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Preliminary communication

The enzymic utilization of sucrose in the synthesis of amylose and derivatives of amylose, using phosphorylases*

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The synthesis of polysaccharides constitutes a challenge to organic chemists. This class of compounds is of current interest because of its importance in biological systems[1,2]. Synthetic methods based on classical chemical manipulations are limited in applicability: using these methods it is difficult to form glycosidic linkages stereospecifically, or to generate polymers of high molecular weight[3,4].

Enzymic catalysis has proved to be an attractive alternative to classical chemical methods for the synthesis of polysaccharides[2,5]. In particular, (1→4)-α-D-glucan phosphorylase from potatoes (EC 2.4.1.1; potato phosphorylase; pph) catalyzes the reversible, degradative phosphorylation of amylose to D-glucosyl phosphate (G-1-P), and has been used in the presence of primers to synthesize polysaccharide chains[5,6]. This approach has two major disadvantages: the cost (~$1300/mol, Sigma) of the starting monomer G-1-P, and the ~60–80% yield of polymer based on this monomer[7]. We now report a new, coupled, enzymic system using sucrose phosphorylase[8] (EC 2.4.1.7; sph) and pph that utilizes inexpensive sucrose as the source of G-1-P, and gives >90% yields of high-molecular-weight amylose, using a variety of primers (see Fig. 1).

In a representative experiment, sucrose (**1**; 1.71 g, 5 mmol), maltoheptaose (**4**; 8 mg), and NaH_2PO_4 (34.5 mg; 5 mol%, relative to sucrose) were dissolved in 0.1M sodium citrate buffer (40 mL) at 37°. The pH was adjusted to 7, and sph (Sigma, 30 U) and pph[5] (15 U) were added. After stirring for 24 h, enzymic assay[9] for glucose, glucosyl phosphate, and fructose indicated that 94% of the sucrose had been converted into amylose (**5**). The mixture was heated to 90°, the precipitate removed by filtration, and 1-butanol (4 mL) was added to the supernatant liquor. After 24 h at 24° the precipitated amylose was filtered off, successively washed with water (10 mL), methanol (30 mL), and ether (50 mL), and dried *in vacuo*.

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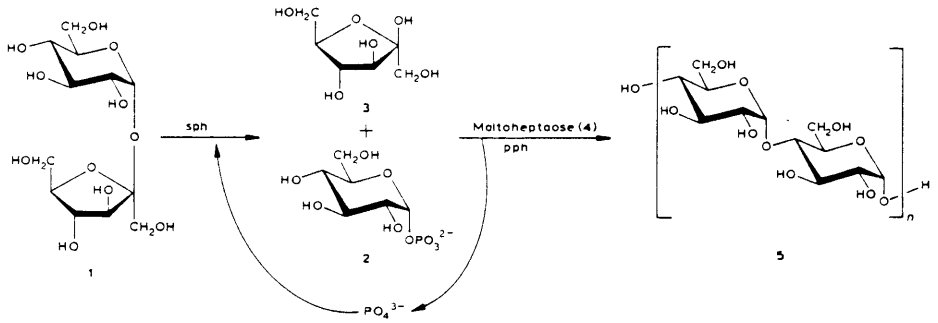

Fig. 1. The sucrose phosphorylase (sph)–potato phosphorylase (pph)-coupled enzyme system. [The molecular weights of the carbohydrate polymers obtained in our studies were determined by high-performance gel-permeation chromatography, using dextrans of known molecular weight as reference compounds.]

Additional polysaccharide could be obtained from the mother liquor after dialysis against water. The overall yield of amylose was 750 mg (92%). The amylose was identified by the formation of a blue complex with iodine, and by its ^{13}C-n.m.r. spectrum[10]: ^{13}C-n.m.r. (125 MHz, 1:1 Me_2SO-d_6 D_2O): δ 100.9, 79.4, 74.1, 72.6, 72.3, and 61.4.

An interesting feature of this system is that, in contrast to the single-enzyme method, the inorganic phosphate liberated by pph is recycled by sph. This removal of phosphate helps to drive the formation of polymer. The coupled-enzyme system also allows for regulation of the molecular weight of the amylose by control of the concentration of the primer[5,7] (see Fig. 2), and it is not restricted to maltoheptaose as the primer. The pph can utilize malto-oligomers bound chemically to various polymers[11]. We have synthesized several unnatural primers (see Fig. 3) and demonstrated that, using sph and pph, high yields of polymer bound to these

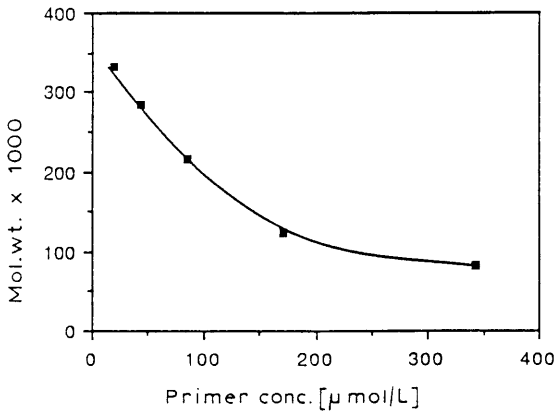

Fig. 2. Correlation between the molecular weight of the amylose and the primer concentration in the sph–pph-coupled enzyme system.

