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Relationship between the protein surface hydrophobicity and its partitioning behaviour in aqueous two-phase systems of polyethyleneglycol–dextran

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Abstract

In order to develop possible correlations to predict partioning behaviour of proteins, five mammalian albumins (goat, bovine, equine, human and pig ones) with similar physico-chemical properties (molecular mass and isoelectrical point) were chosen. Evaluation of the relationship between hydrophobicity and partitioning coefficient (Kr) in polyethylenglycol–dextran (PEG–DxT500) systems formed by polyethyleneglycols of different molecular mass (3350, 6000 and 10,000) was investigated by estimating relative surface hydrophobicity (So) with a fluorescent probe, 1 anilino-8-naphthalene sulfonate. No relationship between Kr and So was found for systems formed by PEG3350, while aqueous two-phase systems with PEG6000 and PEG10,000 gave better correlations. The results obtained may be explained on the basis of an increase in the interaction between the latter PEGs and the protein due to their higher hydrophobic character which increases as the PEG molecular mass does so. In this way, systems with PEGs of higher molecular mass give the highest resolution to exploit hydrophobicity in partitioning.

Keywords: Protein surface hydrophobicity; Partitioning; Aqueous two-phase systems; Polyethyleneglycol–dextran

1. Introduction

Aqueous two-phase systems (ATPSs) are widely used in the biotechnology of the isolation and purification of proteins. These systems are formed by mixing aqueous solutions of two flexible chain polymers or one polymer and a salt above a defined concentration. In the ATPSs, both the top and the bottom phases contain 80–90% (w/w) of water and can be buffered; their interfacial tension is low, thus giving rise to phases that are gentle to most biological materials [1,2]. The partitioning of proteins in aqueous two-phase systems mainly depends on the physico-chemical properties of the protein such as isoelectric point, surface hydrophobicity, molecular mass and other medium variables like as polymer molecular mass, pH, added salt type and its concentration. By controlling these factors, the selective partitioning and recovery of a target protein can be expected. This is the base of the application of this system as a method of isolation and purification of proteins, the most used aqueous biphasic system being the one formed by polyethyleneglycol and dextran.

Factors and mechanisms that govern protein partitioning in ATPSs are poorly understood. Several theories about this process were derived from polymer solution classical thermodynamics [3,4]. However, many areas remain to be further studied. A more complete description of the nature of the interaction between globular proteins and random coil phase polymers is needed to understand the mechanism of protein partitioning [5].

In particular, models which examine the influence of protein surface properties such as surface hydrophobicity must be developed. The partition coefficient (Kr) has been empirically found to depend on several factors which act roughly

Abbreviations: PEG3350, PEG6000 and PEG10,000, polyethyleneglycols of average molecular masses: 3350, 6000 and 10,000; BSA, bovine serum albumin; PSA, pig serum albumin; GSA, goat serum albumin; HAS, human serum albumin; ESA, echine serum albumin; DxT500, dextran of average molecular mass 500,000; ANS, 1 anilino-8-naphthalene sulfonate; So, protein surface hydrophobicity; ATPS, aqueous two-phase system

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independently as [5]:

$$\mathrm{Kr} = K_{\mathrm{hphob}} K_{\mathrm{el}} K_{\mathrm{size}} K_{\mathrm{polymer}} \quad (1)$$

where K_{polymer} is the contribution from phase-forming polymer characteristics and K_{hphob}, K_{el} and K_{size} express the effect of hydrophobicity, charge and size of the protein on the overall partition coefficient. Several authors [6] attempted to correlate the physico-chemical properties of a protein to its partition coefficient in ATPSs using model proteins with clear differences in their molecular masses, isoelectrical points and hydrophobicities. The individual effect of protein hydrophobicity could not be analyzed because by selecting proteins with different hydrophobicity, their molecular masses and isoelectric points were also different.

The aim of this work is to study the effect of surface hydrophobicity as a single parameter, on the protein partitioning in ATPSs of polyethyleneglycol–dextran. This was achieved by selecting albumins from different mammalian species which differ from each other in a few aminoacids but have similar molecular masses and isoelectric points. Since surface hydrophobicity effects on protein partitioning behaviour depend on the type of system used, ATPSs formed by dextran and polyethyleneglycols of different molecular mass were selected.

2. Materials and methods

2.1. Chemicals

Serum albumins of different mammalian species: bovine (BSA), pig (PSA), goat (GSA), human (HSA) and equine (ESA); polyethyleneglycols of the following average molecular masses: 3350 (PEG3350); 6000 (PEG6000) and 10,000 (PEG10,000); dextran of average molecular mass 500,000, (DxT500) and 1-anilino-8 naphthalene sulfonate (ANS) were purchased from Sigma Chemical Co. and used without further purification. All the other reagents were of analytical grade.

