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SHORT COMMUNICATION

SULFHYDRIL NATURE OF GALACTOSYL TRANSFER ENZYMES OF SPINACH CHLOROPLASTS

S. B. CHANG

Department of Chemistry, Western Washington State College, Bellingham, Washington, U.S.A.

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Abstract—The sulfhydril nature of galactosyl transfer enzymes in the biosynthesis of galactolipids has been established with the chloroplasts of *Spinacia oleracea*.

INTRODUCTION

DURING the course of the studies of galactolipids biosynthesis with spinach (*Spinacia oleracea* chloroplasts) some divalent ions, such as cobalt and manganese, were found to be inhibitory.[1] In the present communication the results obtained from the studies made on the inhibitory effect of various sulfhydril reagents such as para-chloromercuribenzoate, iodoacetic acid, and mercury ion are reported. The effect of a reducing agent, mercaptoethanol, on the inhibitory action of the above reagents is also described.

RESULTS

When uridine diphosphate galactose (^{14}C) is incubated with spinach chloroplasts (*Spinacia oleracea*) radioactive galactose was actively incorporated into galactolipids.

The enzyme system which catalyzes the galactose transfer reactions was inhibited almost 100% with the addition of as low as 0·5 μmole of Hg^{2+} to the reaction mixture as shown in Table 1. Para-chloromercuribenzoate and iodoacetate also almost completely inhibited the

TABLE 1. EFFECT OF Hg^{2+} ON THE INCORPORATION OF GALACTOSE INTO GALACTOLIPIDS

	Galactolipids (counts/min)
Control	12,200
+Hg^{2+} (10 μmole)	340
+Hg^{2+} (1·0 μmole)	350
+Hg^{2+} (0·5 μmole)	260
+Hg^{2+} (0·1 μmole)	8800

enzyme activity (Table 2). A reducing agent, mercaptoethanol, recovered more than 60% of the original enzyme activity from the Hg^{2+} inhibition, as shown in Table 3. All these results indicate the presence of sulfhydril group(s) in the active site of the galactosyl transfer enzyme.

[1] S. B. CHANG and N. D. KULKARNI, *Phytochem.* 9, 927 (1970).

TABLE 2. EFFECT OF SULFHYDRIL REAGENTS ON THE INCORPORATION OF GALACTOSE INTO GALACTOLIPIDS

	Galactolipids (counts/min)
Control	8400
+*P*-Chloromercuribenzoate (10 μmole)	260
+Iodoacetate (100 μmole)	330

TABLE 3. EFFECT OF MERCAPTOETHANOL ON Hg^{2+} INHIBITORY ACTION ON GALACTOLIPID BIOSYNTHESIS

	Galactolipids (counts/min)
Control	3900
+Hg^{2+} (0·5 μmole)	280
+Hg^{2+} (0·5 μmole) and Mercaptoethanol (10 μmole)	1100
+Hg^{2+} (0·5 μmole) and Mercaptoethanol (50 μmole)	2500

EXPERIMENTAL

Isolation of Chloroplasts

Chloroplasts were isolated from fresh, washed spinach leaves according to the method of Whatley and Arnon[2] with the modification of the chloroplast solution to sucrose–phosphate buffer, pH 7·4 (0·5 M sucrose and 0·01 M KH_2PO_4).

Enzyme Assay

The chloroplasts equivalent to 5~10 mg protein, and equivalent to 2~5 mg chlorophylls in 0·5 ml were incubated with 0·1 ml of uridine diphosphate [^{14}C] galactose (U) (20,000~40,000 counts/min/0·6~1·2 mμmole) and 0·4 ml of 0·1 M Tris–HCl buffer, pH 7·4, at 37° for 1 hr with constant shaking. The reaction was stopped and the lipid products were extracted according to the method of Lennarz[3] with some modifications. At the end of the incubation time, the reaction was stopped by adding 4·0 ml $CHCl_3$–MeOH (2:1, v/v) to the reaction mixture in 12-ml centrifuge tubes. Tubes were mixed on a Vortex mixture for a minute and placed in a 55° water bath for 3 min. Tubes were mixed again and returned to the bath for an additional 7 min. Then the tubes were taken out of the bath and were mixed again before the reaction mixtures were filtered through a wad of glass-wool to remove denatured proteins. The tubes and funnels were rinsed with 2·0 ml $CHCl_3$–MeOH and 2·5 ml of 0·9% aq. NaCl was added to the filtrate. The mixture was mixed well for a minute and was chilled in an ice bath for 5 min. After centrifugation for 5 min the upper aqueous layer was removed and the lower $CHCl_3$ layer was washed three more times with 2·5 ml NaCl soln. For the assay of total galactolipid products, an appropriate aliquot of the $CHCl_3$ solution was transferred to a planchet, dried and counted.

Analytical Methods

Chlorophylls were determined by the method of Arnon[4] and protein was determined by the method of Lowry.[5] TLC of galactolipid products was done by the method of Chang.[1] Lipids were applied on silica gel G thin-layer plates and two-dimensional chromatograms were developed by solvent systems of $CHCl_3$–CH_3OH–7 N NH_4OH (65:25:4, by vol.) (S_1) and $CHCl_3$–CH_3OH–CH_3CO_2H–H_2O (170:30:20:7, by vol.) (S_2).

[2] F. R. WHATLEY and D. I. ARNON, in *Methods in Enzymology* (edited by S. P. COLOWICK and N. O. KAPLAN), p. 308, Academic Press, New York (1963).
[3] W. J. LENNARZ and B. TALAMO, *J. Biol. Chem.* **241**, 2707 (1966).
[4] D. I. ARNON, *Plant Physiol.* **24**, 1 (1949).
[5] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. Biol. Chem.* **193**, 265 (1951).