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Enzymic Synthesis of Indole-3-Acetyl-1-*O*-β-D-Glucose[1]

II. METABOLIC CHARACTERISTICS OF THE ENZYME

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ABSTRACT

The synthesis of indole-3-acetyl-1-*O*-β-D-glucose from indole-3-acetic acid (IAA) and uridine diphosphoglucose (UDPG) has been shown to be a reversible reaction with the equilibrium away from ester formation and toward formation of IAA. The enzyme occurs primarily in the liquid endosperm of the corn kernel but some activity occurs in the embryo. It is relatively specific showing no glucose ester formation with oxindole-3-acetic acid or 7-hydroxy-oxindole-3-acetic acid, and low activity with phenylpropene acids, such as ρ-coumaric acid. The enzyme is also specific for the nucleotide sugar showing no activity with UDPGalactose or UDPXylose. The enzyme is inhibited by inorganic pyrophosphate, by phosphate esters and by phospholipids, particularly phosphatidyl ethanolamine. The enzyme is inhibited by zeatin, by 2,4-dichlorophenoxyacetic acid, by IAA-*myo*-inositol and IAA-glucan, but not by zeatin riboside, and only weakly by gibberellic acid, abscisic acid, and kinetin. The reaction is slightly stimulated by both calcium and calmodulin and, in some cases, by thiol compounds. The role of this enzyme in the homeostatic control of indole-3-acetic acid levels in *Zea mays* is discussed.

The preceding paper (13) describes a method for the extraction and partial purification of the enzyme catalyzing the formation of indole-3-acetyl-1-*O*-β-D-glucose from UDPG[3] and IAA. In addition, certain physical parameters of the enzyme including its mol wt, pH optimum and stability were provided and an assay method described. In this work, we describe the substrate specificity of the enzyme, its sensitivity to inhibitors, the reversibility of the reaction, how this enzyme could function in the control of levels of free IAA, and our attempt to prepare antibodies to the enzyme.

MATERIALS AND METHODS

The enzyme assay, purification procedure, and all analytical techniques were as described previously (13). Briefly, the assay depends upon separating the uncharged reaction product, 1-[^{14}C]IAA-glucose, from the negatively charged substrate, 1-[^{14}C]IAA, by means of a DEAE column and measuring the radioactivity not retained by DEAE. This neutral product was shown to be indole-3-acetyl-1-*O*-β-D-glucose by comparison with the authentic compound on HPLC and by GC/MS.

Uridine diphospho-D-[U-^{14}C]glucose (200 mCi·mmol^{-1}) was from Amersham as was 1-[^{14}C]indole-3-acetic acid (50 mCi·mmol^{-1}). All other materials used were as for the preceding paper (13).

RESULTS

SUBSTRATE SPECIFICITY

Nucleotide Sugar. As shown by the data of Table I-A, the enzyme is specific for UDPG and will not make an ester from labeled IAA and UDPGal or UDPXylose, the activity with these two latter substrates being zero. A previously published study (14) established that the reaction would only use the uridine nucleotide, and would not use ADP, CDP, or GDP-glucose. Thus, specificity for the nucleotide sugar is stringent.

IAA Oxidation Products. Table I-B shows the specificity of the enzyme for the aromatic acid acceptor for labeled glucose using UDP-[U-^{14}C]glucose as glucosyl donor. The enzyme uses IAA as the glucosyl acceptor but will not use the oxidation products of IAA, either OxIAA, or 7-OH-OxIAA (12, 15, 18, 19) as acceptors. Thus, although conjugates of IAA oxidation products have been shown to occur in nature (20), this enzyme will only use the unoxidized hormone as glucose acceptor.

Aromatic Acids. The ability of the enzyme, at stage 4 purification, to use the phenylpropene, lignin-related, acids as glucose acceptors was investigated. For these studies, UDP-[U-^{14}C]glucose was used as donor and the amount of labeled ester formed was determined. As can be seen from Table I-C, all of the phenyl propene acids showed between 4 and 10% of the activity of IAA. Parahydroxycinnamic acid (coumaric acid) most closely resembles IAA in physical properties and is, in fact, so difficult to separate from IAA, by any commonly used chromatographic procedure, that it is sometimes mistakenly misidentified as IAA, as has previously been discussed (3). Even coumaric acid shows only 10% of the reactivity of IAA. A related experiment, using labeled IAA, and unlabeled UDPG and phenylpropene acid, and measuring inhibition of IAA ester formation, was also performed with nearly identical results as is shown in Table II.

