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Amino-acid recovery using ionic liquids: Partitioning in water + ionic liquid systems

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Abstract

The partition of a series of dinitrophenylated amino-acids is studied experimentally in two (Ionic Liquid + Sodium Phosphate Buffer) biphasic systems at 23 ºC. The ionic liquids used in these experiments are 1-hexyl-3-methylimidazolium tetrafluoroborate, [C_6mim][BF_4], and 1-decyl-3-methylimidazolium tetrafluoroborate, [C_{10}mim][BF_4]. pH of the initial aqueous solution is fixed at 7.4 using a suitable sodium phosphate buffer. DNP-amino-acids distribute preferentially to the ionic liquid phase, and this partition increases with the length of the alkyl chain of amino-acid. The free energy of transfer for a methylene group is calculated from the partition coefficients of the solutes. This provides an effective measurement for the relative hydrophobicity of the conjugated phases, which is higher than in other biphasic systems used in biotechnology (e.g., polymer-polymer and polymer salt aqueous two-phase systems).

Keywords: ionic liquids, partitioning, amino-acids

1. Introduction

Ionic liquids are defined as electrolytes with melting points around or below ambient temperature. This new class of chemicals are claimed to have some interesting properties: negligible volatility, thermal and chemical stability, non-flammability, wide liquid range… These properties provide important advantages in many processes[1,2]. They have been getting increasing attention from both scientists and engineers in the last decade, and research is expanding to many different areas of knowledge. Special emphasis has been devoted in their application both as reaction media or as solvents for different separation processes[3,4]. The physical and chemical properties can be tailored using different ions, also alkylating or functionalizing existing ions, and thus providing task-specific ionic liquids (the so-called "designer solvents").

Biomolecules (proteins, enzymes…) are usually produced at industrial scale using enzymatic or fermentation processes. Separation and purification steps are difficult and expensive, but high purity is often needed for biomolecules' applications.

Information regarding the use of ionic liquids as solvents for the separation and/or purification of biomolecules is still scarce. The partitioning of biomolecules, and also cells and organelles, between aqueous solutions and ionic liquids are necessary for the use of the latter as extraction solvents in biotechnological applications. We present here some preliminary results on the partitioning of a series of dinitrophenylated amino-acids in two (Ionic Liquid + Sodium Phosphate Buffer) biphasic systems.

2. Experimental

2.1. Reagents

Dinitrophenilated (DNP) amino-acids: N-(2,4-dinitrophenyl)glycine (DNP-Gly), N-(2,4-dinitrophenyl)-L-alanine (DNP-Ala), N-(2,4-dinitrophenyl)-DL-*n*-valine (DNP-Val), N-(2,4-dinitrophenyl)-DL-*n*-leucine (DNP-Leu), and N-(2,4-dinitrophenyl)-DL-α-amino-*n*-caprylic acid (DNP-AOc) were obtained from Sigma. Ionic liquids 1-hexyl-3-methylimidazolium tetrafluoroborate, [C_6mim][BF_4], and 1-decyl-3-methylimidazolium tetrafluoroborate, [C_{10}mim][BF_4] were provided by Green Solutions (Vigo, Spain). Water (GR for analysis) and 1-Propanol (LiChrosolv, >99.8%) were obtained from Merck. Sodium phosphate buffer, NaPB, 0.11*m* was prepared with reagent-grade Na_2HPO_4 and NaH_2PO_4 (Merck) to provide a final pH of 7.4. Aqueous stock solutions of DNP-amino-acids were prepared with a concentration of 0.1-0.2 wt%.

2.2. Procedure

The partitioning experiments were performed at room temperature (23 ± 1 ºC controlled by air conditioning) in 2 ml tubes, mixing 1.4 g of NaPB and 0.25 g of ionic liquid. Five replicates were prepared with different DNP-amino acid concentrations, adding from 0 (blank) up to 40 µl of amino-acid stock solution were added. The corresponding amount of water was also added to maintain ionic liquid and buffer compositions the same in all tubes. The tubes were thoroughly vortex mixed for 2 min and then centrifuged at 10^4 rpm for 30 min to accelerate the phase separation. Samples of top and bottom phases were pipetted and diluted conveniently for analysis by UV-Vis spectroscopy (Absorbance was kept in all cases below 0.8). For the system using [C_6mim][BF_4], water was used for dilution in both phases, and polymethylmethacrylate micro-cuvettes were used in the UV-Vis measurements. The partition coefficient for each solute was calculated as the slope of the straight line obtained when the absorbance in top (aqueous) phase is plotted against that in bottom (ionic liquid) phase, corrected with the corresponding dilution factors (DF):

$$K = Abs(top) \cdot DF_{top} \big/ Abs(bottom) \cdot DF_{bottom} \qquad\qquad 1$$

The increasing concentrations of solutes allowed to check aggregation effects. Preliminary experiments in water + [C_6mim][BF_4] system showed aggregation effects

