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Functional Studies of Human GLUT5: Effect of pH on Substrate Selection and an Analysis of Substrate Interactions

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The adsorption of D-fructose within the lumen of the human small intestine is thought to be mediated by the GLUT5 isoform of the human facilitative sugar transporter family. This isoform has been expressed in oocytes and shown to be capable of D-fructose transport. Some debate remains regarding the absolute substrate specificity of this isoform. To that end, we have undertaken an analysis of the functional properties of this protein when expressed in *Xenopus* oocytes. We have examined the pH dependence of transport activity, the ability to transport D-fructose *versus* deoxyglucose, and employed a range of sugar analogues to probe the nature of the exofacial substrate binding site. Our data show that the human GLUT5 isoform functions exclusively as a D-fructose transporter between pH 4.5 and 8. The Km for D-fructose was found to be 15 ± 4 mM at pH 7.5, and was relatively unaltered even at pH 4.5. Analysis of the effects of a range of compounds on GLUT5 function suggests that this isoform transports D-fructose preferentially in the furanose ring form.

The terminal digestion of dietary carbohydrates takes place in the proximal regions of the small intestine (duodenum and jejunum) by brush border enzymes such as sucrase-isomaltase and glucoamylase. The end products of these enzymatic reactions, glucose, galactose and fructose are then transported into the enterocyte by integral membrane transport proteins, SGLT1 (glucose/galactose) and GLUT5 (fructose) [1-5]. Both of these transporters has been identified in the brush border membrane of enterocytes by immuno-labelling techniques [3, 4]. The human GLUT5 isoform has been expressed in oocytes and shown to be capable of the mediating D-fructose accumulation, but not D-glucose or D-galactose [1]. Studies of GLUT5 cDNAs from other species has suggested that the rat isoform is capable of mediating glucose transport, and that the rabbit isoform may also function in part as a glucose transporter [6, 7]. To date, however, no information is available regarding the nature of the D-fructose binding to any of the GLUT5 isoforms presently cloned.

Recent studies of the functional properties of the rabbit sodium-dependent glucose transporter, SGLT1, have indicated that the pH environment can exert profound effects on the nature of the transport event [8]. At low pH, SGLT1 is proposed to function as a proton-dependent transporter of relatively low affinity for glucose, whereas at higher pH, the carrier is sodium-dependent and exhibits increased affinity for glucose. Given the acidic pH of the lumen of the small intestine, and the present debate regarding the absolute substrate specificity of GLUT5, we set out to examine the effect of pH on GLUT5 function and substrate selection, and to investigate the preferred ring-form of D-fructose for transport. We show that human GLUT5 is exclusively a fructose transporter between pH 4.5 and 8, and that the measured Km for D-fructose transport is not significantly altered by pH. GLUT5 is effectively inhibited by 2,5-anhydromannitol but not by L-sorbose, indicating that the preferred substrate for the transporter is D-fructose in the *furan* ring form.

MATERIALS AND METHODS

Materials. Wild caught *Xenopus laevis* were purchased from the African Xenopus Facility (Noordhoek, Republic of South Africa). All isotopes were from DuPont/NEN (UK.), and sugars purchased from Sigma (Poole, UK.). Reagents for *in vitro* transcription were from Promega (Southampton, UK.). All other reagents were as described [9, 10] [9, 11, 12].

Isolation of oocytes and microinjection. Female *Xenopus laevis* were maintained at 18°C on a 12 h light/dark cycle. Individual oo-

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cytes were dissected and stored in DNOM-buffer [9, 10]. All subsequent procedures were performed in DNOM-buffer, except for the assays of hexose transport which were performed in Barths buffer alone. Oocytes were injected with water or 50 nl of cRNA (usually ~ 50 ng), prepared and purified as described in [10, 13], and incubated in DNOM buffer at 18° for 48 to 72 h prior to assay; the medium was replaced every 12 h.

Hexose transport in oocytes. Groups of 8 oocytes were washed three times in Barths buffer and incubated in 0.45 ml of Barths buffer at pH 7.4 in 13.5 ml centrifuge tubes at room temperature (~18°C). Transport measurements were initiated by the addition of a 50μl aliquot of radiolabelled sugar ([2,6 ^{3}H]deGlc or [^{14}C]D-fructose) to the concentration indicated in the Figures. The reaction was stopped after the requisite time interval (30 minutes unless otherwise stated) by quickly aspirating the media and washing the oocytes with 5 ml of ice-cold phosphate buffered saline (PBS; 150 mM NaCl, 10 mM sodium phosphate, pH 7.4). The oocytes were washed in this fashion a further two times and dispensed to scintillation vials, 1 oocyte per vial. These three washes were completed within 30 sec. 1 ml of 1% sodium dodecyl sulphate was added to each scintillation vial and incubated at room temperature for 1 h with agitation, prior to the addition of scintillant and measurement of radioactivity. Parallel assays were undertaken using oocytes microinjected with water as a control. These transport rates were subtracted from those obtained in oocytes expressing a functional transporter, and here we present only the value of transport rates obtained after such subtraction. Such control assays were performed for every condition in every experiment. In general, the water-injected oocytes exhibit a fructose transport rate of between 5 and 10% of that measured in an identical oocyte population expressing GLUT5.