Inhibition of IAGlu Synthase by Other Plant Hormones. The ability of other plant hormones, a hormone conjugate, and several growth regulators to inhibit the synthesis of IAGlu from IAA and UDPG was determined as shown in Table III. Among the hormones tested, zeatin, when present in equimolar amounts to IAA, caused an almost 40% inhibition. Zeatin riboside, kinetin, abscissic acid, and gibberellin A_3 gave less inhibition. Indole-3-acetyl-*myo*-inositol, the next product in the IAA conjugation

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[3] Abbreviations: UDPG, uridinediphosphoglucose; IAGlu, indole-3-acetyl-1-*O*-β-D-glucopyranoside; OxIAA, oxindole-3-acetic acid; 7-OH-OxIAA, 7-hydroxy-oxindole-3-acetic acid; G-1-P, glucose-1-phosphate; TMS, trimethylsilyl.

Table I. *Substrate Specificity of IAGlu Synthase*

Incubation was for 30 min at 37°C. The incubation mixture of 0.5 mL and at pH 7.6 contained: Experiment A, 200 μL 0.1 M Hepes buffer, 100 μL 50 mM mercaptoethanol, 100 μL Hepes buffer containing 5 μmol of the nucleotide tested, 2 μmol of IAA in 50 μL of [^{14}C]IAA containing 0.05 μCi in 50% 2-propanol, and 50 μL of enzyme to initiate the reaction. Experiment B, 200 μL 0.1 M Hepes buffer containing 1 μmol of [^{14}C] UDPG, 0.1 μCi, 100 μL 50 mM mercaptoethanol, 100 μL 0.1 M Hepes buffer containing 0.5 μmol of the organic acid to be tested, 50 μL of distilled water, and 50 μL of enzyme to initiate the reaction. Experiment C, 150 μL of Tris-HCl buffer containing 0.5 μmol of the organic acid tested, 100 μL of 50 mM 2-mercaptoethanol, 200 μL of distilled water containing 1 μmol of [^{14}C]UDPG, 0.1 μCi, and 50 μL of enzyme.

Substrate Tested	Final Concentration	Enzyme Activity	Relative Enzyme Activity
	mM	*munits/tube*	%
A. Sugar donor			
UDPG	10.0	60.8	100.0
UDPGal	10.0	0.0	0.0
UDPXyl	10.0	0.0	0.0
B. Glucose acceptor			
IAA	1.0	57.6	100.0
OxIAA	1.0	0.0	0.0
7-OH-OxIAA	1.0	0.0	0.0
C. Glucose acceptor			
o-Hydroxycinnamic acid	1.0	4.4	7.6
m-Hydroxycinnamic acid	1.0	2.5	4.3
p-Coumaric acid	1.0	5.9	10.2
3-Hydroxy-4-methoxy cinnamic acid	1.0	5.4	9.4
Ferulic acid	1.0	4.5	7.8
3,4-Dimethoxycinnamic acid	1.0	3.5	6.1
2,5-Dimethoxycinnamic acid	1.0	2.5	4.3

sequence (7; Fig. 1 in Ref. 8), showed less than 15% inhibition. In four times molar excess, 2,4-dichlorophenoxyacetic acid caused an almost 40% inhibition, whereas the tropism inhibitor *N*-1-napthylphtalamic acid (6), caused little inhibition.

Effect of Calcium, Calmodulin, Metal Ions, and Chelators on IAGlu Synthase Activity. Calcium, either alone or with bovine brain or spinach calmodulin, was only weakly stimulatory, but the effect was consistent and reproducible, as shown in Table IV. By contrast, manganese, EDTA, and thiol compounds are stimulatory for the enzyme after DEAE-Sephacel or Sephadex G-100 chromatography, at purity stage 4, as seen in Table V. The degree of response to metal ions, chelators, and thiol compounds varied from preparation to preparation.

Effect of Phospholipids on IAGlu Synthase Activity. The addition of phopholipids inhibited IAGlu synthase activity (Table VI). Owing to poor solubility and the relative impurity of the phospholipids tested, it is impossible to compare them on a molar basis for strength of inhibition. However, as can be seen, phosphatidyl choline, phosphatidyl inositol, and phosphatidyl ethanolamine are all strong inhibitors, and phosphatidyl ethanolamine is a stronger inhibitor than is phosphatidic acid. A sample of dolichol phosphate was only weakly inhibitory.

