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Cell partitioning in two-polymer aqueous phase systems and cell electrophoresis in aqueous polymer solutions Red blood cells from different species

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Abstract

A correlation, with some exceptions, between the partitioning behavior of red blood cells (RBCs) from different species in charge-sensitive dextran–poly(ethylene glycol) (PEG) aqueous phase systems and their relative electrophoretic mobilities (EPMs) in phosphate-buffered saline (PBS) has previously been reported. This relationship has now been further probed by carrying out RBC electrophoresis in media (*i.e.*, dextran-rich bottom or PEG-rich top phases) more closely approximating the environment in which RBC partitioning takes place to see whether a better correlation would ensue.

The ratios of viscosity-corrected EPMs of different species' RBCs in (diluted) dextran-rich *or* PEG-rich phases/EPMs of the respective species' RBCs 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 RBCs. There is a general tendency for EPMs in any of the tested media to correlate with both the cells' relative partition ratios as well as with their relative EPMs in one of the other media. However, examination of the behavior of different species' RBCs taken two species at a time indicates that their relative EPMs in any two suspending media or in one suspending medium and partitioning often differ.

Thus, both the cell partition ratio and the cell EPMs obtained in polymer media must, at least in some cases, reflect surface properties other than or in addition to the charge reflected by EPM measurements in PBS or saline. Cell electrophoresis in polymer solutions thereby provides an additional parameter for discriminating between surface properties of certain closely related cell populations.

1. Introduction

Partitioning in dextran–poly(ethylene glycol) (PEG) aqueous phase systems is an established method for the separation and characterization of biomaterials including cells [1–3]. The separation of cell populations and their fractionation into subpopulations by partitioning depends on the interaction of the cells' surface properties with the physical properties of the phase system. Certain salts (*e.g.*, alkali phosphates) give rise to a Donnan potential between the phases thus rendering the system "charge-sensitive". Surface charge-related properties can then, under appropriate conditions [4,5], be a major determinant of the cells' partitioning behavior.

The current investigation on cell electrophoretic mobilities (EPMs) in aqueous polymer solutions (*i.e.*, dextran-rich bottom or PEG-rich top phases) was initiated to determine whether the previously reported correlation between the partition ratios of red blood cells (RBCs) from

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different species and their relative EPMs in isotonic salt solution [6] would be improved by carrying out measurements in an environment more closely approximating that in which RBC partitioning takes place. Such a result could be envisaged because cell–polymer interactions affect both the cell partition ratio [7] and the cell EPM in polymer solutions [8,9]. Cell electrophoresis in polymer solutions was found, however, to provide an additional parameter, and one distinct not only from partitioning but also from EPM measurements in phosphate-buffered saline (PBS) [8,9], for discriminating between surface properties of cells.

2. Experimental

2.1. Reagents

Dextran T500 (lot No. 01 06905) was obtained from Pharmacia LKB (Piscataway, NJ, USA). PEG 8000 (Carbowax 8000) was from Union Carbide (Long Beach, CA, USA). All salts 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 below and in Table 1 were prepared as previously described [4,5]. PBS contained 0.15 *M* NaCl + 0.01 *M* sodium phosphate buffer, pH 6.8.

2.3. Collection of blood from different species

Blood from nine different species was collected in either acid–citrate–dextrose (ACD) anticoagulant solution or in citrate vacutainers. With ACD the ratio used was 10 ml of blood to 3 ml of ACD. Human blood was obtained by venipuncture from presumably hematologically normal individuals; rhesus monkey blood came from the Oregon Regional Primate Research Center (Beaverton, OR, USA); dog blood was from the femoral vein; and rat and mouse blood were obtained by heart puncture. Beef blood came from Shamrock Meats (Vernon, CA, USA); while horse, pig and sheep blood were from the Animal Resource Facility, University of California, Irvine, CA, USA. Erythrocytes were used within one week of collection.

2.4. Viscosity determinations of suspending media used in EPM measurements

The viscosities of the various suspending media were estimated by means of an Ostwald

Table 1
Viscosity-corrected electrophoretic mobilities of red blood cells from different species in three different suspending media

Species	PBS[a]	Top phase[b]	Bottom phase[b]
Beef	−0.89 ± 0.03 (8)	−1.32 ± 0.05 (8)	−2.90 ± 0.14 (8)
Dog	−1.26 ± 0.02 (4)	−2.14 ± 0.03 (4)	−5.48 ± 0.03 (4)
Horse	−1.24 ± 0.01 (4)	−2.17 ± 0.01 (4)	−5.75 ± 0.04 (4)
Human	−1.08 ± 0.01 (26)	−1.82 ± 0.03 (13)	−4.55 ± 0.07 (26)
Rhesus monkey	−1.03 ± 0.01 (3)	−1.71 ± 0.00 (3)	−4.35 ± 0.01 (3)
Mouse	−1.19 ± 0.01 (4)	−2.14 ± 0.04 (4)	−5.26 ± 0.07 (4)
Pig	−0.81 ± 0.02 (4)	−1.32 ± 0.02 (4)	−3.41 ± 0.05 (4)
Rat	−1.25 ± 0.01 (4)	−1.97 ± 0.01 (4)	−5.00 ± 0.02 (4)
Sheep	−1.15 ± 0.01 (4)	−1.84 ± 0.02 (4)	−4.64 ± 0.04 (4)

