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Role of polymer–protein interaction on partitioning pattern of bovine pancreatic trypsinogen and alpha-chymotrypsinogen in polyethyleneglycol/sodium tartrate aqueous two-phase systems

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ABSTRACT

The partitioning pattern of bovine trypsinogen (TRPz) and alpha-chymotrypsinogen (ChTRPz) was investigated in a low impact aqueous two-phase system formed by polyethyleneglycol (PEG) and sodium tartrate (NaTart) pH 5.00. ChTRPz exhibited higher partition coefficients than TRPz did in all the assayed systems. The decrease in PEG molecular weight and the increase in tie line length were observed to displace the partitioning equilibrium of both proteins to the top phase, while phase volume ratios in the range 0.5–1.5 showed not to affect protein partitioning behaviour. Systems formed by PEG of molecular weight 600 with composition corresponding to a high tie line length (PEG 12.93%, w/w and NaTart 21.20%, w/w) are able to recover most of both zymogens in the polymer-enriched phase. A crucial role of PEG–protein interaction in the partitioning mechanism was evidenced by isothermal calorimetric titrations. The major content of highly exposed tryptophan rests, present in ChTRPz molecule, could be considered to be determinant of its higher partition coefficient due to a selective charge transfer interaction with PEG molecule. A satisfactory correlation between partition coefficient and protein surface hydrophobicity was observed in systems formed with PEGs of molecular weight above 4000, this finding being relevant in the design of an extraction process employing aqueous two-phase systems.

1. Introduction

Aqueous two-phase systems (ATPSs) are formed by mixing aqueous solutions of hydrophilic polymers or polymer/salt combinations above a defined concentration [1]. Liquid–liquid extraction with ATPSs has been developed as a primary purification step in which the high yield of a target product, together with the removal of insoluble and major classes of contaminants, has been paramount. This technique combines several features of the early processing steps in only one or two partitioning operations and exhibits the following advantages: substituting difficult solid–liquid separations (precipitation techniques), linearity of scale-up from the laboratory bench over several orders of magnitude, rapidity using continuous mixers and centrifugal separators [2]. ATPSs involving poly(ethylene glycol) (PEG) and salts have been widely used for the recovery of macromolecules from biological extracts [3,4], those ATPSs formed with a salt of a biodegradable anion–citrate, tartrate, being particularly interesting due to their low environmental impact [5]. However, the wider implementation in many applications has been restrained in part by the limited predictability of the partition behaviour of proteins in ATPSs, thus making this method development wholly empirical [6]. Mechanism that causes the uneven distribution of bio-molecules and therefore, their different partition coefficients (Kp), is poorly understood. The value of partition coefficient of a protein relies on its physicochemical properties, e.g. molecular mass, charge, surface chemistry, and its interactions with those of the chosen system, e.g. composition, ionic strength, addition of specific salt ions, pH.

Bovine trypsin and alpha-chymotrypsin are enzymes widely used both to digest other proteins in food and leather industries and to supplement the diet of patients with pancreatic diseases [7]. They are stored in mammalian pancreas as inactive enzymatic precursors—trypsinogen (TRPz) and chymotrypsinogen (ChTRPz). Both zymogens exhibit structural and chemical similarities such as molecular weights (approximately 23,900 and 25,700 respectively), isoelectric points (close to 9) and amino acid compositions.

In this study, we describe the partitioning features of bovine trypsinogen and alpha-chymotrypsinogen in a non-conventional polyethyleneglycol/sodium tartrate (pH 5.00) ATPS in order to evaluate this system applicability in a purification process. Besides,

Abbreviations: ATPS, aqueous two-phase system; PEG, polyethyleneglycol; TRPz, trypsinogen; ChTRPz, chymotrypsinogen.

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Table 1
Composition of aqueous two-phase systems formed by PEGs of different molecular weight (MW) and sodium tartrate (NaTart) pH 5.00.

