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Glucose and nucleoside transporters of human erythrocytes: effects of detergents on immunoadsorption of a membrane protein to its monoclonal antibody

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Immunoadsorption of membrane proteins solubilized in detergents has been used widely for identification, purification and quantitation of transporters and receptors. In an effort to separate the glucose and nucleoside nucleoside transporters of human erythrocytes (GT and NT, respectively) that copurify in a membrane protein fraction band 4.5, we examined in the present study the effects of seven different detergents on the immunoadsorption of GT to its monoclonal antibody, 65D4 (Craik, et al. (1988) Biochem. Cell Biol. 66, 839–852). The following results were obtained. (1) The maximum extent of the immunoadsorption of GT by 65D4 varied between 52 to 98% in different detergents. For non-ionic detergents, there was an apparent inverse correlation between the maximum immunoreactivity of GT and the aggregation number or micellar size of detergents. (2) The immunoprecipitate of GT by 65D4 was contaminated with nucleoside transporter to an extent that varied from 2 to 35 mol% in different detergents. There is an inverse correlation between the extent of the contamination and the detergent aggregation number. However, this contamination was quantitatively accounted for by a time-dependent, non-specific aggregation of NT with GT in detergents. (3) A high degree of purification of NT in band 4.5 by immunoadsorptive removal of GT with 65D4 was achieved in $C_{12}E_8$ as predicted by the observed low NT-GT aggregation and the relatively high epitope-accessibility of GT in this detergent. Based on these findings, we conclude that certain detergents can reduce the immunoreactivity of membrane proteins significantly by modulating epitope accessibility, and may also produce a false immuno-cross-reactivity by inducing nonspecific protein aggregation.

Introduction

Carrier-mediated transport of sugars and nucleosides across the plasma membrane of human erythrocytes involves specific membrane proteins (transporters) that catalyze translocation of permeant across an otherwise practically impermeable membrane diffusion barrier [1,2]. The glucose and nucleoside transporters co-purify in a membrane protein fraction (band 4.5) of M_r 45 000–60 000 [3–10], suggesting physical and chemical similarities between them [11]. Nevertheless, the two transporters can be identified by photolabeling with tight-binding inhibitors, [^{3}H]cytochalasin B and [^{3}H]nitrobenzylthioinosine (NBMPR), respectively [5,7,10,11]. In band 4.5 of human erythrocytes, more than 80% of the protein mass represents glucose transporter, while < 3% is the nucleoside transporter [11].

Monoclonal antibody 65D4 was raised against band 4.5 of human erythrocytes [12]. 65D4 recognizes the glucose transporter of human erythrocytes and mouse and pig neonatal erythrocytes. 65D4 did not recognize the nucleoside transporter of adult and neonatal pig erythrocytes [12]. When immunoprecipitation of band 4.5 of human erythrocytes was assessed in Triton X-100, however, 65D4 precipitated not only [^{3}H]cytochalasin-B-labeled polypeptides but also small amounts of

Abbreviations: NBMPR (nitrobenzylthioinosine), 6-[(4-nitrobenzyl) thio]-9-(β-D-ribofuranosyl)purine; $C_{12}E_8$, octaethyleneglycoldodecyl ether; SDS, sodium dodecyl sulphate.

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[^{3}H]NBMPR-labeled polypeptides [12]. This apparent cross-reactivity raised the possibility of common antigenic determinants, thus structural similarity between the two transporters. However, it is also possible that this apparent cross-reactivity is an artifact, as membrane proteins in detergents form complex protein-detergent mixed micelles [13], which may modify protein activities, including immunoreactivity to antibodies. This important subject has not been systematically addressed to in literature.

