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Glucose affinity for the glucose transporter Glut1 in native or reconstituted lipid bilayers
Temperature-dependence study by biomembrane affinity chromatography

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Abstract

The affinity of D-glucose and the transport inhibitor cytochalasin B (CB) for the glucose transporter Glut1 was studied at 5–42°C by quantitative frontal affinity chromatography on sterically immobilized human red cell membrane vesicles, and on proteoliposomes containing reconstituted red cell membrane proteins. Glut1 in the vesicles showed the highest glucose affinity; the dissociation constant K_d(glc) was nearly constant (16±3 mM) from 15°C to 37°C. For Glut1 in proteoliposomes K_d(glc) decreased from 56 mM at 5°C to 26 mM at 42°C. The CB-Glut1 affinity was strongest around 20°C and was mostly higher with the vesicles, K_d(CB) being 49 nM at 19°C. The entropy and enthalpy changes for the interactions were calculated. © 1997 Elsevier Science B.V.

Keywords: Temperature effects; Cytochalasin B; Glut1; Glucose

1. Introduction

The affinities of D-glucose and cytochalasin B (CB) for the human red cell glucose transporter (Glut1), a transmembrane protein, have been determined by an array of biophysical techniques, for example, fluorescence quenching [1,2], centrifugation [3,4], vacuum filtration [5] and equilibrium dialysis [4,6,7]. Our research group has extended this array with quantitative zonal [8] and frontal [9,10] affinity chromatography on sterically immobilized membrane structures. We denote this method biomembrane affinity chromatography (BAC), which particularly in the frontal mode is a useful tool in studies of ligand interactions with Glut1 under various experimental conditions [9–11]. This protein retains its activity for months at room temperature in the immobilized state [9–11].

In the present work we studied the temperature dependence of glucose and CB interaction with Glut1 in red cell membrane vesicles stripped of peripheral proteins and with Glut1 reconstituted into egg phospholipid liposomes. The question addressed was whether the affinity of glucose and CB for Glut1 and the number of CB binding sites differ with temperature in a manner dependent on the lipid bilayer composition. The results were in line with previous findings for intact red cells [12,13] and for reconstituted Glut1 [2] and provide further evidence that BAC is well suited for this type of experiment.

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2. Experimental

2.1. Preparation of stationary phases

[4-^{3}H]CB was bought from NEN Life Science Products (Boston, MA, USA), dithioerythritol, CB, glucose, scintillation liquid and other materials were purchased from commercial sources as listed earlier [9,10] or as described below. Human red cell concentrate (5–6 weeks old), tested for absence of viral infection, was bought from the Blood Bank of the Uppsala University Hospital (Uppsala, Sweden). Red cell membrane vesicles were prepared and stripped of peripheral proteins by treatment at alkaline pH [9,14]; Glut1, other membrane proteins and lipids were solubilized from this material, isolated by centrifugation [15] and reconstituted with egg phospholipids to form proteoliposomes [15,16]. Stripped vesicles or proteoliposomes were sterically immobilized in Superdex 200 prep grade gel beads (Pharmacia Biotech, Uppsala, Sweden) as described in [8–10] with some modifications: The centrifugal washes after freeze–thaw immobilization were done in buffer A (150 m*M* NaCl, 10 m*M* Tris-HCl and 1 m*M* Na_2EDTA, pH 7.4 at 22°C) at 22°C for 5×3 min at 350 *g*. The amounts of immobilized phospholipids were determined by phosphorus analysis of gel suspension aliquots [9,17]. The gels were packed at 1.5 ml/min for 1 h in 0.5 cm (I.D.) glass columns (HR 5/2 or 5/5, Pharmacia Biotech) to obtain bed volumes of 0.58 ml (vesicles) and 0.87 ml (proteoliposomes). The immobilized material was stabilized during the first 3 days by running the columns for 8 h per day at 23°C at 1 ml/min.

2.2. Quantitative frontal affinity chromatography

Competitive interactions between glucose, CB and Glut1 were analyzed, starting on day 4 after immobilization [9]. The flow-rate 1.0 ml/min was used giving the same elution volumes as with 0.5 ml/min. Water from a temperature-controlled bath (Julabo F20, Julabo Labortechnik, Seelbach, Germany) was passed through a Plexiglass mantle around the Superloop (Pharmacia Biotech) and through channels in close-fitting copper jackets surrounding the inlet tubing and the analytical column. The temperature in the jacket was measured (accuracy of ±0.5°C) and was found to be the same in the gel bed. The eluent was buffer A (pH 7.4 at each temperature, I=0.15–0.16) supplemented with 1 m*M* dithioerythritol and different concentrations of glucose [9]. Before the runs the columns were kept at the chosen temperature for 60 min. The determination of the glucose and CB dissociation constants, K_d(glc) and K_d(CB), at a given temperature comprised two series of 4–6 runs each [9,10] on each of the two columns over a period of 12–14 h. The temperature did not affect the shape of the fronts, which were similar to those illustrated in [9]. For simplicity, the way of data evaluation in [9] was applied. This way was adapted from [18]. Another approach is described in [19] and gave the same results for the interactions studied [10]. The linear plots corresponding to those in Figure 1 in [9] showed correlation coefficients r^2 above 0.993. The changes in solute affinities with temperature were reversible in the range 5–37°C. This was verified by analyses at room temperature before and after analyses at other temperatures, including 5°C and 37°C (see the legend to Fig. 1). Runs below 5°C failed due to eluent leakage over the O-ring of the Superloop piston. The thermodynamic properties of the interactions with Glut1 was evaluated by using standard thermodynamic relationships: $\Delta G^o = -R\mathrm{T} \ln K$ and $\Delta G^o = \Delta H^o - T\Delta S^o$, similarly as described in [2].

