BIOSYNTHESIS OF LACTOSE AND ITS DEOXY DERIVATIVES

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The biosynthesis of lactose (O-β-D-galactopyranosyl-(1 → 4)-D-glucopyranose was investigated in a reaction catalyzed by lactosynthetase (UDP-D-galactose: D-glucose-4-β-D-galactosyltransferase) isolated from milk. The reaction donor in this reaction was UDP-D-galactose, the acceptor D-glucose or its monodeoxy derivatives. As found, monodeoxyglucose — excepting 4-deoxy-D-glucose — enter the transglycosylation reaction of biosynthesis of lactose. The affinity of the enzyme and the rate of biosynthesis of lactose and its deoxy derivatives decrease in the following order: lactose, 2^G-deoxylactose, 6^G-deoxylactose, 3^G-deoxylactose.

As we already reported, replacement of a hydroxyl group for a hydrogen atom in the molecule of a reaction donor is seen both in the slowing-down of the transglycosylation reaction and in the decrease of affinity of the appropriate transglycosylation enzyme in such a modified substrate. None of the hydroxyl groups of the glucose moiety of the molecule nucleoside-diphosphate-glucose[1,2], or α-D-glucopyranosyl phosphate[3] was, however, essential for preservation of the substrate features.

This paper deals with the kinetics of incorporation of 2-deoxy-D-glucose (2-dGlc), 3-deoxy-D-glucose (3-dGlc), 4-deoxy-D-glucose (4-dGlc) and 6-deoxy-D-glucose (6-dGlc) into lactose (O-β-D-galactopyranosyl-(1 → 4)-D-glucopyranose) by lactosynthetase (UDP-D-galactose : D-glucose-4-β-D-galactosyltransferase, EC 2.4.1.22) in an enzyme system isolated from goat milk (*Capra domestica*) in the presence of α-lactalbumine. Results obtained in this paper were compared with those of our preceding studies dealing with the biosynthesis of glycogen[1] and amylopectin[3], modified with deoxy derivatives of glucose and deoxy derivatives of α,α'-trehalose[2] and gentiobiose[4,5]; the importance of hydroxyl groups in various positions of D-glucose as a galactosyl acceptor in reactions of biosynthesis of lactose is discussed.

EXPERIMENTAL

Material and Methods

[U-^{14}C]-D-glucose is a commercial product of Institute for Research, Production and Evaluation of Radioisotopes, Prague. 2-Deoxy-D-glucose was prepared by the Overend's[6] method modified in that the hydratation of glucal was done with 2% solution of trichloroacetic acid, which offered

higher yields. 3-Deoxy-D-glucose was prepared according to[7], 4-deoxy-D-glucose according to[8], 6-deoxy-D-glucose according to[9] by a reductive splitting of the tosyl group bound to the primary hydroxyl group of hexose with lithium aluminium hydride. [2-^{3}H]-2-Deoxy-D-glucose, [3-^{3}H]--3-deoxy-D-glucose, [4-^{3}H]-4-deoxy-D-glucose and [6-^{3}H]-deoxy-D-glucose were prepared by Soukupová and coworkers[10]. 2^G-Deoxylactose, as a reference substance, was kindly supplied by Dr Bílik of this Institute, lactose and UDP-D-galactose (Na-salt) were preparations of Koch-Light (England).

Lactosynthetase (UDP-D-galactose: D-glucose-4-β-D-galactosyltransferase) was prepared from goat milk by a method reported[11] for cow milk (specific activity 791 1U). β-D-Galactosidase (β-D-galactosid galactohydrolase EC 3·2·1·23) was a product of Koch-Light.

Chromatography

For descending paper chromatography on paper Whatman No 1 following solvent systems were used: ethyl acetate–pyridine–water (8 : 2 : 1, v/v) S_1, 1-propanol–ethyl acetate–water (7 : 1 : 2) S_2.