In the present work, we studied the effects of several commonly used detergents on the immunoreactivity of the human erythrocyte glucose transporter to 65D4. Glucose and nucleotide transporters were covalently labeled with [^{3}H]cytochalasin B or [^{3}H]NBMPR, respectively. Immunoadsorbed polypeptides were separately identified by TSK gel-filtration chromatography, and the effects of various detergents on the ability of 65D4 to interact with two transporters were quantitated. We demonstrate that detergents induce nonspecific aggregation of two transporters in varying extents, producing a false cross-reactivity of the monoclonal antibody with nucleoside transporter. Our results also demonstrate that certain detergents significantly reduce the immunoreactivity of the glucose transporter, and this reduction is inversely proportional to the protein-detergent micellar size in non-ionic detergents.

Experimental procedures

Materials. Cytochalasin B and [^{3}H]cytochalasin B were from Sigma (St. Louis, MO). NBMPR and [^{3}H]NBMPR were from Moravek Biochemicals (Brea, CA). Octyl glucoside, Triton X-100, $C_{12}E_8$, SDS, dodecyl maltoside, and sodium deoxycholate were from Calbiochem (San Diego, CA).

Preparation of band 4.5 polypeptides. Band 4.5 polypeptides were prepared from octyl-glucoside-solubilized, white ghosts of human erythrocytes of DEAE-cellulose chromatography, followed by removal of detergent by dilution (by 2–3-fold) and centrifugation (180 000 × *g* for 60 min) exactly as described previously [10].

Photoaffinity labeling of band 4.5 polypeptides. Band 4.5 preparations (100 to 200 μg) were suspended in 1 ml Tris buffer (50 mM Tris-HCl (pH 7.4); hereafter termed T50 buffer) containing 50 mM dithiothreitol and either 1 μM NBMPR or 0.1 μM cytochalasin B plus 10 μM cytochalasin E in the presence of the corresponding radioactive tracer (1.2 μCi [^{3}H]NBMPR; 0.6 μCi [^{3}H]-cytochalasin B). The resulting mixtures were irradiated [11] at a distance of 6.5 cm at ice temperatures for 60 s with a 450 W mercury arc lamp (Canrad-Hanovia, Newark, NJ) through a quartz water-jacket. The irradiated suspensions were immediately diluted with 20 vol. of T50 buffer containing either 20 μM nitrobenylthioguanosine (for [^{3}H]NBMPR labeling) or 10 μM cytochalasin B (for [^{3}H]cytochalasin B labeling) and centrifuged (120 000 × *g*, 45 min, 4°C). The resulting pellets were resuspended in 20 ml T50 buffer and extensively washed (four successive cycles of centrifugation, resuspension and 1 min sonication), yielding labeled vesicles with 2–4% free NBMPR or 10–13% free cytochalasin B.

Immunoprecipitation. The origin and characteristics of murine monoclonal antibody 65D4 (IgG_{2B}), which recognizes the glucose transporter of human erythrocytes, have been described [12]. In this work, antibody 65D4 was purified from ascitic fluids obtained from hybridoma-bearing mice as described [12].

Band 4.5 preparations, photolabeled with [^{3}H]NBMPR or [^{3}H]cytochalasin B, were suspended in T50 buffer containing 1% of the detergent under study and the resulting mixtures were then centrifuged (185 000 × *g*, 15 min, 4°C) to remove any particulate materials. Aliquots (2 μg protein) of detergent-treated preparations were incubated (3 h, 4°C) with 0.2 ml T50 buffer that contained antibody 65D4 (80 μg), 1% bovine serum albumin and 1% of the detergent. A preparation of fixed *Staphylococcus aureus* cells (Pansorbin, Calbiochem, San Diego, CA) was added (150 μl, capable of binding 300 μg IgG), and the mixtures were incubated (1 h, 4°C) with stirring and then separated by centrifugation (10 000 × *g*, 5 min, 4°C) into supernatants and immunoprecipitates. Preliminary studies (not shown), in which ionic strength and incubation times were varied, demonstrated that maximal immunoprecipitation was obtained when detergent-treated band 4.5 preparations were incubated with antibody 65D4 for 3 h in T50 buffer. Where specified, the resulting immunoprecipitates were washed twice by centrifugation (10 000 × *g*, 5 min, 4°C) with T50 buffer containing 0.5% detergent.