3. Results

The Glut1 affinities for glucose and CB varied with the temperature as shown in Fig. 1. K_d(glc) decreased steeply from 53 m*M* at 5°C to 20 m*M* at 15°C and then gently to 12 m*M* at 42°C for Glut1 in membrane vesicles (Fig. 1A). The corresponding decrease for Glut1 in proteoliposomes was essentially linearly from 56 m*M* at 5°C to 26 m*M* at 42°C, i.e., the affinity increased with increasing temperature and was highest with the vesicles. The temperature dependence of the affinity of CB for Glut1 in membrane vesicles differed from that of the glucose affinity. The lowest K_d(CB) values were obtained at 15–23°C (average 50±2 n*M*, n=5). For the reconstituted Glut1, the CB affinity was lower than with the vesicles below 25°C but equal or higher above this temperature. The binding to Glut1 seems to involve

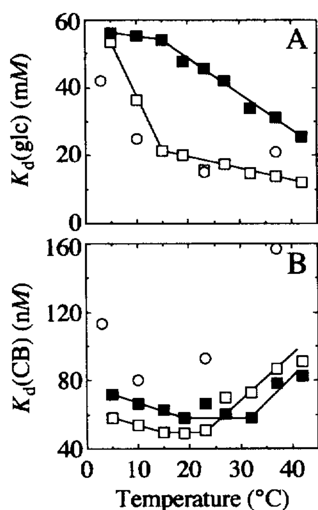

Fig. 1. The temperature dependence of (A) K_d(glc) and (B) K_d(CB). □, Glut1 in membrane vesicles (present work); ■, Glut1 in proteoliposomes (present work); O, Glut1 in intact red cells (values from fluorescence quenching experiments done by May [12]). Our values (□,■) at 23°C are averages of three series of analyses giving standard errors of ±5 m*M* (A) and ±5 n*M* (B). All series (□,■) at other temperatures were run once or twice, with error limits estimated to be as at 23°C. Our series of runs (□,■) were done at 23, 15, 32, 15, 5, 23, 37, 23, 27, 10, 19 and 42°C, in that order.

both enthalpy and entropy effects changing over the studied temperature range as revealed by calculations based on plots of $-RT \ln K$ versus T (Fig. 2). The ΔH and ΔS values for the linear parts of the graphs in Fig. 2 are given in Table 1.

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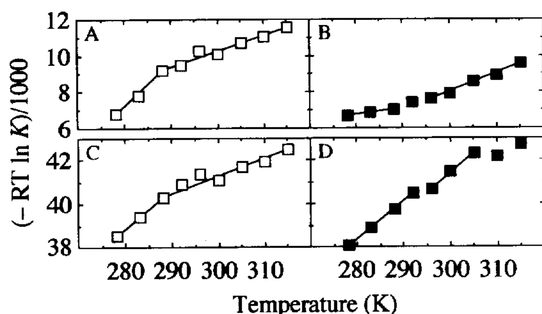

Fig. 2. Thermodynamic evaluations of temperature dependence on the affinities of glucose for Glut1 in (A) membrane vesicles, and (B) proteoliposomes, and of CB for Glut1 in (C) membrane vesicles, and (D) proteoliposomes.

CB-binding sites [9,10] did not vary significantly with the temperature up to 37°C for the proteoliposome column (Fig. 3), but increased slightly for the vesicle column. This indicates that essentially no protein was lost, hidden or inactivated in terms of CB-binding at the different temperatures, except that the number of sites became slightly lower at 42°C.

The nonspecific interaction of CB with the endogenous lipid bilayer (5 μmol phospholipid/ml gel bed) decreased almost linearly from 3.6 ml at 5°C to 2.0 ml at 42°C, whereas the corresponding interaction with the egg phospholipid bilayer (50 μmol phospholipid/ml gel bed) was nonlinear with a minimum at 23°C (Fig. 4).

