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Cell partitioning in two-polymer aqueous phase systems and cell electrophoresis in aqueous polymer solutions. Human and rat young and old red blood cells

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Abstract

It has recently been found that electrophoresis in solutions of appropriately selected polymers in phosphate-buffered saline (PBS) can differentiate between some closely related cell populations which have identical electrophoretic mobilities (EPM) in PBS (e.g., human young and old red blood cells (RBC); RBC from Alzheimer patients and normal individuals). The EPM differences detected in polymer solutions are most likely a consequence of cell- and polymer-specific interactions. Aspects of the relation between the electrophoresis in aqueous polymer solutions of native and in vitro treated young and old RBC (from human and rat) and their partitioning in a charge-sensitive dextran-poly(ethylene glycol) (PEG) aqueous phase system (i.e., a system with a Donnan potential between the phases, top phase positive) have been examined further and are discussed. Unlike the behavior of RBC from Alzheimer patients and normal individuals in which an EPM difference can be detected in PEG solutions but not in dextran, differences in EPM between human young and old RBC are detectable in solutions of either polymer. Selected enzyme treatments of human young and old RBC or their fixation with aldehyde eliminates the EPM differences in dextran; while neuraminidase treatment or formaldehyde fixation of rat young and old RBC retains EPM differences in dextran between these cells. In these latter cases partitioning differences are also in evidence and are in the same direction as the cells' relative EPM (i.e., old RBC < young RBC). The earlier hypothesis that cell partitioning is 'more sensitive' than cell electrophoresis in detecting differences in surface charge between cells bears reexamination because human young and old RBC, which cannot be differentiated by single-tube partitioning in a charge-sensitive phase system, have different EPM in polymer solutions. The difference between these cells can be detected by partitioning but only by use of a multiple-extraction procedure. It is then found to be in a direction similar to the cells' relative EPM in dextran (i.e., human old RBC > young RBC). Rat young and old RBC have different partitions (rat old RBC < young RBC) and different EPM (also rat old RBC < young RBC). Thus, while cell partitioning in a charge-sensitive dextran-PEG aqueous phase system and cell electrophoresis in polymer solution seem to reflect, at least with these cell subpopulations, qualitatively analogous differences in surface properties (in that increasing partitions and EPM are concomitant), there are instances in which either of these physical measurements discerns surface differences which escape detection by the other.

Key words: Cell system; Partitioning; Erythrocyte; Electrophoresis; Aqueous phase

1. Introduction

We have, for years, been interested in probing membrane surface properties that determine the partitioning behavior of cells in dextran-poly(ethylene glycol) (PEG) aqueous phase systems [1,2] and have often used red blood cells (RBC) from different species as a model. In charge-sensitive dextran-PEG phase systems there is a correlation [3] (with some exceptions [3,4]) between the partitions of such RBC and their relative electrophoretic mobilities (EPM) in phosphate-buffered saline (PBS). Furthermore, rat RBC recovered along the extraction train, subsequent to countercurrent distribution in a charge-sensitive phase system, show a concomitant increase in partition and EPM [1]. Partitioning has also been able to discriminate among some cell populations (e.g., beef RBC from different animals

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having differing sialic acid content [4]) which have identical EPM. The latter results suggested that while cell EPM in PBS measures charge at the shear plane, cell partitioning also gauges charge deeper into the membrane [4]. Thus, the surface charge reflected by cell partitioning in dextran-PEG phases and by cell electrophoresis in PBS can but need not be the same.

A question that arose was whether differences between the partitioning behavior of cells and their relative EPM stem from the fact that EPM measurements were carried out in media (PBS, saline) devoid of polymers that constitute the cells' environment in partitioning. We therefore examined cell electrophoretic behavior in solutions of selected polymers in PBS. This approach was rendered more enticing by the results of Nash et al. [5] who reported that human young and old RBC, which have different partitions [6] and are known to have identical EPM in PBS [7], have different EPM in a dextran solution; and by the finding that the partitioning of cells in two-polymer aqueous phase systems depends, among other things, on the polymer to which they are first exposed (i.e., that an interaction with that polymer occurs) [8].

