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Short communication

Partitioning of high-density lipoproteins in charge-sensitive two-phase systems

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Abstract

Aqueous polymer two-phase systems characterized by a difference in the electrical potential between the upper and the lower phase (charged systems) are useful tools for the detection of changes in the surface charge and hydrophobicity of high-density lipoproteins (HDL). While the large particle size of low-density lipoproteins (LDL) leads to accumulation at the interface, the smaller diameter and the higher surface charge density of the native HDL particles allows partitioning without aggregation at the interface. Charged two-phase systems can be used to check the native state of HDL samples. Moreover, these systems would be suitable for investigating the hydrophobicity and surface charge of modified HDL.

1. Introduction

Two-phase partitioning in aqueous polymer solutions represents a sensitive method for investigation of the surface properties of both dissolved macromolecules and suspended molecular complexes, cell organelles as well as intact cells [1–3].

By using buffer solutions containing phosphate and chloride, a difference in potential can be created between the two phases, dextran and polyethylene glycol (PEG), such that the top phase is positive relatively to the bottom phase (charge-sensitive systems) [4]. The partition coefficient largely depends on the size, surface charge and hydrophobicity of the particles and the magnitude of the difference in potential [5].

Although these techniques were often used to detect changes in surface properties of cellular and subcellular particles of high fragility [6,7], no reports are known on the partitioning of high-density lipoprotein, either for native or for chemically modified species.

Systematic investigations of the partitioning of charged particles were carried out with liposomes [6–9]. From these studies, it seems that especially the structure of the exterior surface of molecules and particles determines their partitioning behaviour. Molecules projecting outwards from the particle surface are most accessible to the phases [8]. This however, complicates the interpretation of the experimental results obtained with biological objects of high complexity.

Alteration in the lipoprotein structure by oxidation resulting from lipid peroxidation is cited as the main source of chemical modifications of low-density lipoproteins (LDL) and high-density

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lipoproteins (HDL) and the development of atherogenic processes [10–12]. Changes in the surface charge of modified lipoproteins [13] can be detected by measurement of the electrophoretic mobility of lipoproteins from patients with atherogenic and cardiovascular diseases [14]. Alteration in the physico-chemical properties of the particle surface of LDL and HDL results in the loss of their native character [15,16].

The interaction between lipoproteins and cells is based on the specificity of the interactions between exposed groups in apo-proteins and the molecular structures of the cell surface. Therefore, two-phase partitioning techniques have the potential to become a useful tool for the investigation of cell–particle interactions.

In previous papers we have discussed the application of two-phase systems for the characterization of the surface properties of LDL. Partitioning experiments were carried out both with native and chemically modified LDL [17–19]. In the present paper the partitioning of native HDL in charged and uncharged two-phase systems is studied. It was the aim of this study to investigate the following aspects: (i) the dependence of the partitioning of HDL in an aqueous dextran/PEG two-phase system on the magnitude of the potential difference, (ii) the influence of the ionic strength on the partitioning in charge-sensitive ($\Delta\varphi > 0$) and non-charge-sensitive systems ($\Delta\varphi = 0$) and (iii) the influence of the polymer concentration on the partitioning of HDL.

2. Experimental

2.1. Materials

High-density lipoprotein was obtained from freshly taken plasma of healthy volunteers by sequential ultracentrifugation according to Havel et al. [20]. After separation of very low-density lipoprotein (VLDL) and LDL the remaining plasma was adjusted with solid NaBr to a density of 1.215 g/ml, covered with a solution of the same density and centrifuged at 14°C and 100 000 *g* for 48 h (centrifuge UP 65, rotor 8 × 11). After centrifugation HDL was concentrated as a yellow layer at the top of the tube. HDL was recentrifuged and dialyzed for 24 h against Tris-HCl buffer (10 m*M* Tris-HCl, 100 m*M* NaCl, 1 m*M* Na_2EDTA, pH 7.4) at 4°C. The buffer was changed twice during dialysis. The HDL concentration was determined using the procedure of Lowry et al. [21].

