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Erythrocyte nucleoside and sugar transport

Endo-β-galactosidase and endoglycosidase-F digestion of partially purified human and pig transporter proteins

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Nucleoside- and glucose-transport proteins isolated from human erythrocyte membranes were photoaffinity-labelled with [^{3}H]nitrobenzylthioinosine and [^{3}H]cytochalasin B, respectively, and subjected to endo-β-galactosidase or endoglycosidase-F digestion. Without enzyme treatment the two radiolabelled transporters migrated on SDS/polyacrylamide gels with the same apparent M_r (average) of 55000. Apparent M_r (average) values after endo-β-galactosidase digestion were 47000 and 48000 for the nucleoside and glucose transporters respectively, and 44000 and 45000 respectively after endoglycosidase-F digestion. In contrast, endo-β-galactosidase had no effect on the electrophoretic mobility of the nucleoside transporter isolated from pig erythrocytes. This transport system exhibited a higher M_r than the human protein, endoglycosidase-F treatment decreasing its apparent M_r (average) from 64000 to 57000. It is concluded that the human and pig erythrocyte nucleoside transporters are glycoproteins containing *N*-linked oligosaccharide. The data provide evidence of substantial carbohydrate and polypeptide differences between the human and pig erythrocyte nucleoside transporters, but evidence of molecular similarities between the human erythrocyte nucleoside and glucose transporters.

INTRODUCTION

Nucleosides and glucose cross the plasma membrane of human erythrocytes by functionally similar facilitated-diffusion mechanisms which are selectively inhibited by nitrobenzylthioinosine (NBMPR) and cytochalasin B respectively [1,2]. At the molecular level it has been established that the membrane proteins responsible for these two processes co-migrate as diffuse bands in the 'band-4.5' region of SDS/polyacrylamide gels [apparent M_r (average) 55000] [3–7]. The nucleoside and glucose transporters (10^4 and 10^5 copies per cell respectively) also co-purify during DEAE-cellulose ion-exchange chromatography of detergent-solubilized membrane extracts [3–5]. In contrast with human erythrocytes, erythrocytes from the pig lack glucose-transport activity [8,9] and rely on plasma inosine as the major physiological energy source [9–13]. The pig erythrocyte nucleoside transporter is also a band-4.5 polypeptide, but migrates on SDS/polyacrylamide gels as a sharper band and with a higher apparent M_r than the human erythrocyte nucleoside and glucose transporters [apparent M_r (average) 64000] [7,9]. In addition, the pig erythrocyte nucleoside transporter behaves anomalously during DEAE-cellulose ion-exchange chromatography [14].

The glucose transporter polypeptide from human erythrocytes is heterogeneously glycosylated [15]. Partial or essentially complete removal of oligosaccharide after endo-β-galactosidase or endoglycosidase-F treatment of the isolated transporter result in a sharpening of its band on SDS/polyacrylamide gels and corresponding shifts to lower-apparent-M_r regions of the gel [16,17]. In the present report we extend these studies to a comparison of the effects of endo-β-galactosidase and endoglycosidase-F digestion on the electrophoretic mobilities of partially purified preparations of the human erythrocyte nucleoside and glucose transporters. We also investigated the effects of these enzymes on the pig erythrocyte nucleoside transporter in an attempt to explore the origin of the molecular differences between the human and pig nucleoside-transport proteins. It is established that the human and pig erythrocyte nucleoside transporters are both glycoproteins containing *N*-linked oligosaccharide. The oligosaccharide and polypeptide structures from the two sources are, however, different. Our study also demonstrates that the carbohydrate-depleted human erythrocyte nucleoside and glucose transporters have essentially identical mobilities on SDS/polyacrylamide gels, further evidence of molecular similarities between the two transport systems. A preliminary report of the human erythrocyte experiments has been published [18].

MATERIALS AND METHODS

Materials

[G-^{3}H]NBMPR (sp. radioactivity 35 Ci/mmol) and [4(*n*)-^{3}H]cytochalasin B (sp. radioactivity 17 Ci/mmol)

Abbreviations used: NBMPR, nitrobenzylthioinosine; NBTGR, nitrobenzylthioguanosine.

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were purchased from Moravek Biochemicals (Brea, CA, U.S.A.) and Amersham International (Amersham, Bucks., U.K.) respectively. Endo-β-galactosidase was prepared as described previously [19], and endoglycosidase-F was obtained from New England Nuclear, Boston, MA, U.S.A. *n*-Octyl glucoside was from Sigma (St. Louis, MO, U.S.A.). All other reagents were of analytical grade.

Cell and membrane preparation

Fresh human and pig blood was collected into heparin and the erythrocytes washed three times with a medium containing 140 mM-NaCl, 5 mM-KCl, 20 mM-Tris/HCl, pH 7.4 at 22 °C, 2 mM-$MgCl_2$ and 0.1 mM-EDTA (disodium salt). The buffy coat was discarded. Protein-depeleted membranes were prepared from erythrocyte ghosts by using 0.1 mM-EDTA, pH 11.2, as described previously [3] and resuspended in solubilization buffer (see below).