In a previous investigation we determined that the ratios of viscosity-corrected EPM of RBC from different species in (diluted) dextran-rich or PEG-rich phases/EPM of the respective species' RBC in PBS differ for a number of species, and from each other, reflecting thereby differences in kind (i.e., dextran or PEG) and nature of polymer interaction with these RBC [9]. Thus, both cell partitions and cell EPM obtained in polymer media must, at least with some cell populations, be due to surface properties other than or in addition to the charge measured by EPM in PBS or saline [9].

Here we examine aspects of the similarities and differences in surface properties of the more closely related cell subpopulations comprising human young and old RBC and rat young and old RBC reflected by partitioning, by electrophoresis in polymer solutions and by electrophoresis in PBS.

2. Materials and methods

2.1. Reagents

Dextran T500 (lot No. 01 06905) was obtained from Pharmacia LKB (Piscataway, NJ). Poly(ethylene glycol) 8000 (PEG, Carbowax 8000) was from Union Carbide (Long Beach, CA). Neuraminidase (*Vibrio cholerae*) was a product of Calbiochem (La Jolla, CA) while trypsin, chymotrypsin and paraformaldehyde were from Sigma (St. Louis, MO). All salts and organic solvents used were of analytical reagent grade.

2.2. Preparation of two-polymer aqueous phase systems and other standard solutions

Aqueous two-phase systems having the dextran and PEG concentrations and salt compositions and concentrations indicated in the text were prepared as previously described [1]. Phosphate-buffered saline (PBS) contained 0.15 M NaCl + 0.01 M sodium phosphate buffer (pH 6.8). Solutions containing 4, 8 or 12% (w/w) dextran in PBS or 5% (w/w) PEG in PBS were prepared by weighing out the appropriate amounts of concentrated respective stock solutions [1] (i.e., 20% (w/w) dextran, 40% (w/w) PEG, 0.44 M sodium phosphate buffer (pH 6.8), 0.60 M NaCl).

2.3. Collection of human and rat blood

Human blood, drawn by venipuncture from presumably hematologically normal individuals, and rat blood, obtained by heart puncture, were collected in citrate vacutainers. The blood was fractionated into young and old RBC within 1 h of sample collection and the cells used in the subsequent experiments within 1 week (see below).

2.4. Preparation of young and old red blood cell populations

RBC were fractionated according to age by Murphy's centrifugation method [10] using approximately 2 ml of packed RBC per 10.7 × 77 mm centrifuge tube. About 7% of the upper and lower centrifuged blood cell layers were collected to obtain RBC populations enriched with respect to young (top layer) or old (bottom layer) cells as previously established [7,11]. The cells were washed three times in PBS and used in electrophoresis or partitioning experiments (see below).

2.5. In vitro treatments of human and / or rat red blood cells

Enzyme treatment of red blood cells

Neuraminidase. 1 ml of packed rat RBC was washed three times with 10 vols. of PBS (pH 7.0). A 0.25 ml aliquot of such washed RBC + 1.75 ml of PBS was put into a 12 ml round bottom tube and incubated together with 0.1 ml (1 I.U./ml) of neuraminidase for 90 minutes at 37°C. A similarly treated aliquot of the same cell population was incubated in the absence of enzyme. The cells were then washed three times with PBS (pH 6.8), and used in partitioning and electrophoresis experiments.

Chymotrypsin and trypsin. 0.5 ml of packed rat or 1 ml of packed human RBC were washed three times with 10 volumes of PBS (pH 7.4). 0.05 ml of rat or 0.1 ml of human washed RBC + 10 × the cell volume of

PBS were put into a 12 ml round bottom centrifuge tube and incubated together with 0.025 ml (for rat RBC) or 0.05 ml (for human RBC) of either trypsin (1 mg/ml saline) or chymotrypsin (1 mg/ml saline) at 37°C for 90 min (for rat RBC) or 60 min (for human RBC). At the same time a similarly treated aliquot of the same cell population was incubated in the absence of enzyme. The cells were washed three times with PBS (pH 6.8), and used in electrophoresis experiments.