All samples were checked for purity by horizontal agarose-gel electrophoresis or non-denaturing PAGE [22]. Gels with a gel concentration of 3.5 or 5.0% in the case of PAGE and 1.2% in the case of agarose-gel electrophoresis were used.

HDL diluted with dialysis buffer to a concentration of 2.4 mg protein per millilitre was stored at 4°C and was used within three days after dialysis to be sure that the samples were native.

Buffers used for preparation of polymer mixtures are given in Table 1. The stock solutions of dextran and PEG were filtered before using.

The HDL sample as well as the buffer solutions were filtered through a Sartorius membrane filter with a pore size of 0.1 μm.

Substances for PAGE were obtained from BioRad Laboratories (Munich, Germany), agarose was a product of Serva (Heidelberg, Germany).

If not stated otherwise all reagents were purchased from Fluka (Neu-Ulm, Germany).

Table 1
Composition of phosphate buffers used for preparation of charged two-phase systems

Buffer system[a]	Phosphate (mol/l)	NaCl (mol/l)	$\Delta\varphi$[b] (mV)
I	0.01	0.15	0.0
II	0.03	0.12	0.1
III	0.05	0.09	0.4
IV	0.09	0.03	1.7
V	0.11	0.00	2.7

[a] Buffers II, III and IV were obtained by mixing stock solutions of buffer I (non-charge sensitive) and buffer V (high-charge sensitive). I: 5.34 m*M* KH_2PO_4, 4.66 m*M* Na_2HPO_4, 0.15 *M* NaCl, pH 6.8. II: 58.75 m*M* KH_2PO_4, 51.23 m*M* Na_2HPO_4, pH 6.8.

[b] Data from Ref. [23].

2.2. Methods

Partitioning experiments were carried out in PEG/dextran systems with different polymer concentrations containing pH 6.8 sodium phosphate buffer.

We used different buffer compositions for the preparation of low-potential (non-charge sensitive) and high-potential (charge-sensitive) systems [6]. For all partitioning experiments 7.2 g of a polymer mixture containing special concentrations of PEG and dextran were prepared gravimetrically.

Two-phase systems were prepared by mixing stock solutions of dextran T 500 (20%, w/w; Pharmacia, Uppsala, Sweden) and PEG 6000 (40%, w/w; Ferak, Berlin, Germany) with buffer solution and distilled water. These systems, carefully prepared by shaking the mixture in 10-ml graduated tubes at room temperature, were incubated with 300 μl of dialysis buffer containing HDL (2.4 mg protein per ml). For controls the same volume of HDL-free buffer was added to the two-phase polymer system. HDL or HDL-free dialysis buffers were distributed in the mixture by 40 inversions of the glass tubes. The turbid mixture was allowed to stand over night. After phase separation and equilibration the two immiscible polymer phases are characterized by a sharp interface.

The total volume V and the particular volumes V_b and V_t of the bottom and the top phase were measured in control samples after phase separation of the mixture.

Immediately after mixing of the lipoprotein with the polymer solution, a volume of 1 ml was removed from the center of the turbid mixture for estimation of the total concentration. After phase separation again a 1-ml volume was removed from the center of each phase to determine the top and bottom phase concentration.

Before taking a sample from the bottom phase, the remainder of the top phase has to be removed completely to exclude contamination of the bottom phase.

The UV absorption of the proteins in HDL was used as a measure of the lipoprotein concentration. The samples were diluted 1:2 (w/w) with the buffer used for preparation of the special two-phase system before measuring the absorbance (E) at 280 nm.

By calibration it was checked that the measured absorbance was a linear function of the concentration for the top, bottom and mixed phase. Because the slope of the curves was equal in all cases, the partitioning coefficient could be calculated as the ratio of the top- and bottom-phase concentration C [1].

