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Chromatography on cells: analyses of solute interactions with the glucose transporter Glut1 in human red cells adsorbed on lectin-gel beads

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Abstract

The affinities of the human red cell glucose transporter Glut1 for D-glucose and cytochalasin B (CB) and the stoichiometry of CB binding vary with the Glut1 environment. In order to study the native state of Glut1 we adsorbed human red cells to wheat germ lectin agarose gel beads for frontal affinity chromatographic analyses. Glut1 showed relatively high affinities for D-glucose (K_d 12±1 m*M*) and CB (K_d 59±17 n*M*). The number of CB-binding sites per Glut1 monomer, 0.46±0.16, was approximately doubled upon coating the cells with polylysine, which induced cell association.

Keywords: Glut1; Glucose; Cytochalasin B

1. Introduction

Quantitative analyses of biospecific interactions [1] provide the basis for detailed evaluation of properties of biological macromolecules and cellular functions. The use of chromatographic procedures for analysis of cells and biomolecular assemblies is an approach of considerable potential [2]. We have determined how the affinities of the human red cell glucose transporter Glut1 [3] for D-glucose and the inhibitor cytochalasin B (CB) [4] are affected by the environment of the protein in different kinds of membranes by use of immobilized biomembrane affinity chromatography on cytoskeleton-depleted membrane vesicles or proteoliposomes entrapped in gel beads by freeze-thawing [5–11] and on human red cells adsorbed to *N*-allyldimethylamine-derivatized polymethacrylamide particles [12]. Such data may be useful also in the context of membrane protein crystallization [13].

We have now developed a mild way to immobilize red cells on wheat germ lectin- (WGL-) derivatized agarose gel beads. WGL is also known as wheat germ agglutinin (WGA) and is a dimer of M_r–18,000 subunits [14–16] which binds to *N*-acetylglucosamine and to *N*-acetylneuraminic acid, e.g., on the glycophorin A of red cells [17]. Solubilized Glut1 has been reported to be partially adsorbed to *Ricinus communis* agglutinin [18] and to WGL-agarose gel [19], but the abundance of binding sites on glycophorin presumably results in binding of the lectin to only a small number of the Glut1 copies in the membrane, so that any effects on the Glut1

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ligand affinities and state of association become immeasurably small.

The main advantages of the new procedure compared to the previous one [12] are (*i*) that the lectin-mediated immobilization left the red cells intact, whereas the electrostatic adsorption used in the previous work caused hemolysis of a large proportion of the cells, in both cases as judged by transport retention chromatography (TRC) [12,20], and (*ii*) that the number of binding sites of CB per Glut1 monomer, *r*, could be determined for the lectin-bound cells, since sufficiently accurate binding data were obtained, and since the number of Glut1 copies per cell in [21] has been confirmed.

2. Materials and methods

2.1. Materials

Fresh human red cell concentrate (A Rh+, in SAGMAN solution) was bought from the Blood Bank of the University Hospital, Uppsala; WGL-agarose gel (Sepharose 4B derivatized with 1.96 mg WGL per milliliter of the beaded agarose) and HR 10/2 columns (I.D., 10 mm) from Amersham-Pharmacia Biotech (Uppsala, Sweden); L-[1-^{3}H(N)]-glucose and D-[^{14}C(U)]-glucose from NEN Life Sciences (Boston, MA, USA); [4(n)-^{3}H]-CB from NEN Life Sciences or Amersham (Buckinghamshire, UK); and CB and poly-L-lysine hydrobromide (M_r 150 000–300 000 according to viscosity measurements) from Sigma (St. Louis, MO, USA).

The buffer solution A, 139 m*M* NaCl, 5 m*M* KCl, 50 m*M* mannitol, 3 m*M* NaN_3, and 10 m*M* sodium phosphate buffer, pH 7.4, (I=0.17) was always used unless otherwise stated. All solutions were filtered (0.2 μm cellulose acetate filter) and degassed.