Effect of Phosphate Compounds and Sugar Derivatives on the Activity of IAGlu Synthase. As shown by the data of Table VII, G-1-P, in the presence of UDPG does not inhibit the glucosylation of IAA by UDPG, nor will G-1-P act as a glucosyl donor.

Table II. *Inhibition by Aromatic Organic Acids of IAGlu Synthesis by IAGlu Synthase*

Incubation, volume, and pH was similar to Table I. The incubation mixture contained: 150 μL of Tris-HCl buffer containing 1 μmol of the acid to be tested, 100 μL of 50 mM mercaptoethanol, 100 μL of water containing 3 μmol of UDPG, 100 μL of enzyme after DEAE-Sephacel step, and 50 μL of 50% 2-propanol containing 1 μmol of IAA with 0.05 μCi of [^{14}C]IAA. A zero time blank was prepared with 0.5 mL of 2-propanol and this blank was subtracted from the tabulated values.

Inhibitor	Enzyme Activity	Relative Enzyme Activity
	nmol IAA-glu·tube^{-1}·30 min^{-1}	%
None (control)	80.7	100
o-Hydroxycinnamic acid	73.6	91
m-Hydroxycinnamic acid	75.5	94
p-Hydroxycinnamic acid (coumaric acid)	73.1	91
3-Hydroxy-4-methoxycinnamic acid	77.5	96
4-Hydroxy-3-methoxycinnamic acid (ferulic acid)	74.1	92
3,4-Dimethoxycinnamic acid (*trans*)	75.4	94
2,5-Dimethoxycinnamic acid (*trans*)	66.5	82

Table III. *Inhibition by Plant Hormones and Hormone Analogs of IAGlu Synthesis*

Reaction conditions were as for Table II except that 0.5 μmol of the compound to be tested was converted to the sodium salt, if necessary, and then dissolved in 150 μL of Hepes buffer rather than Tris-HCl.

Compound Tested	Final Concentration	Enzyme Activity	Relative Enzyme Activity
	mM	*nmole-tube^{-1}·min^{-1}*	%
None		72	100
Zeatin	1.0	44	62
Zeatin riboside	1.0	67	93
Kinetin	1.0	63	88
ABA	1.0	63	88
GA_3	1.0	60	82
None		243	100
IAA-*myo*-inositol	2.0	208	86
IAA-*myo*-inositol	4.0	182	75
2,4-D	2.0	196	81
2,4-D	4.0	153	63
Naphthylphthalamic acid	2.0	220	91
Naphthylphthalamic acid	4.0	200	82

G-1-P, plus UTP is a strong inhibitor, but this is possibly owing to the formation of inorganic pyrophosphate since pyrophosphate alone gives a similar inhibition. Of interest is the strong inhibition caused by the naturally occurring, A fraction. The A fraction is an IAA-β-1,4-glucan containing between 8 to 50 glucosyl residues (17) and constitutes as much as 50% of the IAA ester of mature kernels of *Zea mays* (21). The IAA-glucan is very insoluble in aqueous buffer and thus the concentration present in the reaction mixture was approximately one-half the indicated 0.1%.

Table IV. *Effect of Calcium Ion and Calmodulin on IAGlu Synthesis*

The enzyme was used after DEAE-Sephacel chromatography and had 2100 munit·ml^{-1}·30·min^{-1}. All other conditions were as for Table II. Results are the means of triplicate determinations minus a blank.

Compound Tested	Experiment I	
	Concentration	Enzyme activity
	μmol·tube^{-1}	*nmol tube*$^{-1}$·*30 min*$^{-1}$
Control		103
$CaCl_2$	2.5	115
Calmodulin[a]	250 units	113
$CaCl_2$	2.5	
Calmodulin[a]	250 units	116
	Experiment II	
Control		103
$CaCl_2$	2.5	111
Calmodulin[b]	100 units	108
$CaCl_2$	2.5	
Calmodulin[b]	100 units	107

[a] From bovine brain. [b] From spinach.

Table V. *Effect of Various Compounds on IAGlu Synthase Activity*

The compounds tested were dissolved in water and the assay was as in Table II.