For analysis of radioactivity, the immunoprecipitates were dissolved directly in 5 ml of scintillation fluid (Ecoscint, National Diagnostics, Manville, NJ) and analyzed for ^{3}H content. For gel-filtration chromatography, the immunoprecipitates were resuspended in T50 buffer containing 2% SDS to dissociate immune complexes, the Pansorbin was removed by centrifugation (10 000 × *g*, 5 min), and the resulting mixtures were further centrifuged (185 000 × *g*, 15 min) to obtain particulate-free samples.

Gel-filtration chromatography. Bio-Sil TSK 250 columns (Bio-Rad Laboratories, Richmond, CA) were used with medium-pressure liquid chromatographic system (Pharmacia, Piscataway, NJ) as described elsewhere [10].

Immunoaffinity column chromatography. Purified antibody 65D4 was coupled to cyanogen-bromide-activated Sepharose 4B (Sigma) as recommended by the manufacturer. The 65D4-Sepharose was equilibrated with 1% $C_{12}E_8$ in T50 buffer and stored at 4°C. Band 4.5 preparations were solubilized (1% $C_{12}E_8$ in T50 buffer)

and after centrifugation (158000 × *g*, 15 min, 4°C) to remove particulate material, were incubated (6 h, 4°C) with 65D4-Sepharose. The resulting mixtures were passed through disposable polypropylene Econo-Columns (Bio-Rad Laboratories, Richmond, CA), the unbound material was collected, and, after washing columns once with 10 ml of 1% $C_{12}E_8$ in T50 buffer, the bound material was eluted with 10 ml of T50 buffer containing 1% SDS. The 65D4-Sepharose was regenerated by extensive washing with T50 buffer.

Miscellaneous procedures. Assays of equilibrium binding of cytochalasin B [14] and NBMPR [11] to band 4.5 polypeptides were performed as described previously. Protein was determined by the method of Lowry et al. [15].

Results

The effects of detergents on binding of the glucose and nucleoside transporters of human erythrocyte band 4.5 to antibody 65D4 were determined. The glucose and nucleoside transporters were photolabeled with [^{3}H]cytochalasin B or [^{3}H]NBMFR, respectively, prior to immunoprecipitation. Results of a typical set of the experiments is illustrated in Fig. 1. It is evident that the apparent reactivity of the antibody toward the labeled transporters differed with the detergent used during the immunoprecipitation assay. With octyl-glucoside-solubilized band 4.5, 86% and 30% of the radioactivities of [^{3}H]cytochalasin B and [^{3}H]NBMPR, respectively, were precipitated. TSK chromatography revealed that 10-

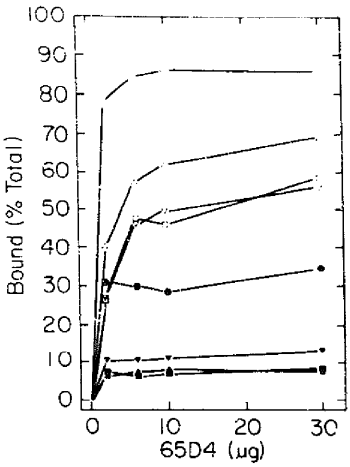

Fig. 1. Immunoprecipitation of band 4.5 polypeptides in various detergents as a function of the concentration of antibody 65D4. Band 4.5 polypeptides were photolabeled with [^{3}H]cytochalasin B (open symbols) or [^{3}H]NBMPR (closed symbols) and incubated with antibody 65D4 in the presence of 1% octyl glucoside (circles), $C_{12}E_8$ (triangles), Triton X-100 (squares) or sodium deoxycholate (inverted triangles) for 3 h prior to addition of Pansorbin. Results are expressed as the percentage of radioactivity associated with the immunoprecipitates relative to total radioactivity present in reaction mixtures. In this particular experiment, 13 and 3% of the radioactivity associated with band 4.5 was free [^{3}H]cytochalasin B and [^{3}H]NBMPR, respectively. Similar results were obtained when the immunoprecipitates were washed (see Methods), with the exception of octyl glucoside, for which the amount of precipitate-associated radioactivity associated was reduced by 50% for both ^{3}H-labeled ligands.