2.2. Preparation of red cell columns

The original frits of the HR 10/2 column endpieces were replaced by thin stainless steel grids (10×30 μm pores). Washed WGL-agarose gel was packed at 2 ml/min to form 0.6–1.3 ml-gel beds in the modified columns. Washed and suspended red cells were sucked into the bed by use of a peristaltic pump (4 min, 0.15 ml/min). The loaded column was incubated for at least 30 min (corresponding to steady-state binding of soluble WGL to suspended cells within 30 min at room temperature [17]). The loading and incubation procedures were repeated 4 times to saturate the bed with cells. After a final incubation of at least 90 min the bed was washed overnight at 0.3 ml/min. The immobilization and all experiments were conducted at 23°C. A flow-rate of 0.1 ml/min (TRC) or 0.3 ml/min (frontal affinity chromatography) was maintained until all analyses were finished.

2.3. TRC

The chromatographic set-up was as shown in Fig. 1 in [8]. An on-line flow-scintillation detector (Radiomatic FLO-ONE Beta A-300 or 525TR), an HPLC pump (2248, Pharmacia Biotech), and a 20 μl sample-loop were used. The samples were prepared by mixing 30 μl of D-[^{14}C]glucose (3.7 MBq/ml) with 3 μl of L-[^{3}H]glucose (37 MBq/ml) and diluting the mixture to 300 μl. The elution volume difference, ΔV_e, between the normalized [^{14}C]D-glucose and [^{3}H]L-glucose elution peaks was determined at half the peak heights. ΔV_e corresponds to the retardation of D-glucose (which is transported through the cells and therefore is eluted at a volume including the aqueous volume of the cells) relative to L-glucose (which does not enter the cells). The detection windows were set at 0–12 keV for ^{3}H detection and at 20–160 keV for ^{14}C detection. Correction for cross-over of ^{14}C counts into the ^{3}H channel was done after calibration with a sample that contained only D-[^{14}C]glucose. Three single TRC analyses were done (Fig. 2, below) on three columns prepared from a cell batch originating from a single blood donor.

The binding affinity of Glut1 for D-glucose does not effect the TRC results, since, according to [22], the specific retention volume is

$$V_{spec} = \frac{N}{K_{d(Glc)} + [Glc]} \quad (1)$$

where [Glc] is the D-glucose concentration. In a typical case of N=400 pmol binding sites, a $K_{d(Glc)}$