PEG MW	Tie line	NaTart	PEG	TLL[a]	PEG MW	Tie line	NaTart	PEG	TLL[a]
		(%, w/w)					(%, w/w)		
600	1	12.17	19.50	24.83	4000	1	12.97	9.37	23.96
	2	12.18	20.00	25.53		2	13.66	9.48	26.75
	3	12.85	20.62	31.33		3	13.91	9.75	28.66
	4	12.93	21.20	42.02		4	14.57	9.95	31.18
1000	1	12.18	20.00	19.31	6000	1	9.50	8.30	15.45
	2	12.85	20.62	28.16		2	10.02	10.00	24.36
	3	12.93	21.20	36.83		3	10.31	10.50	27.56
	4	12.67	20.67	43.59		4	10.65	11.45	32.37
2000	1	10.46	13.39	21.02	8000	1	9.50	10.00	22.81
	2	10.85	15.48	30.09		2	8.79	14.00	27.51
	3	11.15	16.91	39.92		3	10.00	12.00	29.83
	4	11.47	18.29	48.59		4	10.00	14.00	33.53

Each system is named with the PEG molecular weight.
[a] Tie line length.

the mechanism involved in partitioning equilibrium of both zymogens is analyzed in order to explain their differential partitioning behaviour irrespective of their structural similarities. This information could contribute to complete the knowledge about the forces involved in protein partition and therefore, facilitate the design of an extraction process employing aqueous two-phase systems.

2. Materials and methods

2.1. Chemicals

Trypsinogen (TRPz), alpha-chymotrypsinogen (ChTRPz) from bovine pancreas, polyethyleneglycols of average molecular masses: 600, 1000, 2000, 4000, 6000 and 8000 (PEG600, PEG1000, PEG2000, PEG4000, PEG6000, PEG8000) were purchased from Sigma Chem. Co. and used without further purification. Tartaric acid and sodium hydroxide were of analytical quality.

2.2. Preparation of the aqueous biphasic system

To prepare the biphasic aqueous systems, stock solutions of the phase components: PEG of different molecular weight 30% (w/w) and tartaric acid 22% (w/w) were mixed according to the binodal diagram previously obtained in our laboratory [8]. The system pH (5.00) was adjusted by the addition of sodium hydroxide. Low-speed centrifugation was used to speed up phase separation after a thorough gentle mixing of the system components and therefore, phases were withdrawn using syringes. The top phase was sampled first, with care being taken to leave a layer of material at least 0.5 cm thick above the interface. The lower phase was withdrawn using a syringe with a long needle. A tiny bubble of air was retained in the needle tip and expelled once in the bottom phase to prevent contamination from top phase material. One mL of each phase was mixed to reconstitute several two-phase systems in which the protein partition was assayed. The total system composition and the tie line length (TLL) are shown in Table 1.

2.3. Determination of the partition coefficient (Kp)

Partitioning behaviour of TRPz and ChTRPz was analyzed by dissolving a given amount of protein (1.5–2.1 μM total system concentration) in the two-phase systems containing 1 mL of each equilibrated phase. Small aliquots of the protein stock solution (1000 μM) were added to the systems (10–14 μL) in order to make the change of the total volume of each phase negligible. After mixing by inversion for 15 min and leaving it to settle for at least 60 min, the system was centrifuged at low speed for the two-phase separation. Samples were withdrawn from separated phases and after appropriate dilution (with the equilibrated phase free from protein), the protein content in each phase was determined by measuring light absorption at 280 nm. Equally diluted samples from identical phase systems without protein were used as blanks. The partition coefficient was calculated with the following expression:

$$\mathrm{Kp} = \frac{[\mathrm{P}]_{\mathrm{top}}}{[\mathrm{P}]_{\mathrm{bottom}}} \quad (1)$$

where $[\mathrm{P}]_{\mathrm{top}}$ and $[\mathrm{P}]_{\mathrm{bottom}}$ are equilibrium concentrations of the partitioned protein in the PEG and tartrate-rich phases, respectively. Temperature was maintained constant and controlled to within ±0.1 °C by immersing the glass tubes in a thermostatic bath. All the measurements were developed by triplicate.