Data present the mean electrophoretic mobilities ($\mu m\ s^{-1}\ V^{-1}\ cm$) ± S.D. with the number of experiments in parentheses.
[a] PBS was composed of 0.15 *M* NaCl + 0.01 *M* sodium phosphate buffer, pH 6.8.
[b] Top and bottom phases were from a system containing 5% (w/w) dextran T500, 3.5% (w/w) PEG 8000, 0.15 *M* NaCl and 0.01 *M* sodium phosphate buffer, pH 6.8. Top phase is PEG-rich and bottom phase is dextran-rich. Top and bottom phases were diluted 1:1 with the indicated RBC suspension in PBS followed by measuring cell EPMs.

viscometer immersed in a tank thermostated at 25 ± 0.2°C.

2.5. EPM measurements on erythrocytes in various suspending media

RBCs were washed three times with PBS and a suitable cell aliquot was, finally, suspended in PBS.

Phase systems, at 21–24°C, were mixed and permitted to settle in a separatory funnel overnight. 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 12 000 *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 (w/w) with top or bottom phase of the phase system (for phase composition see Table 1).

Cell microelectrophoresis was carried out in a cylindrical chamber (Rank Brothers, Cambridge, UK) at 25 ± 0.2°C with transillumination [10]. 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 RBCs were obtained at the stationary level for the calculation of EPMs in $\mu m\ s^{-1}\ V^{-1}$ cm [10]. The rates of migration were observed in alternate directions.

2.6. Partitioning of erythrocytes in aqueous two-phase systems

The procedure used for partitioning cells has previously been described [7]. The partitioning data in Figs. 1 and 2 were taken from ref. 11 except for rhesus monkey RBCs. The partition ratio for the latter was determined (together with those of a couple of other species' RBCs as reference) and interpolated. The phase system was composed of 5% (w/w) dextran, 4% (w/w) PEG and 0.11 *M* sodium phosphate buffer, pH 6.8, shorthand "5:4 #1" [4,5]. It was selected (for detailed procedure and discussion see ref. 5) so that the polymer concentrations were the lowest at which, in the absence of a Donnan potential, RBCs from the species, shown in the top parts of Figs. 1 and 2, would not partition but would remain at the interface. In this manner partitioning in the presence of a Donnan potential can be considered to be predominantly charge-related [5].

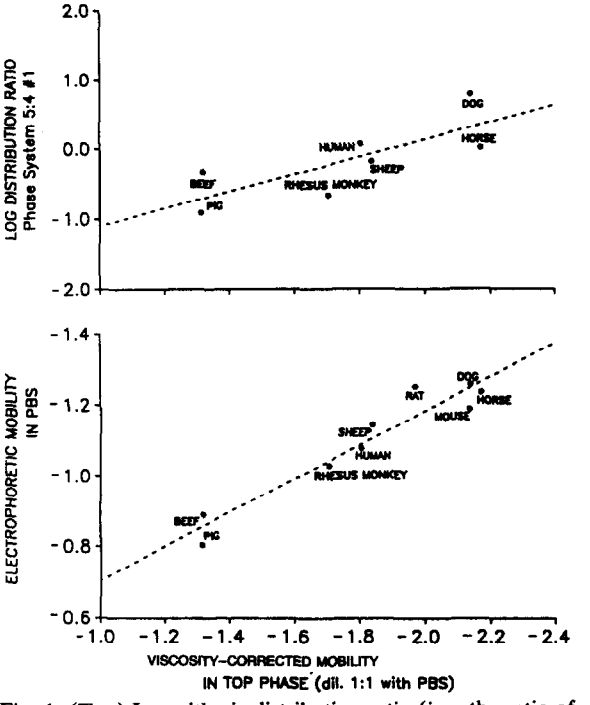

Fig. 1. (Top) Logarithmic distribution ratio (*i.e.*, the ratio of the number of cells in top phase divided by the number of cells at the interface plus bottom phase, taken from ref. 11) in a charge-sensitive dextran–PEG phase system plotted against the viscosity-corrected, relative electrophoretic mobility (EPM) in a top phase (diluted 1:1 with PBS) of erythrocytes (RBCs) from a number of different species. (Bottom) EPMs in PBS of RBCs from different species plotted against their viscosity-corrected EPMs in diluted top phase. For composition of phase systems see text and Table 1. Mobilities in $\mu m\ s^{-1}\ V^{-1}$ cm.