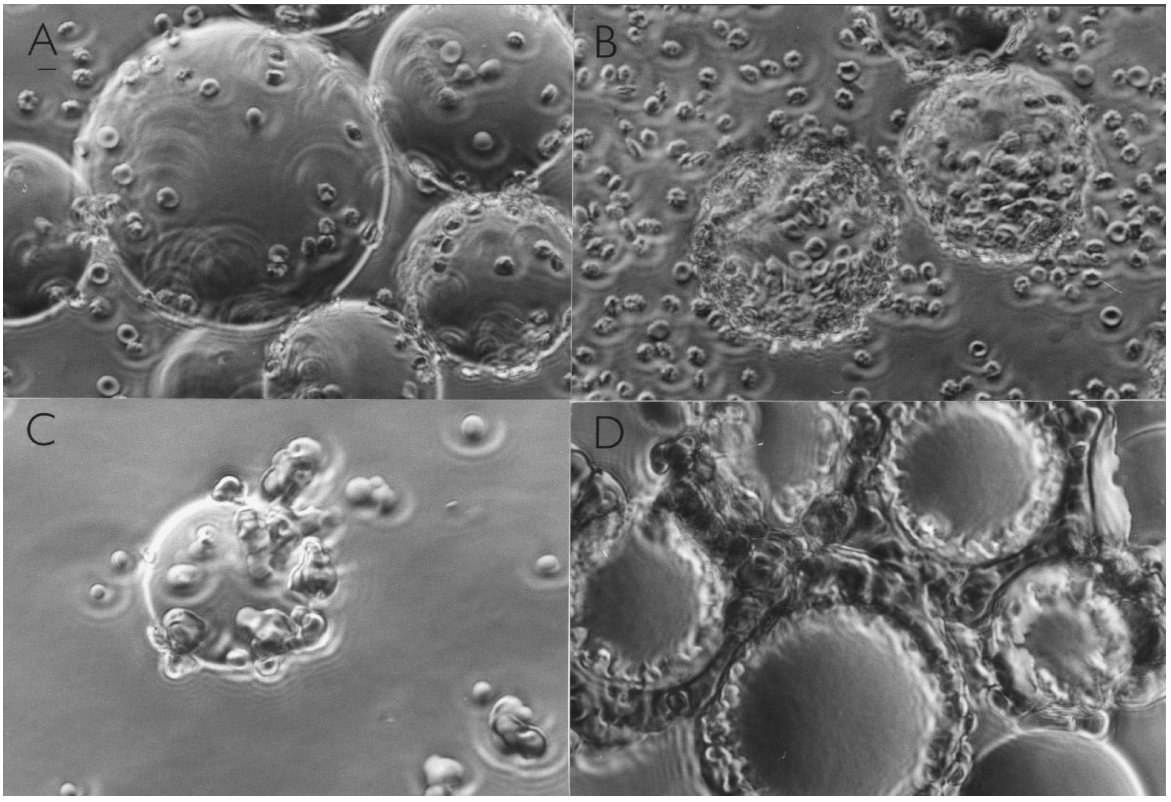

Fig. 1. Light microscopy of human red cells mixed with (A and B) WGA-Sepharose gel beads, and (C and D) with WGA-Sepharose gel beads and polylysine to a final concentration of 0.02 mg/ml. The cell concentration was higher in the samples used for (A and C) than in those used for (B and D). Before microscopy the mixtures were incubated for 30 min at 23°C. Then the sedimented beads were gently resuspended, which released a large number of the bound cells. Each panel shows a 160 μm×210 μm area.

of 12 m*M* and a [Glc] in the micromolar range, V_{spec} only amounts to 0.03 μl.

2.4. Frontal quantitative affinity chromatography

The red cell Glut1 affinities for CB and D-glucose and the *r* values (defined in the Introductory section) were determined by use of frontal affinity chromatography essentially as in [7]. Originally, the procedures were developed for non-membrane components as described in [23]. We ran series of 1 n*M* large-volume [^{3}H]CB samples with 0–50 m*M* concentrations of D-glucose (see below) or 0–100 n*M* concentrations of CB. The experimental set-up was as for TRC, except that large-volume samples were applied by use of a 50 ml Superloop (Amersham Pharmacia Biotech) as in [8]. The frontal elution volumes were measured at half of the plateau height. The dissociation constant for D-glucose binding to Glut1, $K_{d(Glc)}$, was calculated by linear regression analysis of a double reciprocal plot as described in [7], whereas the dissociation constant for CB, $K_{d(CB),}$ was determined by non-linear regression analysis [5,24] (SigmaPlot, Jandel, Erkrath, Germany) according to the equation

$$B = \frac{N[\mathrm{CB}]}{[\mathrm{CB}] + K_{d(CB)}} \quad (2)$$

where *B* is the amount of CB bound to Glut1 at the concentration [CB] of free CB, and *N* is the amount of CB binding sites. *B* equals the product of [CB] and the difference between the elution volume at [CB] and the elution volume for nonspecific inter-