TABLE I

Maximum levels of immunoadsorption of the glucose and nucleoside transporters by antibody 65D4 in different detergents (1%)

Presented are the values (mean ± S.E.) for precipitation of [^{3}H]cytochalasin B-labeled and [^{3}H]NBMPR-labeled polypeptides of band 4.5 obtained from 3–5 separate experiments conducted as described in Fig. 1. Also presented are detergent aggregation numbers with their corresponding micellar molecular masses [16] and the relative amounts of protein present in fraction P0 of the TSK gel filtration column chromatography after incubation for 3 h in various detergents. Decyl glucoside and octyl thiogalactoside (1%) each gave cloud suspension at room temperature and micelles (crystals) came down by centrifugation at the centrifugal forces used in the immunoprecipitation procedure.

Detergent	Aggregation number	P0 fraction [a] (% of total)	Precipitation [b] (% of total)	
			cytochalasin B	NBMPR
Triton X-100	140 (90000) [c]	16 ± 3	58 ± 7	14 ± 2
Octyl thioglucoside		11 [d]	51 ± 4	13 ± 4
Octyl glucoside	84 (24561)	31 ± 6	98 ± 3	35 ± 6
Octyl maltoside		13 [d]	76 ± 8	12 ± 2
Decyl maltoside	98 (49980)	10 [d]	70 ± 5	18 ± 3
$C_{12}E_8$	123 (66300)	9 ± 2	85 ± 7	8 ± 2
Deoxycholate	7 (3000)	9 [d]	55 ± 5	10 ± 4
SDS	62 (18000)	5 ± 1	< 1	< 1

[a] Determined as described elsewhere [10,11] from the relative UV absorption at 254 nm of the P0 fraction after TSK gel filtration chromatography.
[b] Values are the percent of total protein-associated radioactivity precipitated and have been corrected for free (noncovalently associated) ligand.
[c] Molecular mass in Da.
[d] Single determination.

13% of the [^{3}H]cytochalasin B present in the reaction mixtures was free ligand in these experiments (not illustrated). Thus, the precipitation of [^{3}H]cytochalasin-B-labeled polypeptides was almost quantitative (> 95%). In contrast, with Triton X-100 solubilized band 4.5, only 58% and 10% of the labels of [^{3}H]cytochalasin B and [^{3}H]NBMPR, respectively, were precipitated, while less than 5% of the radioactivity was due to free ligand. With $C_{12}E_8$-solubilized preparations, a maximum of about 80% of [^{3}H]cytochalasin-B-labeled polypeptides (or 70% of total radioactivity) was precipitated by 65D4, and the precipitation of [^{3}H]NBMPR-labeled polypeptides was negligible. The results of similar experiments using eight commonly used detergents are summarized in Table I.