Nucleoside- and glucose-transporter purification

Purification of band-4.5 polypeptides from human erythrocyte protein-depleted membranes was performed as described by Baldwin *et al.* [4]. Briefly, membranes (2 mg of protein/ml) were solubilized in 46 mM-*n*-octyl glucoside in 50 mM-Tris/HCl/2 mM-dithiothreitol, pH 7.4 at 4 °C, and 60 ml of the 130000 *g* supernatant was applied at a flow rate of 85 ml/h to a column (2.5 cm × 6.5 cm) of DEAE-cellulose ion-exchange resin equilibrated with the same buffer. Band-4.5 polypeptides were collected in the void-volume fractions, which were pooled, dialysed free of detergent, and concentrated by ultracentrifugation (130000 *g* for 60 min). This procedure resulted in a 19-fold purification of nucleoside transporter on the basis of the assay of high-affinity [^{3}H]NBMPR-binding capacity [3]. The specific activity of NBMPR binding to the preparation was 699 pmol/mg of protein, compared with 36 pmol/mg of protein for the starting human erythrocyte ghosts. The major polypeptide species present in the preparation was glucose transporter [3,4]. The preparation was stored at −70 °C.

The pig erythrocyte nucleoside transporter is adsorbed by DEAE-cellulose under conditions where the human protein is eluted in the column void volume [14]. Pig erythrocyte protein-depleted membranes were therefore solubilized in 46 mM-*n*-octyl glucoside/2 mM-dithiothreitol in 5 mM-Tris/HCl, pH 7.4 at 4 °C. The DEAE-cellulose ion-exchange column was equilibrated in this buffer and eluted with 800 ml of a linear salt gradient (0–0.2 M-NaCl in 34 mM-*n*-octyl glucoside/2 mM-dithiothreitol/5 mM-Tris/HCl). Fractions containing nucleoside-transport protein (0.08–0.11 M-NaCl) were pooled and treated as described above for the human protein. The specific activity of NBMPR binding to the final preparation was 1465 pmol of NBMPR bound/mg of protein compared with 24 pmol of NBMPR bound/mg of protein for the starting pig erythrocyte ghosts, a purification of 61-fold.

Control experiments confirmed that the human band-4.5 preparation exhibited D-glucose-sensitive binding of cytochalasin B. The preparation was also capable of both NBMPR-sensitive uridine transport and cytochalasin B-sensitive glucose transport when reconstituted into soybean phospholipid vesicles as described previously [20]. In contrast, the pig preparation exhibited only NBMPR-sensitive uridine-transport activity. The absence of glucose transporter from the pig erythrocyte preparation accounts for its higher specific activity of NBMPR binding.

Photoaffinity labelling

NBMPR binding to the nucleoside transporter is normally reversible. However, covalent radiolabelling of transport protein occurs when site-bound [^{3}H]NBMPR is exposed to high-intensity u.v. light [5,7]. Partially purified human and pig erythrocyte nucleoside transporters (final protein concn. 0.1 mg/ml) were equilibrated at room temperature for 30 min with a saturating concentration of [^{3}H]NBMPR (50 nM). Samples were then cooled to 4 °C and supplemented with 50 mM-dithiothreitol added as a free-radical scavenger. Photolysis was carried out in 0.4 ml-volume mini-spectrometer quartz cuvettes (1 mm light path) at 4 °C using a 450 W mercury arc lamp (Conrad-Hanovia, Newark, NJ, U.S.A.) U.v. exposure was for 45 s at a distance of 6.5 cm from the lamp's silica cooling sleeve. Samples were then diluted 20-fold with 5 mM-sodium phosphate, pH 8.0, and left at room temperature for 10 min before recovery

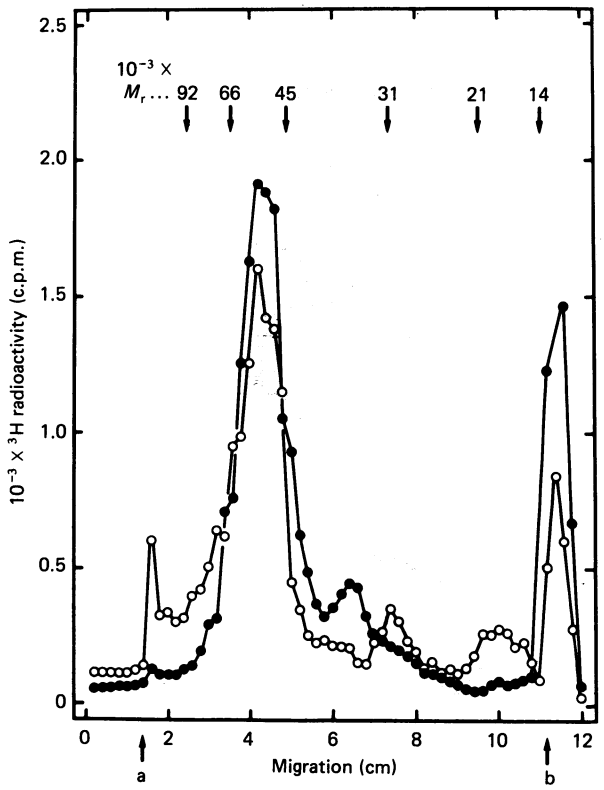

Fig. 1. Photoaffinity labelling of human erythrocyte nucleoside- and glucose-transport polypeptides with [^{3}H]NBMPR and [^{3}H]cytochalasin B

