CONVERSION OF GLUCOSE TO INOSITOL IN PARSLEY LEAVES

Frank A. Loewus and Stanley Kelly

Western Regional Research Laboratory*, Albany, California

Received March 2, 1962

The idea that inositol+ may be formed by ring closure of glucose was clearly formulated by Fischer in 1945. Since then other investigators have demonstrated an over all conversion of glucose to inositol by using isotopically labeled glucose (Daughaday et al., 1955; Halliday and Anderson, 1955; Charalampous, 1957; Eagle et al., 1960). An internal labeling pattern for inositol formed biosynthetically from specifically labeled glucose by yeast cells has been described, but its complex nature suggested that fragmentation of glucose preceded inositol synthesis (Charalampous, 1957).

Higher plants contain appreciable quantities of inositol. A study of the conversion of glucose to inositol in a tissue of this type would be very desirable. This note describes a set of experiments in which this conversion is demonstrated in the detached parsley (Petroselinum) leaf. A highly specific method of obtaining the internal distribution of radioactivity in inositol is provided by the recent observation that labeled inositol is converted by detached ripening strawberries to galacturonosyl residues (pectin) in appreciable yield (Loewus et al., 1962). Very little redistribution of radioactivity occurs during the conversion. Thus inositol-2-C^{14} is converted to galacturonic acid labeled in carbon 5 (Loewus and Kelly, 1962) as well as to L-arabonosyl residues also labeled in carbon 5 (Loewus and Kelly, unpublished observation).

---

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

+ Inositol refers to myo-inositol, glucose to D-glucose, and galacturonic acid and its derivatives to D-galacturonic acid and its derivatives. Inositol is numbered according to Angyal and Anderson (1959).

In each experiment, three to five detached parsley leaves (total weight, approx. 5 g.) were given 15 μc. of glucose-1-C^{14} (4 μc./mg.) in 0.5 ml. of water through their petioles over a one-half hour period. The leaves were then transferred either to water (Expts. 1 and 2) or to 1% inositol (Expts. 3 and 4) for 42 hours in the light. Unlabeled inositol taken up during this period (Expt. 3, 156 mg. and Expt. 4, 90 mg.) was only partially utilized. The unconsumed portion provided a convenient means of recovering labeled inositol that had been formed from the radioactive glucose.

The labeled leaves were frozen, pulverized, and ground in 40 ml. of 0.1% oxalic acid. This slurry was centrifuged at 12,000 x g. for 10 minutes. The clear supernatant was freed of oxalate with an equivalent of Ca formate and then passed through columns of Dowex 50 (H^+) and Dowex 1 (formate). The neutral effluent was reduced to a light sirup. In Expts. 3 and 4, about 50 to 60% of the inositol taken up by the leaves was recovered from this sirup as a radioactive crystalline residue. This residue was brought to constant specific activity after three recrystallizations from water and ethanol.

These sirups (less the crystalline residues removed from Expts. 3 and 4) were streaked on large sheets of Whatman No. 1 paper and developed 24 hours by descending chromatography in ethyl acetate-pyridine-water (8:2:1) (Williams and Bevenue, 1953) along with appropriate controls. Side strips were removed from the dried sheets and treated with $AgNO_3$ followed by a spray of alcoholic KOH (Trevelyan *et al.*, 1950). Inositol appeared in all experiments as a narrow band midway between the origin and sucrose. Autoradiography revealed a dark streak coincident with inositol in Expts. 1 and 2, but the corresponding region in Expts. 3 and 4 was clear since most of the radioactivity associated with inositol in the latter two experiments was removed by diluent inositol and recovered in the crystalline residue prior to chromatography.

The radioactive inositol was eluted from the chromatograms of Expts. 1 and 2 and rechromatographed in the solvent system described above for 72 hours to obtain a clear separation of this compound from other radioactive

constituents. Again the inositol was eluted and assayed chemically (Agranoff et al., 1958). To establish the purity of this material, an aliquot was diluted with authentic non-isotopic inositol, recrystallized, combusted to CO_2, and counted. All of the radioactivity present in the aliquot was accounted for in the recrystallized inositol.

The labeled inositol was administered to detached green strawberries (Fragaria) through their cut stems followed by a period of metabolism in distilled water. The entire berry including stem and hull was ground in cold 70% ethanol. The insoluble residue recovered from this treatment was washed repeatedly with successive portions of 70% ethanol until the radioactivity of the wash was negligible. Pertinent experimental details are given in Table I.

TABLE I

Conversion of Labeled Inositol to Galacturonate by Detached Ripening Strawberries

Experiment Number	1	2	3	4
Amount of inositol administered, mg.	1.9	1.3	65.	30.
C^{14} in inositol administered, dpm.*	34,000	104,000	60,000	68,000
Number of strawberries used	4	4	4	4
Initial fresh weight of berries, g.	9.9	8.7	16.4	14.1
Period of inositol uptake, hours	3	3	45	11
Total period of respiration, hours	51	51	70	70
C^{14} solubilized from washed residues after enzymatic hydrolysis, dpm.*	3,400	7,300	4,300	6,400
C^{14} recovered in galacturonate, dpm.*	1,500	4,000	1,100	3,500
Conversion of inositol to galacturonate (a minimal value), %	4.4	3.9	1.8	5.1

* Disintegrations per minute. Activities of aqueous solutions were measured in a liquid scintillation counter. Specific activities of crystalline materials were obtained with an internal gas phase proportional counter after conversion to CO_2.