To determine the basis of the small but significant precipitation of [^{3}H]NBMPR-labeled polypeptides by antibody 65D4, band 4.5 photolabeled with either [^{3}H]cytochalasin B or [^{3}H]NBMPR was fractionated on a TSK gel-filtration column before and after immunoprecipitation in various detergents. It has been shown [11] that TSK gel-filtration chromatography separates band 4.5 of human erythrocytes into three ultraviolet-absorptive polypeptide fractions (P0, P1, P1a) that exhibit different photolabeling patterns with [^{3}H]cytochalasin B and [^{3}H]NBMPR. Cytochalasin B labeled a 55 kDa polypeptide (the glucose transporter p55GT) in P1 fraction, whereas NBMPR labeled a 50 kDa polypeptide (p50NT) in P1 and a 40 kDa polypeptide (p50NT) in P1a fraction, a truncated form of p50NT, the presumed intact nucleoside transporter [11]. The P0 fraction consists of the mixture of homo- and heterodimers of p55GT, p50NT and p40NT [11]. All of these labeling patterns were reproduced here (as control experiments in Figs. 2 and 3) using Triton X-100 and octyl glucoside. Three ultraviolet-absorptive polypeptide fractions P0, P1 and P1a were obtained and their peak positions are indicated. [^{3}H]Cytochalasin-B-labeled polypeptides were present in two fractions P0, P1 and [^{3}H]NBMPR-labeled polypeptides were present in fractions P0, P1 and P1a. The amount of radioactivity present in the P0 fraction was substantial for both ligands, especially in the octyl-glucoside-solubilized band 4.5. A fourth fraction (P2), which contains lipids [10], also contained radioactivity, apparently as lipid-bound [^{3}H]cytochalasin B or [^{3}H]NBMPR.

In the presence of Triton X-100 (Fig. 2A), immunoprecipitation by 65D4 reduced the amount of [^{3}H]cytochalasin B label in P0 (dimerized p55GT) and P1 (monomeric p55GT) by about 50%, which was quantitatively recovered in the P0 and P1 of the immunoprecipitate. In the presence of octyl glucoside (Fig. 3A), the same immunoprecipitation with antibody 65D4 removed virtually all (> 95%) of the radioactivity associated with the P0 and P1 fractions, most of which was recovered in P0 and P1 fractions of the immunoprecipitate. Little, if any, of the [^{3}H]cytochalasin B associated with the lipid (P2) fraction was affected by the immunoprecipitation, regardless of the detergent used.

The [^{3}H]NBMPR-labeled polypeptides of band 4.5 were affected differently in immunoprecipitation with antibody 65D4 (Figs. 2B, 3B). The amount of the radioactivity associated with P1 or P1a fractions (p50NT and p40NT, respectively) in Triton X-100 or octyl glucoside was unchanged, indicating that the [^{3}H]NBMPR-labeled polypeptides were not immunoabsorbed by antibody 65D4. Antibody treatment was also without effect on lipid-bound radioactivity in the P2 fraction. Only the P0 fraction, the aggregates of p55GT, p50NT and p40NT, was affected by immunoprecipitation. In Triton X-100, there was a 40% reduction in P0-associated radioactivity, most of-which was recovered in the P0 fraction of

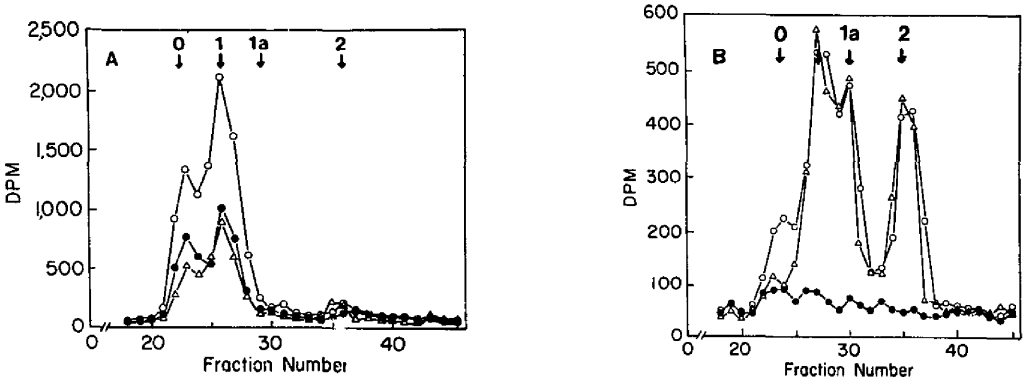

Fig. 2. Analysis of band 4.5 polypeptides by gel-filtration chromatography before and after immunoprecipitation with antibody 65D4 in 1% Triton X-100. Band 4.5 polypeptides were photolabeled with [^{3}H]cytochalasin B (panel A) or [^{3}H]NBMPR (panel B) and subjected to TSK gel-filtration chromatography without immunoprecipitation (open circles) or after immunoprecipitation (supernatant, closed circles; precipitate, open triangles). The positions of maximum ultraviolet absorption (at 254 nm) for fractions P0, P1, P1a and P2 are indicated by 0, 1, 1a and 2, respectively.

