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Short Communication

Isolation and Properties of the Enzyme System Forming Indoleacetic Acid

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The enzymatic conversion of tryptophan to indoleacetic acid (IAA) has been demonstrated in lower and higher plants. In some plants the pathway must be limited to that involving indolepyruvic acid (5) while in others an alternative pathway involving tryptamine also exists (6,9).

Although the enzyme system from the pineapple leaf converting tryptophan and tryptamine to auxin was obtained by precipitation with ammonium sulfate (1), subsequent studies have used the supernatant fraction following centrifugation as the enzyme preparation. Such a preparation has been found inadequate for the precise determination of pathway and the characteristics of the enzymes involved. In the investigation reported here a partial purification of the enzyme system from *Avena* tissue has been achieved and some of its properties have been examined.

Oats (*Avena sativa* cv Victory) are grown in the dark at 24° and 82 % relative humidity for 86 to 90 hours. Five grams of frozen apical segments (1 cm long) of the coleoptiles are macerated in 50 ml of cold buffer (KH_2PO_4-Na_2HPO_4, 0.05 M, pH 7). The homogenate is centrifuged for 40 minutes at 10,000 × g at 0°. The 50 ml of supernatant then is passed through a column of Sephadex G-25 (Pharmacia, Uppsala), which is equilibrated with the buffer, to remove compounds with molecular weights below 5000. The filtrate is saturated with ammonium sulfate and flocculation is allowed to proceed for 16 hours at 0°. The residue is separated by centrifugation at 10,000 × g for 30 minutes at 0°. The resulting pellet is taken up in 50 ml of buffer and dialyzed against diluted buffer (1 liter, 0.025 M) for 18 hours at 0°. The dialysate is passed through a column of Sephadex G-200 (Pharmacia, Uppsala) which is equilibrated with the 0.05 M buffer to remove all molecules with molecular weights below 200,000. The filtrate with volume of 50 ml is used as the enzyme preparation. Throughout the procedure precautions are taken to keep the preparation below 5°. The enzyme preparation gives a negative reaction for peroxidase activity when assayed by the method of Konings (4). When tested with different concentrations of IAA, no change in activity as measured by the curvature test is observed which indicates the absence of any agent that will inactivate or destroy IAA. The preparation converts both tryptophan and tryptamine to IAA. The protein contents of the filtrates from the Sephadex G-25 and G-200 columns were determined by the method of Zamenhof (11) as 150 and 0.75 μg per ml respectively.

The activity of the enzyme is determined by incubating 1 ml of the final filtrate with 1 ml of 1 mM purified tryptophan (Regis) or purified tryptamine (Regis) with or without the addition of 0.3 μmole pyridoxal phosphate (Calbiochem.) and/or 1 ml of 1 mM gibberellic acid (85 % Eastman). The volume is made 3 ml in each determination with glass-distilled water. Incubation is maintained at 27° in a rotary shaker. After 2 hours the mixture is placed in boiling water for 5 minutes. Ten agar blocks (1.25 %) measuring 2 by 2 by 2 mm are placed in the mixture and equilibrated overnight at 0°. The auxin activity is determined by the *Avena* curvature test in which the curvature is compared to that induced by 0.1, 0.3, and 1 μM IAA, 4.5°, 9.4°, and 18° respectively. The curvatures obtained with the different enzyme preparations in the purification procedure are presented in table I together with the equivalent IAA concentrations. Thin-layer chromatography of the concentrated incubation mixtures on cellulose with a butanol-ethanol-water (4:1:1) solvent system shows that the auxin product of the enzyme system is IAA. Chromogenic development with Salkowski reagent gives a spot with an R_F identical to that of authentic IAA and of the same color, with the intensity of the color corresponding to the amount of curvature measured. The enzyme activity is calculated by taking the molarity of IAA equivalents formed in the incubation mixture during 2 hours and converting it to mμmoles per mg of protein per hour. These values show that the enzyme system forming IAA is concentrated in the

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Table I. *Activity of Different Enzyme Preparations in Converting Tryptophan and Tryptamine to Auxin During 2 Hours*

In each determination a control consisted of the enzyme preparation heated in boiling water before the addition of the substrate and other substances. None had auxin activity. The incubation mixtures contained 1.0 μmole of tryptophan or tryptamine, 1.0 μmole of gibberellic acid and 0.3 μmole of pyridoxal phosphate when present.