2.2. Preparation of the aqueous biphasic systems

To prepare the ATPSs, stock solutions of the phase components PEG 40% (w/w), DxT500 20% (w/w), and buffer sodium phosphate 40 mM (pH 7.40) were mixed in order to obtain a total system composition of approximately PEG 8% (w/w) and DxT500 15% (w/w), according to binodal partition diagrams reported by Zaslavsky [7]. Tie lines from the binodal diagrams were selected in order to make both polymer concentrations as similar as possible at the top and bottom phases between the systems with different PEG Mw. Low-speed centrifugation to speed up phase separation was used after thorough gentle mixing of the system components, then 1 ml of each phase was mixed to reconstitute several two-phase systems in which the protein partition was assayed.

2.3. Determination of the protein partition coefficient (Kr)

The partition coefficient was defined as:

$$\mathrm{Kr} = \frac{[P]_{\mathrm{top}}}{[P]_{\mathrm{bottom}}} \quad (2)$$

where $[P]_{\mathrm{top}}$ and $[P]_{\mathrm{bottom}}$ are the equilibrium concentrations of partitioned protein in the PEG (top) and DxT500 (bottom) enriched phases, respectively. Partition coefficient of the protein was determined by dissolving increasing amounts of protein (final protein concentrations were between 5 and 15 μM) in pre-formed ATPSs containing 3 ml of each equilibrated phase. The protein aliquots added to the systems varied from 1 to 10 μl, the change of the total volume of each phase being negligible. After mixing by inversion for 1 min and leaving it to settle for at least 30 min, the systems were centrifuged at low speed for the two phase separation. Samples from separated phases were withdrawn and, after dilution, the protein content in each phase was determined by measuring the native fluorescence emission at 345 nm while exciting at 280 nm. Equally diluted samples from identical phase systems without protein were used as blanks, which were prepared in parallel. A plot of $[P]_{\mathrm{top}}$ versus $[P]_{\mathrm{bottom}}$ showed a linear behaviour, Kr value being its slope. Fluorescence measurements were performed in a Jasco FP770 spectrofluorophotometer.

2.4. Measurements of the protein surface hydrophobicity (So)

The relative surface hydrophobicity of the protein was determined by applying the optical method previously reported [8]. Stock solutions of 8 mM ANS and 3 mM albumin were prepared in 50 mM phosphate buffer (pH 7.4). Aliquots of the protein were added to a sample containing 3 ml of buffer solution or the corresponding equilibrated phase (PEG enriched phase) and 7.5 μl of ANS solution. The final ANS concentration was 20 μM while the protein concentration varied from 0 to 10 μM. The fluorescence emission intensities at 470 nm (while exciting at 360 nm) for each protein concentration were measured at a Jasco FP 770 spectrofluorometer. Under the above mentioned experimental conditions (with ANS excess), the initial slope (So) of the fluorescence intensity versus protein concentration plot has been shown to be correlated to the relative surface hydrophobicity of the protein.

2.5. Effect of PEG presence on chemical stability of proteins

Chemical stability of the protein was assayed by measuring the native fluorescence emission of the protein at 340 nm (while exciting at 280 nm) in media of increasing urea and constant PEG concentrations. We assumed that the urea (denaturant agent) induced unfolding due to the formation of

a complex between the unfolded protein (U) and the denaturant agent (D) according to the previously demonstrated equilibrium equation [9]:

$$U + nD \rightleftarrows UDn$$

Analysis of the data was performed assuming a two-state denaturation model [10] and the unfolded fraction of protein, α, was calculated as:

$$\alpha = \frac{F_N - F_i}{F_N - F_U} \quad (3)$$

where F_N and F_U are the fluorescence of the native (in absence of denaturant) and the unfolded states (at high denaturant concentration) of albumin, respectively; and F_i is the fluorescence of the protein at i denaturant concentration, An α versus the denaturant agent concentration (urea) plot was fitted by using a least squares nonlinear method, and the free energy change of unfolding, ΔG_U was obtained by applying the equation:

$$\Delta G_U = -RT \ln K \quad (4)$$

where K is the unfolding equilibrium constant, calculated as $K = \alpha/(1-\alpha)$.

