BBA 75195

INCLUSION OF L-GLUCOSE WITHIN THE SPECIFICITY LIMITS OF THE ACTIVE SUGAR TRANSPORT SYSTEM OF HAMSTER SMALL INTESTINE

WOLFGANG F. CASPARY AND ROBERT K. CRANE
Department of Physiology, Rutgers Medical School, New Brunswick, N.J. (U.S.A.)
(Received July 15th, 1968)

SUMMARY

Incubation *in vitro* of segments and everted sacs of hamster small intestine have been used to study the mode of absorption of L-glucose. By all current criteria, L-glucose is a substrate of the intestinal transport system for D-glucose. We have examined accumulation against a concentration gradient, substrate inhibition, phlorizin sensitivity, Na^+ dependence, energy dependence and counterflow. The estimated K_m value for L-glucose is 65 mM.

INTRODUCTION

The usual interpretation of the minimal structural requirements for intestinal active transport of sugars holds that the sugar must be in the D-form. A convenient basis for this opinion has been LEFEVRE's correlation of sugar affinity and sugar transport in the red cell[1] based upon accepted methods of conformation analysis[2]. LEFEVRE concluded that substrates with the greatest affinity for the red cell sugar transport system exist in the C1 chair form attributed to D-glucose[1]; those with least affinity, *e.g.*, L-glucose, exist in the 1C chair form[1].

We recently pointed out[3], however, that D- and L-glucose can present the same conformation of the underlying tetrahydropyranose structure and suggested that any differences between these enantiomorphs as substrates should be viewed as a configurational problem related to substituent groups rather than as a conformational problem related to the pyranose ring. At the same time, we reported[3] finding a specific interaction of L-glucose with the sugar transport system of the intestine and concluded on the basis of current transport carrier theory that L-glucose was a substrate, albeit a poor one, of this system.

RUMMEL AND STUPP[4] using the *in vitro* perfusion method of FISHER AND PARSONS[5] reported failure of L-glucose to be transported against a concentration gradient by the rat small intestine. WILSON AND LANDAU[6] confirmed this observation by the *in vitro* everted sac technique.

The purpose of the present paper is to report extensive studies showing that L-glucose is indeed actively transported. During the preparation of this report, NEALE AND WISEMAN[7] have published further preliminary evidence which supports our view.

METHODS AND MATERIALS

Incubation technique

The present experiments were performed by incubation *in vitro* of rings of everted hamster small intestine as described by CRANE AND MANDELSTAM[8]. Hamsters were fasted overnight. Approx. 200–300 mg, wet weight, of tissue were placed in 25-ml erlenmeyer flasks containing 5 ml of KREBS–HENSELEIT phosphate buffer[9] with the appropriate added substrates at 37°. In a number of experiments the buffer was inadvertently gassed with 95% O_2, 5% CO_2 bringing the pH to about 6.8. The major experiments have been performed again using 100% O_2 in the gas space. The differences in results, as will be seen, are minor (Fig. 1).

In some experiments the buffer was modified by replacing Na^+, fully or to the extent indicated in the tables, with $Tris^+$. Isoosmolarity was preserved by adding sugars as a 0.3 M solution.

In the demonstration of counterflow, two successive incubations were performed: (1) Rings of intestine were allowed to accumulate substrate for the time indicated and controls were assayed for the amount of substrate accumulated. (2) Samples of the tissue with accumulated substrate were incubated in fresh media containing the same substrate concentration with appropriate additions.

Some experiments were carried out using the modified everted sac technique described by CRANE AND WILSON[10]. In some of these experiments substrate was added only to the mucosal compartment; in others, it was added to both the serosal and mucosal compartments at equal concentrations. Incubation was as above for 60 min.

