Note

Synthesis of Asymmetrically Labeled Sucrose by a Recombinant Sucrose Synthase

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Received May 22, 1997

About 80% of radioactivity was recovered in asymmetrically labeled sucrose from UDP-[14C]glucose or [14C]fructose with recombinant mung bean sucrose synthase expressed in Escherichia coli harboring pEB-01. This high recovery is due to the fact that the enzyme conserving the activity of sucrose synthase has a similar affinity for UDP-glucose and fructose to an intact enzyme from the mung bean, but a lower affinity for sucrose.

Key words: recombinant enzyme; sucrose; asymmetrically labeling; mung bean

Sucrose synthase (UDP-glucose: fructose 2-α-glucosyltransferase, EC 2.4.1.13) catalyzes the synthesis and cleavage of sucrose according to the following reversible reaction:

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\text{UDP-glucose} + \text{fructose} \rightleftharpoons \text{UDP} + \text{sucrose}
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Sucrose synthase, which occurs only in higher plants, is believed to function to cleave sucrose and generate UDP-glucose, which is used in such diverse pathways as UDP-sugar synthesis, cell wall component synthesis, and energy generation. Kinetic parameters of the purified enzymes from mung bean and rice all indicate that the prime role of sucrose synthase is the biosynthesis of nucleotide sugars at high levels of sucrose present in growing tissues. Mung bean sucrose synthase cDNA contains an open reading frame of 2415 bp that encodes 805 amino acids with a calculated molecular mass of 92,087 daltons. Although sucrose is readily isolated in high purity and in large quantities from natural sources, this synthesis is still difficult chemically. This work describes how recombinant sucrose synthase expressed in E. coli harboring an expression plasmid containing mung bean (Vigna radiata, Wilecek) sucrose synthase cDNA effectively synthesizes an asymmetrically labeled sucrose from UDP-[14C]glucose or [14C]fructose.

To prepare an extract containing a recombinant sucrose synthase, Escherichia coli strain BL21(DE3) cells harboring plasmid pEB-01, which contained mung bean sucrose synthase cDNA, were cultivated in an LB medium at 37°C in a shaker flask. Protein production was induced by isopropyl thio-β-galactoside at a final concentration of 0.3 mM. After harvesting by centrifugation, the cells were suspended in 0.03 M Tris-HCl, pH 7.5, containing 0.1 mM EDTA and 0.1 mM dithiothreitol, and disrupted by sonication. The broken cell suspension was centrifuged and to the supernatant was added ammonium sulfate to 65% saturation. The resulting precipitate was dissolved in 0.03 M Tris-HCl, pH 7.5, containing 0.1 mM EDTA and 0.1 mM dithiothreitol, and portions were used as the source of the enzyme.

For the incorporation of glucose from UDP-[14C]glucose into sucrose, the reaction mixture contained 4.3 μM UDP-[14C]glucose (290 mCi/mmol), 50 mM fructose, 10 mM Tris-HCl, pH 7.5, and the enzyme preparation (usually about 7.5 μg of total protein) in a total volume of 20 μl. For the incorporation of fructose from [14C]fructose into sucrose, the reaction mixture contained 16.7 μM [14C]fructose (300 mCi/mmol), 3 mM UDP-glucose, 50 mM Tris-HCl, pH 7.5, and the enzyme preparation (usually about 7.5 μg of total protein) in a total volume of 20 μl. High specific activity of UDP-[14C]glucose and [14C]fructose were used as substrates at low concentrations to get high specific activities of [14C]glucose-labeled sucrose and [14C]fructose-labeled sucrose, respectively. The reaction was allowed to proceed at 30°C for 10 min, and the mixture was immediately spotted on a 2-cm wide Whatman 3MM filter paper strip, after which the paper was electrophoresed in 50 mM sodium tetraborate, pH 9.6, at 250 V for 3 hours. After electrophoresis, the paper strips were dried and autoradiographed or analyzed by a Fuji Bio-imaging analyzer BAS2000 (Fuji Photo Film Co., Ltd.).

The products formed from UDP-[14C]glucose (4.3 μM) plus cold fructose or [14C]fructose (16.7 μM) plus cold UDP-glucose by recombinant mung bean sucrose synthase gave a saccharide with a paper electrophoretic mobility similar to that of authentic sucrose (Fig. 1A). The area corresponding to sucrose on the paper was excised and eluted with water. The eluate was treated with Dowex 50W (H+ type, Dow Chemical Company) to remove sodium ions.

Fig. 1. Characterization of Products Formed from UDP-[14C]Glucose and [14C]Fructose with Recombinant Sucrose Synthase.