2.4. Determination of thermodynamic functions associated to the protein partitioning

The TRPz and ChTRPz partition coefficients (Kp) were determined at different temperatures (T) in the range 293–310 K. By applying the van't Hoff equation:

$$\frac{\partial \ln \mathrm{Kp}}{\partial(1/T)} = \frac{-\Delta H^{\circ}}{R} \quad (2)$$

the enthalpy change (ΔH°) associated to the protein partitioning was calculated from the slope of a ln Kp vs. $1/T$ plot. The free energy change (ΔG°) was determined from:

$$\Delta G^{\circ} = -RT \ln \mathrm{Kp} \quad (3)$$

and the entropy change (ΔS°) from:

$$\Delta S^{\circ} = \frac{(\Delta H^{\circ} - \Delta G^{\circ})}{T} \quad (4)$$

2.5. Isothermal titration calorimetry (ITC)

Measurements were performed at 20 °C by using a VP-ITC titration calorimeter (MicroCal Inc., USA). The sample cell was loaded with 1.436 mL of TRPz/ChTRPz solutions (50 μM) and the reference cell contained Milli-Q grade water. Titration was carried out using a 0.3 mL syringe filled with PEG solutions. The experiments were performed by adding 25 aliquots of 3 μL of polymer solutions 0.5–1.0% (w/w) to the cell containing the protein solution. The total heat (ΔH_{tot}), associated to each polymer addition, was calculated with MicroCal ORIGIN 7.0 software supplied with the instrument. Besides, titrations of both a protein solution with buffer and a buffer solution with polymer were carried out in order to determinate the

Table 2
Radius of gyration corresponding to PEGs of different molecular weight (MW).

PEG MW	$[\eta]$[a] (cm³/g)	R_G[b] (Å)
600	3.88	7.17
1000	5.38	9.48
2000	8.19	13.75
4000	12.46	19.92
6000	16.93	25.25
8000	18.70	28.73

[a] $[\eta]$, intrinsic viscosity according to Bolognese et al. [13].

[b] R_G, radius of gyration calculated according to: $R_G = \left(\frac{3}{4}\frac{[\eta]MW}{N_{Advogadro}2.5\pi}\right)^{1/3}$.

heat of protein dilution (ΔH_{dil}) and the heat of polymer dissolution (ΔH_{dissol}) respectively. The heat associated to the interaction protein–polymer (ΔH_{int}) was then calculated with the following equation:

$$\Delta H_{int} = \Delta H_{tot} - \Delta H_{dil} - \Delta H_{dissol} \quad (5)$$

The resulting data were fitted to a single set of identical binding sites model. The molar enthalpy change for the binding, ΔH_b°; the binding stoichiometry, n and the affinity constant, K_{af} were thus obtained. The molar free energy change, ΔG_b°, and the molar entropy change, ΔS_b°, for the binding reaction, were calculated by replacing Kp by K_{af} in Eqs. (3) and (4).

2.6. Determination of the protein average surface hydrophobicity, $\langle\phi_{surface}\rangle$

Protein surface hydrophobicity, $\langle\phi_{surface}\rangle$, was calculated with the following equation:

$$\langle f_{surface}\rangle = \sum_{i\in A} r_i f_i \quad (6)$$

where ϕ_i is the hydrophobicity of the amino acid of type i and A is the collection of the 20 possible amino acids. It was proposed by Berggren et al. [9] and assumed that each amino acid in the protein surface contributes, proportionally to its abundance, with properties associated to the protein surface [10]. The hydrophobicity of each amino acid (ϕ_i) was computed according to the scale of Cowan–Whittaker [11] in which values assigned to the most hydrophilic and the most hydrophobic amino acid are −1 and +1 respectively. The fraction of protein superficial area, r_i occupied by the amino acid i is defined by:

$$r_i = \frac{S_i}{\sum_{j\in A} S_j} \quad (7)$$

where S_i is the sum of the accessible superficial area (ASA) for all the amino acids of type i. The value of ASA was calculated using the free software SURFACE RACER [12] by employing the radius of gyration (R_G) corresponding to PEGs of different molecular weights as the "probe" radius (Table 2). The PDB files with the three-dimensional protein-structure were downloaded from the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov/sites/entrez).