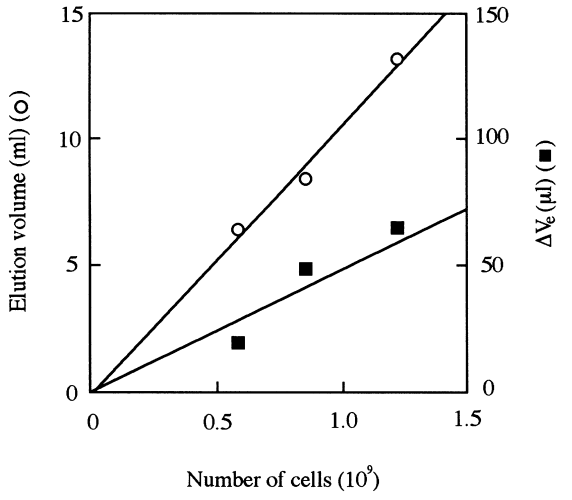

Fig. 2. The frontal affinity chromatographic elution volume of 1 n*M* [^{3}H]CB (○), and the difference ΔV_e between the transport retention chromatography (TRC) elution volumes of D- and L-glucose (■) versus the number of cells in three beds of WGL-agarose gel saturated with human red cells (see text). The TRC line was drawn to begin in origo.

action (V_{min}) alone. To determine V_{min} buffer solution A was supplemented with 7.5, 15, 30 or 50 m*M* D-glucose with corresponding decreases in the mannitol concentration to maintain the isoosmolarity and the data were evaluated by extrapolation in a double reciprocal plot as in [7]. In all, three red cell columns, and three red cell columns with polylysine coating (see section 2.7.), were prepared from two separate blood batches originating from two donors. The K_d and r values below are given with S.E.M. ($n=3$).

2.5. Calculation of the number of Glut1 copies per cell

In [25] the amount of membrane protein per red cell ghost was reported to be 0.74 pg, corresponding to 0.69 pg membrane polypeptide after subtraction of the mass fraction of the oligosaccharides (7.6%) [26]. A value of 1.04 pg dry membrane per ghost in [27] corresponds to 0.51 pg membrane polypeptide per ghost, since the polypeptide fraction of the ghost membrane is 49% by weight [27,28]. The average, 0.60 ± 0.09 pg membrane polypeptide per cell, is consistent with the value 0.60 ± 0.04 pg used in [29]. Since the ratio of integral membrane polypeptide to total membrane polypeptide is $64\pm4\%$ [30] and the Glut1 polypeptide fraction of the red cell integral membrane polypeptides is $12.1\pm2.5\%$ by weight [11], there is 0.046 pg Glut1 per cell, corresponding to $(5.1\pm2.1)\times10^5$ Glut1 copies per cell (or ghost) with a Glut1 M_r of 54,117 (SWISS PROT).

2.6. Determination of the number of immobilized red cells

To determine the number of immobilized cells per gel bed volume, three cell-containing gel beds of different volumes were prepared. On the next day a frontal run of 1 n*M* [^{3}H]CB and TRC of D- and L-glucose were carried out followed by lysis and elution of the cell material with water (Fig. 2). The polypeptide contents of the eluates were determined by automated amino acid analysis. Based on the concentrations of 10 different amino acids and the amino acid composition of hemoglobin (SWISS PROT) the hemoglobin contents of the eluates were calculated. The presence of other proteins was neglected. The hemoglobin amount per cell (in the batches used for determination of r values) was determined by cell counting in a Bürker chamber and amino acid analysis of a cell aliquot to be 31.9 ± 1.5 pg (S.E.M.; $n=2$, two donors), in good agreement with the value of 32 ± 2 pg calculated from data in [31]. The number of immobilized cells was calculated from the amount of hemoglobin in the column and the amount of hemoglobin per cell.

The cells in each gel bed used for frontal affinity chromatography were lysed after the experiments in the same way as were those in the above calibration gel beds. The average of the numbers of immobilized cells calculated on the basis of (*i*) the gel bed volume (section 3.2.) and (*ii*) the hemoglobin value measured after the experiments was used to calculate the r value.

2.7. Red cell column stability

The stability of the WGL-agarose columns containing immobilized cells was monitored by means of daily runs of 1 n*M* [^{3}H]CB over about 10 days. Attempts were made to increase the column stability by use of a nutrient buffer B consisting of buffer

solution A in which the mannitol was exchanged for 1 m*M* adenine, 10 m*M* glutamine, 3 m*M* inosine, 3 m*M* pyruvate, and 40 m*M* fructose, by use of buffer solution A supplemented with 1 m*M* ATP, or by fixation of the immobilized cells with polylysine (5 mg polylysine hydrobromide in 200 ml buffer solution A, applied at 0.15 ml/min for 2 h).