2.6. Quenching of the albumin native fluorescence in the presence of PEG

The quenching of the tryptophan fluorescence residues of the albumin was carried out by titration with acrylamide in the presence and absence of PEG. The data were analysed using the following modified Stern Volmer equation [11]:

$$\frac{F_o}{F_i} = [1 + k_{SV}[Q] \exp([Q]V')] \quad (5)$$

where F_o and F_i are the fluorescence of the protein at 345 nm, while exciting at 295 nm in the absence and presence of quencher respectively, k_{SV}, the Stern Volmer constant being related to the lifetime of the fluorophore and the bimolecular quenching constant, $[Q]$ the quencher concentration and V' expressed as:

$$V' = \frac{vN_A}{1000} \quad (6)$$

where N_A is the Avogadro number and v is the volume of the "sphere of action", i.e, the sphere within which the probability of immediate quenching is unity, and whose radius is only slightly larger than the sum of the radii of the fluorophore and quencher.

3. Results

In order to study the effect of protein surface hydrophobicity on its partitioning behaviour in ATPSs of PEG–DxT500, serum albumins of different mammalian species were selected. These proteins differ from each other in a few aminoacids but have similar molecular masses and isoelectric points. Since some of the different aminoacids are exposed to the solvent, the serum albumins used have different surface hydrophobicity properties.

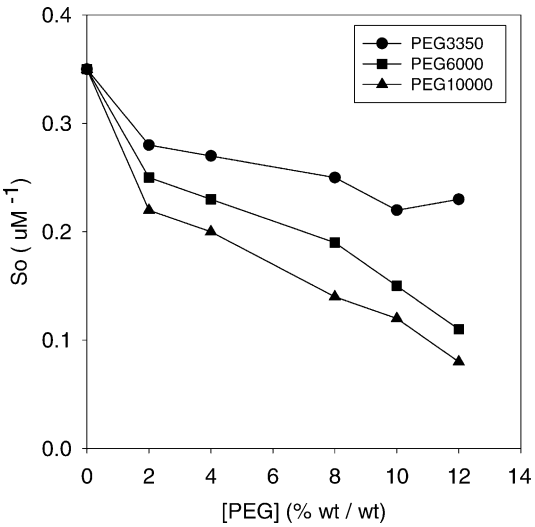

Fig. 1. Effect of PEG concentration on the relative surface hydrophobicity for BSA. Medium of sodium phosphate buffer 50 mM, pH 7.4, temperature: 20 °C.

According to previous reports, the protein partitioning behaviour is very sensitive to surface hydrophobicity properties in ATPSs of PEG–DxT500 [12]. For these systems, the partition coefficient is more affected by PEG than by DxT500 molecular mass changes, therefore, the experiments using different PEGs of molecular masses: 3350, 6000 and 10,000 were carried out.

3.1. Effect of PEG presence on surface hydrophobicity of albumins

Fig. 1 shows the So values obtained for BSA at increasing PEG concentrations for ATPSs formed by DxT500-PEGs of different molecular masses: 3350, 6000 and 10,000. A net decrease in So at increasing PEG concentrations is observed, this effect being strongly marked for PEGs of higher molecular mass. On the other hand, a limit So value for PEG3350 is reached, which suggests the presence of a saturation process linked to the PEG concentration. Similar behaviours were observed for the other albumins assayed (data not shown). Taking into account the dramatic enhancement of the ANS fluorescence when it is placed in a non-polar environment such as the binding sites of a protein [8], these results are in agreement with the presence of an atmosphere of PEG molecules in the surroundings of protein hydrophobic surface. This atmosphere prevents the ANS binding. The magnitude of the observed So decrease is correlated to the hydrophobic character of PEG molecules, which increases as PEG molecular mass does.

Fig. 2 shows the So values for BSA at different temperatures. Both in buffer and PEGs media an increase in So is

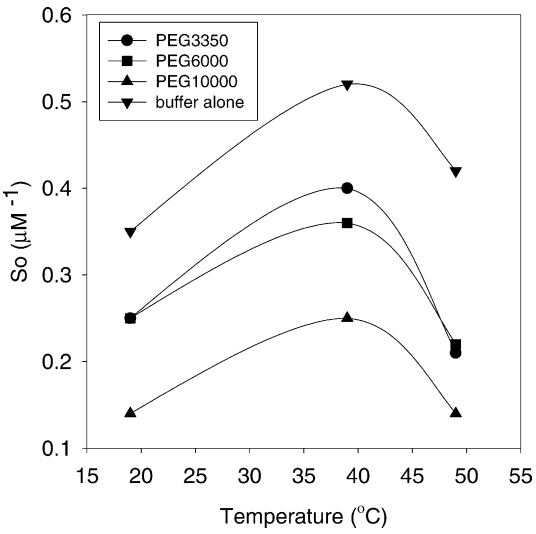

Fig. 2. Effect of temperature and molecular mass of PEG on the relative surface hydrophobicity (So) of BSA in buffer and PEG 8% (w/w). Sodium phosphate buffer 50 mM, pH 7.4, temperature: 20 °C.

