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Identification of L-Tryptophan as an Endogenous Inhibitor of Embryo Germination in White Wheat[1]

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CRAIG F. MORRIS[2], DELBERT D. MUELLER, JON M. FAUBION, AND GARY M. PAULSEN*
Departments of Agronomy (C.F.M., G.M.P.), *Biochemistry* (D.D.M.), *and Grain Science and Industry* (J.M.F.), *Kansas State University, Manhattan, Kansas 66506*

ABSTRACT

An endogenous germination inhibitor(s) in wheat (*Triticum aestivum* L.) grain has been implicated in seed dormancy and germination, but its identity and mode of action have not been elucidated. We isolated and identified an endogenous germination inhibitor in white wheat grain and compared its activity with that of known compounds. A water extract of wheat bran chromatographed on Sephadex LH-20 yielded an inhibitory fraction that was detected by bioassay on embryos excised from dormant wheat seeds. The inhibitor was sequentially purified by DEAE-cellulose, P-2 gel filtration, and C_{18} reversed-phase HPLC and identified as L-tryptophan by carbon and proton nuclear magnetic resonance spectroscopy, mass spectrometry, optical rotation, UV absorption spectrum, and chromatographic co-elution with authentic tryptophan. Extractable tryptophan occurred in wheat grain and bran at about 1.3 to 1.5 and 2.7 to 5.9 milligrams per gram, respectively. Low millimolar concentrations of L-tryptophan similar to physiological levels and D-tryptophan were more inhibitory to embryos from dormant seeds than to embryos from nondormant seeds. Tryptophol and tryptamine, putative intermediates between tryptophan and IAA, were highly inhibitory and noninhibitory of embryo germination, respectively. IAA and 2,4-D also inhibited germination, but embryos from dormant and nondormant seeds responded similarly. L-Tryptophan may be an important germination inhibitor of excised embryos, but its role *in situ* remains to be determined.

Endogenous germination inhibitors in wheat grain are implicated in the control of seed dormancy and germination (3, 11, 13–16, 18). Compounds that inhibit germination of wheat seeds or embryos include ABA (6, 22, 25, 26), catechin and catechin tannin (11, 14, 22), and other partially characterized but unidentified substances (3, 13–16, 18). Flavanol concentration (as catechin equivalents) was not involved in wheat seed dormancy *in vivo* (5), however, and although ABA has been suggested as a controlling factor, data have not supported a direct role in mature seeds (25, 26).

Exogenous inhibitors such as catechin tannin and ABA inhibit germination of embryos excised from dormant wheat seeds much more than germination of embryos from nondormant seeds (22). The endogenous inhibitor(s) acts similarly, inhibiting germination of embryos from dormant or sprouting-resistant wheats but not embryos from after-ripened or sprouting-susceptible wheats (11, 15, 18). Regulation of dormancy and germination results from differential sensitivity of embryos to endogenous inhibitor(s), embryos excised from dormant seeds being sensitive and embryos from nondormant seeds being insensitive (11, 18). Excised embryos of both types, in contrast, germinate readily in the absence of inhibitor(s) (11, 18, 22).

The identity and mode of action of the endogenous germination inhibitor(s) in wheat are not known. Catechin, catechin tannin, and an unidentified alkaloid-like inhibitor occur in the seed coat (testa) of wheat seeds (13, 14), but a regulatory role for them in seed dormancy has not been documented (5). The endogenous inhibitor(s) has been characterized as a water-soluble phenolic derivative that is heat-stable, insoluble in petroleum ether, primarily located in the bran, and unrelated to pericarp color or degree of after-ripening (15, 18).

Identification of the endogenous inhibitor(s) would increase understanding of regulation of wheat seed germination and aid development of cultivars that are resistant to preharvest sprouting. Objectives of this study were to isolate and identify the endogenous germination inhibitor(s) in wheat grain and to compare its activity with known compounds.

MATERIALS AND METHODS

The scheme for isolating and purifying an endogenous germination inhibitor from white wheat (*Triticum aestivum* L.) grain is shown in Figure 1. The inhibitor was extracted from bran with water, separated from other components of the extract by Sephadex LH-20 liquid chromatography, and detected by a wheat embryo bioassay. The inhibitor was purified further by three different types of chromatography and identified by several physical methods. Physiological effects of the inhibitor, related compounds, and ABA were compared by embryo bioassay.