Compounds

The following were obtained from commercial sources: D-glucose, D-galactose, α-methyl-glucoside and α-methyl-xyloside from Pfanstiehl; β-methyl-glucoside and β-methyl-xyloside from Calbiochem; 6-deoxy-L-galactose, 6-deoxy-D-galactose and L-glucose from Sigma Chemical Co.; 2,4-dinitrophenol from Matheson, Coleman and Bell; phlorizin from Nutritional Biochem. Corp.; and L-[^{14}C]glucose from New England Nuclear Corp. 1,5-Anhydro-D-[^{3}H]glucitol and 6-deoxy-D-[^{3}H]glucose were prepared in our laboratory. L-Galactose was kindly provided by Dr. N. K. RICHTMYER, National Institutes of Health.

Analytical methods

Chemical assays for L-galactose and L-glucose were performed by the method of SOMOGYI[11]. Radioactivity was assayed with the Beckman liquid-scintillation system. The purity of L-galactose, L-glucose and 6-deoxy-L-galactose was confirmed by gas chromatography (Beckman GC 4) following silylation by a method similar to that of SWEELEY *et al.*[12] using 6-ft columns with PO-1 packing at 140° and 160°.

Calculation of data

Results are expressed as rates of entry (v) in mmoles of substrate accumulated per ml of tissue water in a given time period, assuming a water content of approx. 80% of the tissue wet weight.

$$\text{Percent filling} = 100 \times \frac{\text{mmoles/ml tissue water}}{\text{mmoles/ml medium}}$$

The results in the tables are averages of duplicates or triplicates. In the figures actual experimental points as well as the means are given.

RESULTS

Time course of L-*glucose transport*

D-Glucose active transport in the intestine is regarded as a Na^+-dependent, phlorizin-sensitive, energy-dependent process of accumulation against a concentration gradient[3]. Accordingly, we tested for these characteristics. First, we incubated rings of hamster intestine for various time intervals. As seen in Fig. 1, L-glucose accumulated over a period of 60 min against a final concentration gradient of about 2-fold. As seen in Table I, addition of phlorizin or omission of Na^+ markedly decreased the entry of L-glucose into the tissue. Addition of 2,4-dinitrophenol also depressed transport of L-glucose.

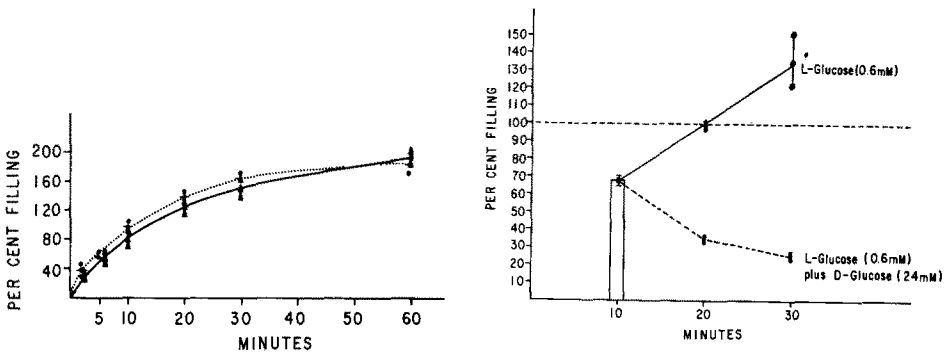

Fig. 1. Uptake of L-[^{14}C]glucose by rings of hamster small intestine. The initial concentration of L-glucose was 6 mM. Incubation medium: KREBS-HENSELEIT phosphate buffer gassed with 100% O_2 (———) and 95% O_2, 5% CO_2(·····).

Fig. 2. Counterflow of L-glucose induced by D-glucose. Rings of hamster small intestine were incubated 10 min with 0.6 mM L-[^{14}C]glucose then transferred to either 0.6 mM L-[^{14}C]glucose or a mixture of 0.6 mM L-[^{14}C]glucose and 24 mM D-glucose and the incubation was continued for additional 10- and 20-min periods.

TABLE I

ACTIVE TRANSPORT OF L-GLUCOSE BY HAMSTER SMALL INTESTINE

Incubation times were 20 min (Expt. a) and 30 min (Expt. b). Inhibition was calculated from the rates in the absence of inhibitors. The initial L-glucose concentration was 6 mM.