observed between 20 and 40 °C, while the opposite effect is observed at higher temperatures. As it was pointed out above, the ANS binding to protein implies the rupture of hydrogen bonds of structured water around the hydrophobic surface on protein and ANS molecules. An increase in temperature between 20 and 40 °C facilitates this mechanism; therefore, the ANS binding and the So increase. When temperature increases above 40 °C, a gradual exposure to polar solvent of protein hydrophobic aminoacids occurs, thus producing a diminution of both ANS quantum extinction coefficient and So value. The latter effect is more noticiable in presence of PEGs. Each ethylene group in the PEG chain interacts with 14 water molecules, thus inducing the structured water formation [13]. Temperature increase (above 40 °C) causes the water molecules to lose their order around the hydrophobic polymer surface, facilitating the interaction protein-PEG and displacing the ANS from protein.

Fig. 3 shows the So obtained for different albumins in the presence of PEGs at 20 °C. All albumins showed a similar behaviour in the PEGs media. The observed So values showed the following sequence:

$$PSA > GSA \geq ESA > BSA \geq HSA$$

These results allow us to obtain an albumin surface hydrophobicity scale where BSA and HSA seem to have less hydrophobical surface than the other three albumins. At 49 °C a dramatic decrease in So is observed (data not shown), adopting similar values for the totality of the albumins assayed.

3.2. Albumin partitioning in PEG–DxT500 aqueous two-phase systems

Fig. 4 shows the partition coefficient of the albumins in ATPSs formed by PEGs of different molecular mass at 20 °C. Large differences between the obtained Kr values which followed the sequence:

$$GSA > ESA > PSA \geq BSA > HSA$$

can be seen for PEG6000 and PEG10,000 systems while an inversion between ESA and GSA positions was observed for PEG 3350. ATPSs of PEG10,000 and PEG6000 seemed to be very sensitive to albumin surface hydrophobicity changes. The comparison of So with Kr sequences shows a good correlation except for PSA. This behaviour may be explained taking into account the fact that the hydrophobic character of PEG increases as its molecular mass does so, thereby, increasing its ability of solubilizing hydrophobic substances.

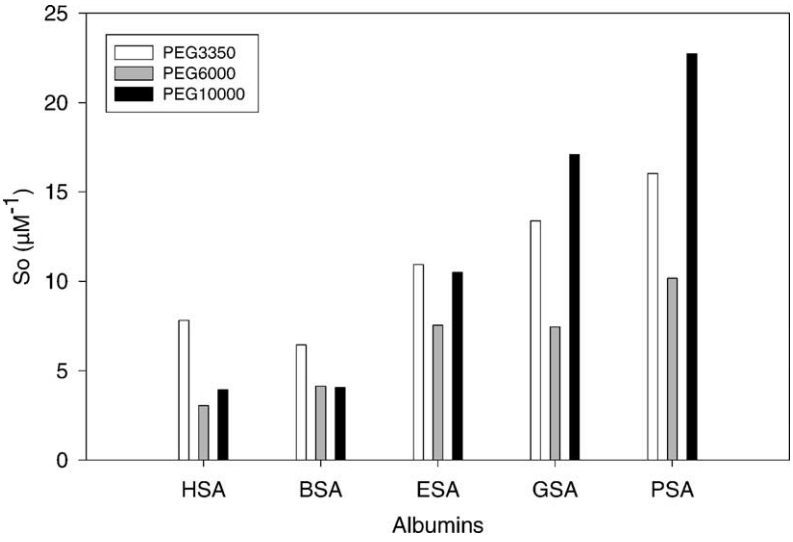

Fig. 3. Relative surface hydrophobicity for albumins of different species determined in PEG of different molecular mass. Temperature: 20 °C, sodium phosphate buffer 50 mM, pH 7.4.

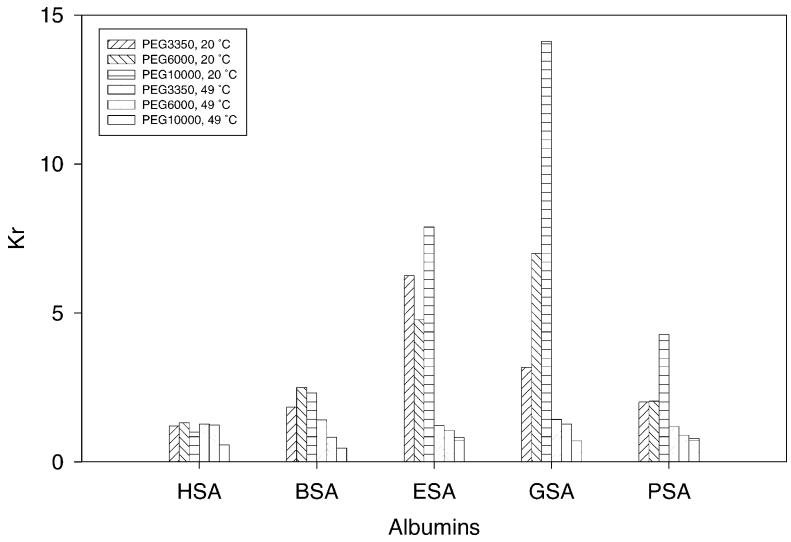

Fig. 4. Partition coeffcient for albumins of different species in aqueous two-phase systems formed by PEG 8%– DxT500 15%, sodium phosphate buffer 50 mM, pH 7.40, temperatures: 20 and 49 °C, respectively.