Formaldehyde fixation of red blood cells

1% paraformaldehyde in PBS was heated at about 80°C for 30 min to obtain dissolution. The solution was allowed to cool. Human and/or rat RBC were washed three times with 10 volumes of PBS (pH 6.8). 18 ml of the 1% formaldehyde solution was put into a 50 ml Erlenmeyer flask. 2 ml of washed packed RBC were added with continuous swirling. The mixture was then permitted to remain at room temperature overnight. The clear formaldehyde solution was poured off and replaced with an identical volume of fresh 1% formaldehyde solution. The cells were resuspended, the flask capped and stored in the cold (4–5°C). Red cells were fixed for a minimum of 1 week. Fixed cells were then removed and washed three times with 10 volumes of PBS before use in experiments.

Lipid extraction of formaldehyde-fixed red blood cells with chloroform / methanol. Fixed cells were washed three times in 10 volumes of PBS. Cells were washed an additional two times with 10 volumes of distilled water and one time with 10 volumes methanol. The cells were then suspended in 7 vols. of methanol, 14 volumes of chloroform were added, the tube was capped and allowed to sit at room temperature for 1 h. The cells were then centrifuged and washed twice with 10 volumes of methanol, twice with 10 volumes of distilled water, twice with PBS and then either suspended in PBS for use in electrophoresis experiments or suspended in top phase of the phase system to be used in a partitioning experiment.

Lipid extraction of formaldehyde-fixed red blood cells with ethanol. Fixed cells were washed as in the previous section. The cells were suspended in 10 volumes of ethanol and permitted to remain at room temperature for 1 h. The cells were subsequently washed and prepared for analysis as in the previous section.

Table 1
Viscosity-corrected electrophoretic mobilities [a] of young and old red blood cells [b] (RBC) from humans and rats in different suspending media

Suspending medium	Human young RBC	Human old RBC	p
PBS [c]	-1.08 ± 0.01 (9)	-1.08 ± 0.01 (9)	n.s.
Dextran [d]	-3.99 ± 0.13 (9)	-4.33 ± 0.13 (9)	$p < 0.01$
Bottom phase [e]	-4.54 ± 0.04 (6)	-4.61 ± 0.06 (6)	$p < 0.03$
PEG [d]	-1.41 ± 0.01 (6)	-1.44 ± 0.03 (6)	$p < 0.04$
Top phase [e]	-1.82 ± 0.03 (6)	-1.86 ± 0.03 (6)	$p < 0.04$
Suspending medium	Rat young RBC	Rat old RBC	
PBS [c]	-1.29 ± 0.01 (9)	-1.23 ± 0.01 (9)	$p < 0.01$
Dextran [d]	-4.91 ± 0.09 (9)	-4.68 ± 0.06 (9)	$p < 0.01$

[a] Data present the mean electrophoretic mobilities, EPM, (μm/s per V/cm), $\pm$S.D. with the number of experiments in parentheses.
[b] Obtained by a centrifugal fractionation method.
[c] Phosphate-buffered saline (PBS) was composed of 0.15 M NaCl + 0.01 M Na-phosphate buffer, pH 6.8 (NaPB).
[d] EPM were measured in a 4% dextran (Dx) T500 solution in PBS and a 2.5% poly(ethylene glycol) (PEG) 8000 solution in PBS.
[e] Top and bottom phases were from a system containing 5% (w/w) Dx T500, 3.5% (w/w) PEG 8000, 0.15 M NaCl and 0.01 M NaPB. Top phase is PEG-rich and bottom phase is Dx-rich. Top and bottom phases were diluted 1:1 with the indicated RBC suspension in PBS followed by measuring cell EPM.

2.6. Viscosity determinations of suspending media used in electrophoretic mobility measurements

The viscosities of the different media were estimated by means of an Ostwald viscometer immersed in a tank thermostated at 25 $\pm$ 0.2°C.

2.7. Electrophoretic mobility measurements on erythrocytes in different suspending media

RBC (fresh, enzyme treated, fixed, fixed and lipid-extracted, etc.) were washed three times with PBS and a suitable cell aliquot was, finally, suspended in PBS.

Top and bottom phases used as suspending media for cell electrophoresis were prepared as previously described [12]. In short, phase systems were mixed and permitted to settle in a separatory funnel overnight at 21–24°C. Top and bottom phases were then separated with the material at the interface being discarded. The PEG-rich top and the dextran-rich bottom phases were centrifuged at 12000 $\times g$ for 15 min to ensure that phase separation was complete. Top phase was removed leaving all remaining bottom phase and some top phase behind in the centrifuge tube. Bottom phase was pipetted out of the latter from the middle of the bottom phase being careful to keep residual top phase from entering the pipette.