3. Results and discussion

3.1. Zymogen partitioning pattern in PEG/NaTart ATPSs

Among the factors which affect protein partitioning, those corresponding to ATPS characteristics such as PEG molecular weight, concentration of phase components and phase volume ratio were investigated. Fig. 1 shows the effect of the molecular weight of PEGs on the partition of TRPz and ChTRPz. Partition coefficients of both TRPz and ChTRPz exhibited a similar pattern, thus decreasing with

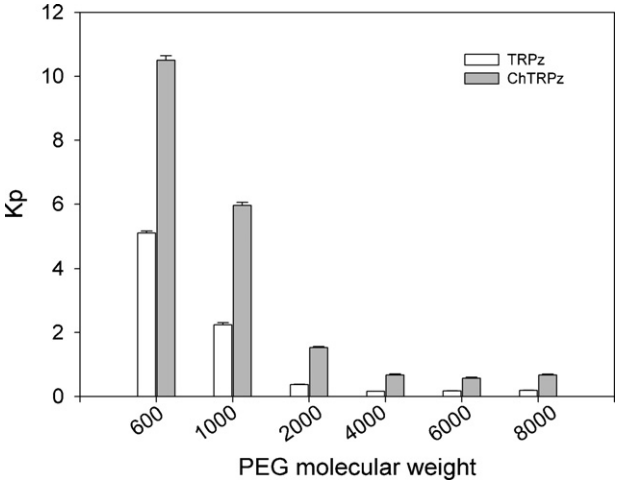

Fig. 1. Effect of PEG molecular weight on the Kp value for TRPz and ChTRPz for systems with tie line 2 composition (see Table 1). Temperature 293 K. Each Kp is the average of three independent measurements. The mean estimated error for the partition coefficients was indicated.

the increase in the PEG molecular weight. This has been shown to be a general rule for many proteins and ATPSs [14] and could be attributed to a reduction of the space available for proteins when the polymer chain length increased. The highest changes in the Kp values were observed when the PEG molecular weight varied from 600 to 2000. This behaviour is predicted by the Flory Huggins' theory which states a linear relationship between the ln Kp and the reciprocal of the flexible chain polymer molecular weight [15].

Systems with compositions corresponding to different tie lines (see Table 1) were also assayed. At increasing tie line lengths, the difference between the PEG at the top and bottom phases (Δ[PEG]) also increases; therefore, Δ[PEG] can be employed to evaluate the response to phase component concentration. Linear ln Kp vs. Δ[PEG] plots were observed for both TRPz and ChTRPz in all the assayed systems, their slopes, $\partial \ln Kp/\partial(\Delta[PEG])$, being considered to depend on the strength of protein–polymer interaction [16]. Three features should be pointed up from visual inspection of Fig. 2: An increase in Δ[PEG] is accompanied by a partition displacement of both zymogens to the top phase (positive slopes) for all assayed systems, thus indicating a favorable PEG–protein interaction; ATPSs formed with PEG of low molecular weight – PEG600, PEG1000 – are more sensitive to system composition and ChTRPz

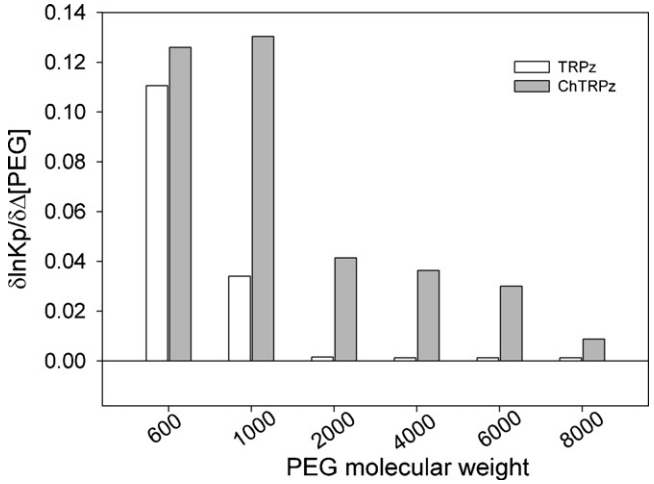

Fig. 2. Effect of PEG concentration (tie line length) on the partitioning of both TRPz and ChTRPz in different PEG/NaTart ATPSs. Temperature 293 K.