Zaslasvky et al. [15] studied the partition behaviour of several hydrophobic substances in PEG–dextran systems. They demonstrated that the partitioning behaviour depends on the relative hydrophobicity of the compound (i.e. the number of carbons present in the aliphatic chain, surface hydrophobicity) and found a linear relationship between the hydrophobic character of the partitioned substance and its partitioning coefficient. A significant increase in Kr for GSA was observed as PEG molecular mass increases, while the observed variations in Kr were lower and did not follow any apparent rule for the other albumins.

Fig. 4 also shows a significant decrease in the observed Kr value for the totality of albumins at 49 °C in comparison with those observed at 20 °C. For systems of a given PEG molecular mass, similar Kr values were obtained for the different albumins (between 0.4 and 1). At high temperature (49 °C), the protein unfolding equilibrium (N-U) is displaced to the unfolded form which possesses a larger hydrophobic surface than the native one. The similar achieved Kr values may suggest that the albumins from different species are interacting in the same way with PEG molecules, due to the similar hydrophobic surface extention (So) showed by the totality of albumins assayed at 49 °C.

On the other hand, the increase of molecular mass of PEG induced a decrease in the Kr value in agreement with the behaviour observed for many other proteins in PEG/Dx systems [14]. When polyethyleneglycol molecular weight increases, its exclusion from protein surface prevails, thus pushing the protein to the dextran enriched phase (bottom).

3.3. Quenching of the albumin tryptophan fluorescence by acrylamide in the presence of PEGs

The fluorescence quenching of a fluorophore by a quencher is a powerful technique that gives information about the quencher accessibility to the fluorophore. This technique has widely been used to determine the number of tryptophan residues accessible to the solvent that a protein possesses. Moreover, this technique gives information about any modification at the tryptophan microenvironment [11] induced by a cosolute presence.

A linear Stern Volmer plot (F_o/F_i versus [Q]) is generally indicative of a single class of fluorophores, all of them equally accesible to the quencher. In our work, positive deviations from the Stern Volmer plot were obtained. We assumed that there existed a sphere of volume v, "*sphere of action*", within which the probability of quenching is unity. This phenomenon frequently occurs when the extent of quenching is large and is intuitively interpreted as an instantaneous fluorophore quenching by the immediately adjacent quencher molecules. In this way, the analysis of the quenching process was made by using a modified Stern Volmer equation (see Eq. (5)). Since the Stern Volmer (k_{SV}) constant is directly related to the diffusion coefficient of the quencher which depends both on its radius and on the media viscosity, we selected the V' values (proportional to sphere action volume) in order to analyze the effect of PEG presence at the protein domain near the accessible tryptophans.

Fig. 5 shows an increase in the V' values in PEG presence for the totality of albumins assayed at 20 °C. These results suggest that the presence of PEG separates the quencher molecules from the fluorophore, thus making the access to the tryptophan difficult. These findings are in agreement with the presence of an atmosphere of PEG molecules in the surroundings of protein surface. V' values obtained for the different albumins were of a similar magnitude except for HSA which showed the lowest V'value. The human serum albumin has only one tryptophan residue which is located in the interior of the protein. Its quenching behaviour can be interpreted as the result of a highly dynamic protein matrix, which fluctuates on the nanosecond timescale so as to permit free diffusion of an uncharged quencher such as oxygen,