for total concentrations of DNP-amino-acids >0.004 wt% (Figure 1). Thus, solute concentrations in partitioning experiments were below 0.004 wt%. For the system using [C_{10}mim][BF_4], water was used for dilutions of the top (aqueous) phase, and 1-propanol for dilutions of the bottom (ionic liquid) phase. 1-Propanol was needed because of the low solubility of [C_{10}mim][BF_4] in water, and quartz cuvettes were needed for measurements in the UV-Vis spectrometer using this solvent. Calibration lines for the concentration range studied were obtained for each solute in each solvent (water and 1-propanol). The calibration lines obtained were linear in all cases. The partition coefficients in this system were then calculated as the slope of the straight line obtained when the concentrations in the top (aqueous) phase is plotted against that in bottom (ionic liquid) phase. The experimental error was below 5% in all cases.
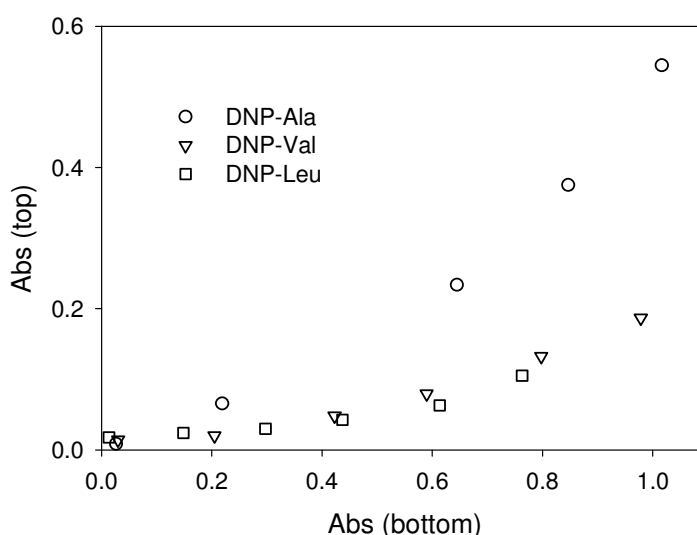

Figure 1: Aggregation effects for DNP-amino-acids in water + [C_6mim][BF_4] system.

3. Results and Discussion

Figure 2 shows the logarithm of the partition coefficients obtained for each DNP-amino-acid, *K*, against the number of equivalent methylene groups in the aliphatic side-chain of each DNP-amino-acid, *n(*CH$_2$*)*. The negative values of the logarithms of partition coefficients indicate the preferences of the solutes for the ionic liquid phase, which increase in both systems with the size of the amino-acid. The crossing of both straight lines in Figure 2 indicates that smallest amino-acids (DNP-Gly and DNP-Ala) partition more towards [C_{10}mim][BF_4] than to [C_6mim][BF_4], but that tendency is inverted for higher amino-acids.

The free energy of transfer of a methylene group between the equilibrium phases, $\Delta G(\mathrm{CH_2})$, can be calculated using the following equations[5]:

*ln K = C + E·n(*CH$_2$*)*   *2*

$$\Delta G(\text{CH}_2) = -R \cdot T \cdot E \qquad\qquad\qquad\qquad\qquad\qquad 3$$

where C and E are constants, R is the universal gas constant and T is the absolute temperature. Parameters C and E are obtained for each system by linear regression ($r^2 > 0.99$), as shown in Figure 2. The free energies of transfer calculated were -0.367 kcal/mol for the system with [C$_6$mim][BF$_4$] ionic liquid, and -0.0678 kcal/mol for the system with [C$_{10}$mim][BF$_4$] ionic liquid. The former is about the same magnitude than free energies found in polymer-salt aqueous two-phase systems; the latter is about the same magnitude than free energies found in polymer-polymer aqueous two-phase systems[6].
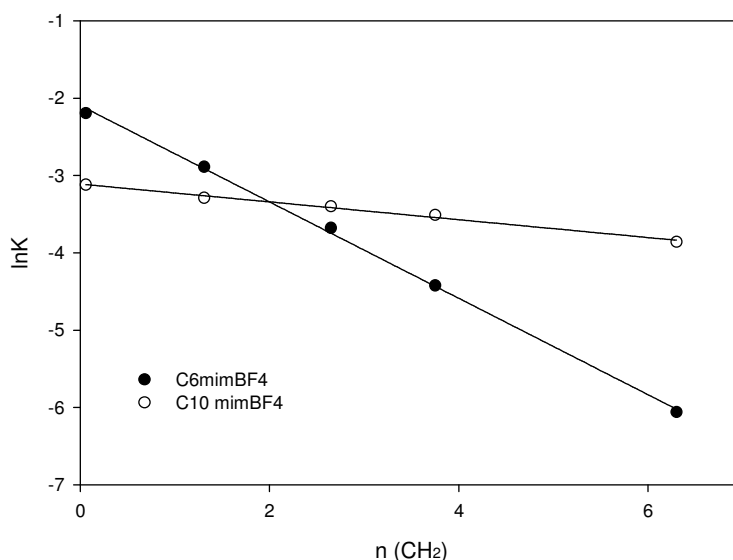

Figure 2: Partition coefficients of DNP-amino-acids in (ionic liquid + phosphate buffer) system at 23 ℃.

4. Conclusions

Partition coefficients for five dinitrophenylated amino-acids in (phosphate buffer + [C$_6$mim][BF$_4$] or [C$_{10}$mim][BF$_4$]) systems were obtained experimentally at 23 ℃. Amino-acids distribute preferentially to the ionic liquid phase, which increases with the length of the amino-acid size chain.

Extraction capacity of [C$_6$mim][BF$_4$] is higher than [C$_{10}$mim][BF$_4$]) (lower partition coefficient, K) for bigger amino-acids (DNP-Val, DNP-Leu and DNP-AOc), but DNP-Gly and DNP-Ala are better extracted by [C$_{10}$mim][BF$_4$].

The free energies of transfer for a methylene group between the conjugated phases were calculated for both systems. Relative hydrophobicity of the phases in system with [C$_6$mim][BF$_4$] ionic liquid is similar to that of polymer-salt aqueous two-phase systems. Relative hydrophobicity of the phases in system with [C$_{10}$mim][BF$_4$] ionic liquid is similar to that of polymer-polymer aqueous two-phase systems.

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