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Note

De novo synthesis of glycosidic linkages by glycosylases: utilization of α-D-glucopyranosyl fluoride by amylosucrase

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We recently reported evidence[1–4] indicating that the biochemical process of glycosyl transfer, contrary to the traditional view[5–7] of this process, is capable of producing glycosidic linkages *de novo*, without the need for pre-existing glycosidic linkages. These findings, obtained with α-, β-, and gluco-amylases[1–3], dextran-sucrase[2,4], and (by others[8]) with sucrose phosphorylase, support the concept that the basis of all carbohydrase-catalyzed reactions is glycosyl–proton interchange[9],

$$\text{glycosyl-X} + \text{HX}' \rightleftharpoons \text{glycosyl-X}' + \text{HX}.$$

This concept postulates that glycosyl transfer is a completely open-ended process, notably one able to be satisfied by donor structures simpler than the glycosidically linked donors customarily assumed[6,7] to be required. The above findings, in verifying this prediction, also affirm the underlying concept that a single type reaction (and a fundamental unity) exists for the various synthetic and degradative actions of the carbohydrases.

A fresh point of support for the concept has been found in the utilization of α-D-glucopyranosyl fluoride by a purified amylopolysaccharide-synthesizing enzyme, namely, amylosucrase [E.C. 2.4.1.4]. Early studies with this enzyme (see ref. 5) indicated that it catalyzes polymerization of the α-D-glucopyranosyl moiety of sucrose to form a glycogen-like α-glucan, with release of D-fructose. No substrate but sucrose was known for amylosucrase, although mixtures of starch or glycogen with D-fructose were found to provide a small degree of sucrose synthesis by the reverse reaction.

Recent development of a method for obtaining amylosucrase in highly purified form has permitted further study of its catalytic properties. Among the new findings are that α-D-glucopyranosyl fluoride, like sucrose, is utilized both for glucosylating D-fructose (to form sucrose) and for synthesizing a highly polymerized, glycogen-like polysaccharide. These results establish the point, until now only assumed[5], that amylosucrase is an α-D-glucopyranosylase that transfers the α-D-glucopyranosyl, not

the α-D-glucopyranosyloxy, group. More important, they show that the catalytic activity of the enzyme does not require the presence of a pre-existing glycosidic linkage in the donor, but extends to the synthesis of glycosidic linkages where none existed before.

EXPERIMENTAL

Materials. — The α-D-glucopyranosyl fluoride was a recrystallized sample, $[\alpha]_D^{24}$ +88° (*c* 2), synthesized as previously described[2]. Sucrose was a commercial reagent (Wako Pure Chemical Industries). Uniformly labeled D-fructose-^{14}C, from New England Nuclear Corp., was purified by paper chromatography before use.

Highly purified amylosucrase was prepared from *Neisseria perflava* 19-34, a Gram-negative coccus isolated from a healthy human throat. Bacterial cells from shallow, still cultures without sucrose were disrupted with a sonic oscillator (Toyoriko Seisakusyo, Model N-50-5) at 10 kc for 15 min. The solubilized enzyme was separated from inert subcellular particles by ultracentrifugation (105,000 × *g*) and freed from maltase, pyrophosphatase, and α-glucan phosphorylase by precipitation with ammonium sulfate and fractionation* on Sephadex G-100. The preparation used assayed 1.04 unit/ml; one unit converts 0.1M reagent sucrose (at pH 6.4 and 30°) into α-glucan and D-fructose at the rate of 1 μmole/min.

Analytical-grade yeast invertase (β-D-fructofuranosidase) was purchased from Difco Corp.; honey invertase (α-D-glucopyranosidase) was prepared from unheated honey according to directions kindly supplied by Dr. J. W. White, Jr. Crystalline, hog-pancreatic *alpha*-amylase and crystalline, sweet-potato *beta*-amylase were from Worthington Corp.; mussel glycogen was purchased from Pfanstiehl; amylopectin was prepared from potato starch by the pentanol method of Schoch.