Inhibitor Extraction. Bran was mill-fractionated from 'Clark's Cream' white wheat grain (break 3 bran) (15), ground to pass a 1.0-mm screen in a Udy Cyclone mill, sealed in plastic bags, and stored at −20°C. For extraction, 100 g of the ground bran was stirred with 500 mL water at about 22°C for 10 min, and the slurry was centrifuged in 250-mL jars at 16,300*g* for 20 min. The supernatant was lyophylized and stored at −20°C; it yielded 8.3 g of water-soluble extract.

Isolation by Sephadex LH-20 Column Chromatography. A subsample of the water-soluble bran extract was redissolved in water (1.25 g in 25 mL) and centrifuged at 27,000*g* for 10 min. The supernatant was collected, and 5 mL was applied to a 2.5 × 55-cm Sephadex LH-20 column; the remaining supernatant was stored at −20°C. The column was eluted with water at a flow rate of approximately 1.5 mL/min, and absorbance of the effluent was continuously monitored at 280 nm (1-mm path). Fractions were collected as individual A_{280} profile peaks that were delimited at the inflection points of the absorbance trace (Fig. 2), immediately frozen, lyophylized, and stored at −20°C. For bioassay, fractions were redissolved in 2 mL water, mixed 1:1

[1] Contribution 88-236-J, Kansas Agricultural Experiment Station.

[2] Present address: Department of Agronomy and Soils, Washington State University, Pullman, WA 99164.

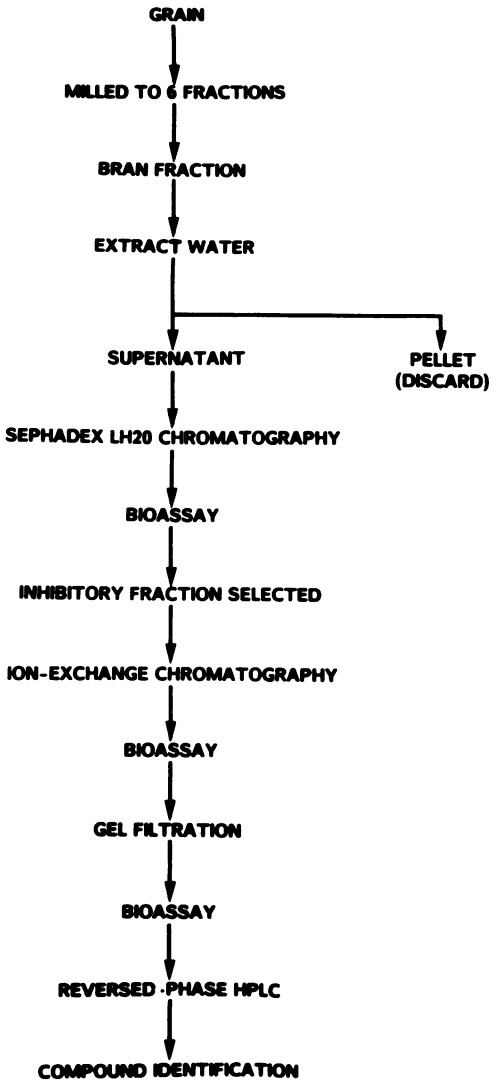

FIG. 1. Schematic representation of the procedure for isolating and purifying an endogenous germination inhibitor from white wheat grain.

(v/v) with double-strength agar, and tested with embryos from dormant ('Clark's Cream' and 'Vona') wheat seeds.

Numerous LH-20 chromatography peaks inhibited embryo germination (Fig. 2). One peak, which inhibited germination completely (PI[3] = 0), eluted considerably later than other peaks that were less inhibitory and closely grouped. The inhibitor contained in this late peak was selected for identification because of its high activity and distinctive separation from the other peaks.

Purification by Ion-Exchange Column Chromatography. A 1 × 6-cm column of DEAE-cellulose in Cl-form was used to further purify the most active fraction from LH-20 chromatography (Fig. 3A). The lyophylized LH-20 fraction was redissolved in water and applied to the water-equilibrated DEAE column. The column was eluted step-wise with water and increasing concentrations of aqueous ammonium formate, and effluent absorbance was continuously monitored at 280 nm. Fractions were collected according to A_{280} profile, immediately frozen, lyophylized, and bioassayed using embryos from dormant seeds.