Aliquots of the cell suspensions in PBS (see above) were diluted 1:1 (by weight) with top or bottom phase (for phase composition see Table 1), 4, 8 or 12% (w/w) dextran in PBS, or 5% (w/w) PEG in PBS for EPM measurements.

Cell microelectrophoresis was carried out as previously described [12]. A cylindrical chamber (Rank Brothers, Cambridge, UK) at 25 $\pm$ 0.2°C with transillumination [13] was used. Measurements were made using an applied voltage of 50.0 resulting in a field strength ranging from 2.28 to 2.58 V/cm depending on the suspension medium used. In each sample the rates of migration of ten RBC were obtained at the stationary level for the calculation of EPM in μm/s per

V/cm [13]. The rates of migration were observed in alternate directions.

2.8. Partitioning of erythrocytes in aqueous two-phase systems

The procedure used for partitioning cells has previously been described [6,14]. In short, the phase system that was to be used, at 21–24°C, was mixed and poured into 50 ml centrifuge tubes. These were centrifuged to speed phase separation and the top and bottom phase volumes were adjusted to be equal. The phase system was then mixed and 3 ml aliquots poured into 12 × 75 mm tubes. A 30 μl volume of the washed, packed cells to be partitioned was pipetted into a mixed 3 ml phase system which was then mixed again. The phase system was permitted to settle 15 min with the tubes in vertical position. An 0.8 ml aliquot was withdrawn from the middle of the top phase. The quantity of fresh or enzyme-treated RBC in the top phase was determined by lysing the cells and measuring hemoglobin absorbance at 540 nm [1,14]. The quantity of aldehyde fixed cells was established by electronic count (see below). The quantity of cells initially added to the partition tubes was similarly ascertained.

2.9. Electronic cell counting

Aliquots of fixed cells were counted on an Electrozone Celloscope (Particle Data, Elmhurst, IL) operating on the Coulter Principle. A 76 μm orifice tube was used.

2.10. Presentation of data

The EPM of the RBC in the different media were corrected to the viscosity of water. The EPM obtained in the different suspending media are presented, in each case, as the mean values ± S.D. with the number of individuals in parentheses. *P* values were obtained by one way analysis of variance (ANOVA).

Partitions are expressed as the quantity of cells in the top phase as a percentage of total cells added [1,14]. Mean values are presented ± S.D. with the number of experiments in parentheses.

3. Results and discussion

3.1. Electrophoretic mobilities of human and of rat young and old RBC in different suspending media and these cells' partitioning behavior

Human young and old RBC (obtained by use of a centrifugation method [10]) have been shown to have the same EPM in PBS [7] but to differ in EPM in a dextran solution in PBS [5]. Fig. 1, top indicates the viscosity-corrected EPM of human young and old RBC in dextran T500 solutions of different concentrations. Note that the viscosity-corrected EPM of young and old RBC differ significantly at higher dextran concentrations (e.g., 4 g%). The difference detected in dextran is limited neither to the molecular weight of the dextran originally used (T70 = M_r 70000 [5]) nor to dextran alone (Table 1, upper part). EPM differences, albeit to a smaller degree, can also be observed in PEG solutions as well as in the dextran-rich bottom or PEG-rich top phases (obtained from an aqueous two-phase system with composition as indicated in Table 1). These cells' behavior differs thereby from the interactions with polymers that pertain to RBC from Alzheimer patients and from normal individuals which display identical EPM in PBS but differ in EPM in PEG yet not in dextran solutions [12].

