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. Interaction of some Disaccharides with the Carrier System for Aldoses in Erythrocytes

BY L. LACKO AND M. BURGER

Institute of Haematology and Blood Transfusion, and Institute of Biology, Czechoslovak Academy of Science, Prague, Czechoslovakia

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At low temperature (0°), when the transport of sugars into erythrocytes is markedly suppressed, exchange transfer of aldoses can take place (Lacko, Burger, Hejmová & Rejnková, 1960). The mechanism of the exchange reaction is visualized as $\text{sugar}_1\text{-carrier} + \text{sugar}_2 = \text{sugar}_2\text{-carrier} + \text{sugar}_1$. Because it operates at 0° it is thought to involve lower activation energy than the reaction $\text{sugar} + \text{carrier} = \text{sugar-carrier}$ (Burger, 1960). Lacko & Burger (1961) used the exchange transfer to study in detail the requirements of the common carrier for aldoses in human erythrocytes with respect to the configuration of transported sugars.

In the present paper we have used exchange transfer to study the interaction with the carrier of some disaccharides which are assumed not to cross the erythrocyte membrane (Tschau-Tjiän Yang, 1950).

MATERIALS AND METHODS

Experimental procedure. Human blood (4 vol.) was diluted with 1 vol. of a solution of 35 g. of glucose, 11 g. of trisodium citrate and 4 g. of citric acid in 1000 ml. of water, and, after 30 min. at room temperature, erythrocytes were separated by centrifuging; in this way erythrocytes were obtained containing 4–5 mg. of glucose/ml. A portion (1 ml.) of these erythrocytes was then suspended in 5 ml. of incubation medium containing 25 mg. of galactose in (unless stated otherwise) 83 mm solution of disaccharide. Iso-osmoticity of the incubation medium was preserved by adding the appropriate amount of sodium chloride. The temperature of incubation was 0°. After 1, 7, 13 and 20 min. erythrocytes were separated from the medium and analysed for glucose and galactose.

Separation of erythrocytes from the incubation medium. Samples (1 ml.) of the erythrocyte suspension were filtered through a layer of Hyflo Super Cel placed on a sintered-glass filter. After the incubation medium was removed by

suction the sugars were removed from the extracellular space by washing the erythrocytes with 8 ml. of ice-cold 0.9% sodium chloride. The washing procedure did not last more than 30 sec. The erythrocytes were then haemolysed directly on the filter with 7 ml. of ice-cold water (Lacko *et al.* 1960).

Glucose determination. The haemolysate was deproteinized according to the usual Somogyi (1945) procedure. Glucose was estimated from the change in the reducing power of the deproteinized filtrates after incubation with glucose oxidase (Wyngaarden, Segal & Folny, 1957). The reducing powers of the filtrates were determined by the colorimetric method of Somogyi (1952) and Nelson (1944).

Paper chromatography. Whatman no. 1 filter paper was used. The solvent system was the upper layer of butanol-benzene-pyridine-water (5:1:3:2, by vol.). The reagents used for the detection of sugars were: silver nitrate in acetone, followed by ethanolic sodium hydroxide, then aqueous ammonia and sodium thiosulphate (Burger, Hejmová & Kleinzeller, 1959).

The sugars used were of analytical purity or recrystallized preparations of Lachema, Brno.

RESULTS

Effect of disaccharides on the exchange transfer of glucose and galactose in erythrocytes. Fig. 1 shows the time-course of glucose exit from erythrocytes when galactose was the exchanging sugar. It may be seen that the presence of maltose in the incubation medium hindered the exit of glucose. When the concentration of maltose in the medium was increased (range 7–83 mM) the inhibitory effect was also increased. The time required at different maltose concentrations for loss of 50% of intracellular glucose (half-time) were: for no maltose 1 min., for 7 mM-maltose 2 min., for 14 mM-maltose 7 min., for 28 mM-maltose 10.5 min., for 83 mM-maltose 15 min. (Fig. 1).

Fig. 1. Time-dependence of glucose exit from erythrocytes into a maltose medium. Centrifuged erythrocytes (1 ml., containing 5 mg. of glucose/ml.) were incubated at 0° in 5 ml. of medium with 25 mg. of galactose and these concentrations (mM) of maltose: ○, 83; ▲, 28; △, 14; □, 7; ●, no maltose. Iso-osmoticity was maintained by adding corresponding amounts of NaCl.

