that should be considered as possible mechanisms of regulation of glucan synthetase activity in response to IAA include product inhibition (32), dependence upon a primer or glucosyl acceptor (6, 21, 33, 35, 49), occurrence of activating and deactivating reactions operating on the enzyme (5, 8, 43), dependence upon a lipid factor (3, 32, 41, 50, 53), and change in permeability of the membrane particles to substrate (35).

The promotive effect of sugars that are utilizable for polysaccharide synthesis speaks against product inhibition as the principal mode of regulation and in favor of regulation by the level of a polysaccharide primer. However, requirement for a primer could constitute a *necessary* condition for activation by IAA via any of the other mechanisms mentioned. The curious transient effect of sucrose alone on synthetase activity suggests a dual mode of regulation first relative to the supply of sugar and second relative to the presence or absence of auxin, as does the failure of higher sucrose concentrations to duplicate the activating effect of IAA. The dependence upon energy metabolism is compatible with a mechanism of phosphorylative activation and dephosphorylative deactivation of glucan synthetase analogous to known mechanisms of regulation of glycogen phosphorylase and glycogen synthetase (6, 39, 43, 47). To distinguish conclusively among these and other possible activation mechanisms requires experiments at the enzymological level, especially with solubilization of enzyme activity, experiments that are currently in progress.

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