Human erythrocyte band-4.5 polypeptides were photoaffinity-labelled with [^{3}H]NBMPR and [^{3}H]cytochalasin B as described in the Materials and methods section and subjected to SDS/polyacrylamide-gel electrophoresis. ^{3}H profiles for the glucose (○) and nucleoside transporters (●) (10 μg of membrane protein) and positions of M_r standards are from the slab gel. The positions of the stacking-gel/running-gel interface and the tracking dye are indicated by a and b respectively

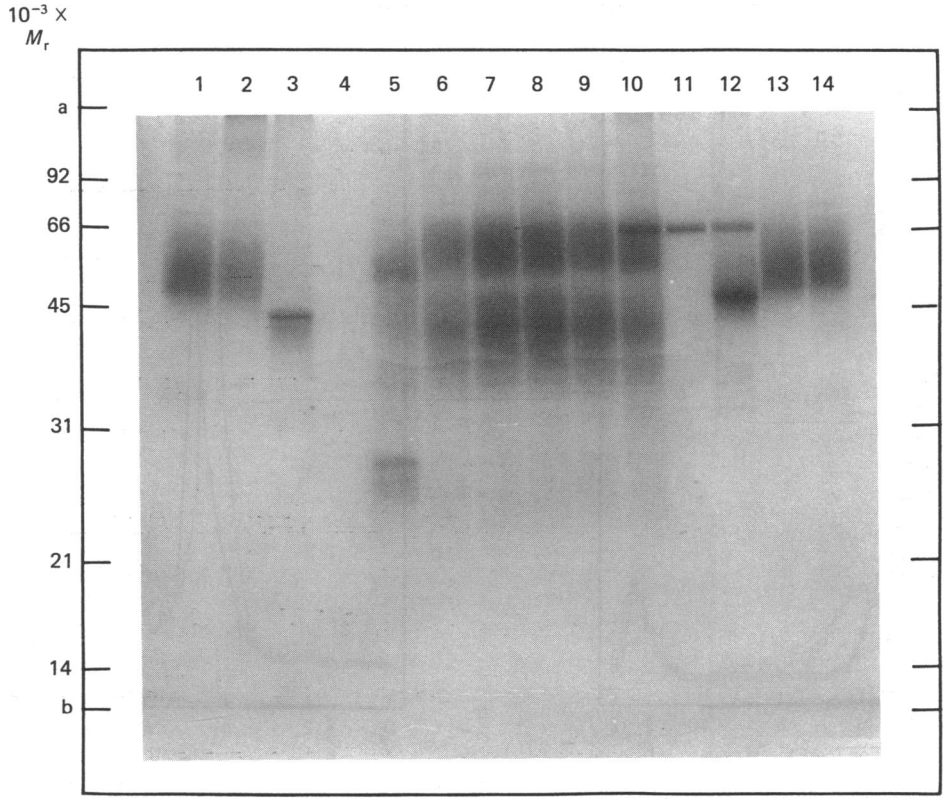

Fig. 2. Effects of endo-β-galactosidase and endoglycosidase-F digestion on the electrophoretic profiles of band-4.5 polypeptides isolated from human and pig erythrocyte membranes

Isolated band-4.5 polypeptides were treated with either endo-β-galactosidase or endoglycosidase-F as detailed in the text, subjected to SDS/polyacrylamide-gel electrophoresis and the gels stained with Coomassie Blue. Lanes were loaded with 10 μg of human protein or 30 μg of pig protein. Lanes 1 and 14, human band 4.5; lanes 7 and 8, pig band 4.5; lanes 2 and 6, human and pig band 4.5 respectively, incubated for 18 h at 22 °C in digestion buffer without endoglycosidase-F; lanes 3 and 5, human and pig band 4.5 respectively, treated with endoglycosidase-F; lane 4, endoglycosidase-F (0.4 unit); lanes 9 and 13, pig and human band 4.5 incubated for 18 h at 37 °C in digestion buffer without endo-β-galactosidase; lanes 10 and 12, pig and human band 4.5 respectively, treated with endo-β-galactosidase; lane 11, endo-β-galactosidase (0.0125 unit) stabilized with 3 μg of bovine serum albumin. Values on the left refer to positions of M_r standards. The positions of the stacking-gel/running-gel interface and the tracking dye are indicated by a and b respectively.

of radiolabelled transporter by ultracentrifugation. The washed pellets were resuspended in the appropriate enzyme-digestion buffer (see below). An essentially identical procedure was used to radiolabel the human erythrocyte glucose transporter with [^{3}H]cytochalasin B [21].