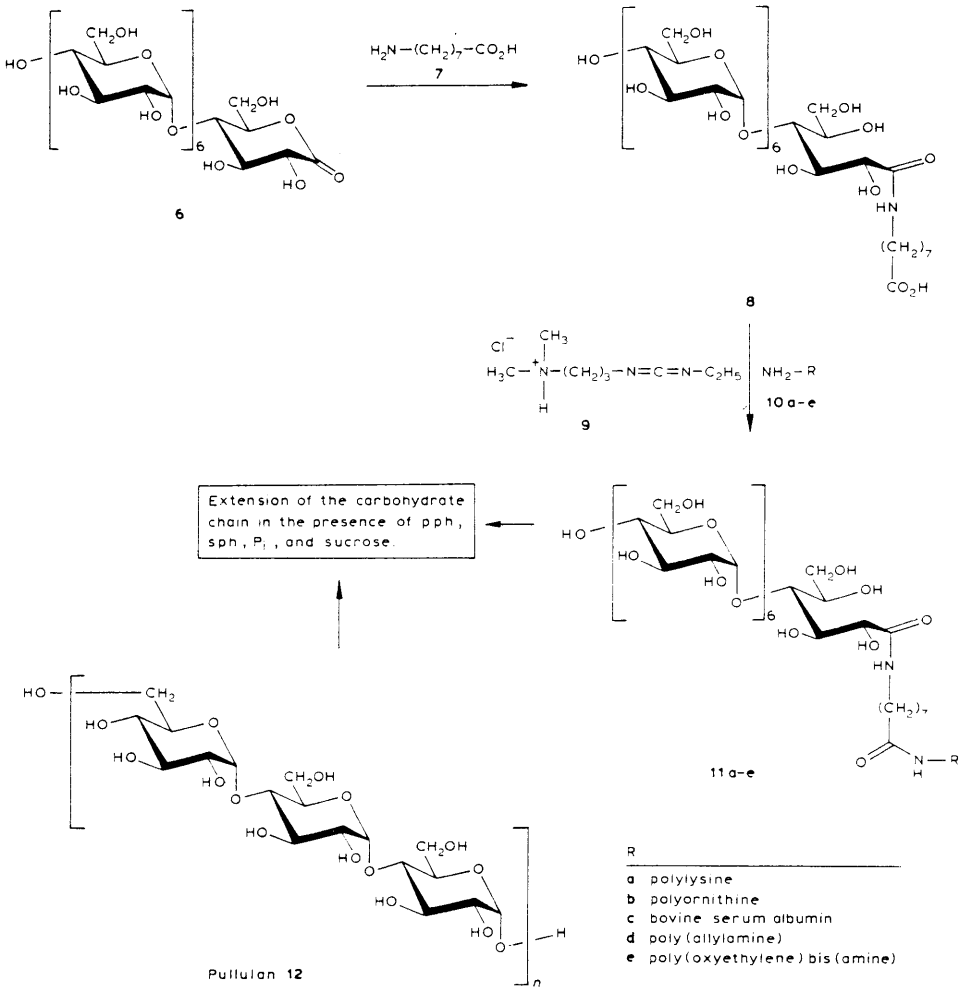

Fig. 3. Synthesis and use of unnatural primers for the sph–pph system.

primers can be obtained. To make a versatile primer, maltoheptaonolactone[12] (**6**) was allowed to react with 8-aminooctanoic acid (**7**). The carboxylic acid **8** thus formed could be coupled to polylysine (**10a**), polyornithine (**10b**), bovine serum albumin (**10c**), poly(allylamine) (**10d**), and poly(oxyethylene)bis(amine) (**10e**) with the aid[13] of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (**9**). Compounds **11a–e** served as primers in the enzymic reaction catalyzed by pph. The yields obtained with the coupled-enzyme system (75–89%) were superior to those observed with G-1-P and pph alone (60–70%).

Pullulan (**12**), a polysaccharide that can be considered a poly(maltotriose)[14], can also be used as a primer (it is itself a primer for initiation of amylose synthesis[15]). Replacement of maltoheptaose by pullulan (mol. wt. ~600,000) in the

foregoing reaction resulted in the formation of a new block-copolymer. Again, the use of sucrose with sph and pph gave higher yields (88%) than did G-1-P and pph (50%).

In summary, the system here described for the synthesis of amylose from sucrose, using a coupled, two-enzyme system (sph–pph) gives higher yields and higher molecular weights of polymer than does that based on pph and G-1-P, and it has the added advantage that its starting material is inexpensive.

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