Purification by Gel Filtration Column Chromatography. The lyophylized A_{280}-absorbing fraction that eluted at V_0 with water

[3] Abbreviations: PI, promptness index; FAB, fast atom bombardment mass spectrometry.

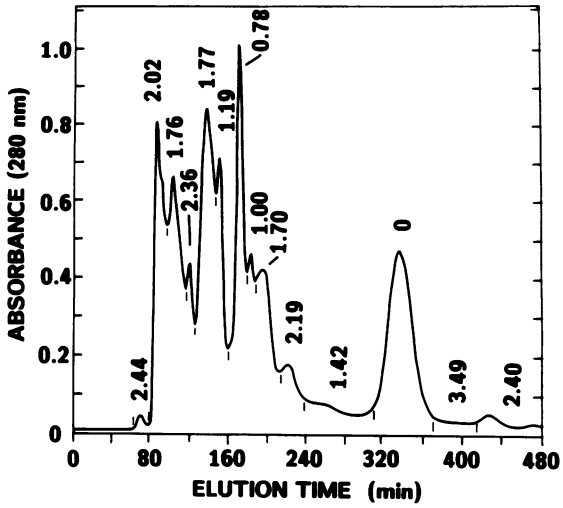

FIG. 2. Sepahdex LH-20 column chromatography of a water-soluble extract from white wheat bran. Fractions were delimited (small vertical bars) and collected according to A_{280} profile, recovered, and bioassayed with wheat embryos from dormant seeds. Numbers above each peak refer to the embryo germination response (promptness index) (see "Materials and Methods"). Promptness index of control embryos equaled 3.35 ± 0.66.

from the DEAE-cellulose column was redissolved in water and chromatographed on a 1.5 × 24-cm Bio-Gel P-2 gel filtration column (Fig. 3B). Elution was with water at approximately 20 mL/h, and absorbance at 280 nm and conductivity were continuously monitored. Fractions were collected according to A_{280} and conductivity profiles, immediately frozen, lyophylized, and bioassayed using embryos from dormant and nondormant ('Parker 76') wheat seeds.

Purification by Reversed-Phase HPLC. The major A_{280} peak from the gel filtration column was chromatographed using an Econosphere C_{18} reversed-phase column (250 × 4.6-mm, 5-μm packing) (Alltech/Applied Science) operated by a Varian 5000 programmable pumping and solvent delivery system (Fig. 3C). Elution was with water and methanol in a programmed solvent system. Effluent absorbance was continuously monitored at 280 nm and recorded using a Hewlett-Packard 3392A integrator and strip chart recorder. Fractions were collected according to A_{280} profile and dried in a Speed Vac (Savant Instruments) concentrator. Dried fractions were redissolved in water and bioassayed using embryos from dormant and nondormant seeds.

Inhibitor Identification. ^{1}H- and ^{13}C-NMR were conducted in ^2H_2O on a Bruker WM-400 instrument using, respectively, about 4,000 or 22,000 Hz spectral regions, 16,384 data points zero-filled to 32,768 before transformation and application of the 0.1- or 1.5-Hz line broadening, and 2.0- or 2.65-s delay times between acquisitions.

Low resolution FAB mass spectrometry of the inhibitor was performed by the Midwest Center for Mass Spectrometry at the University of Nebraska, Lincoln, NE.

Optical rotation of the inhibitor in 23°C water was measured on a model 241 Perkin-Elmer polarimeter with the sodium D-line light source and a path length of 10 cm.

The UV absorption spectrum of the inhibitor was determined against water and compared to authentic L-tryptophan on a Cary 16 spectrophotometer. The cuvette chamber and lamphouse were purged with a constant flow of N_2 gas.

The inhibitor co-eluted with authentic L-tryptophan from P-2 gel filtration and C_{18} reversed-phase HPLC columns operated as described above.