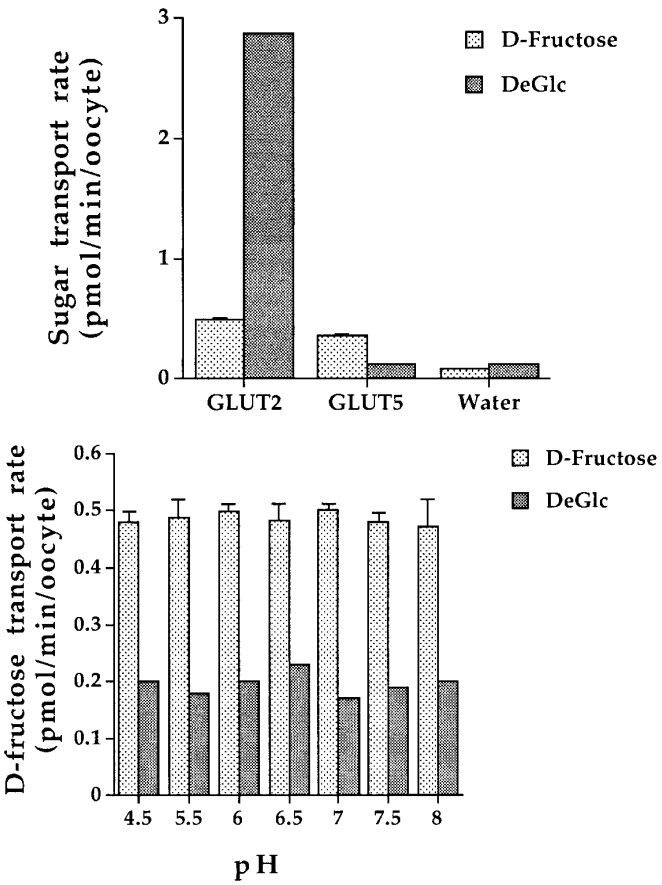

FIG. 1. pH dependence of D-fructose and 2-deoxy-D-glucose transport by GLUT5. *Panel A.* Measurement of D-fructose and 2-deoxyglucose uptake by GLUT2, GLUT5 and in water injected oocytes. Note that GLUT5 expression does not increase fructose uptake by these oocytes. *Panel B.* Using the same oocyte preparation as panel A, we then measured the pH dependence on GLUT5 function. Groups of 10 oocytes were incubated in Barths buffer at the pH shown. After 1h in this buffer, transport rates for 50μM deGlc or 100 μM D-fructose were measured as described in Materials and Methods. Data from a representative experiment are shown, each point is the mean ± s.d. of 10 oocytes. The experiment was repeated three times with similar results. DeGlc transport rates measured in GLUT5 expressing oocytes were not increased over water-injected controls in any experiment (compare with panel A which is data from the same group of oocytes).

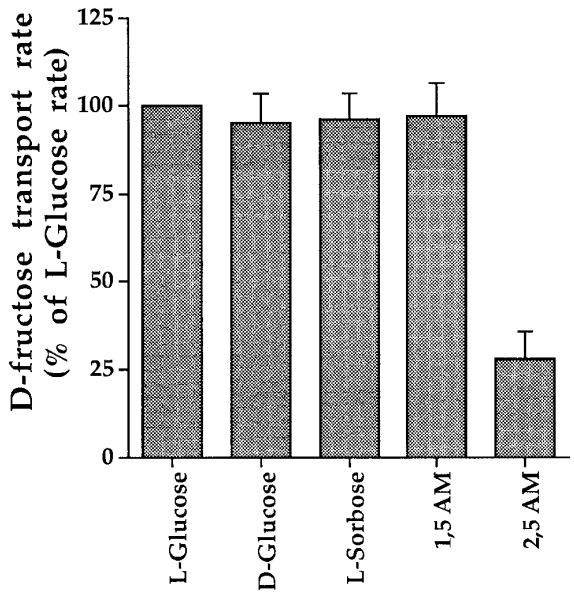

FIG. 2. Effect of fructose and glucose analogues on fructose transport by GLUT5. Groups of 10 oocytes were incubated in Barths buffer for 15 minutes. After this time, an aliquot of the indicated sugar analogue was added such that the concentration of the analogue was 50 mM [D- or L-glucose, L-sorbose, 1,5-anhydromannitol (1,5 AM) or 2,5-anhydromannitol (2,5 AM)]. The transport of D-fructose (100μM) was then initiated as described in Materials and Methods. Data are expressed as transport rates as a % of the rates measured in the presence of L-glucose, and are the mean ± s.d. of three separate experiments.