Preparation of Deoxy Derivatives of Lactose

The reaction mixture consisted of glycine (0·25M, pH 8·5, 0·2 ml), 0·01M-$MnCl_2$ in 2·0M-KCl (0·05 ml), α-lactalbumine (1% solution, 0·01 ml), UDP-D-galactose (5 mM, 0·05 ml), [U-^{14}C]-D--glucose (0·04M, 0·05 ml, specific radioactivity 0·0003 μCi/mmol), or alternatively an appropriate monodeoxy derivative of D-glucose tritium labelled in the deoxy position of the same radioactivity, filled up to 0·5 ml with the enzyme solution and water. The mentioned mixture was incubated at 37°C up to 2 h, during which 0·05 ml samples were withdrawn and spotted on a Whatman No 1 paper. The chromatographic separation in solvent system S_2 lasted 36 h. Products of this transglycosylation reaction were visualized with a diphenylamine–aniline reagent[12] and radiochromatographically. Further purification was achieved by rechromatography in S_1. Zones corresponding to radioactive labelled oligosaccharides were cut out and eluted with water. D-Glucose, lactose and 2^G-deoxylactose were the standards for monitoring the chromatographic separation. The reaction product with D-glucose moved as lactose, that with 2-deGlc as 2^G-deoxylactose.

Hydrolysis of Saccharides

Saccharides cleaved with 0·1M-HCl (at 100°C for 1 h), or by the action of enzyme β-D-galactosidase afforded hexoses in such a way that monodeoxy glucoses, constituting the original reaction mixtures (2-dGlc, 3-dGlc and 6-dGlc), were liberated from the products of transglycosylation reactions in addition to D-galactose. The eluted radioactive disaccharides were reacted with $NaBH_4$ and acid hydrolyzed (0·1M-HCl, 100°C, 1 h) to furnish D-galactose and the corresponding monodeoxy derivative of tritium labelled D-glucitol at the deoxy position.

Kinetic Measurements

The kinetics of biosynthesis of lactose and its deoxy derivatives was measured at the same conditions with [U-^{14}C]-D-glucose, [2-^{3}H]-2-dGlc, [3-^{3}H]-3-dGlc, [4-^{3}H]-4-dGlc and [6-^{3}H]-6-dGlc. The aliquot samples from the reaction miture were spotted on a paper Whatman No 1 and chromatographed (solvent system S_2) overnight. Zones corresponding in mobility to lactose and its derivatives were cut out and the radioactivity was measured by means of a Packard, model 3330, scintillation counter, using the toluene scintillation liquid SLT-31 (Tesla, Czechoslovakia). The content of the reaction mixture is mentioned above.

RESULTS AND DISCUSSION

Comparison of chromatographic mobilities of the reaction products catalyzed by lactosynthetase (Table I) with the mobility of lactose and 2^G-deoxylactose, as well as results of acid hydrolysis, reduction with $NaBH_4$ and hydrolytic cleavage of the isolated disaccharides with β-D-galactosidase indicate that we were able to prepare, besides of lactose, also its deoxy derivatives, namely 2^G-deoxylactose (β-O-D-galactopyranosyl-(1 → 4)-2-deoxy-D-glucopyranose), 3^G-deoxylactose (β-O-D-galactopyranosyl-(1 → 4)-3-deoxy-D-glucopyranose) and 6^G-deoxylactose (β-O-D-glucopyranosyl-(1 → 4)-6-deoxy-D-glucopyranose). We did not succede in isolating any oligosaccharide containing 4-dGlc from the reaction mixture.

K_m and the maximum velocities V for D-glucose, 2-dGlc, 3-dGlc and 6-dGlc as substrates for this transglycosylation reaction were read by means of the graphic plot seen in Fig. 1. As it follows from Fig. 1, deoxy derivatives of D-glucose are substantially less effective acceptors of galactopyranosyl units as is D-glucose. The time dependence of the biosynthesis of lactose and its derivatives D-glucose, 2-dGlc, 3-dGlc and 6-dGlc in equimolar (40 mM) solutions of reaction mixtures, in respect to the hexose acceptor, is represented in Fig. 2. The competitive effect of deoxy derivatives of D-glucose on the transglycosylation reaction of the biosynthesis of lactose from UDP-D-galactose and [U-^{14}C]-D-glucose is seen in Fig. 3.

Studying the transglycosylation reaction of glycogen biosynthesis catalyzed by UDP-glucose-glycogen glucosyltransferase we found that the incorporation of 2-dGlc from UDP-2-dGlc into glycogen proceeds as a repetitive transfer, this being

TABLE I

Chromatographic Mobility of Lactose and Its Deoxy Derivatives

Descending paper chromatography on paper Whatman No 1, (S_2), 36 h, visualization by radiochromatography and diphenylamine–aniline reagent[12].