3. Results and discussion

3.1. Red cell binding to WGL-agarose gel

A fraction of the red cells mixed with WGL-agarose became adsorbed onto the surface of the beads, as illustrated in Fig. 1A and B. However, the interaction was weak; even gentle washing of the beads released cells. The situation was presumably similar in the gel beds saturated with cells. The biconcave disc shape was retained to a large extent, and transformation to elliptocytes, which occurs at a WGA concentration of 100–200 μg/μl in solution [32], was not observed. The presence of polylysine (extended polymer length approximately 0.2–0.4 μm) induced the formation of clusters of cells of undefined shape on the gel bead surfaces and in suspension (Fig. 1C and D), but the immobilization stability did not improve.

3.2. Red cell column properties and TRC

The number of red cells adsorbed to the WGL-agarose gel bed, as determined by amino acid analysis of the hemoglobin content, was proportional to the gel bed volume (0.64 ml, 0.90 ml and 1.30 ml; data not shown) with a linear correlation factor of $R^2 = 0.999$. The slope corresponded to 0.97×10^9 cells per ml gel bed, a cell density approximately 15% lower than in [12].

The frontal elution volume of 1 n*M* CB was proportional to the number of immobilized cells (Fig. 2). TRC showed a retardation of D-glucose relative to L-glucose, ΔV_e, of 48±5 μl (estimated error) per 10^9 cells (slope of the corresponding line in Fig. 2), e.g., 48±5 fl/cell, in good agreement with the total volume of a biconcave-disc-shaped red cell of 90±9 fl [31], which corresponds to 50±8 fl non-hemoglobin volume per cell as calculated from data in [31]. The separation was completely suppressed when 2 μ*M* CB was included in the eluent. TRC on polylysine-coated red cells resulted in a ΔV_e equal to 0, which was probably due to leakiness of the cell clusters or possibly to an inactivation of the glucose transport.

3.3. Glut1 interaction with D-glucose and CB

Non-linear regression analyses of the frontal chromatographic data according to Eq. (2) gave good agreement between the experimental data points and the regression curves, as exemplified in Fig. 3. The *r* values, $K_{d(CB)}$, and $K_{d(Glc)}$ for Glut1 in human red cells (either native or polylysine-coated) are presented in Table 1, together with $K_{d(Glc)}$ and $K_{d(CB)}$ for Glut1 in a mixture of human red cells and ghosts [12]. It is difficult to judge whether the affinities for cells and ghosts adsorbed to cationic particles differ from those in the present work, due to missing margins of error in [12]. The affinity of Glut1 in

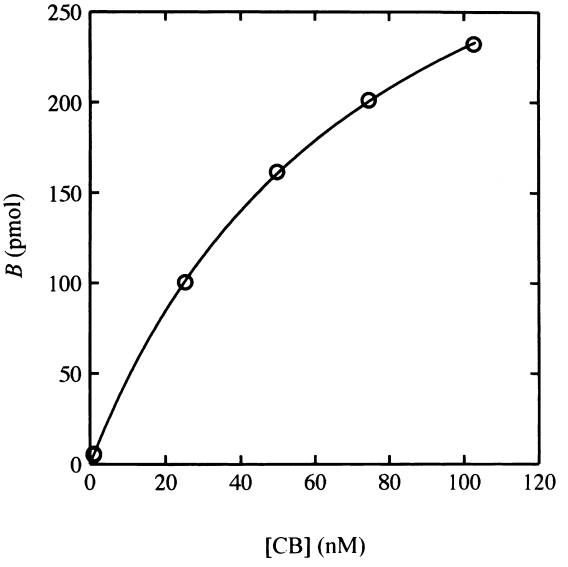

Fig. 3. Example of the non-linear regression analyses of the interaction between cytochalasin B (CB) and Glut1 in human red cells adsorbed to WGL-agarose gel. The amount of CB bound to Glut1, *B*, at the concentration [CB] of free CB was plotted against [CB]. The data were curve-fitted to Eq. (1) to obtain the number of binding sites and the dissociation constant, in this case, $N = 403 \pm 4$ pmol, and $K_{d(CB)}$ 75±2 n*M*. These error limits (S.E.) are those given by the curve-fitting program.