Wheat Embryo Bioassay. Embryos without scutella for inhib-

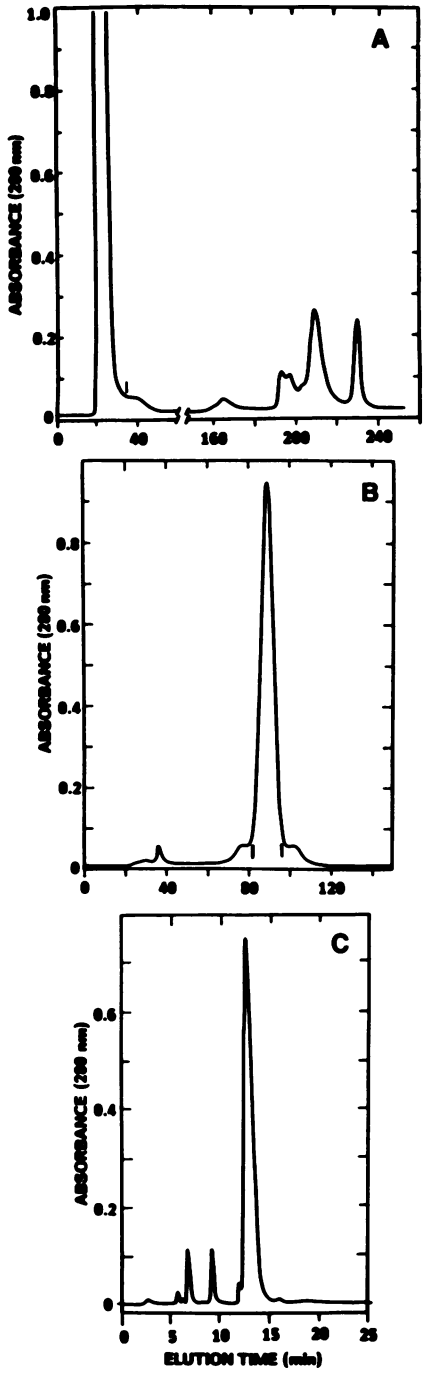

FIG. 3. Sequential chromatographic purification of an endogenous germination inhibitor from white wheat bran. Small vertical bars denote fraction cut-offs. Panel A, DEAE-cellulose chromatography. The inhibitor eluted with water at V_o; additional constituents (>150 min) eluted with 0.005X (v/v) ammonium formate, where X = a saturated solution at room temperature. Panel B, P-2 gel filtration. Panel C, C_{18} reversed-phase HPLC. The sample was injected in water and eluted with a linear methanol-water gradient that increased 1% in methanol concentration per minute.

itor bioassays were excised from dormant harvest-ripe seeds of 'Clark's Cream' and 'Vona' wheat cultivars and from nondormant seeds of 'Parker 76' cultivar that had been after-ripened 4 weeks. Embryo isolation followed a modified procedure of Johnston and Stern (10) as described elsewhere (15).

Excised embryos were germinated in Costar No. 3596 96-well tissue culture plates. Autoclaved agar (double strength) was mixed 1:1 (v/v) with test solutions to give a final concentration of 20 g/L sucrose and 6.8 g/L agar. Each well received 0.2 mL agar-test solution and, after cooling, one embryo that was placed scutellum-side down. Twelve embryos comprised a replication. All operations were conducted in a laminar flow hood. Plates were taped shut and incubated at 22°C in darkness, and embryos were periodically examined under magnification for germination. Embryos were considered germinated if radicle, lateral root, or coleoptile growth exceeded 1 mm.

A PI was calculated as the summation of (n_i/h_i), where n_i is the percentage of embryos germinated on the ith hour of the test and h_i is the ith hour of the test. For analysis of variance, error degrees of freedom were reduced by the number of treatments that produced a PI = 0 (complete inhibition) for all replications. Corrected mean square errors are reported as measures of experimental precision and were used to calculate least significant differences.

Quantification of Endogenous Tryptophan. Duplicate 5-g samples of whole grain meal and six mill fractions (15) were extracted with 25 mL water at about 22°C for 1 h with continuous shaking. Samples were centrifuged at 3000*g* for 15 min. The supernatant (5 mL) was brought to 10% (w/v) TCA, stored at 4°C for 12 h, and centrifuged at 12,000*g* for 20 min. The resulting supernatant was diluted and analyzed for tryptophan by the method of Opienska-Blauth *et al.* (17) using L-tryptophan as the standard. Tryptophan concentrations in the bran extracts were estimated by integrating LH-20 A_{280} chromatograms (*e.g.* Fig. 2).