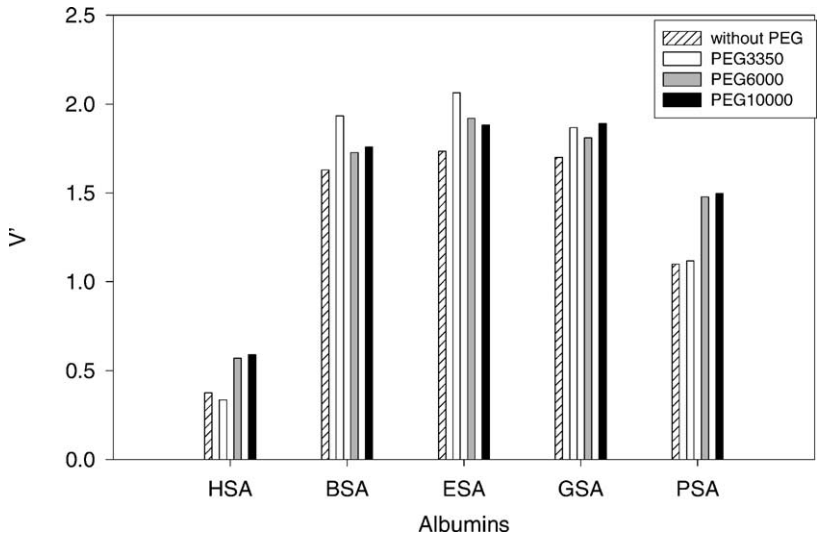

Fig. 5. V' values (proportional to sphere action volume) for albumins of different species. Sodium phosphate buffer 50 mM, pH 7.40, temperature: 20 °C, [albumin] = 10 μM, [PEG] = 8% (w/w), λ_{exc}: 295 nm, λ_{em}: 345 nm.

and acrylamide into the protein structure, thus reaching the fluorophore neighbours to a closer distance [11].

3.4. PEG effect on the thermodynamical stability of albumin

Protein unfolding is an equilibrium process where the compact native form of a macromolecule exposes its buried hydrophobic residues to the solvent and becomes an extended random coil molecule. The protein chemical denaturation may be explained on the basis of an unfolding equilibrium displacement to the unfolded state due to the binding of the denaturant agent (urea) to peptide bonds, which are more exposed as a protein unfolds. Pace [10] has found a linear relationship between ΔG_U and urea concentration according to the following equation:

$$\Delta G_U = \Delta G_W - m[\text{urea}] \quad (7)$$

where ΔG_W is the protein free energy change of unfolding at zero urea concentration (but in PEG presence), and m is the dependence of free energy on the denaturant concentration ($\partial G_U/\partial[\text{urea}]$) which is proportional to the difference in the solvent-exposed surface area between the denatured and native states (ΔA) [16]. The m value depends on the number and type of groups which are exposed to the solvent when the protein unfolds. The chemical unfolding of serum

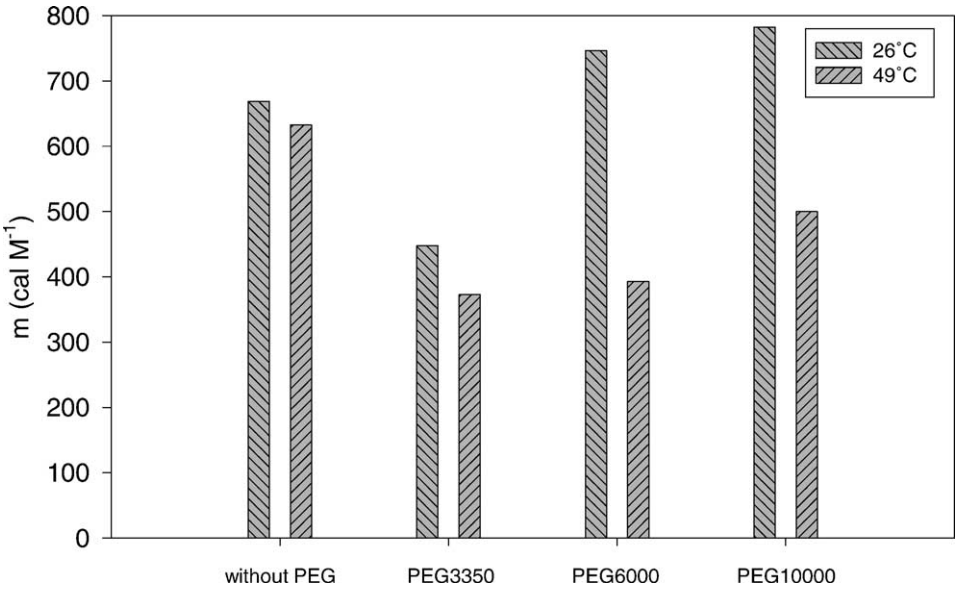

Fig. 6. Dependence of the m value ($\partial\Delta G_U/\partial[\text{urea}]$) on the PEG molecular mass at 26 and 49 °C for BSA. Sodium phosphate buffer 50 mM, pH 7.4, [BSA] = 6 μM, [PEG] = 8% (w/w), λ_{exc}: 280 nm, λ_{em}: 340 nm.