If the total volume V as well as the particular volumes V_t and V_b are known a calculation of the amount of HDL in both the top and the bottom phases is possible. The equation

$$(V \cdot C)_{\text{phase}} / (V \cdot C)_{\text{mixture}} = (V \cdot E)_{\text{phase}} / (V \cdot E)_{\text{mixture}}$$

then allows to express the HDL-partitioning as a percentage of the total amount of HDL added to the two-phase system.

Since the polymer solutions absorb UV light, all measurements were carried out with blank controls.

Samples and controls were prepared in duplicate. The given values for the partitioning coefficient and the relative HDL content in the top and bottom phases are mean values resulting from measurement of two independent samples. The error in the calculation of the partitioning coefficients or in the estimation of the relative mass distribution between the two phases is 20% at most using the experimental techniques as described above.

Gel electrophoresis was carried out with the Multiphor II Electrophoresis System (Pharmacia).

Measurement of the UV absorbance at 280 nm was performed with a Spectrophotometer U-2000 (Hitachi, Tokyo, Japan). Each sample was measured four times under stirring.

3. Results and discussion

Two-phase systems with unchanged composition (5 wt.% PEG, 5 wt.% dextran) but with a variable potential difference between the top and bottom phases can be obtained by mixing the standard buffer solutions I and V (see for compo-

sition Table 1). The decrease in the concentration of phosphate ions is then accompanied by an increase in the concentration of chloride ions. The asymmetric partitioning of phosphate into the bottom phase (dextran-rich) is the main source for the potential produced [6]. The concentration of phosphate was varied from 0.01 to 0.11 mol/l and that of chloride from 0.00 to 0.15 mol/l. The potential difference $\Delta\varphi$ changes from about 3 mV to about zero. The dependence of the potential difference on the concentration of phosphate and chloride was measured according to Reitherman et al. [23].

Since in charge-sensitive systems the top-phase potential is relatively more positive than the bottom-phase potential, partitioning of the negatively charged HDL particles into the top phase is favoured .

Increasing values for $\Delta\varphi$ result in an increase of HDL in the top phase by ca. 30%, whereas the bottom phase shows a corresponding decrease. The partitioning coefficient raises from $k = 0.278$ (buffer I) to $k = 1.00$ (buffer V) (Fig. 1).

The influence of the polymer composition on HDL partitioning was investigated in the high-charge sensitive two-phase system. An increase in the concentration of one of the polymers (PEG or dextran) results in a decrease of the partitioning coefficient for HDL. An increase in the concentration of PEG from 5% to 7% with a constant dextran concentration of 5% has a stronger effect on the decrease of the partitioning coefficient than a corresponding increase in dextran concentration in systems with a constant PEG content (Fig. 2).

Besides an electrostatic influence on the partitioning of particles, a specific polymer–particle interaction has to be taken into account. In systems with $\Delta\varphi = 0$ the dextran-rich bottom phase is favoured for HDL partitioning. Probably hydrogen bonding between the polymer chains (dextran) and molecules constituting the particles (the apo-protein of HDL) is mainly responsible for the polymer–particle interaction and the preferred enrichment of HDL in the bottom phase. Higher amounts of dextran enhance this interaction and cause a progressive attraction of HDL into the dextran-rich more hydrophilic bottom phase.