Enzyme digestion

Treatment of the radiolabelled transporter preparations (0.1 mg of protein/ml) with endo-β-galactosidase was carried out at 37 °C for 18 h in 50 mM-sodium phosphate (pH 6.0)/1 mM-dithiothreitol/1 mM-EDTA, an enzyme concentration of 0.25 units/ml being used [16]. Treatment with endoglycosidase-F (0.4 unit/ml) was carried out at 22 °C for 18 h with agitation in 100 mM-sodium phosphate, pH 6.0, containing 75 mM-β-mercaptoethanol, 50 mM-EDTA, 0.5% (w/v) Triton X-100, 0.05% (w/v) SDS and 0.1 mg of transporter protein/ml [17]. Both enzymic digestions were terminated by addition of equal volumes of SDS/polyacrylamide-gel sample buffer [22].

SDS/polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was carried out in 2 mm-thick 10%-(w/v)-polyacrylamide slab gels by the method of Thompson & Maddy [22], the Laemmli buffer system [23] being used. Radioactivity in the various regions of the gel was determined by slicing the gel lanes into 2 mm fractions. The ^{3}H content of these slices was measured by liquid-scintillation counting [7].

Protein determination

Protein was assayed by the method of Peterson [24].

RESULTS AND DISCUSSION

Exposure of human erythrocyte band-4.5 polypeptides to u.v. light in the presence of [^{3}H]NBMPR or [^{3}H]cytochalasin B under equilibrium binding conditions resulted in substantial radiolabelling of nucleoside- and glucose-transport polypeptides respectively. As shown in Fig. 1, the major peaks of radiolabelling were located in the band-4.5 region of the gel and co-migrated with the single broad band of Coomassie Blue staining given by the preparation [apparent M_r (average) 55000] (Fig. 2, lanes 1 and 14). Most of this Coomassie Blue-stained protein is glucose transporter [3,4]. In agreement with the results from previous photoaffinity-labelling studies with

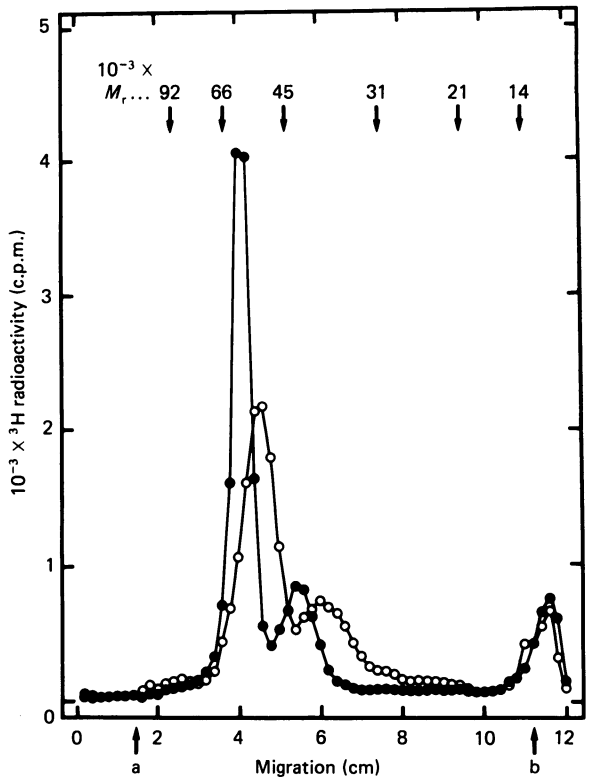

Fig. 3. Photoaffinity labelling of human and pig erythrocyte nucleoside-transport polypeptides with [^{3}H]NBMPR

Partially-purified preparations of the two transporters were photoaffinity labelled with [^{3}H]NBMPR as described in the Materials and methods section and subjected to SDS/polyacrylamide-gel electrophoresis. ^{3}H profiles for the human (○) and pig preparations (●) (10 μg of membrane protein) and positions of M_r standards are from the same slab gel. The positions of the stacking-gel/running-gel interface and the tracking dye are indicated by a and b respectively.

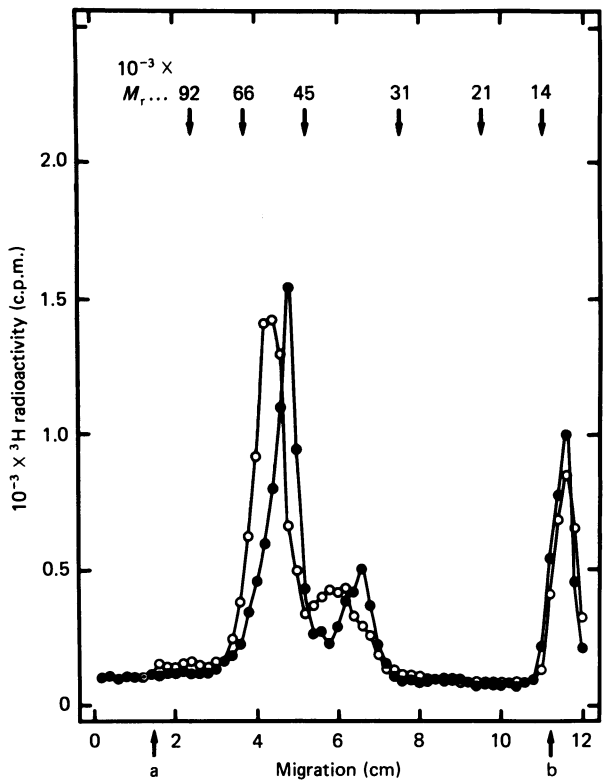

Fig. 4. Effect of endo-β-galactosidase on the electrophoretic mobility of the human erythrocyte nucleoside transporter