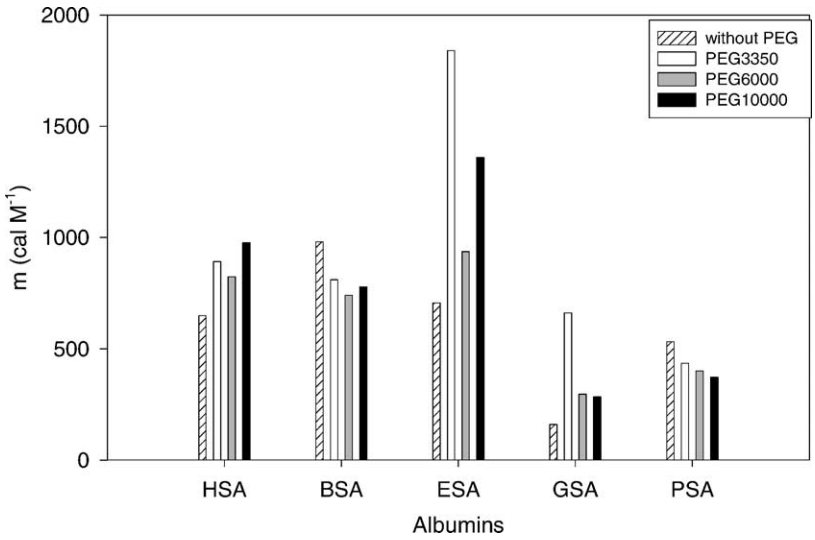

Fig. 7. Slope of ΔG_U vs. urea concentration plot (m) for albumins of different species. Sodium phosphate buffer 50 mM, pH 7.4, temperature: 26 °C, [albumin] = 6 μM, [PEG] = 8% (w/w), λ_{exc}: 280 nm, λ_{em}: 340 nm.

albumins by urea was analysed in the absence and presence of different PEGs at 7% (w/w). By plotting ΔG_U values (calculated from Eqs. (3) and (4)) versus urea concentration, linear behaviours were observed. Fig. 6 shows the m values yielded for the BSA in different PEGs media at 26 and 49 °C, respectively. An increase in the m value is observed, at such temperatures, when the PEG molecular mass increases. By comparing m values for a given PEG at both temperatures, a significant decrease is observed for the higher one.

Fig. 7 shows the m values observed for the different albumins at 26 °C. No correlation was found either for m and PEG molecular mass or for surface hydrophobicity and the m values. These findings suggest that ΔA (which depends appreciably on the protein structure) is very different between the assayed albumins.

4. Discussion

It has been suggested that PEG is essentially an inert polymer which does not interact with proteins due to its steric and hydration properties. The molecule is heavily hydrated and there are few sites to which proteins can bind. In aqueous solution PEG acts as a highly mobile molecule with a large exclusion volume. Although this exclusion effect increases as its molecular mass does, PEGs of higher molecular mass have the capacity to form intramolecular bonds, thus acquiring a more compact conformation. Its rapid motion gives an approaching protein little time in which to form a positive interaction [13]. However, several authors have found that PEG induces the dissociation of the phosphofructokinase tetrameric enzyme [17] and alters the UV absorption spectrum of ribonuclease in the zone of 270–290 nm, due to the modification of the tyrosine residue microenvironment [18]. Farruggia et al. [19] have found that PEG induces a displacement of the fluorescent probe, ANS, from its binding site in human albumin. In this work, the relative surface hydrophobicity of a family of some mammalian albumins was estimated by using a fluorescence probe, the ANS, which has the ability to bind to hydrophobic sites in albumins. In summary, a significant decrease in So at increasing PEG concentrations was observed, such finding may be accounted for by the prevention of ANS binding due to the presence of an atmosphere of PEG molecules in the neighbours of protein hydrophobic surface. This effect is more noticeable for PEGs of higher hydrophobic character, which in fact are those of higher molecular mass. On the other hand, temperature increase (above 40 °C) induces a drastic decrease in So for all the PEGs and albumins assayed, which is compatible with the disruption of the water molecule order around the hydrophobic polymer surface, thus facilitating the protein-PEG interaction and displacing the ANS from the protein. Similar conclusions were achieved by using tryptophan fluorescence quenching by acrylamide experiments. The higher V' values (proportional to the sphere action volume, which is slightly larger than the sum of the radii of the fluorophore and quencher) in PEG presence indicate that PEG molecules in the neighbours of protein domain near the accessible tryptophans separate these tryptophans from the quencher.