Compound Tested	Concentration	Enzyme Activity	Control
	μmol/tube	*nmol·tube*$^{-1}$·*30 min*$^{-1}$	%
Control		41	100
$MgCl_2$	1.3	44	107
$CaCl_2$	1.3	49	119
$MnCl_2$	1.3	70	171
EDTA	1.0	60	146
2-Mercaptoethanol	5.0	77	187
Glutathione (reduced)	5.0	89	216
UDP	0.5	32	77

Table VI. *Inhibition by Phospholipids of IAGlu Synthase*

Reaction conditions were as for Table II. The lipids were dissolved in buffer with warming and sonication in an N_2 gas phase. Assay as in Table II except under an N_2 gas phase.

Addition	Amount Added	Activity	Control
	mg/tube	*munits·tube*$^{-1}$ *·30 min*$^{-1}$	%
None (control)	0.5	74	100
Phosphatidyl choline (14% pure)	0.5	73	98
Phosphatidyl choline (35% pure)	0.5	61	83
Phosphatidyl inositol (50% pure)	0.5	50	68
Phosphatidyl ethanolamine (98% pure)	0.5	24	32
Phosphatidic acid (98% pure)	0.5	39	53
Dolichol monophosphate C_{80}-C_{105} (85% pure)	0.5	62	83

Table VII. *Inhibition by Various Compounds of IAGlu Synthase Activity*

Reaction conditions were as for Table II.

Compound	Amount in Incubation Mix	Enzyme Activity	Relative Enzyme Activity
		nmol·tube^{-1}·*30 min*$^{-1}$	%
None (control)		104	100
Glucose-1-P	2.0 μmol	100	96
Glucose-1-P (no UDPG)	2.0 μmol	0	0
Glucose-1-P	2.0 μmol	26	25
UTP	2.0 μmol		
PPi	2.0 μmol	28	27
IAA-Glucan (Fraction A)	0.5 mg[a]	44	42
Sorbitol	2.0%	110	105
Span-80 (sorbitan monooleate)	0.2%	93	89
Span-85 (sorbitan trioleate)	0.2%	95	91

[a] Due to very low solubility of this compound, the concentration was probably one-half that indicated. The results are the mean of triplicate determinations.

Table VIII. *Inhibition by Reaction Products of IAGlu-Synthesis*

Reaction conditions were as described in Table II.

Compound Tested	Final Concentration in Incubation Mixture	Enzyme Activity	
	mM	*nmol·tube*$^{-1}$·*30 min*$^{-1}$	%
None		81	100
UDP	0.25	61	75
	0.50	53	66
IAGlu[a]	0.25	80	99
	0.50	83	102
IAGlu[a]	0.25	68	84
UDP	0.25		

[a] Synthetic (11).

Effect of Reaction Products On IAGlu Synthase Activity. The effect of the reaction products on the synthesis of IAGlu from UDPG and IAA is shown by the date of Table VIII. It is of interest that UDP is an inhibitor, whereas IAGlu is not. However, the combination of IAGlu and UDP does cause a 16% inhibition, which is close to the 11% inhibition expected by simple dilution of the specific activity of the IAA used as substrate. These results suggest the order of addition for the reverse reaction. Thus, UDP must be present, for the enzyme to bind IAGlu. UDP alone is thus an inhibitor, whereas IAGlu alone is not.

Reaction Stoichiometry and Reaction Reversibility. The reversibility of the reaction was determined by incubating 1 μmol each of IAGlu and UDP together with 200 μL of 0.1 M Hepes buffer and 100 munits of enzyme in a total volume of 0.5 mL. Utilizing Hepes buffer, avoiding Tris, and omitting mercaptoethanol permitted following the reaction quantitatively by chromatography on a PRP-1 (Hamilton) anion exchange column and using a UV detector. After incubation for 30 min at 37°C, the reaction was terminated by the addition of 0.5 mL of 2-propanol. Protein was removed by centrifugation and the resultant solution was concentrated to an aqueous phase *in vacuo*. Glacial acetic