Galacturonic acid was recovered by enzymatic hydrolysis of the washed residue followed by ion exchange procedures already described (Loewus, 1961), and its C^{14} distribution among carbon 1, carbons (2+3+4+5), and carbon 6 determined chemically (Seegmiller et al., 1955). Results are listed in Table II.

TABLE II

C^{14} Distribution in Galacturonate

Experiment Number	1	2	3	4
Total combustion to CO_2 (cpm./mmole galacturonate)*	4,800	17,300	2,300	6,800
Degradation of galacturonate (cpm./mmole of fragment)*				
Carbon 1 as HCHO	3,500	15,100	2,100	5,800
Carbons 2+3+4+5 as HCOOH	800	1,000	100	350
Carbon 6 as CO_2	700	2,200	200	750
Sum	5,000	18,300	2,400	7,000
C^{14} in each fragment (%)				
Carbon 1	70	82	88	84
Carbons 2+3+4+5	16	6	4	5
Carbon 6	14	12	8	11

* Values reported are the observed counts per minute/millimole of carbon (80% efficiency) times the number of carbons in the molecule or fragment.

In all experiments carbon 1 of galacturonic acid (corresponding to carbon 6 of inositol) contained most of the C^{14} (70 to 88%). Since the parsley leaves from which the labeled inositol was recovered had been fed glucose-1-C^{14}, carbon 1 of glucose corresponds to carbon 6 of inositol. The appearance of C^{14} in other carbons of the degraded galacturonic acid, especially carbon 6 (corresponding to carbon 1 of inositol), represents a minor redistribution of label, probably resulting from triose equilibration during the conversion of glucose to inositol. Similar labeling patterns have been observed among other carbohydrate products derived from specifically labeled glucose (Shibko and Edelman, 1957; Loewus and Jang, 1958).

The labeling pattern in inositol recovered from parsley leaves given glucose-1-C^{14} supports a cyclization mechanism like the one proposed by Fischer (1945). Such a process might be catalyzed by an aldolase-type reaction in which the intermediate immediately preceeding the formation of a carbon-carbon bond between carbon 1 and carbon 6 could be a phosphorylated 5-keto glucose or a phosphorylated 6-aldo fructose. The ease with which carbon 2 of

inositol is oxidized and reduced favors the idea of a 5-keto compound with the configuration of glucose.

In higher plants, oxidative cleavage of the 1,6 bond of inositol results in the formation of galacturonosyl residues as well as other related interconversions (Loewus et al., 1962). This means that glucose converted to pectin via inositol will have the same carbon chain sequence (carbon 6 of glucose is oxidized) as that observed when uridine diphosphate glucose is oxidized via uridine diphosphate dehydrogenase (Strominger and Mapson, 1957). Experiments employing specifically labeled glucose-C^{14} will not distinguish between these pathways.

A detailed study of the C^{14} distribution in galacturonosyl residues, the glucose moiety of sucrose, and the L-ascorbic acid recovered from the same parsley leaves is being completed and will be reported elsewhere.

REFERENCES

Agranoff, B. W., Bradley, R. M., and Brady, R. O., J. Biol. Chem., 233, 1077 (1958)
Angyal, S. J., and Anderson, L., Advances Carbohydrate Chem. 14, 135 (1959).
Charalampous, F. C., J. Biol. Chem., 225, 595 (1957).
Daughaday, W. H., Larner, J., and Hartnett, C., J. Biol. Chem., 212, 869 (1955).
Eagle, H., Agranoll, B. W., and Snell, E. E., J. Biol. Chem., 235, 1891 (1960).
Fischer, H. O. L., Harvey Lectures, 40, 156 (1944-45).
Halliday, J. W., and Anderson, L., J. Biol. Chem., 217, 797 (1955).
Loewus, F. A., Ann. N. Y. Acad. Sci., 92, 57 (1961).
Loewus, F. A., and Jang, R., J. Biol. Chem., 232, 505 (1958).
Loewus, F. A., and Kelly, S., Federation Proceedings, 21, in press (1962).
Loewus, F. A., Kelly, S., and Neufeld, E. F., Proc. Natl. Acad. Sci., 48, in press (1962).
Seegmiller, C. G., Axelrod, B., and McCready, R. M., J. Biol. Chem., 217, 765 (1955)
Shibko, S., and Edelman, J., Biochim. Biophys. Acta, 25, 642 (1957).
Strominger, J. L., and Mapson, L. W., Biochem. J., 66, 567 (1957).
Trevelyan, W. E., Procter, D. P., and Harrison, J. S., Nature, 166, 444 (1950).
Williams, K. T., and Bevenue, A., J. Assn. Official Agr. Chem., 36, 969 (1953).