Biochimica et Biophysica Acta, 443 (1976) 284–287

BBA 77389

1-*O*-ACYL DERIVATIVES OF GLUCOSE AS NON-PENETRATING INHIBITORS OF GLUCOSE TRANSPORT BY HAMSTER SMALL INTESTINE IN VITRO

K. RAMASWAMY*, B. R. BHATTACHARYYA** and R. K. CRANE

Department of Physiology, College of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway, N.J. 08854 (U.S.A.)

(Received December 29th, 1975)

SUMMARY

1-*O*-Acyl derivatives of glucose are not transported by the hamster small intestine in vitro. These derivatives, however, are potent inhibitors of the glucose transport system. 1-*O*-Decanoyl glucose is a competitive inhibitor of β-methyl glucoside transport.

INTRODUCTION

Extensive studies of the glucose transport system of hamster small intestine have revealed that basically the carrier site recognises the pyranose ring with affinity being influenced by the identity and configuration of the substituents at each carbon atom [1, 2]. We have reinvestigated the effect of substituent at C-1 using alkyl glucosides [3] and found that glucosides with a long alkyl chain as the aglycone can be transported by carrier-mediated processes in the hamster intestine in vitro. These glucosides interact with the glucose carrier and compete with glucose and analogs for the binding to the carrier. In this communication, we report on studies of a different type of substituent at C-1, namely 1-*O*-acyl derivatives of glucose.

MATERIALS AND METHODS

1-*O*-Acyl derivatives of glucose were prepared in our laboratory by using the carbodiimide-imidazole method described by Keglevic et al. [4] and alkyl β-glucosides were prepared by the modified Koenigs-Knorr method [5, 6]. All were purified by column chromatography and then crystallized. β-Methyl D-glucoside was obtained from Sigma Chemical Co.; β-methyl [U-^{14}C]glucoside with a specific activity of 52.5 Ci/mol was obtained from Calatomic.

* Present address: Gastroenterology Division, University of Texas Medical School at Houston, P. O. Box 20708, Houston, Texas 77030, U.S.A.

** Present address: Polysciences, Inc., Paul Valley Industrial Park, Warrington, Pa. 18976, U.S.A.

The analytical and incubation methods used here have been described earlier [3]. Briefly, everted sacs of hamster intestine tied to polyethylene tubing were incubated with shaking in 10.0 ml of modified Krebs-phosphate buffer [7] containing 4 mM sodium taurocholate and the test compounds. At the end of the incubation periods, the everted sacs were collected and transferred to 80 % ethanol, homogenized and centrifuged. The supernatant liquids were hydrolyzed with 1 M HCl and after neutralization, the amount of glucose liberated was determined by the glucose oxidase method [8]. Recoveries of acyl derivatives in the analytical methods used were between 95 and 100 %. Results are expressed as rate of entry in μmol of sugar accumulated per ml of tissue water in a specified time, assuming a water content of 80 % of the wet tissue weight [9].

RESULTS AND DISCUSSION

1-*O*-Acyl derivatives with chain lengths in the range of 3–14 carbon atoms were synthesized and were used to compare their uptake with 1-*O*-alkyl derivatives with the same chain lengths (Table I). As previously reported [3], alkyl derivatives were transported. However, the corresponding 1-*O*-acyl derivatives were not transported to any significant levels.

1-*O*-Acyl derivatives were tested for their effect on the transport of β-methyl glucoside, which is a good substrate of the glucose transport system [1]. The results are presented in Table II, where it is seen that they are potent inhibitors. Moreover, as shown in Fig. 1, using the representative compound, 1-*O*-decanoyl glucose, the inhibition is competitive with a K_i of 0.16 mM. On the basis of these data, the 1-*O*-acyl derivatives appear to be non-penetrating inhibitors. 1-*O*-Decanoyl glucose is a non-penetrating as well as a competitive inhibitor of β-methyl glucoside transport. The

TABLE I

COMPARISON OF UPTAKE OF ALKYL AND ACYL DERIVATIVES OF GLUCOSE

All the substrates were used at a concentration of 1 mM; time of incubation, 15 min, volume 10 ml; O_2; Krebs-Ringer sodium buffer; 4 mM sodium taurocholate.

	Glucoside or acyl glucose in tissue water (mM)
Propyl glucoside	5.2
1-*O*-Propionyl glucose	0.4
Butyl glucoside	7.4
1-*O*-Butyryl glucose	1.9
Hexyl glucoside	2.9
1-*O*-Henanoyl glucose	0.9
Octyl glucoside	2.9
1-*O*-Octanoyl glucose	0.5
Decyl glucoside	10.9
1-*O*-Decanoyl glucose	0.2
Dodecyl glucoside	7.5
1-*O*-Dodecanoyl glucose	0.2
Tetradecyl glucoside	6.2
1-*O*-Tetradecanoyl glucose	0.9

TABLE II

EFFECT OF 1-*O*-ACYL DERIVATIVES OF GLUCOSE ON UPTAKE OF β-METHYL GLUCOSIDE

Time of incubation, 2 min; volume, 10.0 ml; Krebs-Ringer sodium buffer, 4 mM sodium taurocholate; concentration of acyl derivatives, 1 mM.