After paper electrophoresis (A) of the products, saccharides with mobility similar to sucrose were chromatographed on paper (B) with 1-propanol ethyl acetate-water (3:3:1). The radioactivity was detected by autoradiography on X-ray film and the standard sugars on paper chromatogram were stained with alkaline silver nitrate. Paper electrophoresis A: lane 5, standard sugars and sugar nucleotide; lane 1, products from UDP-[14C]glucose; lane 2, products from [14C]fructose. Chromatogram B: lane 5, standard sugars; lane 1, [14C]glucose-labeled saccharide; lane 2, mild acid hydrolyzate of [14C]glucose-labeled saccharide; lane 3, [14C]fructose-labeled saccharide; lane 4, mild acid hydrolyzate of [14C]fructose-labeled saccharide.

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Fig. 2. Courses of Sucrose Synthesis from UDP-[14C]Glucose and [14C]Fructose with Recombinant or Plant Sucrose Synthase Preparations.

Relative radioactivity was expressed as the recovery of radioactivity in labeled substrate and product sucrose from total radioactivity. Synthesis of [14C]glucose-labeled sucrose from 4.3 μM UDP-[14C]glucose plus 50 mM fructose with recombinant (●) and plant enzymes (○) (panel A) and residual UDP-[14C]glucose with recombinant (■) and plant enzymes (□) (panel B). Synthesis of [14C]fructose-labeled sucrose from 16.7 μM [14C]fructose plus 3 mM UDP-glucose with recombinant (●) and plant enzymes (○) (panel C) and residual [14C]fructose with recombinant (■) and plant enzymes (□) (panel D).

and boric acid was removed by repeated evaporation with methanol. The radioactive sample was hydrolyzed in 0.01 M trifluoroacetic acid at 100°C for 30 min, and the acid was removed by evaporation in vacuo. The sample was then chromatographed on paper with 1-propanol-ethyl acetate-water (3:3:1), followed by exposing X-ray film. Standard sugars were detected on the chromatogram with alkaline silver nitrate. The saccharide also had a mobility similar to that of authentic sucrose on paper chromatography and gave glucose and fructose on mild acid hydrolysis (Fig. 1B). This finding indicates that the saccharide is sucrose that is asymmetrically labeled.

The courses of sucrose synthesis from UDP-[14C]glucose and [14C]fructose with recombinant or plant sucrose synthase are shown in Fig. 2. The plant enzyme was prepared from mung bean seedlings as previously described. When high substrate concentrations of 3 mM UDP-glucose and 50 mM fructose were used, incorporation into sucrose proceeded for about 60 min (data not shown). When low concentrations of UDP-glucose (4.3 μM) and fructose (16.7 μM) were used, incorporation into sucrose from UDP-glucose proceeded rapidly for 2 min and that from fructose for 5 to 10 min. About 80% of radioactivity was recovered in asymmetrically labeled sucrose from UDP-[14C]glucose (Fig. 2A) or [14C]fructose (Fig. 2B) at low substrate concentrations with the recombinant enzyme, after which a steady state was maintained for at least 20 min. When the plant enzyme preparation was used for the reaction, only about 30–45% of radioactivity was recovered in asymmetrically labeled sucrose from UDP-[14C]glucose (Fig. 2A) or [14C]fructose (Fig. 2B) under the same conditions. Degradation of UDP-[14C]glucose into [14C]fructose was found on each paper electrophoresis, probably because of the presence of UDP-glucose-degrading enzyme activities in the enzyme preparation. In addition, much residual [14C]fructose was accumulated in the reaction mixture with the plant enzyme preparation (Fig. 2D).

The kinetic constant for UDP-glucose (0.4 mM) and fructose (7.8 mM) of the recombinant sucrose synthase is similar to that of plant enzyme (0.8 mM and 5.9 mM, respectively), but that for sucrose of the recombinant enzyme (161 mM) is tenfold higher than that of plant enzyme (17 mM). Since the recombinant enzyme is not phosphorylated in E. coli, its kinetic properties may be affected. High levels of incorporation of radioactive glucose and fructose into sucrose with recombinant enzyme were probably due to the lower affinity for sucrose of the recombinant enzyme. On the other hand, eighty-three percent of asymmetrically labeled sucrose derivative (1-deoxy-1-fluorosucrose) has been obtained from UDP-glucose and 1-deoxy-1-fluoro-o-fructose with a barrel sucrose synthase preparation because the sucrose derivative is hardly recognized by invertingase. Nevertheless, the recombinant sucrose synthase is very useful for the synthesis of asymmetrically labeled sucrose due to its low affinity for product sucrose and less invertase activity. This is a kind of carbohydrate engineering that would make valuable contributions to saccharide synthesis.

References