Condition	*Percent filling*	*Inhibition* (%)
Expt. a		
No addition	137	—
Phlorizin (24 mM)	42	69.4
Tris-medium (125 mM $Tris^+$, 16 mM Na^+)	39	71.6
Expt. b		
No addition	179	—
2,4-Dinitrophenol (0.5 mM)	66.5	62.9
Phlorizin (24 mM)	45	74.9

Substrate inhibition of L-*glucose transport*

Tissue was incubated with L-glucose in the presence of several good substrates of the glucose transport system and several sugars which appear not to be substrates[3]. As shown in Table II, accumulation of L-glucose was markedly depressed in the presence of L-fucose, D-galactose and D-xylose but not in the presence of L-galactose, L-rhamnose, L-xylose, D-arabinose or L-arabinose. D-Galactose at 12 mM had a much stronger inhibitory effect than D-xylose at 30 mM, presumably due to the different K_m values of these sugars. In a converse experiment, 30 mM L-glucose reduced the uptake of the transport substrate, 1,5-anhydro-D-glucitol (0.6 mM) from a control percent filling of 234 to 166 during 10 min of incubation.

TABLE II

EFFECT OF OTHER SUGARS ON THE ACTIVE TRANSPORT OF L-GLUCOSE

Incubation times were 30 min. The initial L-glucose concentration in all experiments was 6 mM. Inhibitor concentrations were as indicated. Inhibition was calculated from the rates in the absence of inhibitors. Values are averages of 2 or 3 tests. The number of tests is given in parentheses.

Condition	*Percent filling*	*Inhibition* (%)
No addition	160	—
D-Galactose, 12 mM (2)	59	63.2
D-Xylose, 30 mM (2)	107	33.2
L-Fucose, 18 mM (3)	100	37.5
L-Galactose, 12 mM (3)	156	2.5
L-Xylose, 30 mM (3)	145	9.4
L-Rhamnose, 30 mM (2)	145	9.4
D-Arabinose, 30 mM (2)	155	3.1
L-Arabinose, 30 mM (2)	158	1.3

Counterflow

In order to demonstrate counterflow, the tissue was incubated for 10 min with 0.6 mM L-glucose and then transferred to a second incubation flask containing the same substrate concentration *plus* 24 mM D-glucose. As can be seen in Fig. 2, the presence of D-glucose during the second incubation prevented further uptake of L-glucose and, moreover, induced an outflow of L-glucose against a concentration gradient.

Evaluation of K_m

As shown in Fig. 3, transport of L-glucose exhibited typical saturation kinetics with a K_m on the order of 65 mM. Also, as is characteristic of D-glucose, the uptake of L-glucose was competitively inhibited by 6-deoxy-L-galactose.

Transport of L-*glucose by the everted sac technique*

In order to find out whether earlier reports on the transport of L-glucose resulting in a failure to demonstrate active transport would be consistent with the use of different methods, we used the everted sac technique as modified by CRANE AND WILSON[10].

Adding the substrate to the mucosal compartment alone, as had been done by RUMMEL AND STUPP[4] using the *in vitro* method of FISHER AND PARSONS[5], we did

not find accumulation against a concentration gradient into the serosal compartment after 1 h incubation. However, analysis of the sac tissue showed that L-glucose had been markedly accumulated against the concentration gradient within the intestinal tissue during this time (Fig. 4). When equal substrate concentrations were initially added to both compartments, there was a measurable change in the concentrations of L-glucose in the direction of serosal accumulation. Starting with 3 mM in both, after 1 h of incubation the mucosal concentration was 2.67 mM and the serosal concentration was 3.36 mM. The animals in these experiments were on a normal diet and were not starved overnight.