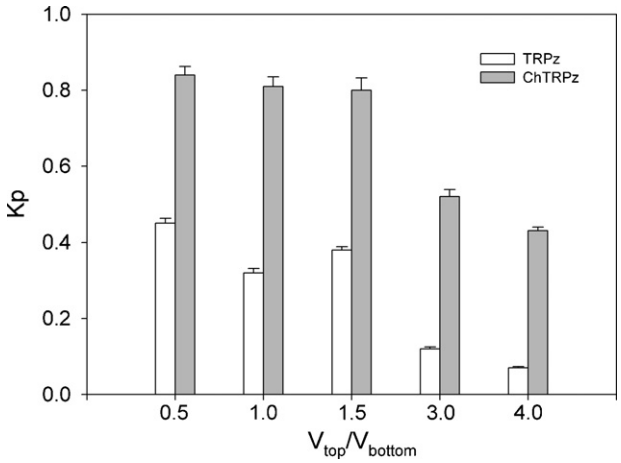

Fig. 3. Effect of V_{top}/V_{bottom} on zymogen partitioning in PEG6000/NaTart pH 5.00 ATPS. Composition corresponding to tie line 2 (see Table 1). Temperature 293 K.

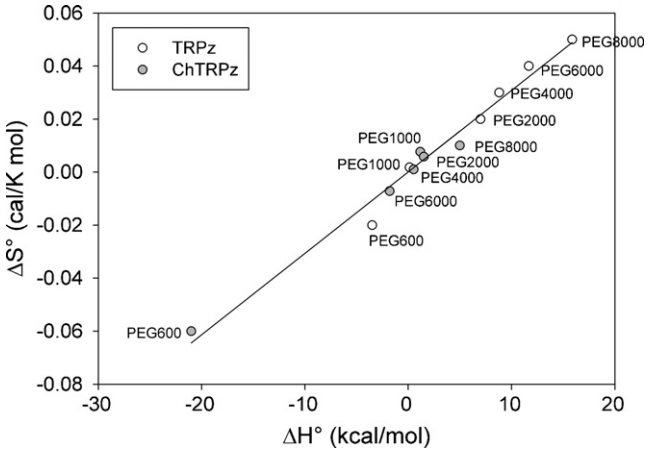

Fig. 4. Entropy–enthalpy compensation plot associated to the partitioning of TRPz and ChTRPz in PEG/NaTart APSs, pH 5.00. Temperature 293 K.

partitioning is more significantly affected by the change in PEG concentration than TRPz is. According to these observations, increasing tie lines length should be an adequate strategy to improve TRPz and ChTRPz recoveries in the top phase of ATPSs formed by PEG600 and PEG1000. Besides, capability of these systems to separate TRPz and ChTRPz from each other is expected to increase due to the higher effect of tie line length on partition equilibrium of ChTRPz.

Fig. 3 shows the effect of phase volume ratio on protein distribution between the two phases. Partition coefficient for TRPz and ChTRPz keeps nearly constant when volume ratios vary from 0.5 to 1.5. However, for V_{top}/V_{bottom} higher than 3, a drastic decrease in Kp is observed, thus suggesting that partition coefficients do not behave as true thermodynamic constants (Kp_{therm}) under this condition. Kp_{therm} depends on temperature exclusively and is linked to the partition coefficient by the following relationship:

$$Kp_{therm} = \frac{a_{P_{top}}}{a_{P_{bottom}}} = \frac{\gamma_{P_{top}}[P]_{top}}{\gamma_{P_{bottom}}[P]_{bottom}} = \frac{\gamma_{P_{top}}}{\gamma_{P_{bottom}}} Kp \quad (12)$$

where $a_{P_{top}}$, $a_{P_{bottom}}$, $\gamma_{P_{top}}$ and $\gamma_{P_{bottom}}$ are activities and activity coefficients for protein in the top and bottom phases. Kp equals to Kp_{therm} at low protein concentrations since activity coefficients tend to unity. When V_{top}/V_{bottom} increases above a critic value, e.g. 3, the resulting V_{bottom} decrease causes an enhancement of protein concentration (from 10 to 50 μM). As a consequence, bottom phase behaviour deviates from ideality, $\gamma_{P_{bottom}}$ decreases and Kp decreases, making Kp_{therm} be unmodified.