Transporter preparation radiolabelled with [^{3}H]NBMPR was incubated for 18 h at 37 °C either in the presence (●) or in the absence of enzyme (○) and electrophoresed as described in the Materials and methods section. ^{3}H profiles and positions of M_r standards are from the same slab gel. Positions of the stacking-gel/running-gel interface and the tracking dye are indicated by a and b respectively.

intact membranes [7,9], the radiolabelled nucleoside transporter in the pig erythrocyte preparation migrated as a sharper peak and with a higher apparent M_r than the human erythrocyte nucleoside transporter (Fig. 3). Similarly, the small amount of radiolabelled proteolytic degradation product present in the pig partially purified preparation also migrated with a higher apparent M_r than the corresponding human cleavage product [21]. Radiolabelling of both the human and pig erythrocyte nucleoside transporters was abolished when photolysis was performed in the presence of 20 μM-NBTGR as competing non-radioactive ligand (results not shown).

Unlike the human preparation, the partially-purified nucleoside transporter preparation from pig exhibited two regions of Coomassie Blue staining (Fig. 2, lanes 7 and 8), one migrating in the same position as the ^{3}H-labelled pig transporter [apparent M_r (average) 64000], the other with a lower apparent M_r (average 43000). The latter protein contributed ~ 60% of the protein present in the preparation as judged from absorbance scans and was not radiolabelled with [^{3}H]NBMPR, the small amount of ^{3}H in this area of the gel being associated with a proteolytic fragment of the nucleoside transporter (see above). The experimentally determined specific activity of reversible [^{3}H]NBMPR binding to the partially purified pig transporter was 1.47 nmol/mg of protein (see the Materials and methods section). Since the low-M_r protein in the preparation is presumably not active in high-affinity [^{3}H]NBMPR binding, we can estimate that the specific activity of [^{3}H]NBMPR binding to the 64000-M_r band would be in the region of 3 nmol/mg of protein. This value compares with a theoretical maximum specific binding activity of 15.6 nmol/mg of protein, calculated by assuming that each 64000-M_r polypeptide binds a single molecule of [^{3}H]NBMPR, or half that value if one molecule of ligand binds to a 64000-M_r dimer. Therefore the pig erythrocyte nucleoside transporter is estimated to contribute 20–40% of the protein present in the 64000-M_r Coomassie Blue-staining band.

Fig. 4 compares the SDS/polyacrylamide-gel ^{3}H profiles of the radiolabelled human erythrocyte nucleoside transporter incubated for 18 h at 37 °C either in the absence or in the presence of endo-β-galactosidase. Enzyme treatment resulted in a significant sharpening of the ^{3}H peak and an increase in electrophoretic mobility. Values of apparent M_r obtained in this and subsequent experiments are summarized in Table 1. As shown in Fig. 4, the ^{3}H-labelled proteolytic cleavage product also increased in electrophoretic mobility after endo-β-

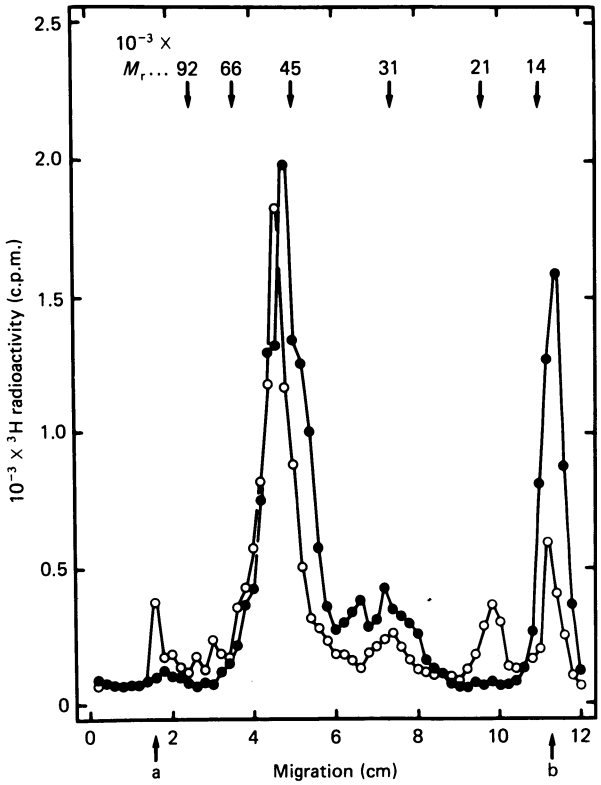

Fig. 5. Effects of endo-β-galactosidase on the electrophoretic mobilities of the human erythrocyte nucleoside and glucose transporters

Band-4.5 polypeptides radiolabelled with [^{3}H]NBMPR (●) and [^{3}H]cytochalasin B (○) were incubated for 18 h at 37 °C in the presence of enzyme and subjected to SDS/polyacrylamide-gel electrophoresis as described in the text. ^{3}H profiles and positions of M_r standards are from the same slab gel. Positions of the stacking-gel/running-gel interface and the tracking dye are indicated by a and b respectively.