RESULTS AND DISCUSSION

Table I shows the ability of purified amylosucrase to catalyze substantial α-D-glucopyranosyl transfer from α-D-glucopyranosyl fluoride (compared with sucrose as donor) to the 2-hydroxyl group of β-D-fructofuranose. Mixtures (0.40 ml) containing α-D-glucopyranosyl fluoride or sucrose (20 μmoles), D-fructose-^{14}C (20 μmoles, 5 μCi), and purified enzyme (0.16 unit in 0.05M maleate buffer, pH 6.4) were incubated for 10 and 60 min at 30°. After inactivation for 10 min at 65°, samples (8 μl) were chromatographed by the descending method on Whatman No. 1 paper with 6:4:3 butanol–pyridine–water. The radiochromatograms, examined with the aid of a windowless scanner (Baird Atomic Model 1-353) equipped with a digital integrator, showed well-defined peaks of radioactivity corresponding to sucrose and D-fructose in each instance. The amounts of labeled sucrose product (0.23 and 1.20 μmoles at 10 and 60 min) present in the mixture with α-D-glucopyranosyl fluoride

*Details of the preparative procedure will be described elsewhere.

were 40–60% of those found with sucrose as the donor (0.53 and 2.02 μmoles). These levels do not, however, provide an accurate measure of the rates of synthesis, as sucrose is, in turn, polymerized to α-glucan. Indeed, both reaction mixtures became opalescent in the course of incubation, indicating the occurrence of polysaccharide synthesis.

TABLE I

SYNTHESIS OF SUCROSE FROM α-D-GLUCOPYRANOSYL FLUORIDE BY AMYLOSUCRASE

Donor substrate	*Incubation time (min)*	*Radioactivity (c.p.m.) under well-defined peaks*		*Sucrose-^{14}C in mixture (μmoles)*
		Sucrose[a] (12–16 cm)	*Fructose* (18–24 cm)	
α-D-Glucosyl fluoride	10	420	35,300	0.23
	60	1,780	27,900	1.20
Sucrose	10	920	34,000	0.53
	60	3,300	29,400	2.02

[a]Identical with authentic sucrose in chromatographic mobility and slow staining with silver nitrate. All counts disappeared when the mixture was treated with yeast β-D-fructofuranosidase or honey α-D-glucopyranosidase.

Further study showed that, in mixtures devoid of D-fructose (sucrose synthesis excluded), α-D-glucopyranosyl fluoride is directly converted into amylopolysaccharide. When purified amylosucrase (1.7 units, in 2.5 ml of 0.05M maleate buffer of pH 6.4) was incubated with α-D-glucopyranosyl fluoride or sucrose (250 μmoles), opalescence developed rapidly, and, after 2 h at 30°, was intense in both mixtures (more so with the sucrose). A water-soluble amylopolysaccharide was recovered from each by repeated precipitation with ethanol (60% by volume) and drying *in vacuo*. The yield from α-D-glucopyranosyl fluoride (5.3 mg) was approximately one-sixth that obtained from the sucrose (32.7 mg, 81% of theory).

The isolated polysaccharides gave solutions (0.5 mg/ml) more highly opalescent than those of mussel glycogen or potato amylopectin; the opalescence disappeared at once on addition of a small proportion of crystalline *alpha*-amylase. Soluble complexes with iodine were formed by both α-glucan products; a solution containing 0.5 mg of the polysaccharide synthesized from α-D-glucopyranosyl fluoride in 5.0 ml of 0.05 M acetate buffer (pH 5.0), treated with 0.1 ml of 0.2% I_2–2% KI, gave optical absorbance values of 0.14 and 0.08 at 500 and 660 nm; these values were intermediate between those found for mussel glycogen (0.05; 0.01) and potato amylopectin (1.12; 0.82). Digestion of the α-D-glucopyranosyl fluoride-derived α-glucan (1.0 mg in 2.0 ml of 0.05 M acetate buffer of pH 5.0) with *beta*-amylase (27 units) for 6 h at 30° yielded 0.224 mg of maltose[10] (identity confirmed chromatographically); 0.47 mg of maltose was released from potato amylopectin under the same conditions.

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