APPENDIX

Inhibition Kinetics of the Glucose Transporter in the Presence of Intracellular Glucose. Because phloretin is a competitive inhibitor, the inhibition of the glucose transporter by this compound may depend on both the extracellular and the intracellular glucose concentration. The kinetics of the inhibitor have been measured in zero-trans-influx experiments. In these experiments, the competition of the inhibitor with extracellular glucose was investigated. Here, the question will be addressed concerning to what extent the intracellular glucose may affect the inhibition of the transporter by phloretin.

Facilitated diffusion can be described by a four-state model (Fig. 6*A*) (1). Phloretin does not enter the cells and can, therefore, only bind to the transporter on the outside (Fig. 6*B*) (2). The steady state kinetics of both reaction schemes were derived by using the King–Altman method (3, 4). The net steady-state uptake rate is given by Eq. **2** in *Results* of the main text. Without inhibitor (Fig. 6*A*), the kinetic constants are given by

$$V^f_{max} = T_{tot}N_1/D_1, \quad [3]$$

$$V^r_{max} = T_{tot}N_2/D_2, \quad [4]$$

$$K_{m,out} = D_0/D_1, \quad [5]$$

$$K_{m,in} = D_0/D_2, \quad [6]$$

$$\alpha = (D_0D_3)/(D_1D_2), \quad [7]$$

in which T_{tot} is the total concentration of the transport protein, and

$$N_1 = k_{-1}k_{-2}k_{-3}k_{-4}, \quad [8]$$

$$N_2 = k_1k_2k_3k_4, \quad [9]$$

$$D_0 = (k_{-1} + k_1)(k_{-2}k_{-3} + k_{-2}k_4 + k_3k_4), \quad [10]$$

$$D_1 = k_{-4}(k_{-2}k_{-3} + k_{-1}k_{-3} + k_{-1}k_{-2} + k_{-1}k_3), \quad [11]$$

$$D_2 = k_2(k_3k_4 + k_1k_{-3} + k_1k_4 + k_1k_3), \quad [12]$$

$$D_3 = k_2k_{-4}(k_{-3} + k_3). \quad [13]$$

In the scheme of Fig. 6*B*, the inhibitor does not affect V^f_{max}. In the presence of the inhibitor, the other kinetic constants become

$$V^{r,app}_{max} = T_{tot}N_2/D^*_2, \quad [14]$$

$$K^{app}_{m,out} = D^*_0/D_1, \quad [15]$$

$$K^{app}_{m,in} = D^*_0/D^*_2, \quad [16]$$

$$\alpha^{app} = (D^*_0D_3)/(D_1D^*_2), \quad [17]$$

in which

$$D^*_0 = D_0 + [I]_{out}K_d(k_{-1}k_{-2}k_{-3} + k_{-1}k_{-2}k_4 + k_{-1}k_3k_4), \quad [18]$$

$$D^*_2 = D_2 + [I]_{out}K_dk_2k_3k_4, \quad [19]$$

$$K_d = \frac{k_{-5}}{k_5}. \quad [20]$$

The effect of phloretin on V^f_{max} and $K_{m,out}$ has been measured in this paper. According to these measurements, V^f_{max} was not affected by the inhibitor, as also was derived above. The effect on $K_{m,out}$ is given by

$$K^{app}_{m,out} = K_{m,out}\cdot D^*_0/D_0$$

$$= K_{m,out}\left(1 + \frac{[I]_{out}K_dk_{-1}}{k_{-1} + k_1}\right)$$

$$= K_{m,out}\left(1 + \frac{[I]_{out}}{K_{i1}}\right). \quad [21]$$

As defined by Eq. **21**, K_{i1} has been measured in this paper. The effect of phloretin on V^r_{max}, $K_{m,in}$, and α does not only depend on D^*_0/D_0, but also on D^*_2/D_2 and must, therefore, be estimated.

Without any inhibitor present, the trypanosome glucose carrier is almost symmetrical (5, 6). In the following, perfect symmetry will be assumed. This means that $k_1 = k_{-1}$, $k_3 = k_{-3}$, $k_2 = k_{-4}$, and $k_4 = k_{-2}$. Consequently, $V^f_{max} = V^r_{max}$ and $K_{m,out} = K_{m,in}$ in the absence of inhibitors. From Eq. **21** and the symmetry assumption, it follows that

$$K_{i1} = \frac{k_{-1} + k_1}{K_dk_{-1}} = \frac{2}{K_d}. \quad [22]$$

Furthermore,

$$\frac{D^*_2}{D_2} = 1 + \frac{[I]_{out}K_d}{1 + \frac{k_1k_{-3}}{k_3k_4} + \frac{k_1}{k_3} + \frac{k_1}{k_4}}$$

$$= 1 + \frac{[I]_{out}K_d}{1 + 2\frac{k_1}{k_4} + \frac{k_1}{k_3}}$$

$$= 1 + \frac{[I]_{out}}{K_{i2}}, \quad [23]$$

which defines K_{i2}. In addition,

$$V^{r,app}_{max} = \frac{V^r_{max}}{1 + \frac{[I]_{out}}{K_{i2}}}, \quad [24]$$

$$K^{app}_{m,in} = K_{m,in}\cdot\frac{1 + \frac{[I]_{out}}{K_{i1}}}{1 + \frac{[I]_{out}}{K_{i2}}}, \quad [25]$$

$$\alpha^{app} = \alpha\cdot\frac{1 + \frac{[I]_{out}}{K_{i1}}}{1 + \frac{[I]_{out}}{K_{i2}}}, \quad [26]$$

$$\frac{K_{i2}}{K_{i1}} = \frac{1 + 2\frac{k_1}{k_4} + \frac{k_1}{k_3}}{2}. \quad [27]$$

Because the elementary rate constants are unknown, the ratio K_{i2}/K_{i1} can have any value between 0.5 and infinity.

In the calculations, the following constants were used: $V^f_{max} = V^r_{max} = 167$ nmol·min^{-1}·mg protein^{-1} (from Table 1); $K_{m,out} = K_{m,in} = 1.2$ mM (from Table 1); $\alpha = 0.75$ (from ref. 6); and $K_{i1} = 21$ μM (from Fig. 2).

The flux control coefficients listed in Table 2 were calculated as follows. The oxygen consumption rate (expressed as a percentage of the uninhibited flux) was plotted against the glucose uptake rate, as derived above (expressed as a percentage of the uninhibited rate). The flux control coefficient was the slope of this plot at 100%. It was calculated by linear regression through the points, excluding the highest inhibitor concentration. An assumption underlying this method is that the transporter works at an internal pseudo-steady state at the time-scale of our transport measurements.

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