Compound	$R_{Glucose}$
Lactose	0·41
2^G-Deoxylactose	0·70
3^G-Deoxylactose	0·62
6^G-Deoxylactose	0·90
D-Glucose	1·00

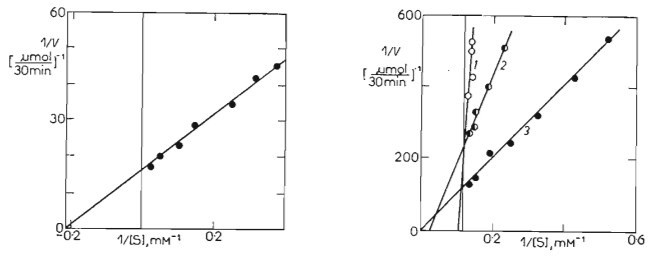
TABLE II

Kinetic Constants of the Reaction of Biosynthesis of Lactose and Its Deoxy Derivatives

The V values are relative to D-glucose. (V for D-glucose is 1·29 μM s^{-1}).

Substrate	K_m mM	K_i	V
D-Glucose	4·8	—	1·00
2-dGlc	11·4	5·6	0·11
3-dGlc	19·6	9·3	0·04
4-dGlc	—	6·1	—
6-dGlc	13·2	5·8	0·06

evidenced by the fact that we isolated 2,2′-deoxy-D-maltose in addition to 2′-deoxy--D-maltose from products of β-amylolysis of 2-dGlc modified glycogen. The repetitive transfer was, however, not evidenced either with 4-dGlc, what we rationalize by termination of the growth of the outer chain due to incorporation of the single 4-dGlc unit, or with 3-dGlc and 6-dGlc, what we rationalize by deteriorated accepting features of outer chains modified by the terminally incorporated respective 3-dGlc and 6-dGlc units for linkage of a further glucosyl unit[1,13].



a *b*

FIG. 1

Determination of Values K_m and V (*a*) for D-Glucose *1*, (*b*) for 2-dGlc *3*, 3-dGlc *1* and 6-dGlc (*2*)

0·05 ml of the reaction mixture contained 0·25M-glycine (pH 8·5, 0·02 ml), 0·1M-$MnCl_2$ in 2·0M-KCl (0·005 ml), 1% solution of α-lactalbumine (0·001 ml), 5 mM-UDP-D-galactose (0·005 ml), [U-^{14}C]-D-glucose of specific radioactivity 0·0003 μCi/mmol and concentration as given in the figure, filled with the solution of enzyme and water up to 0·05 ml. Incubation 30 min at 37°C for D-glucose and 1 h for 2-dGlc, 3-dGlc and 6-dGlc. The rates are expressed in μmol of D-glucose or its deoxy derivatives reacted in the biosynthesis of lactose within 30 min.

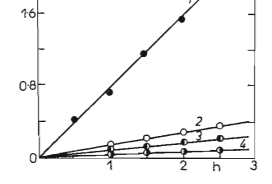


FIG. 2

Time Dependence of the Biosynthesis of Lactose and Its Deoxy Derivatives from D-Glucose *1*, 2-dGlc *2*, 3-dGlc *4* and 6-d-Glc *3*

Composition of the reaction mixture corresponds to that given in the experimental section with exception of the concentration of hexose, which is 40 mM.