acid was added to adjust the pH to 3.5 and the mixture extracted three times with equal volumes of diethyl ether to remove the IAA. IAA in the ether solution was determined by absorbancy at 282 nm using an extinction coefficient of 6060 (3). A recovery of 0.7 μmol of IAA was obtained without correction for manipulative losses. The solution remaining after ether extraction was adjusted to pH 5.0 and applied to the Hamilton HPLC column using water as eluent. All of the IAGlu had been used up. A gradient was then begun from 0.05 M phosphate (pH 5.0) to 0.05 M phosphate (pH 7.0) in 10 min and collecting fractions of 1.0 $ml \cdot min^{-1}$. No UDP remained but 1.0 μmol of UDPG was formed. Extensive studies were not performed to determine an equilibrium constant but it is apparent, since UDP could not be detected, that the equilibrium is far in favor of IAA and UDPG, probably by 1 to 2 orders of magnitude. This would place the free energy of hydrolysis of the IAA-glucose anhydride bond at between 1400 and 2800 calories above that of the UDP-glucose phosphorous anhydride bond. These results are in accord with previous observations concerning the equilibrium of this reaction (13, 14).

Nonenzymic Synthesis of an IAA Ester of TRIS. During the course of experiments to determine the reaction reversibility, using Tris-HCl buffer, an IAA containing compound was eluted at 16.4 mL using a C18 HPLC reverse phase column and 5.0% ethanol in water (v/v) as eluent. This compound was dried and the trimethylsilyl derivative formed using conditions used by Ehmann for derivatization of IAGlu (9). Using a HP 5970 GC/MS and a 11 m OV-17, WCOT, capillary column a peak was observed at 5.3 min (200°C for 1 min, then ramp to 300°C at 150/min) with a molecular ion at 566 and major fragment ions at 551, 246, 202, and 73. These correspond to a tetrakis TMS-IAA-Tris. One TMS is on the indene nitrogen of the IAA to explain the 202 ion while the three remaining TMS must be on the Tris. It is probable the IAA is on a Tris hydroxyl as evidenced by the ion at m/z = 246. The reaction occurred upon simply incubating IAGlu with Tris buffer and so was not further studied. It is of interest that Jenks (10) has previously published on the formation of acyl Tris and acyl imadazole compounds formed from acyl adenylates. This result indicates the strong acylation character of IAGlu and may be of interest in understanding the enzymic synthesis of IAA aspartate (7).

Location of Enzyme in Kernel. The distribution of IAA-UDPG glucosyl transferase in the kernels of *Zea mays*, sweet corn is shown by the data of Table IX. As can be seen, the highest specific activity is in the pasty, semiliquid endosperm, the second highest is in the embryo (plus scutellum), and the lowest activity (if it is not, in fact, owing to contamination by the endosperm) is in the seed coat. The IAA conjugates are primarily located in the endosperm (16). The data for the whole kernel shows that there is no synergetic effect between the tissues. It has also been found (data not shown) that white sweet corn had a 30% higher specific activity than yellow sweet corn.

Chromatographic Behavior of IAGlu Synthase. Further information concerning the activity of this enzyme has been obtained by studying its adsorption to various affinity columns. As shown by the data of Table X the enzyme is strongly adsorbed by cyclohexyl-C_6-Sepharose, by a UDP-glucuronic acid agarose affinity column, and by a UDP-agarose column. It is weakly adsorbed by an IAA-glucan column (17) and by phosphocellulose. The UDPGlucuronic column is an analog of UDPG since the ligand is linked to the gel by the carboxyl moiety. The strong adsorption to the lipophilic IAA-cyclohexyl-Sepharose indicates a lipophilic domain in the enzyme. The adsorption by an IAA-glucan column may indicate recognition of the IAA-$(glucose)_n$ moiety.

Table IX. *The Activity of IAGlu Synthase in Embryo, Liquid Endosperm and Seed Coat*

Assay as for Table II. *N* = number of independent experiments = 2 for embryo; 8 for endosperm; and 3 for seed coat.

Tissue	Protein	Enzyme Activity	Specific Activity
	mg·g wet tissue	*munits·g tissue*$^{-1}$	*munits·mg protein*$^{-1}$
Embryo	53	789	14
Endosperm	31 ± 3	3830 ± 490	126 ± 20
Seed coat	29 ± 5	365 ± 283	12 ± 8

Antibody Production. About 2.4 mg of enzyme purified through stage 4 and then by preparative PAGE were used to immunize a single rabbit using Freund's adjuvant. The intradermal injections were given at 3 week intervals over a 3 month period. Blood was collected from the animal by ear-vein puncture and the IgG fraction prepared by Zeta Chrom 60, DEAE disk chromatography (8). The IgG fraction of this particular animal was less than 5% of the total serum proteins so only a limited amount of IgG was obtained. The titer of the resultant antibody was between 10 and 20 and was not suitable for use for enzyme localization. The low antibody titer observed was probably owing to the failure of the single rabbit involved to respond to the antigen since the rabbit had less than 5% of normal IgG.