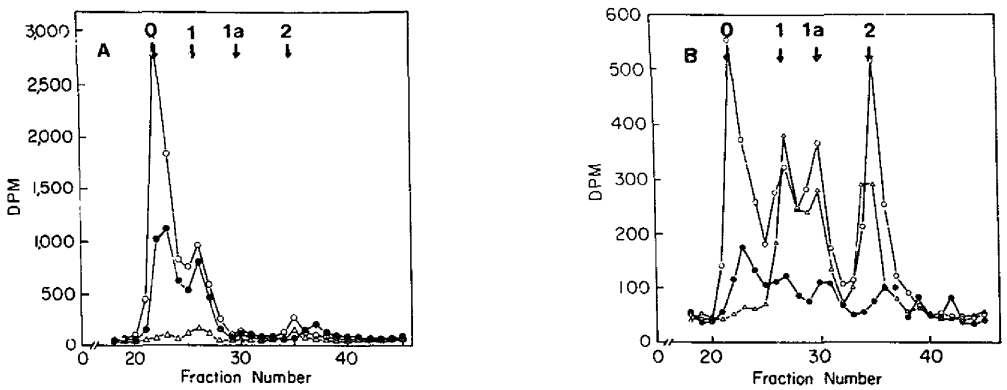

Fig. 3. Analysis of band 4.5 polypeptides by gel-filtration chromatography before and after immunoprecipitation with antibody 65D4 in 1% octyl glucoside. Band 4.5 polypeptides were photolabeled with [^{3}H]cytochalasin B (panel A) or [^{3}H]NBMPR (panel B) and, after enrichment by DEAE-chromatography, were subjected to TSK gel-filtration chromatography without immunoprecipitation (open circles) or after immunoprecipitation (supernatant, closed circles; precipitate, open triangles). The positions of maximum ultraviolet absorption (at 254 nm) are indicated as in Fig. 2.

the immunoprecipitate. In octyl glucoside, there was an 85% reduction in P0-associated radioactivity, 30% of which was recovered in the P0 fraction, with the rest being lost most likely during the washing steps prior to gel-filtration chromatography. It is evident that the precipitation of [^{3}H]NBMPR-labeled polypeptides by antibody 65D4 was due entirely to the reactivity of 65D4 to the p55GT aggregate with p50NT and p40NT in P0 fraction.

Significant purification of nucleoside transporter has been achieved by removing the glucose transporter from band 4.5 solubilized in octyl glucoside by immunoadsorption with polyclonal antibodies specific to glucose transporter [17]. Our results, summarized in Table I, suggest that a higher degree of the purification is expected with $C_{12}E_8$ as with octyl glucoside, particularly since the aggregation of the two transporters is minimal, and the immunoadsorption of the glucose transporter to 65D4 is also nearly stoichiometric in $C_{12}E_8$. In the experiment of Fig. 4, band 4.5 was solubilized in $C_{12}E_8$ and applied to a 65D4-Sepharose column. The [^{3}H]cytochalasin-B-labeled polypeptides (p55GT) present in the P0 and P1 fractions were largely removed (> 90%) by the 65D4-Sepharose column (Fig. 4A). By contrast, most (about 95%) of [^{3}H]NBMPR-labeled polypeptides (p50NT, p40NT) were recovered in the flow-through (Fig. 4B). When this experiment was performed in octyl glucoside (not illustrated), the removal