4. Discussion

Studies of the interactions between glucose, CB and Glut1 by fluorescence quenching have revealed a nonlinear temperature dependence [2,12,13,20]. These observations were confirmed and extended by BAC analyses (Fig. 1). For example, the affinities to Glut1 in the membrane vesicles varied with the temperature as reported for Glut1 in intact human red cells [12]. Our results in the reconstituted system with egg phospholipids differed from the above findings and the affinities were mostly weaker than with the vesicles. The temperature dependence of the affinities may be related to structure and conformational state of Glut1 [12] or lipid bilayer phase transitions [7]. Also, glucose transport in human red cells is dependent on temperature and perhaps lipid bilayer state [21,22] although no clear effects of pressure have been found [23]. For the red cell membrane lipid bilayer transition temperatures have been found at 16°C [24], 23°C [23,25] and at 33 and 38°C [26]. Membrane proteins have been suggested to cluster and loose activity upon lipid bilayer transition to gel-like state [27]. For the membrane vesicles we found a change in CB affinity at 15°C and at 23°C and a change in glucose affinity at 15°C, which may be related to bilayer phase transitions.

The enthalpy and entropy changes for glucose and CB dissociation from Glut1 differ between the temperature ranges studied, as shown in Fig. 2 and Table 1. Walmsley and Lowe [20] reported that the enthalpy and entropy changes for glucose dissocia-

Table 1
Calculated enthalpy and entropy changes in chosen parts of the graphs in Fig. 2

Interaction	Temperature range (°C)	Enthalpy ΔH (kJ mol^{-1})	Entropy ΔS (J mol^{-1} K^{-1})
Glucose–MG1[a]	5–15	−61	244
	15–42	−15	84
Glucose–RG1[b]	5–15	−3	33
	23–42	−22	102
CB–MG1	5–15	−10	175
	15–42	20	71
CB–RG1	5–19	−10	172
	23–32	−12	177

[a] MG1, Glut1 in membrane vesicles.
[b] RG1, reconstituted Glut1.

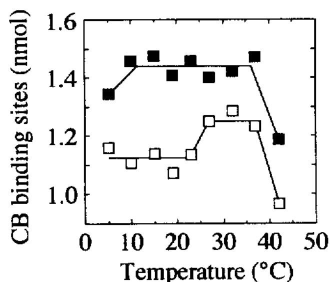

Fig. 3. The amount of CB-binding sites, determined by the use of Equation 2 in [9]. □, Glut1 in membrane vesicles, ■, Glut1 in proteoliposomes. From day 4 to day 48 after immobilization (5–37°C) 10% of the CB-binding sites were lost in the vesicle column, consistent with earlier results [9,10], whereas the loss of protein from the proteoliposome column was 20%. Correction of the decrease in the amount of CB-binding sites owing to this loss was done by repeating the V_{max} runs [9,10] and making the assumption that K_d values were constant for each temperature over the time period over which the columns were used. The error limits were estimated to be ±0.05 nmol.

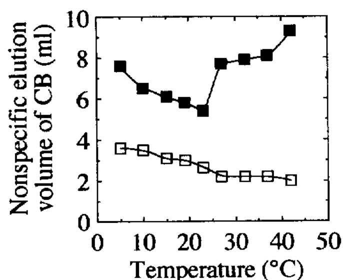

Fig. 4. The nonspecific interaction of CB (essentially partitioning into lipid bilayers) represented by the CB elution volume obtained by extrapolation to infinite glucose concentration [9]. □, Glut1 in membrane vesicles; ■, Glut1 in proteoliposomes. The error limits were estimated to be ±0.2 ml.

tion from Glut1 in red cells differs between the outside binding site (ΔH, 6 kJ mol^{-1} and ΔS, 17 J mol^{-1} K^{-1}) and the inside binding site (ΔH, −5 kJ mol^{-1} and ΔS, 58 J mol^{-1} K^{-1}) as determined in the range 0–50°C. The enthalpy and entropy changes for the CB dissociation from Glut1 reconstituted in endogenous lipids [2], were calculated to be −52 kJ mol^{-1} and −46 J mol^{-1} K^{-1}, respectively, in the range 15–30°C.

The amount of CB binding sites of Glut1, which are equivalent with inward-facing glucose binding sites, was nearly constant up to 37°C, inconsistent with the proposed decrease with increasing temperature in the proportion of these sites compared to the outward-facing glucose binding sites [13,28]. This deserves further investigation as does the fact that the dissociation constants in this work are somewhat lower than those we have reported previously [9,10], probably owing to differences in raw material (red cell concentrate) and improvements in stationary phase preparations.

Even the interaction between CB and the lipid bilayers was temperature-dependent, but in entirely different ways for the vesicles and the proteoliposomes, presumably reflecting different lipid compositions and different types of phase transitions. In general, hydrophobic interaction between amphiphilic surfaces like lipid bilayers tends to increase with increasing temperature [29], since more hydrophobic parts of the phospholipids become exposed, but this phenomenon explains only part of the observed effects.

The present work further illustrates the applicability of BAC. Quantitative chromatographic interaction

analysis of immobilized cells or subcellular particles has to our knowledge hitherto been limited [9–11,30,31]. We are confident that this field will expand and that the application areas will be extended shortly.

Acknowledgments

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