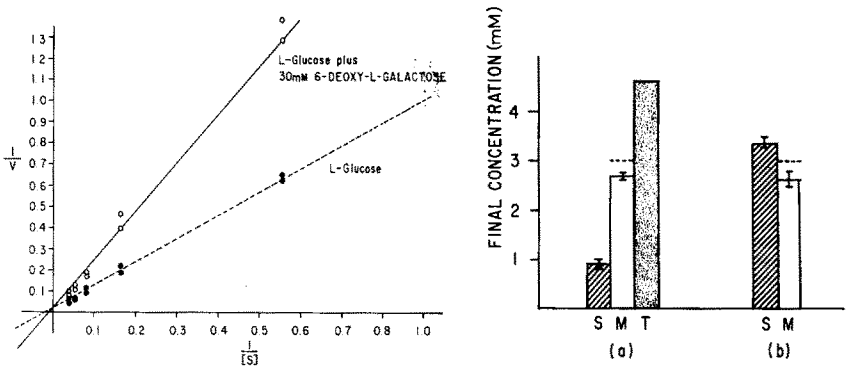

Fig. 3. Rate *vs.* concentration relationships for L-glucose. Also shown is the competitive inhibition by 6-deoxy-L-galactose.

Fig. 4. Demonstration of active transport of L-glucose using everted sacs. Everted sacs were incubated for 60 min with 3 mM L-[^{14}C]glucose. In Experiment a substrate was added to the mucosal compartment only. In Experiment b substrate was added to both the mucosal and serosal compartments at the same concentrations: 3 mM. M, mucosal compartment; S, serosal compartment; T, tissue. The values shown were obtained at the end of incubation.

DISCUSSION

Our results show that L-glucose exhibits all the criteria needed for it to be accepted as a substrate for the D-glucose active transport system; namely, accumulation against a concentration gradient, substrate inhibition, energy dependence, phlorizin sensitivity, Na^+ dependence and counterflow. L-Glucose has, however, a relatively low affinity for the system, $K_m = 65$ mM, which is the most likely reason why active transport of this sugar was not seen earlier. Also, previous investigators[4,6] assayed only the fluid compartments and did not assay the tissue.

As is well known[13–15], the accumulation of actively transported sugars occurs first within the mucosal cell and only subsequently does it diffuse across the serosal barriers. Lengthy incubation times are usually necessary to observe accumulation in the serosal compartment and become longer with lesser extents of substrate accumulation within the mucosal cell. The tissue–medium concentration ratio achieved by L-glucose is not very great.

The results reported here do point up the need in studying specificity to take into account the variety of family relationships which any one monosaccharide can have. The diagrams in Fig. 5 show that so far as concepts of substrate binding to a

specific site are concerned, D- and L-glucose differ only in the interchange of two substituents: –OH and $-CH_2OH$. Since it is known[3] that the $-CH_2OH$ at C-6 of glucose is not an absolute requirement for transport and that large substituents at C-1 are consonant with good transport qualities, it was expected that L-glucose would prove to be a substrate.

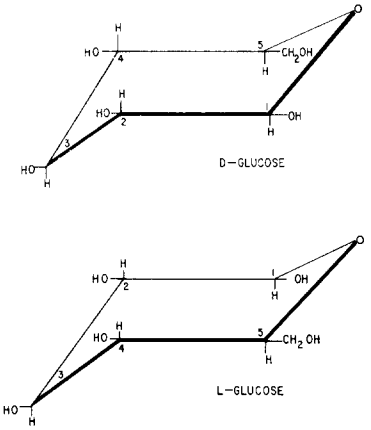

Fig. 5. Comparison of D- and L-glucose conformations to accentuate their configurational relationships.

Evidence that L-glucose interacts with L-histidine during intestinal transport has been presented by HINDMARSH, KILBY AND WISEMAN[16]. HUANG and his associates[17–19] have recently shown by clearance and stop-flow techniques that D- and L-glucose interact in the renal tubular cell of rats and dogs and have concluded that the enantiomorphs share a common pathway.

ACKNOWLEDGEMENT

This work was supported by a grant from the National Science Foundation.

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