Effects of Tryptophan and Other Compounds on Embryo Germination. L- and D-tryptophan, six L-amino acids, and glycine were bioassayed at 1 and 10 mM using embryos from dormant wheat seeds. Concentration-dependent effects of tryptophan on embryo germination were investigated further by bioassaying L-tryptophan at 10 μM and L- and D-tryptophan at 50, 100, 500, 1,000, and 10,000 μM with embryos from dormant and nondormant wheat seeds. The possible relationship of L-tryptophan to IAA was investigated by bioassaying the IAA precursors, tryptophol and tryptamine, at 2.5, 50, and 100 μM using embryos from dormant wheat seeds. The natural and synthetic auxins IAA and 2,4-D were bioassayed on embryos from dormant and nondormant wheat seeds. For comparison, 2,4-dichlorophenol was also bioassayed. As a final comparison, tryptophol, IAA, 2,4-D, and ABA were bioassayed at 1 μM using embryos from dormant and nondormant seeds.

SEM documented the effects of L- and D-tryptophan on embryo germination and callus growth after 12 d. Embryos were fixed in FAA solution (formalin:glacial acetic acid:ethanol:water 4:2:19:10) at about 22°C for 1 h and sequentially dehydrated in 30, 50, 70, 85, 95, and 100% (v/v) ethanol for 15 to 20 min each. Embryos were then critical point-dried using CO_2 and were sputter-coated with gold on aluminum stubs. Micrographs of the dorsal side of embryos were taken from the radicle end.

Reagents. D-tryptophan (T-9753), IAA (I-1250), tryptamine (T-2891), tryptophol (T-1504), (±)-2-*cis*-4-*trans*-ABA (A-2784), and 2,4-dichlorophenol (D-6023) were from Sigma Chemical Co. L-Tryptophan and the remaining amino acids were part of a standards kit (22, 20065, Pierce Chemical). 2,4-D and purified agar were from Eastman Kodak and Difco, respectively. Unless noted, all other chemicals were reagent grade or better. Distilled, resin-deionized water was used throughout.

RESULTS

Inhibitor Isolation. Fourteen fractions were collected from LH-20 liquid chromatography and tested for inhibitory activity by a wheat embryo bioassay (Fig. 2). The peak eluting at about 340 min was most inhibitory; it completely stopped germination of embryos from dormant seeds (PI = 0). A second sample of the

same extract and the water-soluble extract of another bran fraction with high germination inhibition (break 3 medium) (15) were also chromatographed on LH-20 and bioassayed. The data confirmed the activity of the selected fraction, which was used for the subsequent procedures.

Nearly all of the inhibitor selected from the LH-20 chromatography effluent eluted at V_0 with water from the DEAE-cellulose column (Fig. 3A). The inhibitor fraction was collected and chromatographed on the P-2 column, where it eluted as a single peak with small leading and trailing shoulders (Fig. 3B). The inhibitor was recovered and chromatographed on the C_{18} reversed-phase HPLC column; it eluted as a well-defined single peak that was distinctly separated from two minor contaminants (Fig. 3C). Inhibitory activity of the major fraction that eluted from the DEAE-cellulose and P-2 columns was confirmed using embryos from dormant seeds for bioassays. The quantity of inhibitor from HPLC, however, was too small for conclusive results.

Inhibitor Identification. The sequential column chromatographic procedures illustrated in Figure 3 yielded a nearly pure inhibitor, and rechromatography of the HPLC fraction containing the major peak resulted again in a single peak. Because of the small quantity of inhibitor collected in the HPLC fraction, however, the P-2 column product was used for most of the physical identification procedures.

The ^{13}C-NMR spectra of the unknown inhibitor purified through the P-2 column and pure L-tryptophan disclosed the same number of carbon atoms, and the peak positions were essentially superimposable. The absence of extra resonances in the inhibitor spectrum indicated, at the signal/noise ratio achieved, that the inhibitor was at least 80% pure at this point. Further confirmation that both compounds are tryptophan can be seen in the nearly identical chemical shifts of the carbon and hydrogen resonances and in the proton coupling constants (Table I). The carbon atoms were assigned according to Allerhand *et al.* (1) and Richarz and Wuthrich (19), and the proton assignments are those of Wuthrich (27). All of the carbon resonances were within 0.1 ppm of each other, and most differed by 0.05 ppm or less. Considering the differences in concentration and the possible differences in pH (the dry samples were dissolved in 100% ^2H_2O without pH adjustment), the agreement is well within the range expected for identical ionizable compounds. Similarly, all of the ^{1}H chemical shifts (Table I) except H5 and H6 agreed within 0.02 ppm, and even the latter were within 0.2 ppm. The only substantial differences in coupling constants were those involving the Hα and Hβ protons that are closest to the ionizable groups and, thus, the most sensitive to differences in solution pH. Overall, the agreement in 2- and 3-bond coupling constants substantiated the identity of the inhibitor as tryptophan.