The results in Fig. 2 show that different disaccharides of glucose do not inhibit exchange transfer to the same extent. The sequence of disaccharides (most inhibiting first) at a concentration of 83 mM was: maltose (half-time 15 min.), cellobiose (8 min.), isomaltose (2.5 min.). Trehalose (Fig. 2), lactose and sucrose (Fig. 3) did not inhibit exchange transfer.

These results appear to show that some disaccharides can interact with the carrier system for monosaccharides, though it has been previously claimed that they and other oligosaccharides do not penetrate erythrocytes (Tschau-Tjiän Yang, 1950; Kozawa, 1914). Chromatographic analysis revealed that under conditions of the experiments a

Fig. 2. Time-dependence of glucose exit from erythrocytes into medium containing different disaccharides of glucose. Centrifuged erythrocytes (1 ml., containing 5 mg. of glucose/ml.) were incubated at 0° in 5 ml. of 83 mM-disaccharide containing 25 mg. of galactose and 32.5 mg. of NaCl. ○, Maltose; ▲, cellobiose; ■, isomaltose; □, trehalose; ●, 25 mg. of galactose + 45 mg. of NaCl/5 ml. of medium with no disaccharide.

Fig. 3. Time-dependence of glucose exit from erythrocytes into medium containing some disaccharides. Centrifuged erythrocytes (1 ml., containing 4 mg. of glucose/ml.) were incubated at 0° in 5 ml. of 83 mM-disaccharide containing 25 mg. of galactose and 32.5 mg. of NaCl. ○, Maltose; △, lactose; □, sucrose; ●, 25 mg. of galactose + 45 mg. of NaCl/5 ml. of medium with no disaccharide.

significant amount of isomaltose but not of maltose penetrate erythrocytes. Moreover, only trace amounts of cellobiose and lactose could be detected in haemolysates after incubation with these sugars.

Effect of the configuration of the reducing group of disaccharides on exchange transfer. It has been claimed that the β -anomer of glucose has a greater affinity for the carrier than the α -anomer (Reinwein,

Kalman & Park, 1957). We have therefore investigated the influence of the configuration of the reducing group on the inhibitory effect of disaccharides in exchange transfer. If this concept were applicable it might be expected that maltose (which is predominantly in the β form in freshly prepared solutions) would exert a greater inhibitory effect on exchange transfer than aged solutions consisting of 38 % of α and 62 % of β . (No mutarotase was found in erythrocytes; Willbrandt, 1960.) The opposite holds for lactose, which is predominantly in the α form in freshly prepared solutions. To elucidate this problem we compared the inhibitory effect of fresh solutions of maltose or of lactose or both on exchange transfer with that of solutions of the sugars kept for 24 hr. at room temperature.

Fig. 4 shows that the inhibitory effect of freshly prepared solutions of these sugars is very similar to that of solutions aged for 24 hr. This would suggest that the configuration of the reducing group does not exert a significant effect on the interaction of disaccharide and carrier.

Effect of glucose tri- and poly-saccharides on exchange transfer. Results given in Fig. 5 show that the inhibitory effect on the exchange transfer of galactose decreases with increasing molecular weight of the inhibitory sugar in the order: glucose, maltose, maltotriose (83 mm). In this experiment the values shown were obtained by densitometry of the chromatogram.

A dextran with number-average molecular weight 2700 was also tested; it did not exert any inhibitory effect at the same concentration (w/v) as maltose.

DISCUSSION

Separation of erythrocytes from the incubation medium presents a special problem. The high rate of sugar movement across the erythrocyte membranes precludes ordinary centrifuging, and according to the findings of LeFevre & McGinnis (1960) a high-speed air-driven microcentrifuge is not suitable, as it brings about haemolysis of the erythrocytes and moreover only permits analysis of the medium and not of the cells. They therefore proposed the application of a fixing mixture blocking the movement of sugars and permitting the use of an ordinary centrifuge. The filtration technique used in the present work is suitable for studying transport of sugars in erythrocytes and particularly exchange transfer as it makes it possible to follow the shifts of several sugars across the erythrocyte membrane and to remove sugars from the extracellular space without incurring losses to the intracellular sugar content.