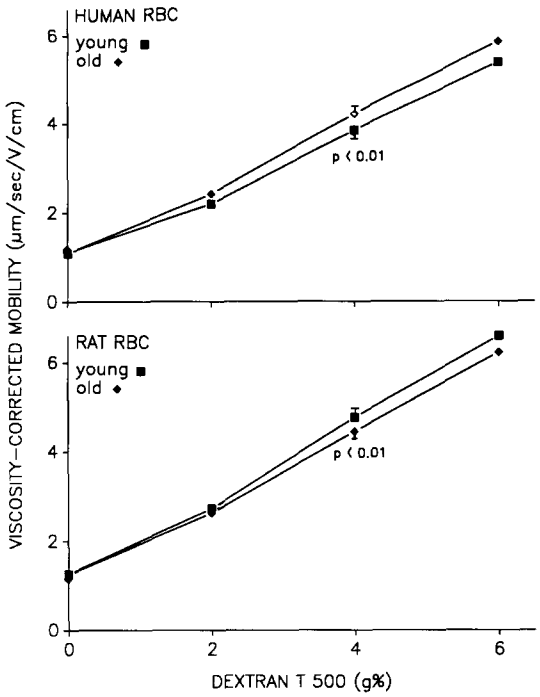

Fig. 1. Human young and old red blood cells (RBC), obtained by a centrifugal method, have the same electrophoretic mobilities (EPM) in PBS (see Table 1 and Ref. [7]). A difference in EPM between human young and old RBC can be detected in dextran (as previously reported by Nash et al. [5]) with the old cells having the higher mobility (top). Young and old rat RBC differ in mobility in PBS (see Table 1) and in dextran with the young cells having the higher mobility (bottom). See text for discussion and a comparison of these data with those obtained by partitioning the respective cells in a charge-sensitive dextran-PEG aqueous phase system.

Human young and old RBC cannot be differentiated by single-tube partitioning in charge-sensitive dextran-PEG phase systems. They can be shown to differ when a cell isotopic labeling technique is combined with multiple extraction (i.e., countercurrent distribution) in such systems [1]. The change in the partitioning of RBC as they age is then found to be in the same

direction as their relative EPM in dextran (i.e., human old RBC > young RBC). From these results it appears not only that cell electrophoresis in polymer solutions can, under appropriate circumstances, detect surface differences not detectable by electrophoresis in PBS but also that said detection can, as in the present case, be more sensitive than that attained by partitioning.

Rat young and old RBC (obtained as above) have different EPM in PBS (old RBC < young) as well as in dextran solution in PBS (Fig. 1; Table 1, lower part). These cells can be readily differentiated, even by single-tube partitioning, in charge-sensitive dextran-PEG phase systems and also have partitions which are directionally analogous to their relative EPM (i.e., rat old RBC < young RBC) [1]) – which sequence is oppposite to that associated with human young and old RBC (see above).

Whether correlations between cell EPM in polymer solutions and cell partitioning behavior 'within' fractionated cell populations (i.e., cell subpopulations such as young and old RBC) are, in general, better than among different cell populations (e.g., RBC from different species [9]) is, at present, subject to conjecture.

3.2. In vitro treatments of human young and old RBC and their effect on the cells' relative electrophoretic mobilities in different suspending media

The difference between the EPM of human young and old RBC detected in dextran solution (Fig. 1, Table 1) disappears when the young and old cells are first subjected to any of a number of in vitro treatments (i.e., chymotrypsin, trypsin, formaldehyde-fixation, formaldehyde-fixation followed by lipid extraction with chloroform/methanol or with ethanol alone, Table 2) indicating a differential effect of these treatments on the young and old cells. The fact that the EPM of treated (e.g., formaldehyde-fixed) cells in PBS and in dextran solution relative to untreated cells in the respective suspending medium can differ (Table 2) reemphasizes that the latter measurements can uncover surface alterations that escape detection by the former [9].

3.3. In vitro treatments of rat young and old RBC and their effect on the cells' relative electrophoretic mobilities in different suspending media and on their relative partitions

The last point in the previous section is reinforced by the findings with treated and untreated rat young and old RBC (Table 3). Here all treatments (i.e., chymotrypsin, trypsin, formaldehyde-fixation, formaldehyde-fixation followed by lipid extraction with chloroform/methanol or with ethanol alone), except the one with neuraminidase, eliminate EPM differences between these cells when measured in PBS. In dextran solution, however, EPM differences between young and old RBC are clearly in evidence not only with neuraminidase treated but also with formaldehyde-fixed cells and fixed cells extracted with chloroform/methanol. (The EPM of rat RBC subsequent to chymotrypsin- or trypsin-treatment are too low in the viscous dextran solution to be measured.)

Neuraminidase treatment of cells results in their reduced EPM, when compared to untreated cells, both in PBS and dextran solution while formaldehyde-fixation appears to affect the EPM of young cells, relative to the respective untreated cells, more in PBS than in dextran (Table 3).

