DECARBOXYLATION OF METHIONINE BY AN ENZYME SYSTEM FROM CABBAGE LEAF

Mendel Mazelis

Western Regional Research Laboratory,* Albany, California

Only two amino acid decarboxylases have been described in higher plants. L-glutamic acid decarboxylase has been found widely distributed in the plant kingdom (Schales, Mims, and Schales, 1946), and a γ-methylene-L-glutamic acid decarboxylase has been found in extracts from peanut plants, red pepper, and barley roots (Fowden and Done, 1953). Although several other amino acid decarboxylases have been demonstrated in bacteria or animal tissues (Meister, 1957), the decarboxylation of methionine has not been observed.

This note is a preliminary report of an enzyme system obtained from cabbage leaves which will convert the carboxyl group of methionine to CO_2 in the presence of Mn^{++} and pyridoxal phosphate.

The enzyme was prepared from washed cytoplasmic particles obtained from cabbage leaf homogenates by differential centrifugation as described previously (Mazelis, 1959). Two volumes of a 1% digitonin solution were added to the particulate suspension. After 60 minutes at 4° C., with constant stirring the suspension was centrifuged for 30 minutes at 27,000 x g. A clear green supernatant solution was obtained. The solution was made 75% saturated to $(NH_4)_2SO_4$. The precipitate was collected by centrifugation, dissolved in a small volume of cold distilled H_2O and then dialyzed for 1.5 to 2 hours at 10° C. The precipitate which formed in the dialysis sack was discarded and the clear solution used as the source of enzyme.

The enzyme assay consisted of measuring the amount of $C^{14}O_2$ released from DL-methionine-$C^{14}OOH$ after incubation for 90 minutes at 30° C. The

* A Laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

reaction was carried out in standard Warburg vessels with 0.2 ml. 20% KOH in the center well. It was terminated by tipping in 0.3 ml. 5 N H_2SO_4 from the sidearm. The KOH was collected after shaking for at least 10 minutes more. Absorbed CO_2 was precipitated and counted as $BaCO_3$ (Stumpf, 1955).

The decarboxylase nature of the solubilized enzyme is shown in Table I. The only requirements for activity are the presence of Mn^{++} and pyridoxal phosphate. Early experiments, in which the enzyme was dissolved in and dialyzed against dilute phosphate buffers, had demonstrated a stimulation on the addition of ATP, DPN, and TPN. Increasing the ATP concentration eliminated any stimulation by added DPN and TPN. This implied that the ATP and pyridine nucleotides had some secondary effect on the decarboxylation. The presence in cabbage leaves of an apyrase, nucleotide pyrophosphatase, and a non-specific phosphatase active at pH 7.0 has been well documented (Mazelis, 1959). It seemed a reasonable explanation of the nucleotide effects that the added nucleotides protected against the dephosphorylation of pyridoxal phosphate by competing as substrates for any phosphatases present after undergoing either apyrase or nucleotide pyrophosphatase action. Therefore, only at low concentrations of ATP would the pyridine nucleotides have any effect. The belief that dephosphorylation of pyridoxal phosphate occurs is given some substantiation in Table I. When F^- was added, the decarboxylation was stimulated by 11%.

Table I

Cofactor Requirements for Decarboxylation of Methionine by Soluble System

System	Total counts as $BaC^{14}O_3$
Complete	4485
No $MnCl_2$	297
No pyridoxal phosphate	53
Complete plus ATP	4381
Complete plus F^-	4985
Heated, complete	70

Complete system contained 2 µmoles DL-methionine-$C^{14}OOH$ (140,000 total counts); 100 µmoles phosphate buffer pH 7.0; 0.4 µmoles pyridoxal phosphate; 5 µmoles $MnCl_2$; 1 mg. protein as enzyme; and distilled water to a final vol-

ume of 2 ml. in the main compartment. Where indicated 10 μmoles ATP and 50 μmoles NaF were added. The enzyme was heated for 5 minutes in a boiling water bath for use in the heated control.

The addition of ATP did not increase the activity. Recent modifications in the preparation of the soluble system have eliminated any large effects of added ATP. The enzyme is quite stable as it retained almost its full activity after being stored for 2 months at -10° C.

The intracellular localization of the enzyme was studied. A comparison of the activity of the soluble system with the particles from which it was extracted and with the supernatant fluid obtained after the particles were removed by centrifugation is given in Table II. When freshly washed cytoplasmic particles were used as the enzyme source, wide fluctuations in specific activities were found. Stimulation of the activity by ATP, DPN, and TPN was inconsistent. However, Mn^{++} and pyridoxal phosphate were obligatory cofactors. In the experiment reported, the specific activity was increased 13-fold by solubilization. The low activity of the particles may be due to the difficulty of access of methionine to the enzyme sites.

Further studies are in progress to purify the enzyme and characterize its properties.

Table II

Intracellular Distribution of Methionine Decarboxylase Activity

Enzyme source	Specific activity (Total counts per mg. protein)
Soluble system from digitonin-treated particles	1581
Washed cytoplasmic particles	126
Cytoplasmic protein	71

Reaction system contained phosphate buffer and DL-methionine-$C^{14}OOH$ as in Table I; 0.2 μmoles DPN; 0.2 μmoles TPN; 2 μmoles ATP; 5 μmoles $MnCl_2$; 0.4 μmoles pyridoxal phosphate; enzyme preparation; and distilled water to a final volume of 2 ml. in the main compartment. Enzyme sources: 2 mg. protein as the soluble system from digitonin-treated particles; 9.3 mg. protein as washed cytoplasmic particles; 2.4 mg. of cytoplasmic proteins remaining after removal of the cytoplasmic particles.

References

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