Since non-reducing disaccharides did not inhibit the exchange transfer of glucose and galactose in erythrocytes the reducing group and hence the

Fig. 4. Time-dependence of glucose exit from erythrocytes into maltose and lactose media. Centrifuged erythrocytes (1 ml., containing 5 mg. of glucose/ml.) were incubated at 0° in 5 ml. of medium with 25 mg. of galactose and 32.5 mg. of NaCl. Disaccharide concentration was 83 mm. Δ , Maltose 24 hr. after dissolving; $\blacktriangle$, maltose immediately after dissolving; $\square$, lactose 24 hr. after dissolving; $\bullet$, lactose immediately after dissolving; $\bullet$, medium with 25 mg. of galactose + 45 mg. of NaCl in 5 ml. of medium with no disaccharide.

Fig. 5. Time-dependence of galactose entry into erythrocytes in the presence of glucose, maltose or maltotriose in the medium. Centrifuged erythrocytes (1 ml.) were incubated at 0° in 5 ml. of medium containing 25 mg. of galactose and 32.5 mg. of NaCl. Sugar concentration was 83 mm. Δ , Maltose; $\blacktriangle$, maltotriose; $\circ$, glucose; $\bullet$, medium with 25 mg. of galactose + 45 mg. of NaCl/5 ml. of medium without inhibitory sugar.

reducing moiety of the disaccharides may be important in the combination of sugars with the carrier. The significance of the free reducing group is confirmed by another finding, that methyl glucoside has a relatively weak inhibitory effect on the exchange transfer of aldoses (unpublished work). If the effect depended solely on the reducing unit of the disaccharide then all disaccharides containing the same reducing moiety should display the same inhibitory effect. However, even reducing disaccharides composed only of glucose differed considerably in their affinity for the carrier system. This indicates that the nature of the linkage between glucose residues forming the disaccharide may also exert an important influence on the interaction with the carrier. Since maltose and isomaltose differ the aldose carbon atom contributing to the glycoside link is one factor of importance. Others are the nature of the second sugar and whether the linkage is α or β . This is indicated by different effects of maltose and cellobiose, which are 1 $\rightarrow$ 4-linked disaccharides of glucose differing only in that maltose is an α - and cellobiose a β -glycoside. Further, lactose which is also a 1 $\rightarrow$ 4-linked β -glycoside does not inhibit the exchange transfer even though galactose and glucose are transported by the same carrier system (Lacko & Burger, 1961).

The suggestion was made by LeFevre & Marshall (1958) that the affinity of monosaccharides for the carrier system is related to the conformation of the sugars. The conformations of the pyranose rings of maltose and cellobiose have been studied in detail by Bentley (1959), who found that the reducing group of both disaccharides is expressed by the conformation formula C1 of Reeves (1950). The different inhibitory effect of the two disaccharides may result therefore from different conformations of the non-reducing unit. In cellobiose, a β -glycoside, the chair-form conformation C1 predominates [in common with lactose (Wallenfels, Lehmann & Malhotra, 1960)]; in maltose, an α -glycoside, the conformation is between the B1 and 3B boat forms (Bentley, 1959). It may be that the flexible boat form of the non-reducing unit of maltose fits the steric configuration of the carrier better than the relatively rigid chair-form of the non-reducing unit of cellobiose.

There was no correlation between penetration of disaccharides and their inhibitory effects on the exchange transfer of glucose and galactose. Maltose, which exhibited the most marked effect, did not appear to penetrate at all; cellobiose, with a weaker interaction, was demonstrable in such small amounts that they might be ascribed to adsorption on the erythrocyte membrane; isomaltose, which displayed a still weaker effect on the exchange transfer, appeared to penetrate the erythrocytes significantly.

SUMMARY

1. The inhibitory effect of disaccharides composed of two glucose moieties on the exchange transfer of glucose and galactose in erythrocytes at 0° decreases in the order maltose, cellobiose and isomaltose. Trehalose, lactose and sucrose do not affect the exchange transfer.

2. The configuration of the reducing group does not affect the affinity of the disaccharide for the carrier system.

3. The interaction of sugars with the carrier system decreases in the order glucose, maltose, maltotriose, i.e. with increasing molecular weight.

4. Exchange transfer was not inhibited by the non-reducing disaccharides sucrose and trehalose; this suggests participation of the reducing unit in the interaction of these sugars with the carrier. The possibility is discussed that the conformation of the non-reducing residue in disaccharides may be of importance in the interaction of disaccharides and carrier.

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