Table 1
Binding parameters for CB and D-glucose interaction with Glut1 in immobilized human red cells, as determined by frontal affinity chromatography

Material	CB-sites/Glut1 monomer (r value)	$K_{d(CB)}$ (nM)	$K_{d(Glc)}$ (mM)
Cells on WGL-gel[a]	0.47±0.16	59±17	12±1
Polylysine-coated cells on WGL-gel[a]	0.99±0.27	79±16	15±1
Cells/ghosts on cationic gel particles[b]	–	33	7

[a] Values±S.E.M.; $n=3$.
[b] Values from [12]; $n=1$.

human red cells for D-glucose (Table 1) was higher than for Glut1 in cytoskeleton-depleted membrane vesicles and in proteoliposomes [11].

In Fig. 4 the cell data are compared with previous data for cytoskeleton-depleted membrane vesicles and proteoliposomes [11] (see also Figure legend). It is illustrated that Glut1 in immobilized cells offered about 1 CB-binding site per 2 Glut1 monomers ($r=0.5$), in agreement with the suggestion by Hebert and Carruthers [33] that this association state reflects a native state of Glut1. On the other hand, polylysine-coated immobilized cells showed 1 binding site per Glut1 monomer ($r=1$). This may represent a transition that could occur also under physiological conditions. In interesting analogy, the removal of the cytoskeleton also doubled the r value, whereas the gentle change in the environment of the depleted vesicles upon entrapment in a dextran-grafted agarose gel matrix again lowered the value to 0.5 [11], as illustrated in Fig. 4. Therefore, the shift of the r value is reversible.

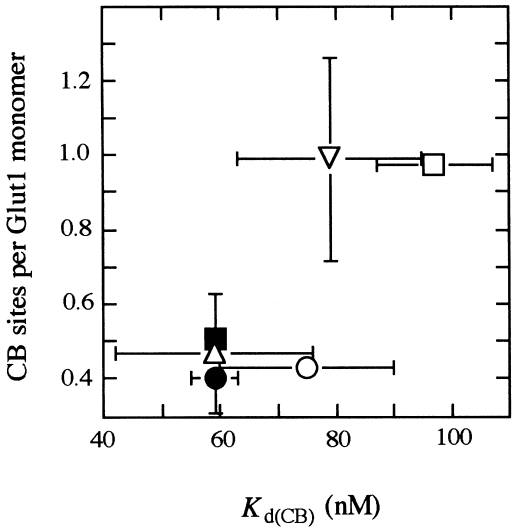

Fig. 4. The number of cytochalasin B (CB) binding sites per Glut1 monomer (the r value) for human red cells adsorbed to WGL-lectin agarose gel (△), and for human red cells adsorbed to WGL-lectin agarose gel and coated with polylysine (▽), versus the dissociation constant for Glut1 interaction with CB. For comparison the corresponding data from [11] are shown: (□), Free cytoskeleton-depleted human red cell membrane vesicles (Hummel and Dreyer chromatography [38]); (■), cytoskeleton-depleted human red cell membrane vesicles entrapped in gel beads by freeze-thawing; (○), free Glut1 proteoliposomes (Hummel and Dreyer chromatography and ultracentrifugation); (●), Glut1 proteoliposomes entrapped in gel beads by freeze-thawing. In the latter cases, Glut1 was reconstituted with the human red cell membrane lipids copurified with Glut1 to form the proteoliposomes.

