SYNTHESIS OF GALACTOSYLINOSITOL BY EXTRACTS FROM PEAS*

Rosalia B. Frydman and Elizabeth F. Neufeld

Department of Biochemistry, University of California
Berkeley, California

Received May 27, 1963

Oligosaccharides containing D-galactose are of common occurrence in higher plants (Courtois, 1960). While most of these are galactosides of sucrose (e.g., raffinose, stachyose), a galactoside of inositol (galactinol) has been isolated from sugar beets (Brown and Serro, 1953), and shown to possess the structure 1-O-α-D-galactopyranosyl myoinositol (Kabat et al., 1953). A preparation has now been obtained from unripe pea seeds, which catalyzes the transfer of galactosyl residues from UDP-D-galactose to myoinositol to yield a compound with the properties of galactinol. The same preparation also catalyzes galactosyl transfer to scyllo-, dextro-, and levoinositol to yield galactosides different from the galactinol of sugar beets. The existence of such enzyme(s), which may be formally designated as UDP-D-galactose:inositol galactosyltransferase(s), in peas suggests that galactosides of inositol may have a much wider distribution in plants than has been suspected.

Preparation of transferase. Fresh peas obtained in a local market were shelled and the seeds used as source of the D-galactosyl transferase either immediately or after rapid freezing and storage at -10° for several months. Fifty g of peas were ground in a chilled mortar with 50 ml of buffer (0.5 M sucrose, 0.05 M phosphate pH 7.0, 0.05 M mercaptoethanol).

*This investigation was supported in part by a research grant (No. A-1418) from the National Institutes of Health, U. S. Public Health Service, and by a research grant (No. G-23763) from the National Science Foundation.

After centrifugation at 10,000 x g for 10 minutes, the supernatant solution was fractionated with solid $(NH_4)_2SO_4$. The 30-50% fraction was dissolved in 0.01 M Tris-HCl, pH 7.5, dialyzed against 3 liters of the same buffer, and clarified by centrifugation (typical yield: 12 ml, containing 360 mg protein). A 5 ml portion of this fraction was refractionated further by the sequential addition of 2.5, 1, 1.5, and 5 ml of a saturated neutralized solution of $(NH_4)_2SO_4$. The precipitates were dissolved in 1 to 2 ml of 0.01 M Tris, pH 7.5, and dialyzed against the same buffer. The most active fraction, usually the second or third, was used for subsequent work. This preparation was stable at -10° for at least two months.

Assay of Transferase. The ability of this preparation to catalyze the transfer of D-galactosyl residues to inositol was routinely assayed as follows. UDP-D-Galactose-C^{14} (0.06 μc, of 6 μc/μmole unless otherwise specified), 0.5 μmole myoinositol, 2.5 μmole sodium acetate buffer, 0.5 μmole $MnCl_2$, and enzyme, were incubated at pH 5.6 in a total volume of 45 to 65 μl. A control reaction mixture from which inositol was omitted was set up concurrently. After incubation at 30° for 15 to 120 minutes, the reaction mixtures were subjected to paper electrophoresis in 0.2 M ammonium formate, pH 3.7. The neutral material was eluted and subjected to descending chromatography on Whatman 4 paper in n-propanol-ethyl acetate-water (7:1:2). The radioactivity in areas corresponding to the location of galactinol was measured. The rate of incorporation of radioactivity into the product, catalyzed by a typical preparation, and the linear relationship between enzyme concentration and product formation are illustrated in Fig. 1. Optimal pH was found to be 5.6 in 0.05 M acetate or phosphate buffers. K_m for inositol was found to be 5×10^{-3} M.

Identification of Reaction Product as Galactinol. The identification of the radioactive reaction product as galactinol is based on the following criteria: (1) co-chromatography with authentic galactinol in n-propanol-ethyl acetate-water (7:1:2), phenol-water (80:20), n-butanol-

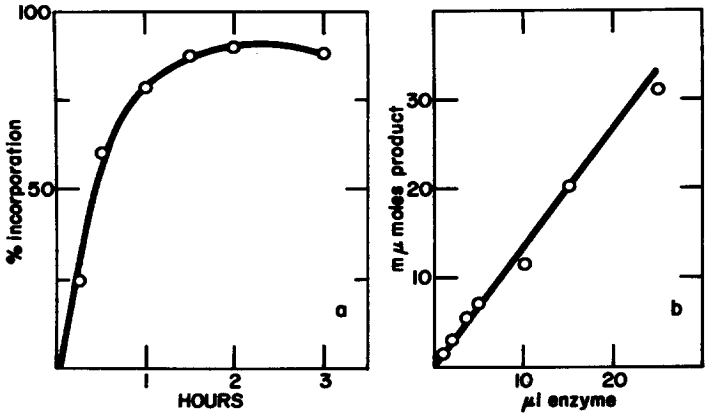

Fig. 1. (a) Rate of incorporation of galactosyl residues from UDP-D-galactose into product, assayed as described in text. (b) Effect of increasing enzyme concentration, assayed as described in text, except for the use of 0.14 μmole (0.01 μc) UDP-D-galactose.

pyridine-water (6:4:3); (2) co-electrophoresis in 0.05 M sodium tetraborate; (3) hydrolysis by α-galactosidase (a preparation of invertase, containing melibiase, was used for this purpose without purification) to yield a radioactive compound behaving chromatographically as galactose; (4) co-crystallization with galactinol; the specific activity increased at first and remained constant from the fourth through the seventh recrystallization.