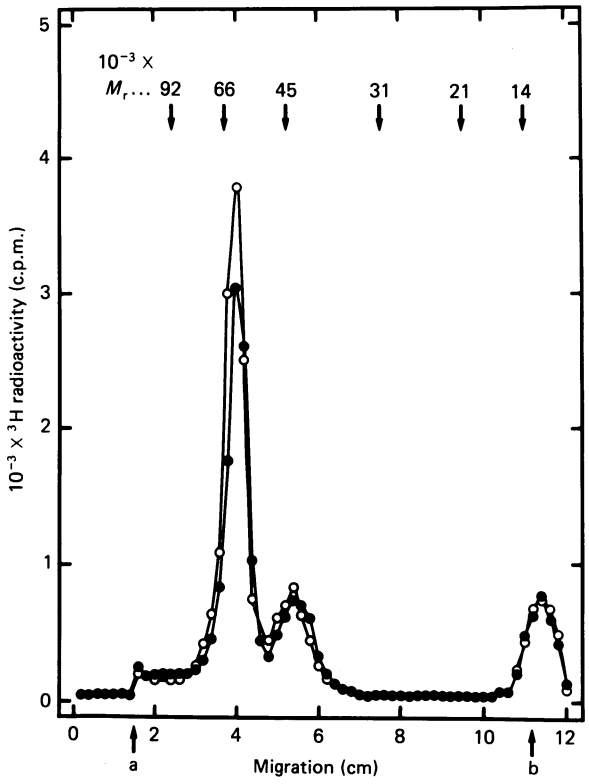

Fig. 6. Effect of endo-β-galactosidase on the electrophoretic mobility of the pig erythrocyte nucleoside transporter

Transporter preparation radiolabelled with [^{3}H]NBMPR was incubated for 18 h at 37 °C either in the presence (●) or in the absence of enzyme (○) and electrophoresed as described in the Materials and methods section. ^{3}H profiles and positions of M_r standards are from the same slab gel. Positions of the stacking-gel/running-gel interface and the tracking dye are indicated by a and b respectively.

Table 1. Aparent M_r values for erythrocyte nucleoside- and glucose-transport polypeptides treated with endo-β-galactosidase and endoglycosidase-F

Values are mean results from two independent experiments. See the text and Figs. 3 and 4 for experimental details. The small difference in electrophoretic mobility of the endoglycosidase-F-treated human glucose and nucleoside transporters was confirmed in two additional experiments.

	Apparent M_r		
Polypeptide	Control	Endo-β-galactosidase treated	Endoglycosidase-F-treated
Glucose transporter (human)	55000	48000	45000
Nucleoside transporter			
Human	55000	47000	44000
Pig	64000	64000	57000

galactosidase digestion, providing evidence that this fragment of the transporter contains carbohydrate. As expected from previous studies [16], endo-β-galactosidase produced a parallel change in the electrophoretic mobility of the Coomassie Blue-stained band-4.5 protein (Fig 2, lane 12). The sharp protein band observed in endo-β-galactosidase-treated samples at apparent M_r 66000 is bovine serum albumin (included to stabilize the freeze-dried enzyme preparation) (Fig. 2, lane 11). A parallel experiment directly comparing the electrophoretic mobilities of the endo-β-galactosidase-treated human erythrocyte nucleoside and glucose transporters is

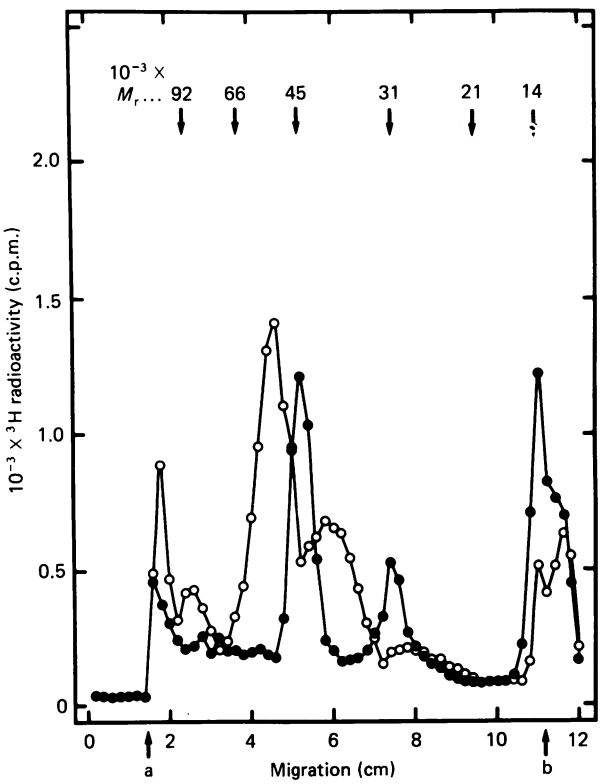

Fig. 7. Effect of endoglycosidase-F on the electrophoretic mobility of the human erythrocyte nucleoside transporter

Transporter polypeptide radiolabelled with [^{3}H]NBMPR was incubated for 18 h at 22 °C either in the presence (●) or in the absence (○) of enzyme and electrophoresed as described in the Materials and methods section. ^{3}H profiles and positions of M_r standards are from the same slab gel. Positions of the stacking-gel/running-gel interface and the tracking dye are indicated by a and b respectively.

