Ethanolamine Metabolism in Plant Tissues[1, 2]

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Summary. Ethanolamine is readily metabolized by oat, pea, wheat, apple and carrot tissue preparations. Ethanolamine-1,2 ^{14}C was incorporated into the lipid fraction, and ^{14}C activity was distributed in the organic acid, sugar, acid volatile, carbon dioxide and insoluble residue fractions. The distribution varied with the particular tissue. Incorporation into the lipid fraction occurred in tissue homogenates in the absence of ATP by a Ca^{++} activated system similar to that reported for animal preparations. The initial step in ethanolamine oxidation involves an amine oxidase. Glycolaldehyde and glyoxylic acid are metabolic intermediates, the former in the conversion of ethanolamine to carbon dioxide. No evidence was obtained for the operation of an ethanolamine transaminase or for the involvement of phosphorylated intermediates in the conversion of ethanolamine to carbon dioxide.

The detailed metabolism of ethanolamine in plants has not been established despite its wide distribution in the plant kingdom in both free and combined forms. Limited evidence suggests that in animal systems ethanolamine is oxidized to CO_2 via glycolaldehyde, glycolate and glyoxylate (1, 2). This evidence is indirect since none of the postulated intermediates have been isolated and identified as products of ethanolamine metabolism. Phosphorylated intermediates may be involved and must be considered. The oxidation of phosphoethanolamine to phosphoglycolaldehyde has not been observed in plant tissues, but has been reported in rat liver homogenates (3). The oxidation of phosphoglycolaldehyde to phosphoglycolate has been reported in etiolated pea seedling mitochondria (4) and in spinach preparations (5). A phosphatase specific for phosphoglycolate has been isolated and partially characterized from spinach leaves (6). However, the oxidation of unphosphorylated ethanolamine (7) and glycolaldehyde (8) has been observed in pea seedling preparations.

In this investigation a series of plant tissues have been studied with respect to their ability to metabolize ethanolamine-1,2-^{14}C. Since each tissue examined demonstrated ethanolamine-1,2-^{14}C utilization, the general distribution of ^{14}C activity in organic acids, sugars, lipids, amino acids and CO_2 was established for each plant system and specific products considered. Although significant conversion of ethanolamine to CO_2 was observed in each plant tissue, no specific involvement of phosphorylated intermediates is indicated.

Materials

Peas (*Pisum sativum* L. var. Early Alaska), oats (*Avena sativa* L.) and wheat (*Triticum vulgare* L.) were surface sterilized with 0.5 % sodium hypochlorite and germinated in sterilized vermiculite at 25° for 7 to 10 days. Tobacco (*Nicotiana tobaccum* L.) was grown to maturity in a garden plot. Apples (*Pyrus malus* L.) and carrots (*Daucus carota* L.) were obtained from local commercial sources.

Ethanolamine-1,2-^{14}C was purchased from Volk Radiochemical Company and New England Nuclear Corporation. The commercial preparation was purified by paper chromatography in ethanol:ammonia (95:5).

Methods

Feeding Experiments. Apples and carrots were cut into cylinders 12 mm in diameter with a cork borer and sliced into 1 mm thick slices with a razor blade. The slices were washed 3 times with distilled water, blotted dry with filter paper and weighed. Approximately one g of tissue was placed in a 30 ml serum bottle and vacuum infiltrated with approximately 1 μc of ethanolamine-1,2-^{14}C (2 μmoles) according to the method of Cossins and Beevers (8). The tissues were incubated from 4 to 24 hours in the excess infiltration solution in the dark at 28°. Wheat, pea and oat seedlings were sliced into 1 to 3 mm sections and similarly vacuum infiltrated with ethanolamine-1,2-^{14}C.

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Carbonate free 10 % NaOH (w/v) or hyamine was placed in a separate compartment in the reaction vessel to absorb $^{14}CO_2$. Radioactivity of the $^{14}CO_2$ was measured with a Packard TriCarb liquid scintillation spectrometer, Series 314E. The $^{14}CO_2$ trapped with hyamine was solubilized in 10 ml of toluene phosphor solution and the $^{14}CO_2$ trapped in NaOH was solubilized in a toluene-ethanol (2:1) phosphor solution.