Activators and Inhibitors. Transfer of galactosyl residues to myoinositol was reduced by about 80% when $MnCl_2$ was omitted from the system. Mn^{++} could be replaced, albeit less effectively, by Co^{++} or Fe^{++}, but not by Mg^{++}, Ca^{++}, Zn^{++}, or Ni^{++}. Optimal concentrations for Mn^{++} or Co^{++} were found to be 7×10^{-3} M. In the absence of Mn^{++}, EDTA at a concentration of 2.5×10^{-4} M completely inhibited transfer.

UTP, dTTP, and UDP (8×10^{-3} M) inhibited the reaction completely. Similar concentrations of ATP, ADP, GTP, or UMP were half as inhibitory, while uridine and uracil had no effect.

Specificity of the Galactosyl Acceptor. In addition to myoinositol, three naturally-occurring cyclitols (scyllo-, dextro-, and levoinositol)

were found to be acceptors of galactosyl residues in the system used. Although the relative effectiveness of these substrates varied somewhat from preparation to preparation, generally they reacted in the order myo > dextro = levo > scyllo.

A number of other compounds were tested and found not to function as galactosyl acceptors. These include neo-, muco-, and alloinositols, pinitol, quercitol, phosphorylated inositol derivatives, phosphoinositide, D-glucitol, D-mannitol, galactitol, D-allitol, D-talitol, D-glucose, D-galactose, sucrose, and lactose.

The products of galactosyl transfer to scyllo- and dextroinositol are readily hydrolyzed by α-galactosidase. The product of transfer to levoinositol, however, proved completely resistant to the action of both yeast α-galactosidase and to E. coli β-galactosidase. Treatment with 1 N HCl at 100° for 30 minutes released radioactive material chromatographically indistinguishable from galactose. The resistance of the compound to enzymatic hydrolysis merits further study.

The galactosides of dextro- and levoinositol are chromatographically separable from galactinol ($R_{galactinol}$:galactosyldextroinositol, 1.6; galactosyllevoinositol, 1.4). The product of galactosyl transfer to scylloinositol could not be separated from galactinol in a number of systems, but was shown to be a galactoside of scylloinositol by the following experiment. Scylloinositol-$2H^3$ was incubated with pea enzyme, $MnCl_2$, and unlabeled UDP-D-galactose. The neutral compounds were subjected to chromatography. The area corresponding to galactosylinositol was eluted, purified by rechromatography, and treated with α-galactosidase. The tritium-labeled product of this hydrolysis was shown to migrate as scylloinositol upon electrophoresis in 0.05 M sodium tetraborate, in which galactosylinositol, myoinositol, and scylloinositol are clearly separable.

Specificity of the Galactosyl Donor. As shown in Table 1, dUDP-D-galactose and dTDP-D-galactose (Neufeld, 1962) function as galactosyl donors to the four cyclitol substrates at a rate much lower than UDP-D-

galactose. No detectable galactoside was formed from α-D-galactose-1-phosphate or ADP-D-galactose.

TABLE I.

Specificity of the Galactosyl Donor

Inositol	% Radioactivity Transferred from UDP-D-galactose	dTDP-D-galactose	dUDP-D-galactose
Myo	88	5	15
Dextro	71	4	7
Levo	75	3	7
Scyllo	53	2	1

The reaction was carried out under conditions similar to those in the routine assay. Galactosyl nucleotides had a specific activity of 6 μc/μmole. Incubation was carried out for 30 minutes for myoinositol and for 2 hours for the other cyclitols.

Acknowledgments

The authors are greatly indebted to Dr. C. E. Ballou for gifts of scyllo-, dextro-, levo-, neo-, muco-, alloinositols, phosphorylated inositols and phosphoinositide, and for a sample of galactinol; to Dr. E. Rorem for galactinol; and to Dr. F. Loewus for 2-H^3-scylloinositol and 2-H^3-myoinositol.

References

Brown, R. J., and Serro, R. F., J. Am. Chem. Soc. 75, 1040 (1953).

Courtois, J. E., Bull. Soc. Chimie Biol. 42, 1451 (1960).

Kabat, E. A., MacDonald, D. L., Ballou, C. E., and Fischer, H. O. L., J. Am. Chem. Soc. 75, 4507 (1953).

Neufeld, E. F., Biochem. Biophys. Res. Commun. 7, 461 (1962).