BIOSYNTHESIS OF SUCROSE AND ITS DEOXY DERIVATIVES

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Received February 4th, 1987

The biosynthesis of sucrose (β-D-fructofuranosyl-α-D-glucopyranoside) was studied in the reaction catalyzed by partialy purified sucrose synthases (UDP-D-glucose: D-fructose 2-glucosyltransferase) isolated from pea seedlings, bean seedlings and sugar beet roots using UDP-D--glucose and its deoxyglucosyl derivatives as donors and D-fructose and its deoxy-analogues as acceptors. It was found that none of the hydroxyl groups either of the reaction donor or the acceptor is essential for the substrate properties in the transglycosylation reaction. The affinity of these plant enzymes and the rate of hexose incorporation into sucrose decreases however in the following sequence: sucrose, 2^G-deoxysucrose, 6^G-deoxysucrose, 4^G-deoxysucrose, and 3^G-deoxysucrose for the donor and sucrose, 6^F-deoxysucrose, 1^F-deoxysucrose, 4^F-deoxysucrose, and 3^F-deoxysucrose for the acceptor.

As we reported in our previous papers, replacement of a hydroxyl group of glucose for a hydrogen atom in the molecule of the reaction substrate slows substantially down the course of transglycosylation. These results dealing with the transglycosylation reactions of biosynthesis of glycogen[1,2], α,α'-trehalose-6-phosphate[3], starch[4], and lactose[5] pointed out that the effect of hydroxyl groups of D-glucose on the course of transglycosylation is of the same importance, whether the donor or the reaction acceptor is involved. A certain nonspecificity of the plant and microbial enzymes towards absence of the hydroxyl group at C-2 position of glucose was further demonstrated in transformation of 2dGlc into O-β-fructofuranosyl-(2→6)-2--deoxy-D-glucopyranose and 2-deoxyglucosyl analogues of gentiobiose[6,7], and in formation of deoxy-analogues of trehalose in yeast[8,9]. Biosynthesis of 2^G-deoxysucrose described Weidemann and Fischer[10] in vivo and Farkaš et al. in vitro[11]. Guthrie et al.[12] describes organic synthesis of 1^F-deoxy, 6^F-deoxy-, and $1,6^F$-dideoxysucrose. Jenkins and Thang Sun prepared $6^G,6^F$-dideoxysucrose[13]. The chemoenzymatic synthesis of some 1^F-deoxy, 4^F-deoxy-, and 6^F-deoxysucroses via sucrose synthase and corresponding analogues of the reaction acceptor was described recently[14,15].

* Abbreviations: 2dGlc, 2-deoxy-D-glucose; 3dGlc, 3-deoxy-D-glucose; 4dGlc, 4-deoxy-D--glucose; 6dGlc, 6-deoxy-D-glucose; 1dFru, 1-deoxy-D-fructose; 3dFru, 3-deoxy-D-fructose; 4dFru, 4-deoxy-D-fructose; 6dFru, 6-deoxy-D-fructose.

The present study deals with the kinetics of D-glucose, 2dGlc, 3dGlc, 4dGlc, and 6dGlc incorporation into sucrose from UDPG and its deoxyglucosyl analogues as reaction donors and D-fructose, 1dFru, 3dFru, 4dFru, and 6dFru* as reaction acceptors, catalyzed with plant sucrose synthases isolated from pea seedlings, bean seedlings and mature sugar beet roots. The kinetic data obtained are compared with those resulting from the studies with above mentioned transglycosylation enzymes. Beside this, the significance of the hydroxyl group at the particular positions of D-glucose and D-fructose molecules in the sucrose biosynthesis is discussed.

EXPERIMENTAL

Material and Methods

UDPG (sodium salt) was a Koch–Light product (Colnbrook, England). UDP-(U-^{14}C)G (ammonium salt) was purchased from The Radiochemical Center (Amersham, England). UDP2dGlc, UDP4dGlc, and UDP6dGlc were prepared by the methods of Kochetkov et al.[16,17]. D-Fructose was a Koch–Light product. (U-^{14}C)D-Fructose was prepared according to Kučár et al.[18]. 1dFru was prepared according to Haylock[19]. 3dFru, 4dFru, and 6dFru were prepared according to Kučár et al.[20–21].