Tissue Fractionation. Plant tissue slices used in the radioactive distribution studies with ethanolamine-1,2-^{14}C were homogenized in 80 % ethanol and extracted by the procedure of Cossins and Beevers (8). After removal of the total lipid fraction with diethyl ether the extracts were further fractionated into organic acids, sugars, and amino acids by ion exchange chromatography according to the method of Wang (9). Distribution of ^{14}C in each fraction was determined by counting at infinite thinness triplicate 1 ml aliquots dried on metal or glass planchets at 60° on a Nuclear Chicago Ultrascaler Model 192A. All activities were corrected for background and counting efficiency against a calibrated ^{14}C standard.

Total activity in each experiment is reported as the sum of the activity of the $^{14}CO_2$, the insoluble residue and the 80 % ethanol extract. From 90 to 100 % of this total radioactivity was accounted for in the final fractions. The variation in total radioactivity between individual experiments is thus due to a variation in the initial ethanolamine-1,2-^{14}C concentration of the infiltrate.

Glycolaldehyde-^{14}C and glyoxylic acid-^{14}C were isolated from pea seedling tissue slices metabolizing ethanolamine-1,2-^{14}C by the addition of 200 mg of carrier glycolaldehyde or 100 mg of carrier glyoxylic acid. Pea seedling slices (1 g) were vacuum infiltrated with ethanolamine-1,2-^{14}C as described previously and incubated in the dark at 28° for 4 hours. The reaction was stopped by homogenizing the tissue slices in 10 ml of 10 % trichloroacetic acid. The protein precipitate was removed by centrifugation and the carrier glycolaldehyde or glyoxylic acid added. The aldehydes were isolated as their 2,4-dinitrophenylhydrazone derivatives, washed, and recrystallized from hot ethanol. The intermediates were identified by cochromatography in 2 solvent systems, methanol:chloroform (2:1, v/v) and ethanol:petroleum ether (4:1, v/v) for glycolaldehyde and methanol:benzene:*n*-butanol:H_2O (4:2:2:2, v/v) and *n*-butanol:water:ethanol (5:4:1, v/v) for glyoxylic acid (10).

Homogenates. Homogenates (20 % w/v) were prepared by grinding the plant tissues in 0.1 M potassium phosphate buffer, pH 7.0, with a mortar and pestle. Cellular debris was removed by centrifugation at 500 × g for 5 minutes. Assays of ethanolamine oxidation were performed in a Warburg apparatus at 25°. Reaction mixtures contained 1 ml of homogenate, 0.3 ml of 0.1 M ethanolamine and 0.1 M potassium phosphate buffer, pH 7.0, to a total volume of 3.3 ml. Where other reagents were included, adjustment of total volume was made by decreasing the volume of phosphate buffer.

Results

Distribution of Radioactivity in Plant Tissue Slices. The major consideration of ethanolamine in plant systems has been limited to its well established role as a precursor for choline and phospholipids. However, other areas of metabolic involvement are significant as indicated by the distribution of ^{14}C labeling in plant tissue slices vacuum infiltrated with ethanolamine-1,2-^{14}C (table I). Radioactivity was incorporated into organic acids, sugars, lipids, amino acids, insoluble residues and CO_2. In each instance from 60 to 90 % of the radioactivity was found in the amino acid fractions. Since in the fractionation procedure employed the exogenous ethanolamine-1,2-^{14}C is held with the amino acids, an estimate of the extent of metabolism may be made by summing the activity of the other fractions. Thus, depending on the specific tissue employed, 10 to 40 % of the ethanolamine-1,2-^{14}C is metabolized in a 4 to 24 hour incubation period.

The extent of ^{14}C distribution in each fraction varies from tissue to tissue. Ethanolamine-1,2-^{14}C

Table I. *Metabolism of Ethanolamine-1,2-^{14}C by Plant Tissue Slices*

Plant tissues were vacuum infiltrated with ethanolamine-1,2-^{14}C and incubated at 28° in the dark for the indicated time period.