Effect of an Unidentified Heat-Stable Factor on the Activity of IAGlu Synthase. The unexplained, precipitous losses of enzyme activity following certain column chromatographic procedures suggests that the enzyme requires an as yet unidentified cofactor. This is particularly evident following chromatography on a Sephacryl S-300 or on a hydroxyapatite column. In both cases 100% of the activity is lost during a 1 or 2 h chromatographic procedure at 4°C, although the enzyme is stable during storage at 4°C for more than 30 d (13). In the following experiments, boiled 5% PEG-25 mM Tris-HCl buffer, eluates of the fraction emerging from a DEAE-Sephacryl column before the bulk of the enzyme activity (13) were added to the enzyme incubation mixture. In four experiments, with each assay in triplicate, the stimulation observed was as follows: experiment I, 24%; experiment II, 56%; experiment III, 23%; experiment IV, 36%; for the samples to which column wash was added. We conclude that there is an unknown, heat-stable factor which can, in part, restore enzyme activity.

DISCUSSION

The synthesis of IAA-glucose from free IAA and UDPG is the first step in a series of reactions leading to the characteristic IAA-*myo*-inositol conjugates in plants such as *Zea mays* (4, 5, 7, 13, 14, 18). Thus, this enzyme may represent a control point for the regulation of the relative amounts of free and esterified IAA. We are, as yet, uncertain as to whether the IAA conjugates have activities as growth hormones (1, 2, 4, 18) or whether they serve purely as part of a homeostatic mechanism for control of hormone levels (2). Nonetheless, the increased stability (7) and the difference in lipophillicity between IAA and the IAA sugar conjugates will act as a kind of zip code to govern hormone distribution (4), and thus it is important to understand the control of hormone conjugation.

This work demonstrates many possible chemical regulators for the activity of the IAA-UDPG-glucosyl transferase enzyme. The enzyme is inhibited by phospholipids, by UDP, by other plant hormones, by pyrophosphate, and, very slightly, by phenolics. The enzyme is faintly stimulated by calcium-calmodulin, and strongly by thiol reagents and by manganese. These compounds then serve as potential regulators of IAA conjugate synthesis.

There is evidence for an unknown cofactor for this enzyme. The enzyme is almost totally inactivated by many column chromatographic procedures, particularly, Sephacaryl S-300 and hydroxylapatite even during a 1 h chromatography at 4°C despite

Table X. *Chromatographic Behavior of IAGlu Synthase on Columns With Different Ligands*

Stage IV enzyme of the indicated amount (2.2 mg protein, 563 munits of activity) was dissolved in 2 mL of 25 mM Tris-HCl (pH 7.3) and was applied to a column of 2 mL gel bed volume and the column washed with 3 mL of Tris buffer. Elution was with 2.5 mL of 50 mM Tris, 0.15 M NaCl.

Gel or Ligand Tested	Protein Adsorbed	Protein Recovered[a]	Activity Unadsorbed	Activity Adsorbed	Activity Eluted	Activity Recovered[a]
	mg			%		
None (control)		100				100
IAA-Glucan (A-Fraction)	0.45	96.3	35	65	8.3	43.3
IAA-cyclohexyl (C_4) Sepharose Cl 4B	1.1	85	0	100	26.7	26.7
UDP-Agarose	1.3	94	3	97	30	33
UDPGlucuronic acid-agarose	1.4	78	0	100	24	24
Phosphocellulose	0.6	103	33	67	7	40

[a] Sum of unadsorbed protein/activity and protein/activity eluted from the column with 0.15 M NaCl.

the fact that the enzyme is stable for more than 30 d at 4°C (13). In most cases a portion of the activity can be regained by adding a portion of the eluate which emerges from the column just after the enzyme activity. Characterization of this cofactor, and thus being able to purify this enzyme to a homogeneous state, must remain for future studies.

We wished to test as many compounds as possible for their effect as activators or inhibitors of IAGlu synthesis. Thus, most compounds were tested at only one concentration, that is 1 mM, and this high concentration does not indicate the compound would be an inhibitor *in vivo* at physiological concentrations.

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