The values for cells in Fig. 4 fit well with the earlier data for other materials, both with regards to r values and dissociation constants. The affinities for CB were equal among native cells, immobilized vesicles and immobilized proteoliposomes. Free vesicles showed lower affinity as determined by Hummel and Dreyer chromatography [34]. These affinities are probably correlated to the r values [11]. The K_d value for polylysine-coated cells did not differ significantly from the values of the low- and high-affinity materials owing to large error limits.

These observations imply that both the lipid environment and the presence of polymers, proteins or other macromolecules at the membrane surfaces may affect the function of integral membrane proteins.

3.4. Red cell column stability

In general the stability, homeostasis and function of cells depend on the pH, ionic strength, osmolarity, temperature, nutrients, solvent, and the presence of

hormones, proteins and enzymes. The requirements of red cells are relatively moderate, owing to their simple glycolytic metabolism. They are suited for chromatographic analysis due to their small size, and can be coupled to surfaces even by weak interactions, provided that many bonds can be formed [35].

In our experiments, lysis was largely avoided upon immobilization of the red cells on WGL-agarose gel, whereas the electrostatic coupling of cells to *N*-allyldimethylamine-derivatized particles [12] and to polylysine-covered polyacrylamide beads [36] caused release of the cell contents to various degrees. The cell binding to the lectin gel rather than the integrity of the cells seemed to govern the chromatographic stability, since the nutrient buffer B, which contained moderate amounts of inosine, glutamine, pyruvate, fructose and adenine, did not improve the stability. The supplements used do improve the maintenance of red cell physiological properties during blood storage [37–40].

In daily frontal runs of 1 n*M* [^{3}H]CB the columns retained 80–100% of the initial elution volume over a period of one week (data not shown). Supplementation of the buffer A with ATP, or polylysine-coating of the cells did not improve the column stability. The stability of the cell immobilization was sensitive to pressure variations, e.g., those occurring upon connection of the column to the flow scintillation counter. The series of runs for frontal affinity chromatographic analyses were performed during the first two days after column preparation and caused, on the average, a decrease in the elution volume of 1 n*M* [^{3}H]CB to 87±3% (S.E.M., $n=5$) of the initial elution volume (or 82±3% of the initial specific elution volume). When a WGL column was reused after storage for one month the capacity for cell immobilization had decreased to 60% of the original value.

