MEMBRANE SURFACE PROPERTIES REFLECTED BY CELL PARTITION IN TWO-POLYMER AQUEOUS PHASES

Classes of beef erythrocytes having different membrane lipid, charge and affinity for a ligand

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1. Introduction

Partition behavior of cells and membranes in two-polymer aqueous phase systems of different polymer and ionic composition and concentration reflects differences in membrane surface properties (e.g., charge, lipid, receptor) [1]. Recently it has been shown that, as judged by partition, human erythrocyte ghosts and membrane vesicles have surface properties that differ from those of the red cells from which they are derived [2]. Beef erythrocyte membranes are, next to human red cell membranes, perhaps the most studied as models of membrane composition and structure. In the work reported here the partition coefficient of beef red cells from different animals has been determined in phase systems that measure different surface properties. It was found that beef erythrocytes fall into numerous classes that differ not only with respect to membrane charge-associated properties (as has previously been shown [3,4]) but also with regard to lipid-related characteristics and affinity for a ligand.

Aqueous solutions of dextran and of polyethylene glycol when mixed above certain concentrations give rise to immiscible, liquid two-phase systems [5] with a polyethylene glycol-rich top and a dextran-rich bottom phase. Such systems can be buffered and rendered isotonic and are suitable for the partition of cells and membranes [1,5]. Although both dextran and polyethylene glycol are non-ionic polymers certain salts (e.g., phosphates) have different affinities for the two phases [6]. An electrostatic potential difference between the phases results [7] and cells or membranes added to such systems will partition according to surface charge-associated properties [8]. If the salt chosen is one which has essentially equal affinity for the two phases (e.g., NaCl) there will be no electrostatic potential difference between them and most cells will accumulate in the interface (i.e., they will not partition). When, under these circumstances, the polymer concentrations are reduced (resulting in a concomitant decrease in interfacial tension [9]) the interaction of cells with polyethylene glycol will prove adequate to 'pull' some of the cells out of the interface and into the top phase [10] and a measurable partition coefficient results. The latter has been shown, at least in the case of erythrocytes from different species, to give an excellent correlation with the ratio of membrane poly/mono-unsaturated fatty acids [10].

If a phase system with high polymer concentration and no electrostatic potential difference between the phases is used the cells, as stated above, will be in the interface. Incorporation of a polymer-ligand (e.g., the ester of polyethylene glycol and palmitic acid, polyethylene glycol-palmitate) which itself favors one of the phases (in this case the top phase), causes those cells which interact with the ligand to be extracted from the interface into the phase containing the ligand. An affinity partition method results [11–14].

Beef erythrocytes from different animals have been partitioned in charged phase systems, uncharged phase systems with low polymer concentration, and

uncharged phases with higher polymer concentration containing a small quantity of polyethylene glycol-palmitate. Numerous classes of beef erythrocytes could thus be differentiated.

2. Materials and methods

2.1. *Beef erythrocytes*

Beef blood was collected from a local packing house using acid–citrate–dextrose (ACD) solution as anticoagulant and used in the experiments to be described on the same day. The red cells were washed 3 times with aqueous isotonic salt solution and once in the top phase of the system in which they were to be partitioned (see below).

2.2. *Preparation of phase systems*

Three systems were used. They were prepared as previously described [1] and had the following compositions: System 1 – 5% (w/w) dextran T500, lot No. 3936 (Pharmacia Fine Chemicals, Piscataway, NJ), 4% (w/w) polyethylene glycol 6000 (PEG, tradename 'Carbowax', Union Carbide, New York) and 0.11 M Na-phosphate buffer, pH 6.8; System 2 – 4.5% (w/w) dextran, 3.6% (w/w) PEG, 0.15 M NaCl and 0.01 M Na-phosphate buffer, pH 6.8; System 3 – 5% (w/w) dextran, 3.5% (w/w) PEG, 0.15 M NaCl, 0.01 M Na-phosphate buffer, pH 6.8 and 0.0005% (w/w) polyethylene glycol-palmitate (a generous gift of Dr G. Johansson [15]).

2.3. *Partition of beef erythrocytes*

Red cells were partitioned as previously described [1] in each of the above-indicated phase systems. After addition of a known aliquot of cells to a phase system, the latter was mixed and permitted to settle by the clock. A 20 min settling time with tubes in vertical position was used for phase system 1; a 20 min settling time with the tubes in horizontal position [1] for phase system 2; and a 7 min settling time with the tubes in horizontal position for phase system 3. Because phase system 2 is very close to the critical point (i.e., the polymer concentrations below which a homogeneous solution is obtained) the glass-stoppered tubes were allowed to settle in a water bath set at 23°C. The times chosen for phase settling of the various systems reflect differences due to polymer concentrations (see refs [1,5]).

2.4. *Presentation of data*

The partition coefficient of cells is defined as [1]: Quantity of cells in the top phase (percent of total cells added).