Enzyme preparation	Protein content	Curvature		Equivalent IAA conc.		Enzyme activity	
		Tryptophan	Tryptamine	Tryptophan	Tryptamine	Tryptophan	Tryptamine
	μg/ml	degrees		$\times 10^{-7}$ M		mμmoles mg^{-1} protein hr^{-1}	
Supernatant		11.6	10.4	4.8	3.8		
Filtrate from sephadex G-25	150 0	11.6	10.0	4.8	3.5	4.8	3.5
Dialysate		9.0	7.0	2.8	2.0		
Filtrate from sephadex G-200	0.75	9.2	6.8	2.9	2.0	580.0	400.0
Filtrate from sephadex G-200 + pyridoxal phosphate		12.6	9.6	5.6	3 1	1126.0	620.0
Filtrate from sephadex G-200 + gibberellic acid		14.8	10 1	7.4	4.4	1480 0	880.0
Filtrate from sephadex G-200 + gibberellic acid + pyridoxal phosphate (Incubation volume diluted 1:1)		14.3	11.1	7.0	4.6	2800.0	1840.0

Table II. *Effects of α-Ketoglutaric Acid and Other Additives on the Conversion of Tryptophan to Auxin During 1 Hour*

In each determination a control consisted of the enzyme preparation heated in boiling water before the addition of the substrate and other substances. None had auxin activity. The incubation mixtures contained 1.0 μmole of tryptophan, 1.0 μmole of α-ketoglutaric acid and gibberellic acid when present, and 0.3 μmole of pyridoxal phosphate when present.

Additives	Curvature	Equivalent IAA conc
	degrees	$\times 10^{-7}$ M
None	5.6	1.5
α-Ketoglutaric acid	9.1	2.8
Pyridoxal phosphate	7.3	2.2
Gibberellic acid	9.2	2.8
α-Ketoglutaric acid + pyridoxal phosphate	11.6	5.0
α-Ketoglutaric acid + gibberellic acid	11.3	4.8

protein fraction from Sephadex G-200 by a factor of 120 for tryptophan as the substrate and 114 for tryptamine as the substrate.

Gel filtration with Sephadex G-25 or Sephadex G-200 does not alter the activity of the enzyme system. Dialysis decreases the activity. Since pyridoxal phosphate is a possible co-factor for the diamine oxidase of pea seedlings (2) and for the oxidative decarboxylation of tryptophan to indoleacetamide in cabbage seedlings (7), it was added to the filtrate from Sephadex G-200. As shown in table I, the addition restored the activity lost by dialysis and indicates the probable role of pyridoxal phosphate as a co-factor in this system. The aldehyde group of pyridoxal phosphate reacts with the amino group of tryptophan and removes it from the acid leaving an α-keto acid (3). The amount of auxin formed by the system after gel filtration then would depend on the amount of pyridoxal phosphate present.

Since some evidence indicates that gibberellic acid promotes the conversion of tryptophan to auxin in plant tissue (8), the effect of gibberellic acid on the purified enzyme system was examined. Gibberellic acid also restores the activity lost by dialysis (table I). The preparation of gibberellic acid

used in this investigation is only 85 % pure and the possibility exists that the effects observed are due to the impurities. However, when the preparation was purified by thin-layer chromatography on Silica Gel G with butanol:ethanol:water (4:1:1) as the solvent system, the purified material had exactly the same effect as the original preparation.

In transamination reactions involving pyridoxal phosphate the molecule is ordinarily regenerated by the transfer of the amino group to a keto acid such as α-ketoglutaric acid. For this reason the activity of the enzyme system in the presence of 1.0 μmole of the acid (Nutritional Biochem) was determined. The data of 1 experiment are given in table II. The curvatures obtained in a duplicate experiment were within 1° of those given in the table. To maintain the curvatures in the range for standard solutions of IAA the time of incubation was reduced from 2 hours to 1 hour. The results show that α-ketoglutaric acid and gibberellic acid are equally effective in restoring the activity of the enzyme system which is lost by dialysis. The greater yield of IAA with tryptophan as the substrate also indicates that the conversion depends on a transaminase component of the system rather than a decarboxylase changing tryptophan to tryptamine. The conversion of tryptamine to IAA depends on another component of the preparation, perhaps an amine oxidase.

The retention of nearly equivalent activity of the enzyme system in the preparation that has been passed through Sephadex G-200 indicates the enzymes of the system, whatever the number involved, all have molecular weights of 200,000 or more. The molecular weight of amine oxidase which may well be one of these is reported to be 252,000 (10).

The enzyme system has also been isolated from apices of peas, tomato ovary tissue and Savoy cabbage seedlings.

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