In Table 4 the partitions in selected charge-sensitive dextran-PEG phase systems are given for rat fresh, neuraminidase-treated or formaldehyde-fixed young and old RBC. (The phase systems were chosen in a manner such that the polymer concentrations are the lowest at which, in each case, all cells are at the interface in the absence of a Donnan potential. A discussion of the rationale for phase selection can be found in Ref. [14]).

Table 4 shows that neuraminidase-susceptible sialic

Table 2
Viscosity-corrected electrophoretic mobilities [a] of untreated and variously treated human young and old red blood cells (RBC) in different suspending media

Treatment	PBS [b]		p	Dextran [c]		p
	young RBC	old RBC		young RBC	old RBC	
None	-1.08 ± 0.01 (9)	-1.08 ± 0.01 (9)	n.s.	-3.99 ± 0.13 (9)	-4.33 ± 0.13 (9)	$p < 0.01$
Chymotrypsin	-0.94 ± 0.02 (3)	-0.94 ± 0.03 (3)	n.s.	-3.61 ± 0.14 (3)	-3.62 ± 0.16 (3)	n.s.
Trypsin	-0.83 ± 0.03 (3)	-0.84 ± 0.03 (3)	n.s.	-3.08 ± 0.08 (3)	-3.08 ± 0.08 (3)	n.s.
Formaldehyde fixed	-1.08 ± 0.01 (3)	-1.09 ± 0.01 (3)	n.s.	-4.15 ± 0.04 (3)	-4.12 ± 0.03 (3)	n.s.
Fixed, $CHCl_3$/MeOH extracted	-1.07 ± 0.01 (3)	-1.06 ± 0.01 (3)	n.s.	-3.46 ± 0.04 (3)	-3.49 ± 0.07 (3)	n.s.
Fixed, EtOH extracted	-0.92 ± 0.01 (3)	-0.92 ± 0.01 (3)	n.s.	-3.15 ± 0.11 (3)	-3.14 ± 0.10 (3)	n.s.

[a] Data present the mean electrophoretic mobilities, EPM, (μm/s per V/cm), $\pm$S.D. with the number of experiments in parentheses.
[b] Phosphate-buffered saline (PBS) was composed of 0.15 M NaCl + 0.01 M Na-phosphate buffer (pH 6.8).
[c] EPM was measured in a 4% dextran T500 solution in PBS.

Table 3
Viscosity-corrected electrophoretic mobilities [a] of untreated and variously treated rat young and old red blood cells (RBC) in different suspending media

Treatment	PBS [b]		p	Dextran [c]		p
	young RBC	old RBC		young RBC	old RBC	
None	−1.29 ± 0.01 (8)	−1.23 ± 0.01 (8)	$p < 0.01$	−4.91 ± 0.08 (8)	−4.69 ± 0.05 (8)	$p < 0.01$
Chymotrypsin	−0.65 ± 0.01 (3)	−0.64 ± 0.00 (3)	n.s.	not determined		
Trypsin	−0.65 ± 0.01 (3)	−0.64 ± 0.01 (3)	n.s.	not determined		
Neuraminidase	−0.96 ± 0.02 (8)	−0.94 ± 0.02 (8)	$p < 0.02$	−3.85 ± 0.04 (8)	−3.76 ± 0.04 (8)	$p < 0.01$
Formaldehyde fixed	−1.26 ± 0.01 (5)	−1.25 ± 0.02 (5)	n.s.	−4.90 ± 0.04 (5)	−4.80 ± 0.03 (5)	$p < 0.01$
Fixed, $CHCl_3$/MeOH extracted	−1.23 ± 0.02 (5)	−1.21 ± 0.02 (5)	n.s.	−4.20 ± 0.01 (5)	−4.10 ± 0.02 (5)	$p < 0.01$
Fixed, EtOH extracted	−1.07 ± 0.02 (3)	−1.05 ± 0.04 (3)	n.s.	−3.99 ± 0.10 (3)	−3.96 ± 0.07 (3)	n.s.