Plant sucrose synthase (UDPG: D-fructose 2-glucosyltransferase, EC 2·4·1·13) was isolated from pea seedlings (*Pisum sativum*)[22], from bean seedlings (*Phaseolus aureus*)[23] and from mature sugar beet roots (*Beta vulgaris* ssp. *esculenta*)[24] and further purified on DEAE cellulose column (100 × 5 cm) adjusted to pH 7·7 with phosphate buffer (50 mmol l^{-1}). The proteins were eluted in the same buffer (pH 6·5). Specific activity of the enzyme obtained from pea seedlings was 52 nkat mg^{-1}, of the enzyme from bean seedlings 45·5 nkat mg^{-1} and of that isolated from sugar beet roots 17·5 nkat mg^{-1}. β-D-Fructofuranosidase (EC 3.2.1.26) from bakers yeast was purchased from Koch–Light. α-D-Glucosidase (EC 3.2.1.20) from brewers yeast was product of Sigma (St. Louis, U.S.A.).

Descending paper chromatography was carried out on Whatman No. 1 paper using following systems: S1 ethyl acetate–pyridine–water (8 : 2 : 1, v/v/v), S2 ethyl acetate–2-propanol–water (65 : 24 : 11, v/v/v), and S3 2-butanone, saturated with water,

Preparation of Sucrose and Its Derivatives Containing Incorporated Deoxyhexoses

The incubation mixture 0·5 ml contained UDPG (20 mmol l^{-1}) or its deoxy-analogue. Further components of the reaction mixtures were EDTA (1 mmol l^{-1}), NaF (5 mmol l^{-1}), phosphate buffer (50 mol l^{-1}; pH 7), and sucrose synthase (0·3 nkat). When the effect of UDPG and its deoxyglucosyl analogues was studied D-(U-^{14}C) fructose (spec. radioactivity 196 GBq mol^{-1}) was used as an acceptor. On the other hand when the effect of deoxyfructoses was studied, UDP-(U-^{14}C)G (spec. radioactivity 296 GBq mol^{-1}) was introduced as a donor. The reaction was carried out at 30°C for 5 h. The mixtures were applied on a strip of Whatman No. 1 paper, chromatographed in S1 for 16 h (Fig. 1) and the spot corresponding to a disaccharide of sucrose type was cut out and eluted with distilled water. Further purification of the eluates was done by repeated descending chromatography in the system 2 overnight. A separate paper was sprayed with 0·0025 mol l^{-1} HCl and detected with an $AgNO_3$ reagent[25].

Kinetic Measurements

The transglycosylation of a hexose into sucrose was carried out as described above. The reaction velocities were estimated by measuring the radioactivity incorporated into sucrose either from UDP-(U-^{14}C)G or from D-(U-^{14}C) fructose. The reaction mixture was applied on a strip of Whatman No. 1 paper, chromatographed in S1 for 16 h and the spot corresponding to the analogue of sucrose was cut out and measured directly in a liquid scintillation counter Packard 3330 using a toluene fluid SLX-31 (Tesla, Czechoslovakia).

RESULTS

It was found that the deoxyglucosyl analogues of UDPG and the deoxy analogues of D-fructose can act as substrates in the transglycosylation reaction of sucrose biosynthesis catalyzed by sucrose synthase from pea seedlings, been seedlings and sugar beet roots. The disaccharides obtained remained unaffected after treatment with $NaBH_4$ (result not demonstrated). The disaccharide obtained from the reaction mixture containing UDPG and D-fructose moved in S1 and S3 as sucrose. When