Alonso and Dill [16] have developed a statistical mechanical theory for the effects of denaturing agents on protein stability. They reached a physical interpretation of the slope of the free energy change of unfolding (ΔG_U) versus denaturant agent concentration curve ($\partial G_U/\partial$[denaturuant]) according to the following equation:

$$\left(\frac{\partial \Delta G_U}{\partial [\text{denaturant}]}\right) = -kT\,\Delta A \left(\frac{\partial \chi}{\partial [\text{denaturant}]}\right) \quad (8)$$

where k is the Boltzman constant and T is the absolute temperature, χ is the free energy of transfer of an average hydrophobic residue from an aqueous medium to a solution of

denaturant. χ has been shown to depend on both the temperature and the chemical composition of the solution but it also depends slightly on the protein structure, while ΔA depends largely on the protein structure because it is related not only to the number of aminoacids per protein molecule but also to the change of the hydrophobic residue fraction on the protein surface associated to the unfolding process. Therefore, the size and composition of the protein affect $\partial\Delta G_U/\partial$[denaturant] because they influence the amount of hydrophobic surface area that can be exposed upon unfolding (ΔA).

In our work, ΔG_U versus [denaturant agent (urea)] plots followed a linear behaviour; therefore, their slopes, m, can be used as a measurement of the $\partial\Delta G_U/\partial$[urea] magnitude. Since the albumins assayed have approximately the same number of aminoacid residues, differences in the *m* values will be due to differences between their hydrophobic surface change upon unfolding. No relationship between the observed m and either So or Kr values was found. This behaviour may be due to the fact that the m parameter includes differences in the hydrophobic surfaces of mammalian albumins not only in the folded but also in the unfolded state as well (ΔA).

By comparing the observed So for the proteins in all the PEGs assayed, the following sequence was found:

$$\text{PSA} > \text{GSA} \geq \text{ESA} > \text{BSA} \geq \text{HSA}$$

which represents a hydrophobicity scale for the mammalian albumins assayed. A similar sequence was observed for their partition coefficient, Kr. An evaluation of the relationship between hydrophobicity and protein partitioning behaviour was investigated in detail by plotting ln Kr vsersus ln So (Fig. 8) A non linear correlation ($r^2 = 0.33$) was found for systems formed by PEG 3350 while ATPSs with PEG6000 and PEG10,000 gave better correlations ($r^2 = 0.95$ and $r^2 = 0.92$, respectively). Partitioning of proteins in ATPSs that mainly exploit hydrophobicity follows a similar equation which has been proposed for smaller molecules and peptides [20]:

$$\ln \text{Kr} = A \ln \text{So} + B \tag{9}$$

where *A* and *B* are constants for a given ATPS, which depend mainly on the phase polymer composition and molecular mass. The ln Kr versus ln So plot slope, *A* describes the system resolution power. Fig. 8 clearly shows that systems with PEG10,000 and PEG6000 give high resolutions to exploit hydrophobicity. Zavslasky et al. [15] have studied the partitioning behaviour of a homologous series of dinitrophenylated aminoacids with aliphatic side-chains of a different size. They found a linear relationship between the ln Kr and the number of carbon atoms in the aliphatic side-chain, whose slope increased as the relative hydrophobicity between the phases did so. By looking at the protein partitioning in ATPSs as a result of multiple interactions between phase polymers-protein-water, the higher sensitivity to exploit protein hydrophobicity showed by ATPSs of higher molecular mass PEGs is a consequence of an increase in the PEG-protein interaction. On the other hand, the absence of a correlation between So and Kr in ATPSs of PEG3350 could be due to a poorer ability of this PEG to interact with the protein.

In summary, it is important to point out that the correct choice of a family of proteins allows us to study the effect of surface hydrophobicity as a single parameter, on the protein partitioning in ATPSs of polyethyleneglycol–dextran, thus developing possible correlations for the prediction of protein partitioning behaviour.

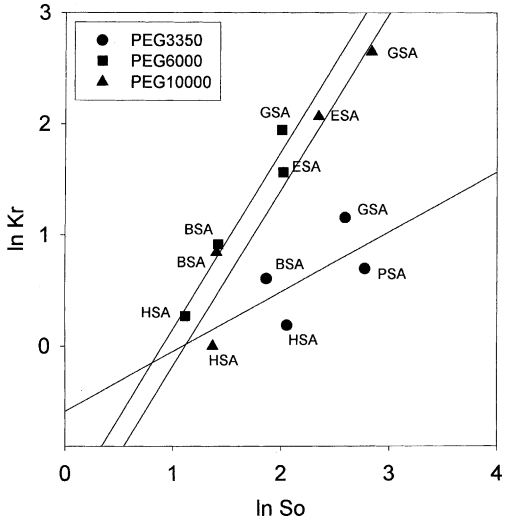

Fig. 8. The logarithm of the partition coefficient (ln Kr) vs. logarithm of relative surface hydrophobicity (ln So) for ATPSs of DxT500 and PEGs of different molecular weight. Sodium phosphate buffer 50 mM, pH 7.4, temperature: 20 °C.

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