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References

[1] P. Lundahl, A. Lundqvist, E. Greijer (Eds.), Quantitative Analysis of Biospecific Interactions, Harwood Academic Publishers, Amsterdam, 1998.
[2] A. Lundqvist, P. Lundahl, J. Chromatogr. B 699 (1997) 209.
[3] M. Mueckler, Eur. J. Biochem. 219 (1994) 713.
[4] J.F. Griffin, A.L. Rampal, C.Y. Jung, Proc. Natl. Acad. Sci. USA 79 (1982) 3759.
[5] A. Lundqvist, E. Brekkan, L. Haneskog, Q. Yang, J. Miyake, P. Lundahl, in: P. Lundahl, A. Lundqvist, E. Greijer (Eds.), Quantitative Analysis of Biospecific Interactions, Harwood Academic Publishers, Amsterdam, 1998, p. 79.
[6] Q. Yang, P. Lundahl, Biochemistry 34 (1995) 7289.
[7] E. Brekkan, A. Lundqvist, P. Lundahl, Biochemistry 35 (1996) 12141.
[8] A. Lundqvist, E. Brekkan, C. Lagerquist, L. Haneskog, P. Lundahl, Mat. Sci. Eng. C 4 (1997) 221.
[9] L. Lu, A. Lundqvist, C.-M. Zeng, C. Lagerquist, P. Lundahl, J. Chromatogr. A 776 (1997) 81.
[10] A. Lundqvist, P. Lundahl, J. Chromatogr. A 776 (1997) 87.
[11] A. Lundqvist, C.-M. Zeng, C. Lagerquist Hägglund, E. Brekkan, S. Zuo, D. Eaker, and P. Lundahl in A. Lundqvist, Chromatographic Characterization of the Human Red Cell Glucose Transporter Glut1 in the Cells, in Membrane Vesicles and in Proteoliposomes, Thesis, Uppsala University, Uppsala, 1999.
[12] C.-M. Zeng, Y. Zhang, L. Lu, E. Brekkan, A. Lundqvist, P. Lundahl, Biochim. Biophys. Acta 1325 (1997) 91.
[13] C.H. Simons, A.B. Weinglass, S.A. Baldwin, Biochem. Soc. Trans. 25 (1997) 463S.
[14] R.H. Rice, E. Etzler, Biochemistry 14 (1975) 4093.
[15] Y. Nagata, M.M. Burger, J. Biol. Chem. 249 (1974) 3116.
[16] I.J. Goldstein, C.E. Hayes, Adv. Carbohydr. Chem. Biochem. 35 (1978) 127.
[17] W.L. Adair, S. Kornfeld, J. Biol. Chem. 249 (1974) 4696.
[18] F.R. Gorga, S.A. Baldwin, G.E. Lienhard, Biochem. Biophys. Res. Commun. 91 (1976) 955.
[19] G. Fröman, P. Lundahl, F. Acevedo, FEBS Lett. 129 (1981) 100.
[20] Q. Yang, M. Wallstén, P. Lundahl, Biochim. Biophys. Acta 938 (1988) 243.
[21] E. Mascher, P. Lundahl, Biochim. Biophys. Acta 945 (1988) 350.
[22] K.-i. Kasai, Y. Oda, M. Nishikata, S.-i. Ishii, J. Chromatogr. 376 (1986) 33.
[23] D.J. Winzor, in: P.D.G. Dean, W.S. Johnson, F.A. Middle (Eds.), Affinity Chromatography, a Practical Approach, IRL Press, Oxford, 1985, p. 149.

[24] L. Haneskog, C.-M. Zeng, A. Lundqvist, P. Lundahl, Biochim. Biophys. Acta 1371 (1998) 1.
[25] J.T. Dodge, C. Mitchell, D.J. Hanahan, Arch. Biochem. Biophys. 100 (1963) 119.
[26] S.A. Rosenberg, G. Guidotti, J. Biol. Chem. 243 (1968) 1985.
[27] P.J. Bjerrum, J. Membr. Biol. 48 (1979) 43.
[28] G. Guidotti, Annu. Rev. Biochem. 41 (1972) 804.
[29] P.E. Coderre, E.K. Cloherty, R.J. Zottola, A. Carruthers, Biochemistry 34 (1995) 9762.
[30] P. Lundahl, Biochim. Biophys. Acta 379 (1975) 304.
[31] L.R. Dixon, J. Perinat. Neonat. Nurs. 11 (1997) 1.
[32] R.E. Lovrien, R.A. Anderson, J. Cell Biol. 85 (1980) 534.
[33] D.N. Hebert, A. Carruthers, J. Biol. Chem. 267 (1992) 23829.
[34] P. Lundahl, C.-M. Zeng, C. Lagerquist Hägglund, I. Gottschalk, E. Greijer, J. Chromatogr. B 722 (1999) 103.
[35] A. Kobayashi, A. Koyama, M. Goto, K. Kobayashi, C.-W. Chang, K. Tomita, T. Akaike, Proc. Japan Acad. 69 (1993) 88.
[36] B.C. Jacobson, D. Branton, Science 195 (1977) 302.
[37] R.B. Dawson Jr., T.J. Ellis, Transfusion 10 (1970) 113.
[38] B. Deuticke, J. Duhm, R. Dierkesmann, Pflügers Archiv 326 (1971) 15.
[39] R.B. Dawson, Z. Levine, T. Zuck, R.T. Hershey, C. Myers, Transfusion 18 (1978) 347.
[40] U.J. Dumaswala, M.J. Wilson, T. José, D.L. Daleke, Blood 88 (1996) 697.