Plant	Incubation time hrs	Fractions: Organic acids		Sugars		Lipids		Amino acids		CO_2		Residue		Acid volatiles	
		^{14}C (cpm)	% of ^{14}C	^{14}C (cpm)	% of ^{14}C	^{14}C (cpm)	% of ^{14}C	^{14}C (cpm)	% of ^{14}C	^{14}C (cpm)	% of ^{14}C	^{14}C (cpm)	% of ^{14}C	^{14}C (cpm)	% of ^{14}C
Oat	4	45,825	1.6	2695	0.1	353.600	12.5	2,081,600	73.5	5100	0.2	27.150	1.0	283.000	10.0
Oat	24	5110	0.8	12,075	1.8	25,200	3.7	493,103	72.8	10.452	1.5	6560	1.0	109,250	15.9
Tobacco	4	12,000	0.5	17,145	0.7	154 300	6.2	1,661.630	66.8	5545	0.2	37,900	1.5	84,150	3.4
Tobacco	24	38,850	0.9	37,060	0.9	24,300	0.6	2,857,140	68.0	29,705	0.7	141.200	3.4	793,250	18.9
Apple	4	4995	0.4	4940	0.4	600	0.1	1,243,125	87.2	3300	0.2	29 931	2.2	129,000	9.6
Wheat	24	24,375	1.2	30.245	1.5	164,400	7.9	1,623,347	78.2	4908	0.2	67.550	3.3	91.575	4.4
Pea	24	15,600	4.4	17.600	4.9	6880	1.9	206.762	51.5	23,028	6.4	15 130	4.2	36,750	10.3

incorporation into lipids in 4 hours ranged from 0.05 % in apple to 12.5 % in oats. Acid volatiles varied from 3.4 % in tobacco leaves to 20 % in carrot slices while organic acids ranged from 0.37 % in apple to 1.62 % in oats. While significant differences in percent total activity exist in fractions from different plant tissues, significant activity was found in each fraction. Two-dimensional paper chromatography and radioautography of the organic acid, amino acid, and sugar fractions yielded identical patterns, differing only in the extent of labeling of a given compound. No consistent radioautography pattern or trend distinguished the 4-hour from the 24-hour incubation experiments.

Glycolaldehyde-^{14}C and glyoxylic acid-^{14}C were isolated and identified as intermediates of ethanolamine-1,2-^{14}C metabolism by pea seedlings.

Ethanolamine Metabolism by Pea Seedling Homogenates. Pea seedling homogenates readily oxidized ethanolamine, the rate being directly proportional to the concentration of homogenate employed (fig 1). Pyridoxal phosphate ($1 \times 10^{-3} \times$ M) and Mn^{++} (1×10^{-5} M) were required for optimum activity which occurred between pH 6.5 to 7.0 at 25°. Higher concentrations of Mn^{++} (1×10^{-3} M) were inhibitory. The addition of oxalacetate (3×10^{-3} M), pyruvate (3×10^{-3} M) α-ketoglutarate (3×10^{-3} M), ATP (3×10^{-3} M), Mg^{++} (1×10^{-4} M) and thiamine pyrophosphate (3×10^{-4} M) had no effect on ethanolamine-1,2-^{14}C oxidation or its conversion to $^{14}CO_2$. No oxidation of phosphoethanolamine by pea seedling homogenates could be demonstrated under the conditions employed for ethanolamine oxidation.