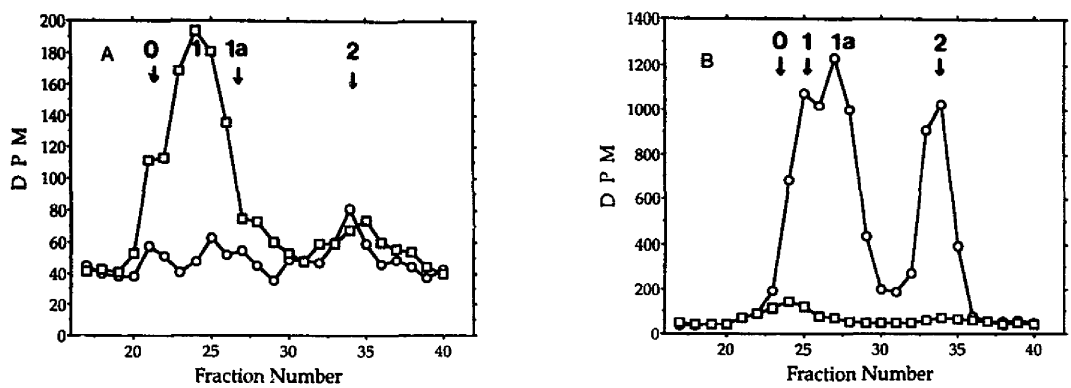

Fig. 4. Removal of glucose transporter polypeptides from band 4.5 by immunoadsorption with antibody 65D4 in 1% $C_{12}E_8$. Band 4.5 polypeptides photolabeled with [^{3}H]cytochalasin B (panel A) and [^{3}H]NBMPR (panel B) were subjected to immunoadsorption chromatography in 1% $C_{12}E_8$ using antibody 65D4-Sepharose. The flow through (open circles) and the immunoadsorbed materials (open squares) from the column were subjected to TSK gel filtration chromatography. The positions of maximum ultraviolet absorption (at 254 nm) are indicated as in Fig. 2.

of p55GT was highly effective, while the recovery of P50NT plus P40NT in the flow through was only about 70%.

In parallel experiments, immunoadsorption chromatography of unlabeled band 4.5 was conducted in $C_{12}E_8$ as described in Fig. 4. The flow through was concentrated, the detergent was removed and the resulting material was reconstituted into vesicles. Results of the equilibrium binding assay using [^{3}H]cytochalasin B and [^{3}H]NBMPR revealed that the reconstituted material bound NBMPR (with a K_d and B_t of 102 nM and 6–8 nmol/mg protein, respectively,) but no detectable amount of cytochalasin B (data not shown).

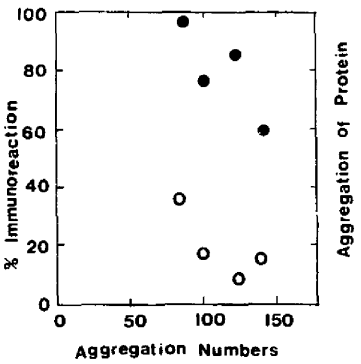

Fig. 5. Apparent dependencies of nonspecific protein aggregation and epitope accessibility of glucose transporter to 65D4 in detergents to detergent aggregation number. The data of four nonionic detergents (octyl glucoside, decyl maltoside, $C_{12}E_8$ and Triton X-100) in Table I were used. Protein aggregation (open circles) is estimated by the size of P0 fraction. Epitope accessibility (closed circles) represents the maximum level of immunoprecipitation of polypeptide labeled with cytochalasin B.

Discussion

Our results of the immunoprecipitation studies with a monoclonal antibody 65D4 illustrate that certain detergents can modify interaction of a membrane protein to its antibodies to a significant extent. Both stoichiometry and selectivity of the immunoadsorption were affected in this modification. For the effect on selectivity, the results of gel-filtration chromatography before and after immunoprecipitation (Figs. 2 and 3) demonstrate that neither intact nor turnicated nucleoside transporter monomer (p50NT and p40NT) binds to 65D4. Each of these nucleoside transporters in detergents, however, forms a nonspecific aggregate with glucose transporter (55GT) and is immuno-precipitated indirectly. For all the detergents tested, the extent of this nucleoside transporter aggregation to glucose transporter (P0 formation) quantitatively accounted for the amount of nucleoside transporters in the precipitate (Table I). These results clearly demonstrate that antibody 65D4 is specific for the glucose transporter, and its apparent cross-reactivity to the nucleoside transporter seen in the immunoprecipitation experiments is an artifact resulting from detergent-induced aggregation of two transporters (Figs. 2, 3).