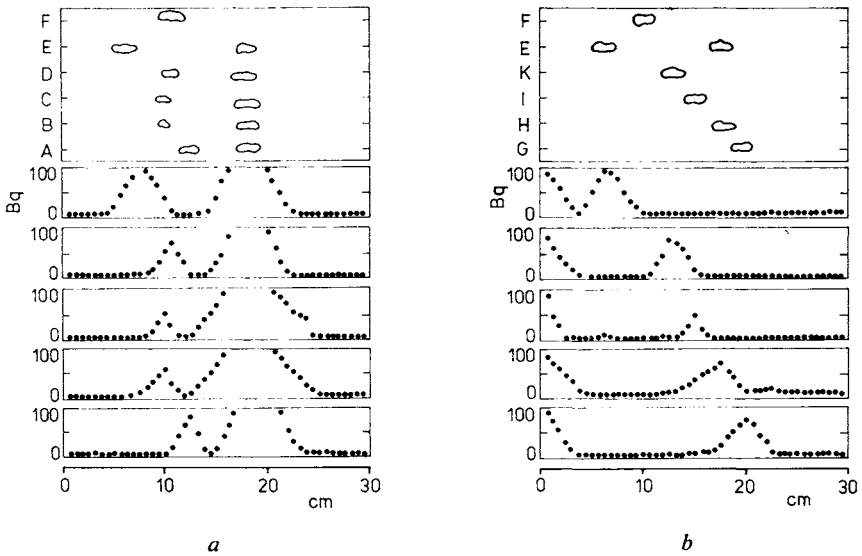


FIG. 1

Chromatographic separation of monodeoxy analogues of sucrose. *a* Experiment with UDP deoxy glucoses. Upper part: detected with $AgNO_3$ reagent[25]; lower part: radioactivity of 5 mm strips measured in a liquid scintillation counter. Developed in S1 for 16 h. UDPG and its analogues remained under these conditions on the starting line. *b* Experiment with deoxyfructoses. A–E unreacted D-fructose as a faster moving sugar: A 6^{G}-deoxysucrose, B 4^{G}-deoxysucrose, C 3^{G}-deoxysucrose, D 2^{G}-deoxysucrose, E sucrose, F D-glucose, G 6^{F}-deoxysucrose, H 4^{F}-deoxysucrose, I 3^{F}-deoxysucrose, K 1^{F}-deoxysucrose

treated with 0·1 mol l^{-1} HCl (50°C; 2 h, ref.[10]) the disaccharides were split into D-glucose and a corresponding deoxy-analogue of D-fructose or into D-fructose and a corresponding deoxy-analogue of D-glucose. Similarly, when treated with β-D--fructofuranoside fructohydrolase (10 nkat in 0·05 mol l^{-1} phosphate buffer pH 6 at 30°C for 3 h), some of the disaccharides were split into D-glucose, D-fructose or their deoxy-analogues (not shown). All the reaction products of the sucrose synthase reaction were however recognized as substrates by α-glucosidase (not shown). The hydrolyzates were chromatographed in S3 on Whatman No. 1 paper. Sugars were detected using diphenylamine–aniline reagent[26]. Based on chemical and physical properties the disaccharides were found to be sucrose and its monodeoxy-glucosyl and monodeoxyfructosyl analogues (Fig. 1). The reaction rates of D-glucose, 2dGlc, 3dGlc, 4dGlc, and 6dGlc incorporation into sucrose from equimolar (20 mmol. $.l^{-1}$) concentrations of glycosyl donors as well as that of the second substrate (D-fructose, 1dFru, 4dFru, 3dFru, and 6dFru) were linear up to 5 h (Fig. 2). The values of the Michaelis constants, K_m, and the maximal velocity, V, for UDPG and its monodeoxyglucosyl analogues were determined in Fig. 3*a*. The K_m and V values for the reaction acceptor and its monodeoxy-analogues were obtained as demonstrated in Fig. 3*b*. The competitive inhibitory effect of deoxyglucosyl analogues of the donor on the reaction of sucrose biosynthesis was demonstrated in a graphical plot according Dixon[27], Fig. 4*a*. The competetive inhibitory effect of the acceptor analogues was calculated in the same way as demonstrated in Fig. 4*b*.

The kinetic constants of hexose incorporation into sucrose for enzymes from three plant sources obtained in the same manner as demonstrated above are summarized in Table I.

DISCUSSION

We compared the properties of sucrose synthases isolated from pea seedlings, been seedlings and from sugar beet roots, using either UDPG and a monodeoxy-D--fructose or D-fructose and a UDP-monodeoxyglucose as substrates. Radiochromatographic analysis showed that all of the expected disaccharides were obtained (Fig. 1). This finding supports our suggestion raised for glycogen synthase[1,2], α.α'-trehalose 6-phosphate synthase[3], and phosphorylase[4] that there is no essential hydroxyl group in the glucosyl donor of the reaction. The affinity of sucrose synthases towards the substrate and its analogues decreases in the sequence: UDPG, UDP2dGlc, UDP6dGlc, UDP4dGlc, and UDP3dGlc. Gabrielyan et al.[28] evaluated roughly the substrate properties of some UDP-monodeoxyglucoses and the enzyme isolated from pea seedlings according to the optical density given in the thiobarbituric assay. The substrate properties and the affinity of the sucrose synthase to UDP4dGlc is higher than that of UDP6dGlc according to these authors following the above mentioned unaccurate assay[28]. The role of the hydroxy group of the glycosyl ac-

ceptor D-fructose, has not been systematically studied in the reaction yet. Using the sucrose synthase preparations described above we found that no one of the hydroxy