The partitioning data for beef RBCs plotted are those belonging to partition class 1 (deemed the most suitable comparison because (see ref. 12) these cells have the lowest partition ratio but

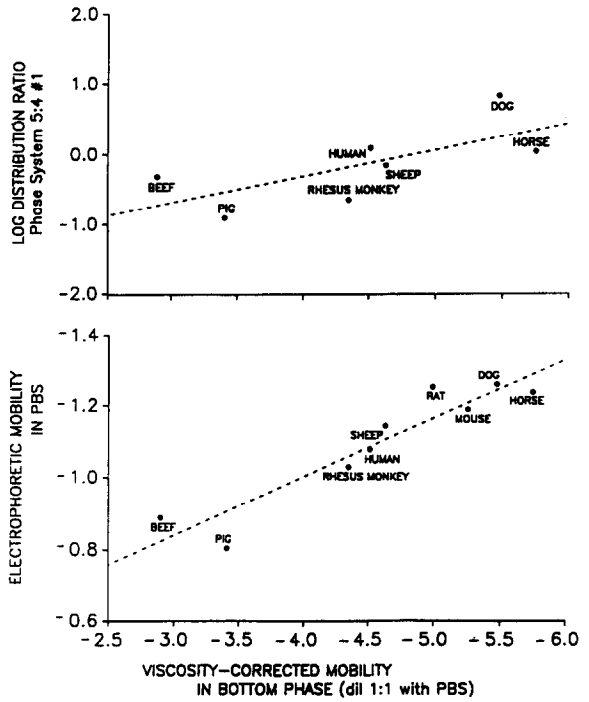

Fig. 2. (Top) Logarithmic distribution ratio (*i.e.*, the ratio of the number of cells in top phase divided by the number of cells at the interface plus bottom phase, taken from ref. 11) in a charge-sensitive dextran–PEG phase system plotted against the viscosity-corrected, relative electrophoretic mobility (EPM) in a bottom phase (diluted 1:1 with PBS) of erythrocytes (RBCs) from a number of different species. (Bottom) EPMs in PBS of RBCs from different species plotted against their viscosity-corrected EPMs in diluted bottom phase. For composition of phase systems see text and Table 1. Mobilities in $\mu m\ s^{-1}\ V^{-1}$ cm.

the same EPM in saline as the other two beef RBC partition classes).

2.7. Presentation of data

The EPMs of the RBCs in the different media were corrected to the viscosity of water. The EPMs obtained in the different suspending media are presented, in Table 1, as the mean ± S.D. with the number of individuals or animals in parentheses. p values (Table 2) were obtained by one-way analysis of variance (ANOVA).

Partitions are expressed in terms of log distribution ratios (*i.e.*, the ratio of number of cells in top phase/number of cells in bottom phase + interface).

3. Results and discussion

3.1. Electrophoresis of RBCs from different species in PBS and in selected polymer solutions

In Table 1 we present the viscosity-corrected EPMs of RBCs from a number of species in three different suspending media (PBS, diluted PEG-rich top phase and diluted dextran-rich bottom phase of the system indicated). The cell mobilities, in top and bottom phase, are plotted (in lower parts of Figs. 1 and 2, respectively) against their respective EPMs in PBS. It is apparent that the EPMs of some species' RBCs differ in PBS but not in top phase (*e.g.*, beef, pig) and that the relative mobilities of RBCs from some species' reverse depending on whether PBS or top phase is the suspending medium used (*e.g.*, rat and mouse) (Fig. 1, bottom). Comparison of Figs. 1 and 2 further indicates that the relative EPMs of some of the different species' RBCs are differentially affected by the top and bottom phases (*e.g.*, beef and pig RBCs which have the same EPM in top but differ in mobility in bottom phase).

The significance, in terms of p, of the difference between the ratios of one species' RBCs EPM in top phase/EPM in PBS *or* EPM in bottom phase/EPM in PBS to those of another species' ratios is indicated in the top and bottom parts, respectively, of Table 2. Note that there is a significant difference in most cases but that a difference in one phase for a given comparison of two species' RBCs does not necessarily mean that a difference will be found in the other (*e.g.*, dog and mouse).