3. Results and discussion

We have previously reported that when beef erythrocytes from different animals are partitioned in a dextran–polyethylene glycol phase system containing phosphate (i.e., a system with an electrostatic potential difference between the phases, see system 1, table 1) they fall into three classes. Cells with the lowest partition coefficient (Class I) release far less sialic acid when treated with neuraminidase or with trypsin than do cells having the highest partition coefficient (Class III) [4]. All beef erythrocytes have the same electrophoretic mobility, however, indicating that the membrane charge measured by cell electrophoresis and that measured by partition is not necessarily the same [4]. Appropriate manipulation of phase system composition yields, in addition to (or instead of) information on membrane charge-associated properties, an insight into membrane lipid-related characteristics [10]. By substituting NaCl for phosphate in the phases the electrostatic potential difference between the phases is reduced almost to zero [7,10]. When, in addition, the polymer concentrations are diminished, resulting in a lowered interfacial tension [9], the interaction between the polyethylene glycol and cell surface is adequate to pull cells out of the interface and into the polyethylene glycol-rich top phase. The partition coefficient which results (see phase system 2, table 1) correlates well with the cells' ratio of membrane poly/monounsaturated fatty acids [10]. The correlation is probably a consequence of the less dense packing of membrane lipids with an increase in the indicated ratio [16] permitting a greater intercalation of the polyethylene glycol with the membrane surface [10].

Table 1 shows that beef erythrocytes belonging to class I in the charged phase system (system 1) split into at least three classes in phase system 2. Class III beef erythrocytes gives rise to one partition class in phase system 2 which appears to have a lower partition coefficient than any of the beef erythrocytes belong-

Table 1
Partition coefficient[a] of beef erythrocytes from different animals in charged[b] and in uncharged two-polymer, aqueous phase systems measuring different surface properties

Erythrocyte 'charge' classes	Erythrocyte 'lipid' classes		
	Phase system 1[c]	Phase system 2[c]	Phase system 3[c]
Class I	32 ± 9 (28)	10 ± 3 (22)	30 ± 10 (9)
		21 ± 2 (4)	72 ± 8 (4)
		55 ± 12 (2)	94 ± 4 (11)
Class II	61 ± 10 (4)	9 ± 7 (4)	
Class III	83 ± 7 (12)	3 ± 1 (12)	6 ± 3 (5)
			24 ± 7 (2)

[a] Partition coefficient is defined as the quantity of cells in the top phase (% total cells added) and is given ± SD with the number of animals in parentheses
[b] See Walter, Tung, Jackson and Seaman [4]
[c] Phase system 1 contained 5% dextran, 4% polyethylene glycol (PEG) and 0.11 M Na-phosphate buffer, pH 6.8. Phase system 2 contained 4.5% dextran, 3.6% PEG, 0.15 M NaCl, 0.01 M Na-phosphate buffer, pH 6.8. Phase system 3 contained 5% dextran, 3.5% PEG, 0.15 M NaCl, 0.01 M Na-phosphate buffer, pH 6.8, and 0.0005% polyethylene glycol-palmitate. Partitions were done at 23°C.

ing to class I. It is clear that beef erythrocyte classes based on membrane charge-associated properties, as measured by partition, display additional surface heterogeneities related to membrane lipid-related parameters.

Beef red cells were also partitioned in a phase system containing a higher polymer concentration than phase system 2, no electrostatic potential difference between the phases and a small quantity of the polymer-ligand, polyethylene glycol-palmitate. The latter has been shown to interact with red cells in a species-specific [13,14], cell-age [14] and membrane-lipid dependent manner [12–14]. Again class I beef erythrocytes gives rise to at least three classes of cells with low, intermediate and high partition coefficients (system 3, table 1). It is important to note that while the majority of beef red cells belonging to class I had the lowest partition coefficient in phase system 2 (i.e., 22 out of 28 animals), in phase system 3 the number of animals having the highest and lowest partition coefficients are about equally divided (i.e., 9 and 11 animals, respectively).

It follows that the interaction of the cell membrane with the palmitoyl moiety of polyethylene glycol-palmitate does not reflect the same properties observed in phase system 2. Hence the partition coefficients in phase system 3 reflect still additional classes of beef red cells beyond those revealed by partition in phase system 2.

Partition of class III erythrocytes in phase system 3 also shows at least two classes of red cells whereas partition in phase system 2 showed only one class. The latter may, however, be due to the fact that the partition coefficient in phase system 2 is so low that detection of subclasses is not feasible in this system.

In conclusion, we have found that partition of beef erythrocytes from different animals in two-polymer aqueous phase systems reveals that the cells fall into numerous classes. These reflect not only membrane charge but also membrane lipid composition and affinity for a ligand (i.e., palmitate). We have, so far, not noted any analogous partition behavior of red blood cells from different animals belonging to other mammalian species.

Our results should be considered in studies on membrane composition and structure being pursued with 'beef' erythrocyte membranes from different animals.

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