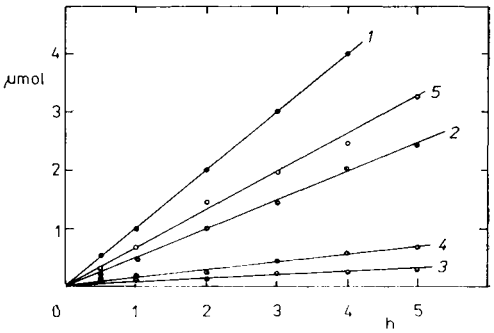
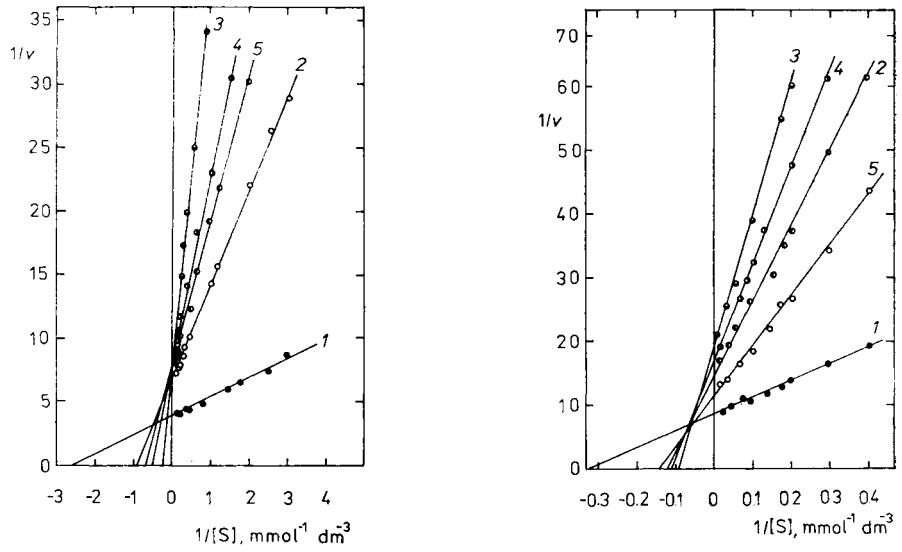


FIG. 2

Time dependence of the biosynthesis of sucrose and its deoxy derivatives from UDPG and Fru (*1*), 1dFru (*2*), 3dFru (*3*), 4dFru (*4*), and 6dFru (*5*) catalyzed with the enzyme from pea seedlings. Composition of the reaction mixture corresponds to that given in the Experimental



a *b*

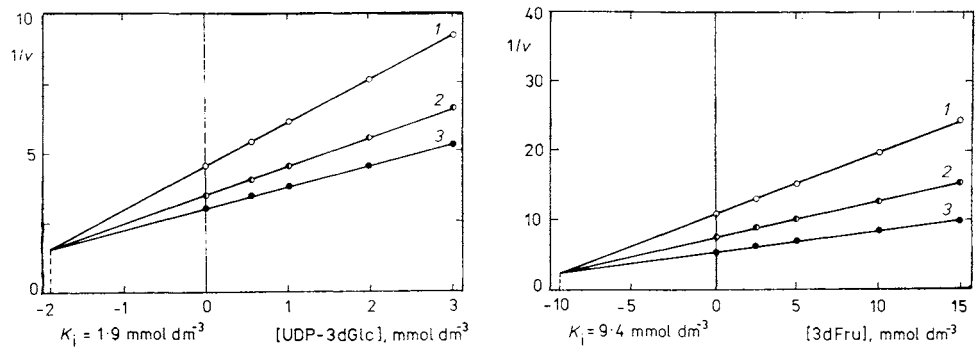
FIG. 3

Determination of the K_m and V values of the sucrose synthases in reaction with the deoxy--analogues of substrates. *a* UDPG (*1*), UDP2dGlc (*2*), UDP3Glc (*3*), UDP4dGlc (*4*), UDP6Glc (*5*) in the reaction catalyzed with the enzyme from sugar beet roots. *b* D-fructose (*1*), 1dFru (*2*), 3dFru (*3*), 4dFru (*4*), 6dFru (*5*) in the reaction catalyzed with the enzyme from pea seedlings. The 0·05 ml reaction mixtures had the same composition as described in Experimental except that concentrations of substrates varied as indicated. Incubation was carried out at 30°C for 30 min with UDPG and 2 h with their deoxy-analogues Velocities are expressed as μmol of hexoses incorporated into sucrose during 30 min