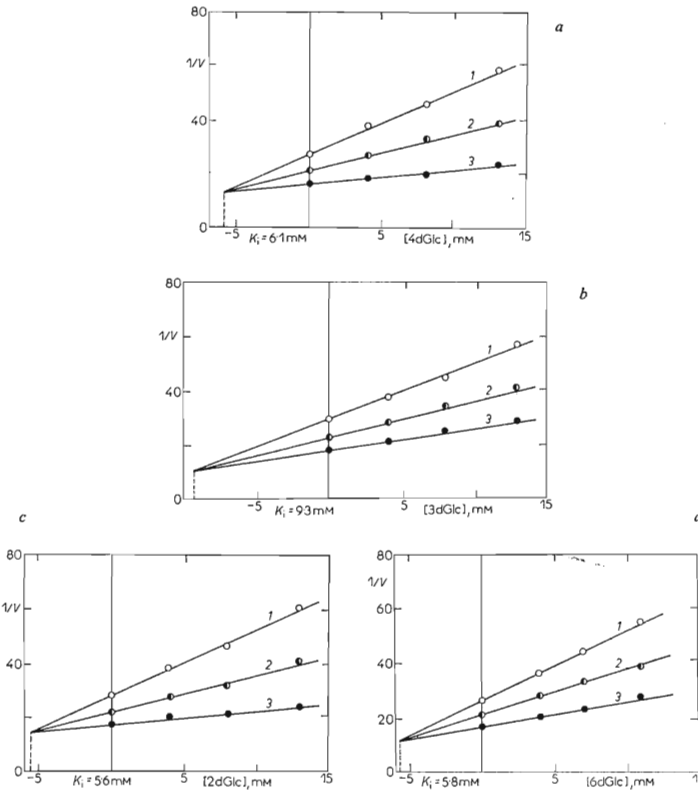


FIG. 3

The Competitive Effect of Deoxy Derivatives of D-Glucose on the Transglycosylation Reaction of the Biosynthesis of Lactose from UDP-D-Galactose and [U-^{14}C]-D-Glucose

Concentrations of 2-dGlc (*3a*) [U-^{14}C]-D-Glucose, mM, 3-dGlc (*3b*), [U^{14}C]-D-Glucose, mM, 4-dGlc (*3c*), [U-^{14}C]-D-Glucose, mM, 6-dGlc (*3d*), [U-^{14}C]-D-Glucose, mM are within the range given in the figure. Concentration of the 0·05 ml of reaction mixtures are shown in Fig. 1. Incubation 30 min at 37°C. The rates are given in μmol of D-galactose, which reacted within 30 min in the reaction of biosynthesis of lactose.

The influence of a hydroxyl group of aldose of the reaction acceptor on the transglycosylation reaction course has so far not been systematically investigated. Based upon presented results concerning the biosynthesis of deoxy derivatives of lactose it is evident that, excepting the hydroxyl group at $C_{(4)}$ of glucose necessary for the formation of $\beta(1 \rightarrow 4)$ linkage, none of further hydroxyl groups is essential for the course of transglycosylation reaction of the biosynthesis of lactose. The rate of this transglycosylation reaction, as well as the affinity of the enzyme system responsible for the biosynthesis of lactose towards the reaction acceptor, decreases as follows (Table II):

$$\text{D-glucose} > \text{2-dGlc} > \text{6-dGlc} > \text{3-dGlc} .$$

The course of incorporation of 2-dGlc is in agreement with results reporting[14] that 2-dGlc is more than 10 times slower incorporated into lactose. The 2-dGlc was shown to be an efficient reaction acceptor of glucose units during the biosynthesis of deoxy derivatives of gentiobiose in tissue cultures of tobacco[4] and spruce[5].

Even though the replacement of the only hydroxyl group of glucose of the reaction acceptor slows substantially down the course of this transglycosylation reaction, it provisionally follows – on the basis of comparison with results obtained from transglycosylation reactions of biosynthesis of glycogen[1], α,α'-trehalose[2] and $\alpha(1 \rightarrow 4)$ glucans of starch[3] – that the effect of hydroxyl groups of D-glucose on the course of the transglycosylation reaction is of the same importance, whether the donor or acceptor of the transglycosylation reaction is involved. 4-dGlc cannot be the reaction acceptor in the biosynthesis of oligo and polysaccharides linked by $1 \rightarrow 4$ bonds. The affinity of this deoxy derivative of glucose towards UDP-D-galactose : D--glucose-4-β-D-galactosyltransferase is, however, great enough as to manifest the competitive inhibition of the biosynthesis of lactose from D-glucose (Fig. 3*c*, Table II).

Similarly as in our preceding papers on enzymes cleaving hydrolytically the $\alpha(1 \rightarrow 4)$ bond of glucan (β-amylase, α-D-glucosidase), it was found that also β-D-galactosidase is nonspecific towards the absence of hydroxyl groups at $C_{(2)}$, $C_{(3)}$, $C_{(6)}$ of the glucose moiety of the lactose molecule[1,3,13].

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