It thus appears that the EPMs of cells in polymer solutions depend on the interaction of the particular polymer used with the particular cell examined. Since the EPMs of different cell populations can be differentially affected by the same polymer, and that of some cell populations can be differentially affected by different polymers, the nature of the surface properties re-

Table 2
Significance (p values) of difference between ratios of electrophoretic mobilities of red blood cells from different species compared in top phase/phosphate-buffered saline (top) *or* bottom phase/phosphate-buffered saline (bottom)

	Beef	Dog	Horse	Human	Rhesus monkey	Mouse	Pig	Rat	Sheep
Beef		$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$
Dog	$p<0.01$		$p<0.01$	N.S.	$p<0.01$	$p<0.01$	$p<0.02$	$p<0.01$	$p<0.01$
Horse	$p<0.01$	$p<0.01$		$p<0.01$	$p<0.01$	N.S.	$p<0.01$	$p<0.01$	$p<0.01$
Human	$p<0.01$	$p<0.01$	$p<0.01$		N.S.	$p<0.01$	N.S.	$p<0.01$	$p<0.01$
Rhesus monkey	$p<0.01$	$p<0.05$	$p<0.01$	N.S.		$p<0.01$	N.S.	$p<0.01$	$p<0.01$
Mouse	$p<0.01$	N.S.	$p<0.01$	$p<0.01$	$p<0.01$		$p<0.01$	$p<0.01$	$p<0.01$
Pig	$p<0.01$	N.S.	$p<0.01$	N.S.	N.S.	$p<0.05$		$p<0.05$	N.S.
Rat	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$		$p<0.01$
Sheep	$p<0.01$	$p<0.01$	$p<0.01$	N.S.	$p<0.01$	$p<0.01$	$p<0.05$	N.S.	

N.S. = Not significant.

flected, though unknown, need not be related directly to the cell surface charge estimated by cell electrophoresis in PBS or saline.

3.2. Relationship between the EPMs of RBCs from different species in polymer solutions and the cells' relative partition ratios in a charge-sensitive dextran–PEG phase system

The mobilities in top and bottom phase are plotted (in upper parts of Figs. 1 and 2, respectively) against the partition ratios of the cells obtained in charge-sensitive phase system 5:4 #1 [4,11]. It is apparent that some species' RBCs differ in partition ratios but have the same EPM in top phase (*e.g.*, beef and pig) while others show the reverse behavior (*e.g.*, beef and sheep, human and horse) (Fig. 1, top).

The partitions of RBCs from different species in a charge-sensitive dextran–PEG aqueous phase system tend to display an overall correlation with the cells' relative EPMs in top or bottom phase (as has previously been found for EPMs measured in saline [4–6]). However, while such a tendency exists, examination of the behavior of RBCs from two species at a time reveals that these may have the same mobility and differ in partition ratio or the reverse. Thus, just as in the cases of EPM measurements in PBS and in top or bottom phases (see above), partitioning in a charge-sensitive phase system can reflect yet other surface properties than those indicated not only by the cell EPM in PBS but also those discriminated by cell EPM in top or bottom phases.

3.3. Relationship between the EPMs of beef RBCs from different animals in polymer solutions and the cells' relative partition ratios in a charge-sensitive dextran–PEG phase system

It is known from previous work that cell partitioning in charge-sensitive dextran–PEG phase systems and cell electrophoresis in saline (or PBS) do not necessarily reflect the same surface charge [12]. This can be illustrated by the fact that beef RBCs from different animals have the same EPM but fall into three partition classes: classes comprised of cells having low (class 1), intermediate (class 2) or high (class 3) partition ratios [12]. The classes reflect, to some extent, the quantity of charge-bearing groups on the respective cells' surfaces [12]. EPM in saline measures charge at the shear plane while partitioning may be able to gauge charge also deeper into the membrane.

The EPMs of beef RBCs presented in Table 1 were obtained from eight animals of which three belonged to partition class 1, four to class 2 and one to class 3. The EPMs in dextran-rich bottom

and PEG-rich top phases of these RBCs did not discriminate among the partition classes to which the cells belonged. This result reinforces the conclusion that partitioning in charge-sensitive phase systems and EPM measurements even in top and bottom phases can reflect different cell surface properties.

4. Conclusions

While both the (red blood) cell partition ratio obtained in a charge-sensitive dextran–PEG phase system and the (red blood) cell EPMs measured in polymer media are surface charge-related they must, at least in some cases, also reflect surface properties other than or in addition to the charge determined by EPM measurements in PBS or saline. Thus, the possibility that polymer solutions serve to amplify cell EPM differences too small to detect in PBS (see ref. 9) seems unlikely since EPM differences in polymer solutions can also be qualitatively unlike those measured in PBS.

Cell electrophoresis in polymer solutions provides a useful and distinct parameter with which to test for differences in surface properties between closely related cell populations. This is borne out by the EPM differences found between Alzheimer and normal RBCs [9] and between human young and old RBCs [8] in appropriately selected polymer solutions, differences which cannot be observed in PBS.

5. Acknowledgements

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