RESULTS AND DISCUSSION

Studies of the sodium-dependent glucose transporter SGLT1 have shown that the kinetic properties of the protein are significantly different when measured under acidic conditions (such as those observed in the lumen of the small intestine) compared to those measured under neutral pH [8]. We therefore examined the ability of GLUT5 to transport either deoxyglucose or D-fructose over the pH range 4.5 to 8, and the results of a typical experiment are presented in Figure 1. As shown, we observed no significant changes in the rate of D-fructose transport over this pH range. Similarly,

at all pH values examined, we observed no transport activity using deoxyglucose as substrate, indicating that under conditions of pH approximating those observed in the small intestine, GLUT5 is exclusively a fructose transporter and does not transport glucose. Some confusion regarding the substrate specificity of GLUT5 between species has been recently highlighted with the observations that both rat and rabbit GLUT5 isoforms may exhibit some degree of deoxyglucose transport [1, 6, 7]. Our data demonstrate that under all conditions of pH employed (pH 4.5 to 8), human GLUT5 is unable to transport deoxyglucose (figure 1B).

The K_m for fructose measured in oocytes expressing the human isoform of GLUT5 has been reported to be ~6 mM [1]. We measured the K_m for GLUT5 for D-fructose over a range of pH values (4.5, 6 and 7.5). At pH 7.5, the observed K_m for D-fructose for the human GLUT5 isoform was 15 ± 4 mM, a value similar to that reported by Burrant et al (data not shown). Similar values were recorded at both pH 4.5 and 6 (13 ± 2.3 and 14 ± 3.1, respectively; data not shown), suggesting that unlike SGLT1 [8], the affinity of the transporter for its primary substrate is not modulated by pH.

In effort to determine whether GLUT5 accepts fructose in either the *pyran* or *furan* ring form, we measured the ability of a series of fructose analogues to inhibit D-fructose transport in oocytes expressing GLUT5 (Figure 2). Consistent with previous studies, we have observed no inhibition of D-fructose transport by either L- or D-glucose at concentrations up to 50 mM [1]. Similarly, both L-sorbose and 1,5-anhydromannitol did not significantly inhibit fructose transport by GLUT5. L-sorbose exists primarily in the pyranose ring conformation, and 1,5-anhydromannitol is a fused ring *pyran*-analogue of D-fructose. In contrast, the locked *furan*-ring fructose analogue 2,5-anhydromannitol was an effective inhibitor of fructose transport mediated by GLUT5, suggesting that GLUT5, like the dual specificity glucose/fructose transporter GLUT2 [11], accepts fructose preferentially in the *furan* ring form.

In summary, we show that under conditions of pH similar to those encountered in the small intestine, human GLUT5 is an efficient fructose transporter with no detectable ability to transport D-glucose. The affinity of the transporter for fructose is not modulated by pH. Finally, we have shown that GLUT5 preferentially accepts fructose in the five membered furan-ring form, and has only low affinity for fructose analogues locked in the pyran-form.

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REFERENCES

1. Burant, C. F., Takeda, J., Brot-Laroche, E., Bell, G. I., and Davidson, N. O. (1992) *J. Biol. Chem.* **267,** 14523–14526.
2. Castello, A., Guma, A., Sevilla, L., Furriols, M., Testar, X., Palacin, M., and Zorzano, A. (1995) *Biochem. J.* **309,** 271–277.
3. Davidson, N. O., Hausman, A. M. L., Ifkovits, C. A., Buse, J. B., Gould, G. W., Burant, C. F., and Bell, G. I. (1992) *Am. J. Physiol.* **262,** C795–C800.
4. Hwang, E. S., Hiraayama, B. A., and Wright, E. M. (1991) *Biochem. Biophys. Res. Comm.* **181,** 1208–1217.
5. Martin, M. G., Turk, E., Lostao, M. P., Kerner, C., and Wright, E. M. (1996) *Nature Genetics* **12,** 216–220.
6. Miyamoto, K., Tatsumi, S., Morimoto, A., Minami, H., Yamamoto, Y., Sone, K., Taketani, E., Nakabou, Y., Oka, T., and Takeda, E. (1994) *Biochem. J.* **303,** 877–883.
7. Rand, E. B., Depaoli, A. M., Davidson, N. O., Bell, G. I., and Burant, C. F. (1993) *Am. J. Physiol.* **264,** G1169–G1176.
8. Hirayama, B. A., Loo, D. D. F., and Wright, E. M. (1994) *J. Biol. Chem.* **269,** 21407–21410.
9. Arbuckle, M. I., Kane, S., Porter, L. M., Seatter, M. J., and Gould, G. W. (1996) *Biochemistry* **35,** 16519–16527.
10. Colville, C. A., Seatter, M. J., Jess, T. J., Gould, G. W., and Thomas, H. M. (1993) *Biochem. J.* **290,** 701–706.
11. Colville, C. A., Seatter, M. J., and Gould, G. W. (1993) *Biochem. J.* **194,** 753–760.
12. Gould, G. W., Thomas, H. M., Jess, T. J., and Bell, G. I. (1991) *Biochemistry* **30,** 5139–5145.
13. Kayano, T., Burrant, C. F., Fukumoto, H., Gould, G. W., Fan, Y.-S., Eddy, R. L., Byers, M. G., Shows, T. B., Seino, S., and Bell, G. I. (1990) *J. Biol. Chem.* **265,** 13276–13282.