3.2. Thermodynamics of zymogen partitioning

ln Kp vs. $1/T$ plots showed linearity for both zymogens in the temperature range assayed, thus indicating that the enthalpy change, calculated from its slope, keeps constant within this interval. ΔH° and ΔS° associated with the TRPz and ChTRPz partition equilibrium in systems containing PEGs of different molecular weight are shown in an entropy vs. enthalpy plot (Fig. 4). The transfer of both proteins from salt to PEG-rich phase follows a similar trend, being exothermic for PEG600 ATPS, but endothermic for the other systems. The linear relationship between ΔS° and ΔH°, indicates that both variables change in parallel and compensate each other to produce minor changes in the free energy of the process. This pattern, observed in several processes, is considered to be as an evidence of the participation of water molecules in the process mechanism [17] and appears to be the thermodynamic manifestation of "structure making" and "structure breaking". This type of interaction involves van der Waals forces (as a result of the fluctuations in the charges of the proteins and dipoles or multipoles in the polymer) between hydrophobic surfaces from PEG and protein molecules, which would imply the rupture of hydrogen bonds from the structured water in their neighbours [18,19]. From inspection of Fig. 4, it results clear that entropic and enthalpic changes associated to ATPSs of PEG600 do not correlate so well as the other systems, thus indicating the presence of an additional mechanism different from the hydrophobic effect, involved in protein transfer to the polymer phase.

3.3. Characterization of PEG–protein binding

The complex formation between both zymogens and PEGs of low and high molecular weight – 600 and 8000 – was investigated by isothermal titration calorimetry. Fig. 5 shows the calorimetric titration curve of TRPz with PEG8000 in a 50 mM sodium tartrate buffer, pH 5.00. A saturation behaviour, which indicates a specific ligand–protein interaction, was observed for all the assayed systems. The data were fitted by assuming a binding model of equivalent and independent protein sites in which the polymer molecules bind to several protein sites, all with the same intensity [20].

The equilibrium constant (K_{af}) associated to the polymer–protein binding and the number of PEG molecules

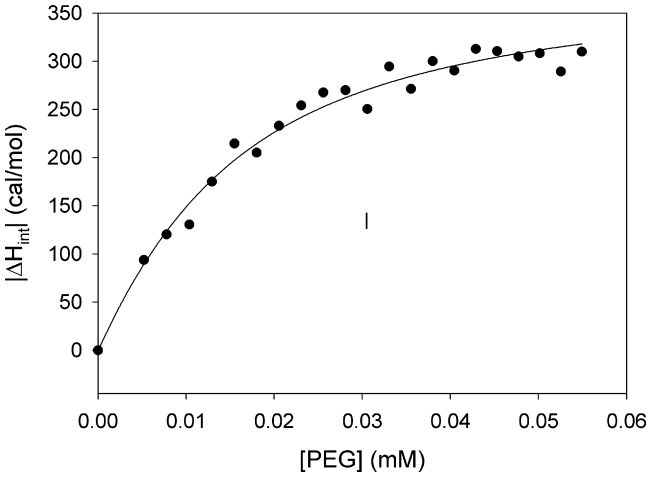

Fig. 5. Binding curve for the calorimetric titration of TRPz with PEG8000 (1%, w/w). Protein concentration 50 mM. Medium sodium tartrate buffer 50 mM, pH 5.00. Temperature 298 K.

Table 3
Affinity constant (K_{af}), number of binding sites (n) and thermodynamic parameters corresponding to the interaction between zymogens (TRPz, ChTRPz) and PEGs of molecular weights 600 and 8000 respectively.