Low resolution FAB mass spectrometry of the inhibitor gave an $[M + H]^+$ of 205, and lithiation produced a sharp signal at m/z 217 corresponding to $[M - H + 2\ Li]^+$.

Optical rotation of the inhibitor purified by two passes through the LH-20 column was $[\alpha]_D = -27.9$ (c = 1.4 mg/mL, H_2O), which is in reasonable agreement with that of L-tryptophan, $[\alpha]_D = -31.5$ (c = 10 mg/mL, H_2O) (12).

Identical UV absorption spectra of the inhibitor and L-tryptophan and co-elution of the two compounds from P-2 and C_{18} columns (data not shown) further confirmed the identification.

Quantification of Endogenous Tryptophan. Soluble tryptophan was low in high-flour fractions (0.48–0.59 mg/g) and highest in the bran fraction (5.9 mg/g) of mill-fractionated wheat grain that was previously prepared (15). Multiplying the tryptophan concentration of each mill fraction by its proportional yield gave 1.49 mg tryptophan/g grain, and analysis of whole grain meal gave 1.26 mg tryptophan/g grain.

Integrating the tryptophan peak from two LH-20 chromatograms (*e.g.* Fig. 2) produced results similar to those obtained by colorimetric analysis of tryptophan. Assuming from NMR data that the peak represented nearly pure tryptophan, integration gave a value of 2.7 ± 0.2 mg tryptophan/g bran and a concentration of 9.9 ± 0.6 mM tryptophan in the embryo bioassays. Bioassay of these two fractions completely inhibited germination (PI = 0).

Effect of L-Tryptophan and Other Compounds on Embryo Germination. L- and D-tryptophan were more inhibitory than the

Table I. *Chemical Shifts and ^{1}H Apparent Coupling Constants of an Endogenous Germination Inhibitor from White Wheat Bran and Authentic L-Tryptophan*

NMR Parameter	Compound	Carbon Atom								
		CO	Cα	Cβ	C2 or C5	C3	C4 or C6	C7	C8	C9
						ppm				
Shift[a]	Inhibitor	175.57	55.92	27.31	125.81,122.94	108.39	120.27,119.26	112.74	137.14	127.47
	L-Tryptophan	175.48	55.96	27.30	125.86,122.97	108.40	120.30,119.29	112.79	137.18	127.51
		Hydrogen Atom								
		H	H_1	H_2	H2	H4	H5	H6	H7	
						ppm				
Shift[b]	Inhibitor	4.00	3.43	3.25	7.27	7.69	7.16	7.24	7.49	
	L-Tryptophan	4.01	3.46	3.28	7.28	7.70	6.98	7.05	7.50	
						Hz				
Coupling constant	Inhibitor	$J\alpha\beta_1$	$J\beta_1\beta_2$	$J\beta_1\beta_2$		J_{45}	J_{45}	J_{67}	J_{67}	
		4.79	15.1	15.1		7.92	(7.92)	(8.12)	8.12	
	L-Tryptophan	$J\alpha\beta_1$	$J\beta_1\beta_2$	$J\beta_1\beta_2$		J_{45}	J_{45}	J_{67}	J_{67}	
		4.80	15.3	15.3		7.94	(7.94)	(8.03)	8.03	
	Inhibitor	$J\alpha\beta_2$	$J\alpha\beta_1$	$J\alpha\beta_2$			J_{56}	J_{56}		
		7.56	4.51	8.05			6.96	7.21		
	L-Tryptophan	$J\alpha\beta_2$	$J\alpha\beta_1$	$J\alpha\beta_2$			J_{56}	J_{56}		
		8.04	4.76	8.12			6.98	7.25		

[a] Relative to internal *p*-dioxane in ^2H_2O taken as 67.4 ppm. [b] Relative to internal sodium 2,2-dimethyl-2-silapentanesulfonate in ^2H_2O taken as 0 ppm.

other amino acids except L-cysteine, which appeared to be toxic and bleached embryos from dormant seeds (Table II). The high concentration (10 mM) of the two tryptophan enantiomers completely inhibited embryo germination. L-phenylalanine, L-lysine, and L-glutamic acid were partially inhibitory at 10 mM but not at 1 mM. The other amino acids had little effect.