The aggregation of the two transporter in detergents increases with incubation time in detergents [10,11]. The apparent cross-reactivity of 65D4 to nucleoside transporter was also increased with an increase in incubation time in detergents (data not shown). The physicochemical basis for this nonspecific protein aggregation in detergents is not well understood. It is possible that the protein aggregation may be reduced by increasing the detergent to protein ratio by diluting the proteins in detergent suspension. This interesting possibility is yet to be tested. When data of the four nonionic detergents in Table I were analyzed, there was an apparent, inverse relationship between the tendency of the protein aggregation and the detergent micellar size (or aggregation number) (Fig. 5), the significance of which is yet to be determined.

Our results with 65D4 also illustrate that the immunoadsorption of an integral membrane protein by an antibody can be grossly less than quantitative in some commonly used detergents. For example, only partial (about 50%) precipitation of glucose transporter was obtained in Triton X-100 (Fig. 2A), whereas almost complete precipitation (> 95%) was achievable in octyl glucoside (Fig. 3A). The partial precipitation in Triton X-100 was not caused by an insufficient supply of Pansorbin or by failure to reach equilibrium, as the use of an excess of Pansorbin or longer incubation time in immunoprecipitation experiments did not result in more precipitation.

Molecular basis for the occurrence of this nonreactive glucose transporter subpopulation is not known. Antibody 65D4 is known to react with the glucose transporter only at its cytoplasmic domain [12], and the less than stoichiometric adsorption may be related to limited accessibility of the epitope(s) to antibody in detergents such as Triton X-100. The maximal levels of the immunoadsorption of glucose transporter by 65D4 in four different, noniomic detergents were plotted against aggregation numbers of these detergents (Fig. 5). Although the sample size is small, there is an apparent inverse correlation between the maximum immunoaccessibility of glucose transporter to the antibody and the aggregation number of detergent. The significance of this correlation is to be determined when a large body of data become available in the future.

A significant purification of nucleoside transporter in band 4.5 has been achieved by the immunoadsorption of glucose transporter with polyclonal antibodies [17]. Our results, summarized in Table I, indicate that both the purity and yield of nucleoside transporter in such a purification scheme will be affected greatly by the use

of different detergents. We found that octyl glucoside gave a high purity with a low yield. This is consistent with the nearly quantitative immunoadsorption and the relatively high protein aggregation observed in this detergent. Triton X-100 also gave a low purity (unpublished data), as expected from the partial immunoadsorption of glucose transporter to 65D4 in this detergent. $C_{12}E_8$, on the other hand, gave the best immunopurification of nucleoside transporter (Fig. 4), as predicted from the high immunoreactivity of 65D4 with low protein aggregation of transporter proteins in this detergent.

In conclusion, the monoclonal antibody 65D4 is specific to the glucose transporter. 65D4 does not cross-react with the nucleoside transporter of human erythrocytes, thus there is no immunological indication that two transporters show any structural identity. Nucleoside and glucose transporter polypeptides aggregated in detergents, which resulted in an artifactual cross-reactivity of 65D4 to nucleoside transporter. This aggregation-induced artifact is substantial in certain detergents (such as octyl glucoside for 65D4), and was less so in others. The reactivity of antibody 65D4 to the glucose transporter was reduced significantly in some detergents, and this is most likely due to limited accessibility of the epitope to immunoreaction secondary to its steric or conformational hindrance in the protein-detergent micelles formed in these detergents.

Acknowledgments

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