Protein-PEG	K_{af} (10^5 M^{-1})	n	ΔH_b° (kcal/mol)	ΔG_b° (kcal/mol)	ΔS_b° (cal/K mol)
ChTRPz-PEG600	2.6 ± 0.5	3.2	−1.40	−7.44	20.3
TRPz-PEG600	1.5 ± 0.3	0.9	3.50	−7.09	41.1
ChTRPz-PEG8000	1.1 ± 0.1	0.2	−3.50	−6.90	11.4
TRPz-PEG8000	0.91 ± 0.09	0.2	−0.39	−6.80	21.5

(n) bound per zymogen molecule, estimated by non-linear fitting, are listed in Table 3. A comparison among the affinity constant values offers the following picture: K_{af} values are in the order of 10^5 M^{-1}, thus indicating that PEG molecules bind quite tightly to zymogens. Results also show that ChTRP exhibit higher affinity for PEG molecules than TRPz do. The PEG–protein stoichiometry is highly dependent on PEG molecular weight. Fractional n values nearly 0.2, observed for PEG8000 (with both zymogens) indicate that one PEG molecule binds on average to 4–5 protein molecules which agree with the pattern observed for other polymer–protein interactions [21]. Sequence observed for K_{af} and n values is similar to that corresponding to the values of both Kp (Fig. 1) and $\partial \ln Kp/\partial(\Delta[PEG])$ (Fig. 2) for the differently assayed ATPSs, thus suggesting that the strength and the stoichiometry of PEG–protein binding could be considered as the main mechanism that determines protein partition equilibrium. Thermodynamic binding analysis suggests that PEG–protein interaction is entropically driven. Positive ΔS° values suggest the participation of "hydrophobic effect" component in the interaction. Consequently, one question to be answered is: Is there any relationship between PEG-affinity and the protein hydrophobicity? The hydrophobicity value of a protein can be assigned by many different methodologies which can be experimental or theoretical. Lienqueo et al. [22] showed that retention times in hydrophobic interaction chromatography were well correlated with the protein average surface hydrophobicity, $\phi_{surface}$. Is it possible to predict these zymogens partition behaviour based on this property? Fig. 6 shows that Kp values (for all the assayed systems) are not well correlated with $\phi_{surface}$ values. However, a linear relationship (slope 13.82 ± 1.27, intercept 2.38 ± 0.17) is appreciated for PEGs of molecular weight higher than 4000, giving certain predictability to the partition coefficient in these systems. This behaviour would indicate that "hydrophobic effect" is the main force involved in protein transfer from the tartrate-rich phase to that enriched in PEG (for ATPSs of higher PEGs). A poor correlation was observed for PEGs of low molecular weight in agreement with the behaviour observed in the enthalpic–entropic compensation plot. This may be due to the presence of additional forces, elsewhere hydrophobic, in protein partition, e.g. electrostatic interaction.

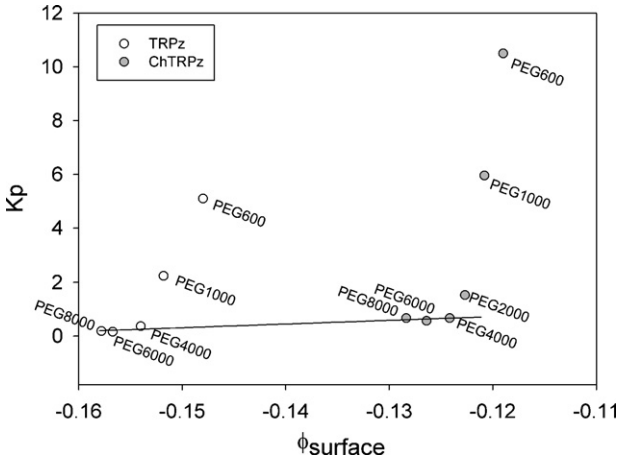

Fig. 6. Correlation between partition coefficients (Kp) for TRPz and ChTRPz (in ATPS formed with PEGs of different molecular weight) and $\phi_{surface}$ calculated for each zymogen with probe radius of Table 2.