With a constant amount of dextran, an increase in the PEG concentration reduces the amount of HDL partitioned into the upper phase. The stronger decrease of the partitioning coefficient as a function of increasing PEG content compared with the decrease observed when the concentration of dextran is increased,

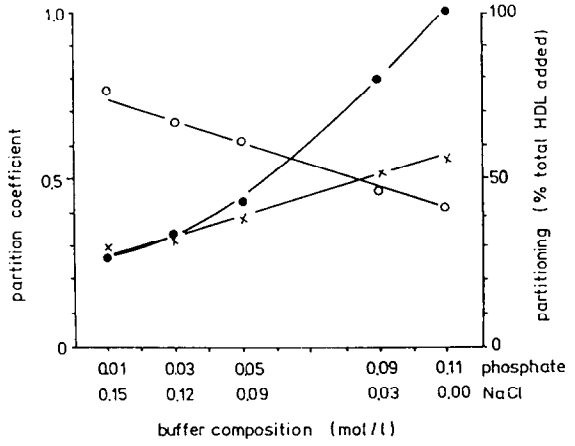

Fig. 1. Dependence of the partitioning coefficient (●) and relative partitioning of HDL in the top phase (×) and bottom phase (○) on the buffer composition (pH 6.8).

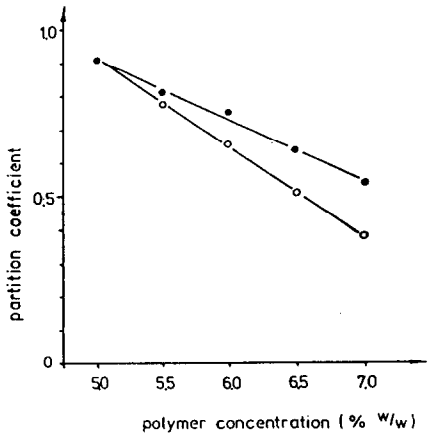

Fig. 2. Dependence of the partitioning coefficient of HDL in the high-charge sensitive system (0.11 mol/l PBS, pH 6.8) on the concentration of dextran (●) and PEG (○). The concentration of each other polymer remains constant at 5% (w/w).

indicates a stronger influence of the more hydrophobic polymer on the partitioning of HDL.

A simultaneous increase in the concentration of both polymers causes an additive effect on HDL partitioning. Fig. 3 shows the very strong decrease of the partitioning coefficient as a function of the polymer concentration. The ability of the upper and lower phases for particle accumulation becomes more and more unequal such that the partitioning coefficient drops from 1 to lower values.

In charge-sensitive systems the large particle size of LDL leads to a strong accumulation of aggregated lipoproteins at the interface independent of the ionic strength of the buffer used (results not given). While partitioning of LDL in such systems raises a lot of experimental problems, the smaller particle size of HDL combined with a higher charge density on the particle surface allows partitioning without particle aggregation. In accordance with its higher surface charge, HDL shows a higher electrophoretic mobility in agarose gels than LDL.

Freshly prepared HDL in its native state shows partitioning without any aggregation and accumulation at the interface. In contrast, samples with partly denaturated HDL or samples contaminated with LDL, as well as samples with an increased sample age show partitioning with aggregation at the interface. From these results it can be seen that the interface accumulation represents a sensitive criterion for the purity and the native state of HDL preparations. The behaviour of HDL partitioning corresponds to the properties of charged liposomes in aqueous polymer two-phase systems [9].

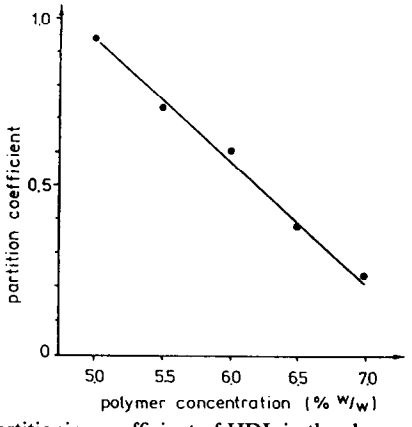

Fig. 3. Partitioning coefficient of HDL in the charge-sensitive system (0.11 mol/l PBS, pH 6.8) as a function of total polymer concentration for equal concentrations of PEG and dextran.

The results obtained permit the conclusion that charge-sensitive aqueous polymer two-phase systems would be suitable for the investigation of surface charge and hydrophobicity of modified high-density lipoprotein.

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