[a] Data present the mean electrophoretic mobilities, EPM, (μm/s per V/cm), ±S.D. with the number of experiments in parentheses.
[b] Phosphate-buffered saline (PBS) was composed of 0.15 M NaCl + 0.01 M Na-phosphate buffer (pH 6.8).
[c] EPM was measured in a 4% dextran T500 solution in PBS.

acid is responsible neither for the partitioning differences between rat young and old RBC [15] nor for the differences in their EPM in PBS or dextran. Formaldehyde-fixation of rat young and old RBC, as has previously been found with their glutaraldehyde-fixed counterparts [15], also does not eliminate the partitioning difference between them while ethanol-extraction of glutaraldehyde-fixed cells does [15]. These and results with a number of other in vitro treatments have previously led to the conclusion that the partitioning difference between rat young and old RBC is most likely due to ganglioside-bound, neuraminidase-resistant membrane sialic acid [15].

Note that the partitions of rat young RBC are higher than those of old RBC just as the EPM of the corresponding young cells are higher than those of old cells (Table 4).

4. Concluding remarks

Cell partitioning behavior in dextran-PEG aqueous phase systems depends on surface properties that can neither be simply stated nor unequivocably defined. Still, cell partitioning in charge-sensitive dextran-PEG aqueous phase systems (i.e., those having a Donnan potential between the phases) has been thought to be, both on theoretical and practical grounds, more sensitive than electrophoresis to differences and changes in cell surface charge [1,16,17]. That is because parameters which contribute to partitioning behavior are related *exponentially* to the partition coefficient [17]; while in electrophoresis charge is related *linearly* to the EPM [13].

Thus, the fact that human young and old RBC, obtained by a centrifugation method, which have the same EPM in PBS [7], yet different EPM in a dextran solution in PBS [5], cannot be differentiated by single-tube partitioning in a charge-sensitive phase system (i.e., because the difference between them is too small) requires reexamination of the above-indicated premise. The young and old RBC can be shown to differ by partitioning, but only by combination of multiple-extraction (countercurrent distribution) and an isotope technique, and in a direction similar to their relative EPM in dextran (i.e., human old RBC > young RBC) [6]. Rat young and old RBC have different partitions (rat old RBC < young RBC) and different EPM in PBS

Table 4
Viscosity-corrected electrophoretic mobilities in dextran [a] and partitions in a dextran-poly(ethylene glycol) phase system [b] of fresh, neuraminidase treated, formaldehyde-fixed, and formaldehyde-fixed, lipid extracted rat young and old red blood cells (RBC)

Treatment	EPM [b]		p	Partitions [c]		p
	young RBC	old RBC		young RBC	old RBC	
None	−4.91 ± 0.08 (8)	−4.69 ± 0.05 (8)	$p < 0.01$	54 ± 4 (5)	29 ± 3 (5)	$p < 0.01$
Neuraminidase	−3.85 ± 0.04 (8)	−3.76 ± 0.04 (8)	$p < 0.01$	55 ± 1 (5)	28 ± 2 (5)	$p < 0.01$
Formaldehyde-fixed	−4.90 ± 0.04 (5)	−4.80 ± 0.03 (5)	$p < 0.01$	62 ± 3 (5)	31 ± 2 (5)	$p < 0.01$
Fixed, $CHCl_3$/MeOH extracted	−4.20 ± 0.01 (5)	−4.10 ± 0.02 (5)	$p < 0.01$	not determined		
Fixed, EtOH extracted	−3.99 ± 0.10 (3)	−3.96 ± 0.07 (3)	n.s.	not determined		

[a] Electrophoretic mobilities, EPM, (μm/s per V/cm), ±S.D. with the number of experiments in parentheses, were measured in a 4% dextran (Dx) T500 solution in phosphate-buffered saline (pH 6.8).
[b] Partitions are expressed as the quantity of cells in the top phase as a percentage of total cells added ± S.D. with the number of experiments in parentheses. All phase systems were charge-sensitive and contained 0.11 M sodium phosphate buffer (pH 6.8), 5% (w/w) Dx T500 and 4.7% (w/w) PEG 8000 for fresh RBC, 4.3% (w/w) PEG 8000 for neuraminidase treated RBC and 4.5% (w/w) PEG 8000 for formaldehyde-fixed RBC. Cells were partitioned in 3 gms of phase system adjusted to have equal volumes of top and bottom phase. Settling time was 15 min in the vertical position. Partitions were run with 30 μl packed RBC.