Germination of embryos from dormant seeds was inhibited by increasing concentration of tryptophan (Fig. 4A), and L- and D-enantiomers were equally active. Germination inhibition occurred as a log-linear response, with complete inhibition at 10 mM tryptophan. Germination of embryos from nondormant seeds, on the other hand, was affected much less by either isomer of tryptophan. Only at 10 mM L- or D-tryptophan was germination reduced (Fig. 4B).

One IAA precursor, tryptophol, was highly inhibitory and the other precursor, tryptamine, was noninhibitory at concentrations of 1 mM and higher (Table III). In other tests, tryptophol at 1 μM had no significant effect on embryo germination. The germination response (PI) to the same concentration of ABA was 0.66 and 1.95 for embryos from dormant and nondormant seeds, respectively. The PIs of untreated control embryos from dormant and nondormant seeds were 2.69 and 3.00, respectively. Embryos from dormant and nondormant seeds responded similarly to the auxins IAA and 2,4-D, and both types were less sensitive to IAA than to 2,4-D. Mean germination response (PI) of embryos from dormant and nondormant seeds was 2.84, 3.00, 0.34, and 0.12 for 0, 1, 50, and 500 μM IAA, respectively. Germination response (PI) to 2,4-D was 0.96 for 1 μM and 0 for 50 and 500 μM. The 2,4-D analog, 2,4-dichlorophenol, appeared to be toxic to embryos at 1 and 10 μM and noninhibitory at 50 μM and less (data not shown).

Table II. *Germination Promptness Index (PI) Response of Embryos Isolated from Dormant Wheat Seeds to Amino Acids*

Amino Acid	Concentration	
	1 mM	10 mM
	PI	
L-Tryptophan	1.23	0.00
D-Tryptophan	1.04	0.00
L-Cysteine	1.65	0.00
L-Phenylalanine	3.62	1.60
L-Lysine	3.08	1.86
L-Glutamic acid	3.11	1.84
L-Glutamine	3.24	3.51
L-Serine	3.71	2.65
Glycine	3.45	3.18
Control	3.35	
$LSD_{(0.05)}$	0.63	
MSE[a] (n = 2, 19-3 df)	0.089	

[a] Mean square error.

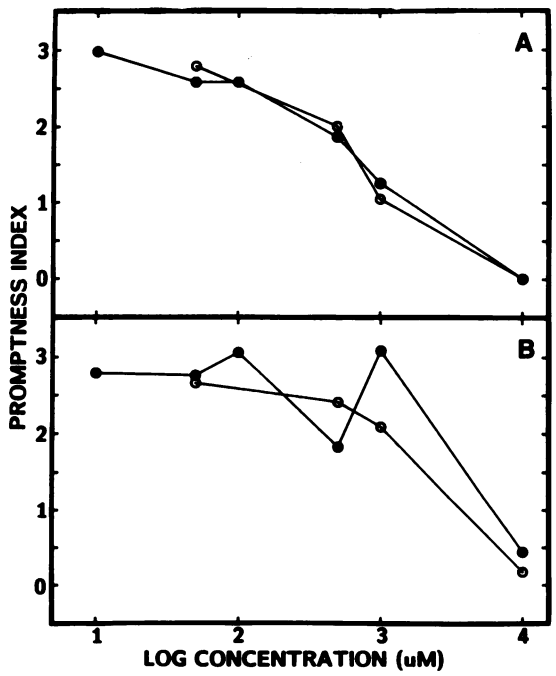

FIG. 4. Concentration-dependent germination response curves of isolated embryos to L- and D-tryptophan. Panels A and B, embryos isolated from dormant and nondormant wheat seeds, respectively.

Table III. *Germination Promptness Index Response of Embryos Isolated from Dormant Wheat Seeds to IAA Precursors Tryptophol and Tryptamine*

Treatment	Concentration	Germination Response (Promptness Index)
Tryptophol	2.5 μM	0
	50 μM	0
	1 mM	0
Tryptamine	2.5 μM	3.75
	50 μM	2.65
	1 mM	2.84
Control	0	2.84
$LSD_{(0.05)}$		0.97
MSE[a] (n = 2, 7-3 df)		0.12

[a] Mean square error.