	β-Methyl glucoside in tissue water (mM)	Inhibition (%)
1 mM β-methyl glucoside	9.1	–
+hexanoyl glucose	6.9	24
+octanoyl glucose	5.8	38
+decanoyl glucose	1.7	81
+dodecanoyl glucose	3.9	57
+tetradecanoyl glucose	4.5	50

other 1-*O*-acyl derivatives were not tested for their kinetics of inhibition.

The results presented above clearly show that a carbonyl function in the substituent at C-1 makes the glucose derivative unable to be transported. It has been seen from our earlier studies on alkyl glucosides [3] that glucosides with lipid-soluble aglycones can be transported but the data presented here clearly indicate that the nature and not the lipid solubility of the substituent is important for transport. 1-*O*-Acyl derivatives, however, bind to the glucose carrier and in the case of decanoyl glucose, competitively with very high affinity. These results might be interpreted to

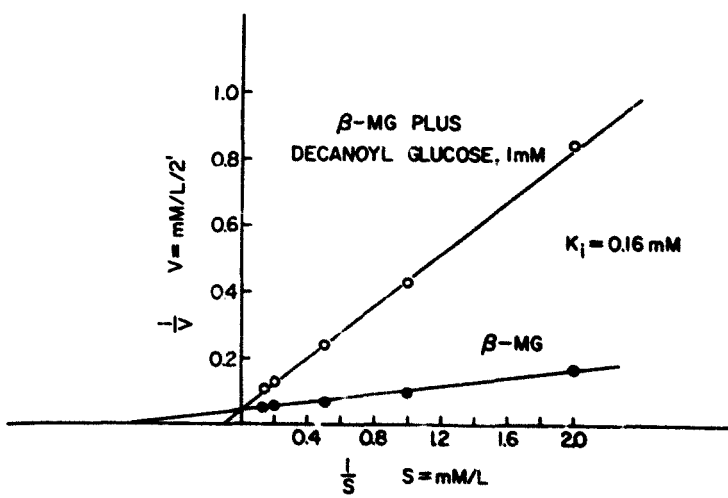

Fig. 1. Lineweaver-Burk plot for β-methyl glucoside uptake in the presence and absence of 1-*O*-decanoyl glucose. Incubations were in 10.0 ml of buffer for 2 min. 1-*O*-Decanoyl glucose was at 1 mM. Corrections for extracellular β-methyl glucoside were made from corresponding mannitol controls.

indicate that the presence of an ester group at C-1 of glucose may possibly render the compound tightly bound but prevent the carrier from undergoing conformational or any other changes needed for transport. Barnett and Munday [2] have interpreted their studies on structural requirement for active intestinal transport to show the presence of hydrogen bonds donated by the carrier to the oxygens at carbons 1, 3, 4, and 6 being important though not individually critical for establishing the fit between the substrate and the carrier. Our data on 1-*O*-acyl derivatives might be interpreted to point out that possibly the presence of an ester group capable of additional hydrogen bonding might give rise to tight binding to the carrier, thereby making the compound unable to be transported.

ACKNOWLEDGEMENT

This work was supported by grants from the National Science Foundation, Grant No. 14464 and the National Institutes of Health, Grant No. AM 10696.

REFERENCES

1 Crane, R. K. (1968) in Handbook of Physiology, Sect. 6, Alimentary Canal (Code, C. F., ed.), Vol. 3, pp. 1323–1351, Physiological Soc., Washington, D.C.
2 Barnett, J. E. G. and Munday, K. A. (1972) in Transport across the Intestine, A Glaxo Symposium (Burland, W. L. and Samuel, P. D., eds.), pp. 111–125, Churchill Livingstone, Edinburgh and London
3 Ramaswamy, K., Bhattacharyya, B. R. and Crane, R. K. (1976) Biochim. Biophys. Acta, in the press
4 Keglevic, D., Kornhauser, A. and Valentekovic, S. (1972) Carbohydr. Res. 22, 245–256
5 Talley, E. A. (1963) in Methods in Carbohydrate Chemistry (Whistler, R. L. and Wolfrom, M. L., eds.), Vol 2, pp. 337–340, Academic Press, New York
6 Flowers, H. M., Levy, A. and Sharon, N. (1967) Carbohydr. Res. 4, 189–195
7 Schafer, J. A. and Jacquez, J. A. (1967) Biochim. Biophys. Acta 135, 741–750
8 Lloyd, J. B. and Whelan, W. J. (1969) Anal. Biochem. 30, 467–475
9 Crane, R. K. and Mandelstam, P. (1960) Biochim. Biophys. Acta 45, 460–476