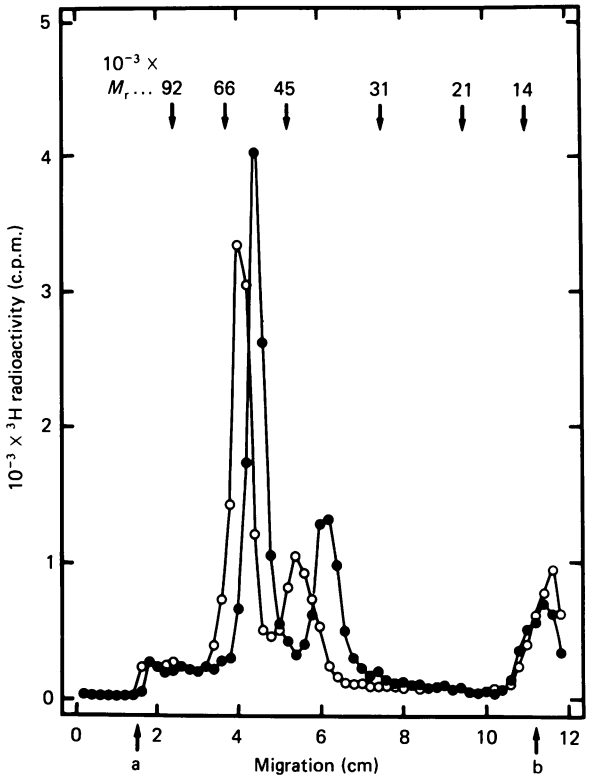

Fig. 8 Effect of endoglycosidase-F on the electrophoretic mobility of the pig erythrocyte nucleoside transporter

Transporter polypeptide radiolabelled with [^{3}H]NBMPR was incubated for 18 h at 22 °C either in the presence (●) or in the absence (○) of enzyme and electrophoresed as described in the Materials and methods section. ^{3}H profiles and positions of M_r standards are from the same slab gel. Positions of the stacking-gel/running-gel interface and the tracking dye are indicated by a and b respectively.

presented in Fig. 5. The results establish a close, but not exact, correspondence in apparent M_r for the two enzyme-treated transporters (Table 1). In contrast with its effect on the human erythrocyte nucleoside and glucose transporters, endo-β-galactosidase digestion under identical conditions had no measurable effect on the electrophoretic mobility of the radiolabelled pig erythrocyte nucleoside transporter (Fig. 6). Similarly, there was no effect on the electrophoretic mobilities of the two Coomassie Blue-stained bands present in the pig preparation (Fig. 2, lane 10).

Endo-β-galactosidase hydrolyses internal β-galactosidic linkages of oligosaccharides belonging to the poly-(*N*-acetyl-lactosamine) series. In a second series of experiments, we studied the effects of endoglycosidase-F, an enzyme which cleaves the glycosidic bond of *N*-acetylglucosamine(1–4)*N*-acetylglucosamine linked to asparagine of the core protein of high-mannose structures. Figs. 7 and 8 compare the SDS/polyacrylamide-gel ^{3}H profiles of radiolabelled human and pig erythrocyte nucleoside transporters incubated (18 h, 22 °C) in the absence or in the presence of endoglycosidase-F. In contrast with endo-β-galactosidase digestion, endoglycosidase-F digestion affected the electrophoretic mobilities of both the human and pig erythrocyte nucleoside transporters. In each case there was a significant sharpening of the ^{3}H peak and a shift to a lower apparent M_r, the human protein showing the larger decrease in M_r (Table 1). The electrophoretic mobilities of both the human and pig degradation ^{3}H peaks were also shifted to lower-M_r regions of the gel, again an indication that these polypeptide fragments are glycosylated.

As shown in Fig. 9 and summarized in Table 1, there was again a close, but not exact, correspondence between the electrophoretic mobilities of the human [^{3}H]NBMPR-labelled nucleoside transporter and the [^{3}H]cytochalasin B-labelled glucose transporter after endoglycosidase-F digestion, a result similar to that obtained in the previous endo-β-galactosidase experiments (Fig. 5). As expected, the ^{3}H peak associated with the carbohydrate-depleted glucose transporter exactly co-migrated with endoglycosidase-F-treated band 4.5 (Fig. 2, lane 3). Similarly, the ^{3}H peak associated with the endoglycosidase-F-treated pig nucleoside transporter co-migrated with the high-M_r band of Coomassie Blue staining present in the preparation (Fig. 2, lane 5). The non-radioactive 43000-M_r protein band present in the pig nucleoside transporter preparation had an apparent M_r of 28000 after endoglycosidase-F digestion. The endoglycosidase-F present in the samples (0.4 unit) was not detected by Coomassie Blue staining (Fig. 2, lane 4). Aggregation of the radiolabelled human erythrocyte