Germination of embryos from dormant seeds recorded by SEM micrographs (not shown) was completely inhibited by 10 mM L- and D-tryptophan for up to 12 d, whereas untreated control embryos had roots 16 to 30 mm long. Callus growth response differed markedly from the root growth response to the two tryptophan enantiomers. With L-tryptophan, callus proliferation occurred at the coleorhiza and lateral root sheaths (lateral root equivalent of the coleorhiza) and the margin of the epiblast. With D-tryptophan, callus was generally absent; when it occurred occasionally, it was at the apical end of the radicle.

DISCUSSION

Endogenous germination inhibitors have been commonly invoked as major factors controlling dormancy and germination in wheat seed (3, 11, 13–16, 18). Elucidating the mode of action of endogenous inhibitor(s) and the control of dormancy and germination in wheat seed has been hampered, however, because the inhibitor(s) has not been chemically defined. Isolation of a germination inhibitor in wheat bran and its identification as L-tryptophan should lead to a better understanding of seed dormancy and germination.

Earlier investigations (3, 13, 14, 16) of wheat seed dormancy were undoubtedly handicapped by the use of nondormant seeds or embryos from nondormant seeds for bioassays. Sensitivity of embryos to inhibitors is lost along with dormancy during afterripening of wheat seeds, making them undesirable for bioassaying inhibitors. More recent studies have used embryos from dormant wheat to detect exogenous inhibitors (22) and endogenous inhibitors (11, 15, 18); these embryos are highly sensitive to the inhibitors and are much more desirable for bioassays.

Identification of L-tryptophan in bran, the major inhibitory fraction in wheat (15), and investigations of its physiological action strongly imply a role for the compound in seed dormancy.

It is apparent from NMR, mass spectrometry, optical rotation, UV absorption, and co-chromatography that L-tryptophan is the predominant component of the major inhibitory fraction. It is likewise apparent that L-tryptophan exerts a strong inhibitory effect on embryos from dormant seeds, which is in accord with previous research on the problem. The presence of germination inhibitors was once associated with pericarp color because dormancy is more profound and persistent in red-colored wheats than in white-colored wheats (4). Recent research suggests, however, that germination inhibitors are independent of pericarp color or dormancy (11, 18). Widespread occurrence of tryptophan probably explains this ubiquitous nature of the inhibitor.

Several amino acids in general and tryptophan in particular are notable inhibitors of plant growth processes (2). Tryptophan from oat (*Avena sativa* L.) hulls, for instance, strongly inhibits root elongation (7). An unidentified derivative of another indole, tryptophanamide, is a marker of seed dormancy in *Petunia* species (9). The mechanism of dormancy appears to differ from that in wheat, however. Accumulation of the tryptophanamide derivative in *Petunia* seeds occurred only after they ripened and was directly associated with the level of dormancy. Absence of the derivative was a necessary but insufficient condition for nondormancy (8).

Two different methods, colorimetric analysis and A_{280} chromatogram integration, measured similar concentrations of tryptophan in wheat bran. The tryptophan content of wheat seeds and estimates of the amount of water imbibed during germination give *in vivo* concentrations of tryptophan that fall easily within the observed physiologically important low-millimolar range. The log-linear response of embryo germination to tryptophan is also typical of growth regulator-plant response interactions (24). Stimulation of germination over controls was not observed during this study, but it may occur at lower concentrations because organs differ in sensitivity to auxin and, presumably, to related compounds (23).

Our physiological studies establish that L-tryptophan is inhibitory to excised wheat embryos, but they do not resolve whether tryptophan acts directly or indirectly. Similar activities of the L and D forms on embryo germination suggest that there may be a direct action. The strong inhibitory activities of IAA and tryptophol, an intermediate between tryptophan and IAA (20), on the other hand, indicate that IAA itself may be the active compound. Auxins such as IAA are commonly included in wheat tissue culture initiation media to inhibit embryo germination and promote callus growth (21). Low levels (10 μM) of IAA also significantly inhibit wheat embryo germination at physiological maturity but not at harvest ripeness (6). The inhibitory activities of IAA and the synthetic auxin, 2,4-D, were nonspecific in our studies, however, because they affected embryos from dormant and nondormant seeds alike.

Embryo germination response to L-tryptophan is in accord with a model of dormancy control in wheat (11, 18). The model proposes that embryos from dormant seeds are receptive, or sensitive, to endogenous inhibitor; that embryos from nondormant seeds are insensitive to endogenous inhibitor so germination proceeds unhindered; and that endogenous inhibitor is located primarily outside the embryo (*i.e.* 'imposed' dormancy in wheat) and moves to the site(s) of action in the embryo during imbibition.

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