(also rat old RBC < young RBC) as well as in dextran solution in PBS. Thus, in these cases in which differences are found, cell partitioning in a charge-sensitive dextran-PEG aqueous phase system and cell electrophoresis in polymer solution reflect differences in surface properties that are, at least 'directionally', analogous in that increasing partitions and EPM are concomitant.

While cell EPM in PBS measures charge at the shear plane, the cell surface properties gauged by EPM measurements in polymer solutions can reflect other or additional parameters rendered detectable through cell interaction with polymer. Partitioning in charge-sensitive dextran-PEG phase systems can depend on still other or additional parameters [9]. Cell electrophoresis in polymer solutions can, in some cases, be more 'sensitive' than cell partitioning to differences in certain surface properties (e.g., those between human young and old RBC) and less 'sensitive' in, or incapable of, measuring others (e.g., beef RBC from different animals having different quantities of membrane sialic acid (or other surface charge components)) [9]. Hence, partitioning, cell electrophoresis in PBS and electrophoresis in appropriately selected polymer solutions represent distinct probes for cell surface differences. Whether, in cases where partitioning and electrophoresis (in PBS or in polymer) do actually measure the same surface properties, partitioning will reflect them more sensitively cannot be answered because we do not know when this occurs. A question that also remains is whether the exponential dependence mentioned above applies to the partitioning of particulates (see [18] and also, for discussion, [19]).

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References

[1] Walter, H. (1985) in Partitioning in Aqueous Two-Phase Systems. Theory, Methods, Uses, and Applications to Biotechnology (Walter, H., Brooks, D.E. and Fisher, D., eds.), pp. 327–376, Academic Press, Orlando.
[2] Walter, H. (1994) Methods Enzymol. 228, pp. 299–320.
[3] Walter, H., Selby, F.W. and Garza, R. (1967) Biochim. Biophys. Acta 136, 148–150.
[4] Walter, H., Tung, R., Jackson, L.J. and Seaman, G.V.F. (1972) Biochem. Biophys. Res. Commun. 48, 565–571.
[5] Nash, G.B., Wenby, R.B., Sowemimo-Coker, S.O. and Meiselman, H.J. (1987) Clin. Hemorheol. 7, 93–108.
[6] Walter, H. and Krob, E.J. (1983) Cell Biophys. 5, 205–219 and 301–306.
[7] Walter, H., Krob, E.J., Tamblyn, C.H. and Seaman, G.V.F. (1980) Biochem. Biophys. Res. Commun. 97, 107–113.
[8] Walter, H., Webber, T.J. and Krob, E.J. (1992) Biochim. Biophys. Acta 1105, 221–229.
[9] Walter, H. and Widen, K.E. (1994) J. Chromatogr. 668, 185–190.
[10] Murphy, J.R. (1973) J. Lab. Clin. Med. 82, 334–341.
[11] Walter, H., Krob, E.J. and Ascher, G.S. (1981) Biochim. Biophys. Acta 641, 202–215.
[12] Walter, H., Widen, K.E. and Read, S.L. (1993) Biochem. Biophys. Res. Commun. 194, 23–28.
[13] Seaman, G.V.F. (1975) in The Red Blood Cell, Vol. II (Surgenor, M.D., ed.), pp. 1135–1229, Academic Press, New York.
[14] Walter, H. and Larsson, C. (1994) Methods Enzymol. 228, 42–63.
[15] Walter, H., Krob, E.J., Pedram, A., Tamblyn, C.H. and Seaman, G.V.F. (1986) Biochim. Biophys. Acta 860, 650–661.
[16] Brooks, D.E., Sharp, K.A. and Fisher, D. (1985) in Partitioning in Aqueous Two-Phase Systems. Theory, Methods, Uses, and Applications to Biotechnology (Walter, H., Brooks, D.E. and Fisher, D., eds.), pp. 11–84, Academic Press, Orlando.
[17] Albertsson, P.-Å. (1986) Partition of Cell Particles and Macromolecules, Wiley-Interscience, New York.
[18] Walter, H., Fisher, D. and Tilcock, C. (1990) FEBS Lett. 270, 1–3.
[19] Walter, H., Johansson, G. and Brooks, D.E. (1991) Anal. Biochem. 197, 1–18.