TABLE I

Constants of the incorporation of deoxy-analogues of D-glucose and D-fructose into sucrose. Kinetic constants of sucrose biosynthesis for sucrose synthases isolated from A pea seedlings, B bean seedlings and C nature sugar beet roots

Substrate	K_m, mmol l^{-1}			K_i, mmol l^{-1}			V, μmol l^{-1} s^{-1}		
	A	B	C	A	B	C	A	B	C
UDPG	1·8	0·21	0·38	—	—	—	1·98	2·35	2·77
UDP2dGlc	5·4	0·46	1·07	2·1	0·25	0·74	1·39	1·42	1·63
UDP3dGlc	11·1	1·52	4·3	4·2	1·05	1·9	1·15	1·19	1·33
UDP4dGlc	8·4	0·95	2·1	3·5	0·71	1·05	1·31	1·30	1·44
UDP6dGlc	6·8	0·77	1·52	2·8	0·58	0·95	1·35	1·35	1·55
D-Fructose	2·92	2·0	3·2	—	—	—	1·27	1·45	1·16
1dFru	8·32	7·16	9·1	5·3	2·2	5·6	0·76	1·50	1·38
3dFru	11·1	9·8	12·4	9·4	3·7	10·2	0·57	0·62	0·44
4dFru	9·1	8·2	9·5	8·1	2·8	9·1	0·64	0·80	1·18
6dFru	7·14	6·3	7·8	4·8	1·9	4·7	0·96	1·15	0·78



a *b*

FIG. 4

Effect of monodeoxy-analogues of reaction substrates on the rate of sucrose biosynthesis. *a* Effect of UDP3dGlc on biosynthesis of sucrose catalyzed by the enzyme from sugar beet roots. Concentration of UDP-(U-^{14}C)G, mmol l^{-1}: *1* 1; *2* 2; *3* 4. *b* Effect of 3dFru on biosynthesis of sucrose catalyzed with the enzyme from pea seedlings. Concentration of (U-^{14}C)D fructose, mmol l^{-1}: *1* 12·5; *2* 25; *3* 50. The 0·05 reaction mixtures had the same composition as shown in Fig. 2. Incubations were carried out at 30°C for 15 min. Velocities are expressed as μmol of hexose incorporated into sucrose during 15 min. Concentrations of deoxy-analogues of substrates varried as indicated

groups of the acceptor, D-fructose, is essential for the transglycosylation. The reaction rate and the affinity of the enzyme to the substrates decreases as follows (Table I): D-fructose, 6dFru, 1dFru, 4dFru, and 3dFru. Role of the hydroxy group at C-1 of D-fructose in the vicinity of the reacting semiacetal group seems to be more important than that of the hydroxy group at C-2 of D-glucose. For recognition of the substrates, the hydroxyls at C-3 and C-4 are of greater importance for both the donor and the acceptor (Table I). Absence of the hydroxy group at C-3 of the sugars resulted in the greatest decrease of the sucrose synthase affinity, probably due to localization of the elements of instability in the idealized axis of symmetry for both hexoses, crossing the oxygen atom and the C-3 atom of the pyranose or the furanose ring. According to equations describing the kinetics of a competitively inhibited reaction and the reaction of one enzyme with two substrates, the values of K_m and the corresponding K_i should be formally identical (Table I). Similarly as we found for yeast glycogen synthase and UDPG (ref.[29]) it seems likely that the low molecular weight natural substrate can activate the transglycosylating enzyme in a way not fully substitutable through its deoxy analogues whether the reaction proceeds in for sucrose synthases suggested the "ping-pong bi-bi"[30] or in the random mechanism[31].

The isolated deoxy-analogues of sucrose were studied as substrates in reactions catalyzed with the β-D-fructofuranosidase and the α-glucosidase. According to our preliminary results, β-fructofuranosidase does not recognize beside 1^F-deoxysucrose[32,33] also 3^F-deoxy- and 4^F-deoxysucrose as substrates. On the other hand beside sucrose all its deoxy-analogues were proved to be substrates for α-glucosidase.

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Translated by the author (J.Z.).