3.4. Structural features of PEG–protein binding

Typical modes of the polyethyleneglycol interaction with proteins will be explored in an attempt to localize the structural features responsible for above observations. Crystallographic studies [23] stated that PEG–protein surface interactions could be categorized into the following groups:

- Linear binding pattern in clefts of protein surface where the predominant contacts are hydrogen bonds to either side chains of amino acid residues or main chain NH groups.
- Multiple coordination contacts to positively charged lysine, arginine and histidine residues.
- A very specific type of interaction, which involves a coordination embedding of the cation from the solution and forming a loop of PEG molecule, whose outer hydrophobic part contacts hydrophobic areas at the protein surface. In this case, the presence of specific amino acid residues, such as hydrophobic tryptophan, exposed in protein surface has been reported [24] to enhance drastically the protein partition to the polymer phase. A selective charge transfer interaction between PEG and tryptophan, in which the pirrole nitrogen of the indole ring would be a hydrogen donor, has been postulated.

Table 4 shows the accessible surface area of above cited amino acids, calculated with SURFACE RACER software for each zymogen. Clearly, ChTRPz exhibits better chances to interact with PEG molecule than TRPz does as a result of two features: the major exposure of positive charged amino acid (Arg, Lys and His) and the double amount of Trp residues (from 4 to 8) highly exposed. The first mechanism, due to the presence of hydrophilic amino acids, is present in protein interaction with PEGs of both low and high molecular weights. The second, involves the presence of hydrophobic residues, such as Trp, and prevails in protein interaction with PEGs of higher molecular weight since these polymer molecules are able to encapsulate cations from solutions and expose hydrophobic outer part of the loop of PEG molecule. This finding is in agreement with the satisfactory correlation observed between partition coefficient and protein hydrophobicity surface in systems formed with PEGs of molecular weight above 4000.

Table 4
The accessible surface area (ASA) of arginine (Arg), Lysine (Lys), histidine (His) and tryptophan (Trp) residues calculated with SURFACE RACER software for each zymogen.

Amino acid	TRPz		ChTRPz	
	Amount	Total ASA (Å^2)	Amount	Total ASA (Å^2)
Arg	2	154.02	3	272.59
Lys	14	1180.46	14	1816.05
His	3	118.74	2	128.18
Trp	4	88.11	8	255.99

4. Conclusions

The areas that address the molecular understanding of partitioning, generally involve the use of model systems. In this sense, we consider that trypsinogen and alpha-chymotrypsinogen partitioning could offer relevant information about structural factors which are crucial in protein partitioning. These proteins show considerable homology and therefore, exhibit similar physico-chemical properties. Although these similarities, ChTRPz exhibited higher partition coefficients than TRPz did in all the assayed systems. According to our results, the strength (K_{af}) and stoichiometry (n) of PEG–protein complex formation would play a crucial role in this behaviour. The major content of highly exposed tryptophan rests, present in ChTRPz molecule, could be considered to be determinant of its higher partition coefficient due to a selective charge transfer interaction with PEG molecule. A good correlation with protein surface hydrophobicity was only observed for systems formed with PEGs of the highest molecular weight.

Finally, as regards the practical aspects of this work, the effect of systems parameters (i.e. tie line length, PEG molecular weight and phase volume ratio) on partitioning of TRPz and ChTRPz in PEG/sodium tartrate pH 5.00 ATPSs was explored. Obtaining pure products from a natural source, involves a downstream process which comprises clarification, enrichment and fractionation. ATPS extraction is mainly employed in the early stages of this process, in which several attempts to maximize the product yield at the expense of retaining major contaminants are made [6]. Systems formed by PEG of molecular weight 600 with composition corresponding to a high tie line length (PEG 12.93%, w/w and NaTart 21.20%, w/w) fulfill these requirements due to their ability of recovering most of both zymogens in the polymer-enriched phase. However, the successful design of ATPS processes for primary recovery and partial purification of either TRPz or ChTRPz needs a further optimization of operating conditions. At present, these experiences are being carried out at our laboratory.

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