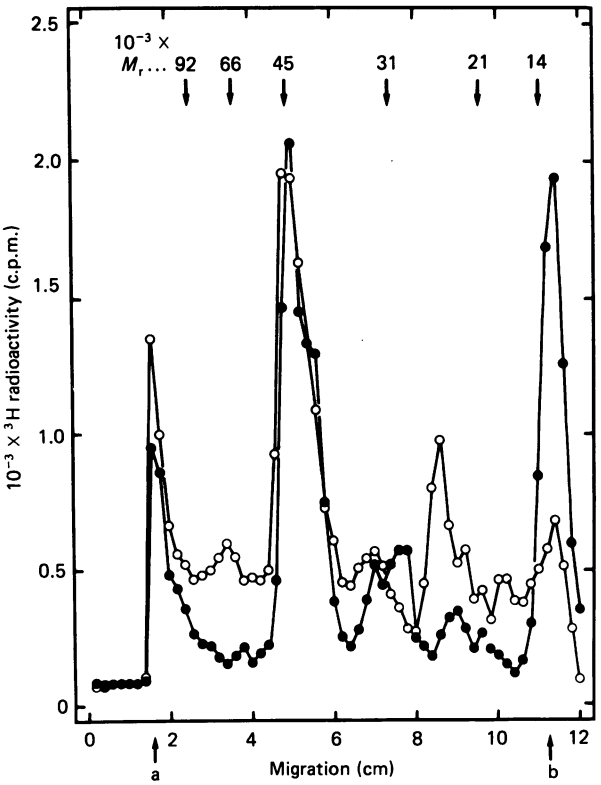

Fig. 9. Effects of endoglycosidase-F on the electrophoretic mobilities of the human erythrocyte nucleoside and glucose transporters

Band-4.5 polypeptides radiolabelled with [^{3}H]NBMPR (●) and [^{3}H[cytochalasin B (○) were incubated for 18 h at 22 °C in the presence of enzyme and subjected to SDS/polyacrylamide-gel electrophoresis as described in the text. ^{3}H profiles and positions of M_r standards are from the same slab gel. Positions of the stacking-gel/running-gel interface and the tracking dye are indicated by a and b respectively.

glucose and nucleoside transporters was more severe than in the endo-β-galactosidase experiments, possibly reflecting the presence of detergent in the digestion buffer [17].

We conclude from the present study that the human and pig erythrocyte nucleoside transporters are both glycoproteins containing *N*-linked oligosaccharide. The observation that the pig nucleoside transporter is resistant to endo-β-galactosidase digestion and the finding that endoglycosidase-F treatment had a more pronounced effect on the apparent M_r of the human protein provide evidence for a marked species difference in the detailed structures of the oligosaccharide present in the two transporter polypeptides. Carbohydrate differences between the human and pig erythrocyte nucleoside transporters might, in part, account for the different electrophoretic mobilities of the native proteins on SDS/polyacrylamide gels and their different elution profiles during DEAE-cellulose ion-exchange chromatography. The finding that endoglycosidase-F actually increased the difference in apparent M_r between the human and pig erythrocyte nucleoside transporters (13000 after enzyme digestion compared with 9000 before) (Table 1) is evidence that there is also a major polypeptide difference between the two transporters. This latter conclusion relies on the assumption that endoglycosidase-F removed most of the carbohydrate attached to the pig protein. Photoaffinity-labelling experiments with [^{3}H]NBMPR have established that nucleoside transporters with an apparent M_r similar to that of the human erythrocyte protein (55000) are present in rabbit erythrocytes [25], various guinea-pig tissues [26–29] and mouse S49 lymphoma cells [30]. Interestingly, rat nucleoside transporters have a consistently higher apparent M_r (average) in the region of 60000 [27,28]. In contrast with the human/pig difference described in the present paper, the difference in apparent M_r between the guinea-pig and rat liver nucleoside transporters is abolished after endoglycosidase-F digestion (S. A. Baldwin, J.-S. R. Wu, and J. D. Young, unpublished work).

The present results also provide further evidence of molecular similarities between the human erythrocyte nucleoside and glucose transporters, the native proteins having indistinguishable electrophoretic mobilities on SDS/polyacrylamide gels. More significantly, the deglycosylated polypeptides exhibited almost identical apparent M_r values of 44000 and 45000 respectively. As detailed in the Introduction and elsewhere [3,5,7,13], there are important functional and physiological similarities between the two transport systems. The correspondence in apparent M_r may therefore not be coincidental and is perhaps an indication of some degree of structural homology between the two proteins. With regard to possible sites of glycosylation, the recently determined amino acid sequence of the human HepG2 hepatoma glucose transporter contains two asparagine residues, one of which (Asn^{411}) is located within a postulated membrane-spanning domain of the transporter [31]. The other asparagine residue (Asn^{45}) has been shown to be glycosylated *in vitro* [31], and its location is consistent with the site of glycosylation of the erythrocyte glucose transporter determined by enzyme- and chemical-cleavage studies [16].

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