The conversion of ethanolamine-1,2-^{14}C to $^{14}CO_2$ by pea seedling homogenates is extremely limited (table II). The enzymic nature of the reaction is indicated by the complete inactivity of homogenate placed in a boiling water bath for 10 minutes. The

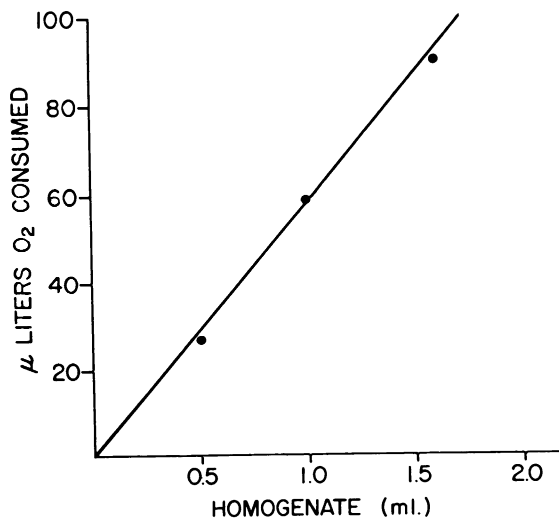

FIG. 1. The effect of pea seedling homogenate on the rate of ethanolamine oxidation.

Table II. *Oxidation of Ethanolamine-1,2-^{14}C to $^{14}CO_2$ by Pea Seedling Homogenate*

The reaction mixture contained 1 μc ethanolamine-1,2-^{14}C, 20 % homogenate prepared in 0.1 M phosphate buffer, pH 7.0, additions as indicated and H_2O to a total volume of 5.0 ml. Incubation was performed at 30° for 4 hours. $^{14}CO_2$ produced was trapped in hyamine.

Pea seedling homogenate (ml)	Additions	$^{14}CO_2$ Formed (cpm)
0	None	0
4	None	1630
4	Glycolaldehyde (30 μmoles)	652
4 (boiled)	None	0

addition of 30 μmoles of unlabeled glycolaldehyde to the homogenate oxidizing ethanolamine-1,2-^{14}C resulted in a 60 % decrease in $^{14}CO_2$ production.

Detailed characterization of the pea seedling homogenate with respect to substrate specificity, pH optimum, inhibitors, and coenzyme and cofactor activation indicates that the enzyme involved in the initial oxidation of ethanolamine is identical to the oxidase enzyme purified and characterized by Mann (7). Semicarbazide, hydroxylamine and KCN inhibited the oxidation of ethanolamine by pea amine oxidase and when vacuum infiltrated into pea leaf slices concurrently with ethanolamine-1,2-^{14}C inhibited $^{14}CO_2$ formation (table III). Since ethanolamine-1,2-^{14}C is the precursor of the $^{14}CO_2$ the inhibition observed in the tissue slices suggests the involvement of the amine oxidase in the in vivo system.

Incorporation of Ethanolamine-1,2-^{14}C into Lipids by Pea Seedling Homogenates. The incorporation of ethanolamine-1,2-^{14}C into the lipid fraction of pea seedling homogenates was linear over a 4-hour incubation period (fig 2.) Incorporation occurred in the absence of ATP. The dialyzed homogenates required the addition of Ca^{++}. Serine competitively inhibited the incorporation of ethanolamine-1,2-^{14}C and, conversely, ethanolamine competitively inhibited the incorporation of serine-3-^{14}C into the lipid fractions by dialyzed pea seedling homogenates.

Discussion

The results obtained in this investigation indicate that plant tissues possess the ability to readily metabolize ethanolamine. This metabolism involves more than the direct incorporation of ethanolamine into phosphatidylethanolamine, although in the case of the 4-hour oat sample, such incorporation may account for as much as 50 % of the total. The incorporation of radioactivity into sugars, organic acids, acid volatiles, and CO_2 indicates the presence of additional pathways of ethanolamine metabolism

Table III. *Effect of Amine Oxidase Inhibitors on Ethanolamine Metabolism by Pea Amine Oxidase and Pea Leaf Slices*

The amine oxidase studies were performed in a Warburg respirometer. The reaction mixture consisted of 0.5 ml pea amine oxidase, 0.3 ml ethanolamine, 0.1 M, inhibitor as indicated, and potassium phosphate buffer 0.1 M, pH 7.0, to a total volume of 3.7 ml. The center well contain 0.2 ml of 20 % KOH. Incubations were performed for 45 minutes at 30° in an air atmosphere. Inhibitors and ethanolamine-1,2-^{14}C were vacuum infiltrated concurrently into the pea leaf slices and the tissue preparations incubated in the dark for 4 hours at 28°. The $^{14}CO_2$ produced was trapped in hyamine.

Tissue preparation	Inhibitor	Conc: In reaction milieu (M)	Conc: In vacuum infiltrate (M)	O_2 Consumed (μmoles)	$^{14}CO_2$ formed (cpm)	% of Control
Pea amine oxidase	None			2.75		
Pea amine oxidase	Semicarbazide	1×10^{-3}		0		0
Pea amine oxidase	Semicarbazide	1×10^{-5}		0.64		23
Pea amine oxidase	Hydroxylamine	1×10^{-3}		0.20		7
Pea amine oxidase	Hydroxylamine	1×10^{-5}		0.40		15
Pea amine oxidase	KCN	1×10^{-3}		2.26		82
Pea amine oxidase	KCN	1×10^{-5}		2.83		103
Pea leaf slices	None				28,000	
Pea leaf slices	Semicarbazide		2×10^{-2}		215	1
Pea leaf slices	Hydroxylamine		2×10^{-2}		1227	5
Pea leaf slices	KCN		4×10^{-2}		1539	7
Pea leaf slices	KCN		2×10^{-2}		4828	21

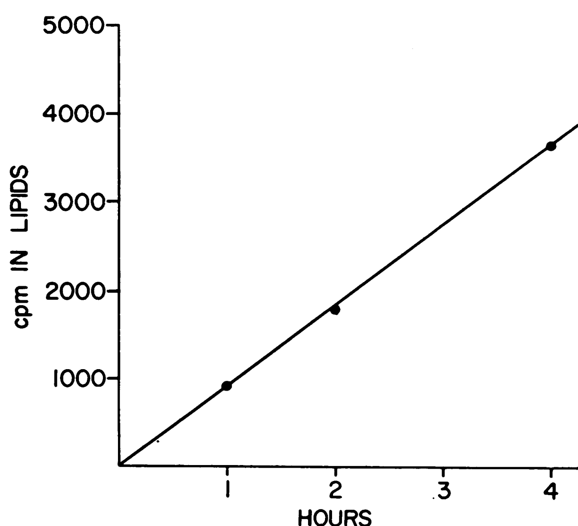

FIG. 2. The incorporation of ethanolamine-1,2-^{14}C into lipids by pea seedling homogenates.

in plant tissues. The extent of in vivo operation of such additional pathways is not immediately apparent. However, the presence of free ethanolamine and ethanolamine phosphate in plants suggest that limited operation may exist (11, 12).

The oxidation of ethanolamine by pea seedling amine oxidase has been previously reported by Mann (7). The participation of this enzyme in ethanolamine oxidation to CO_2 is indicated by the complete elimination of $^{14}CO_2$ production in crude homogenate when pea amine oxidase inhibitors are added. The inhibition of $^{14}CO_2$ formation when semicarbazide, hydroxylamine and KCN are vacuum infiltrated into plant tissue slices suggest the in vivo participation of this enzyme in ethanolamine oxidation. The isolation and partial purification of amine oxidases which preferentially oxidize ethanolamine in mature bean, beet, and spinach leaves tends to indicate that similar amine oxidases are involved in ethanolamine oxidation in the various plant tissues investigated (unpublished data).

The isolation and identification of glycolaldehyde and glyoxylic acid as intermediates in the oxidation of ethanolamine by pea slices further supports the direct involvement of an amine oxidase, glycolaldehyde dehydrogenase, and glycolic acid oxidase.

The incorporation of ethanolamine into the phospholipids of pea seedling homogenates appears to occur by the same mechanism reported for rat liver homogenates (13, 14). The activation by Ca^{++}, the absence of ATP stimulation, and the competitive inhibition between ethanolamine and serine are all characteristic of this system. Sufficient data are not available to determine the extent of the contribution this metabolic pathway may make in plant systems.

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