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Radionuclide and tensiometry approaches to studying lysozyme behaviors in water–ionic liquid systems

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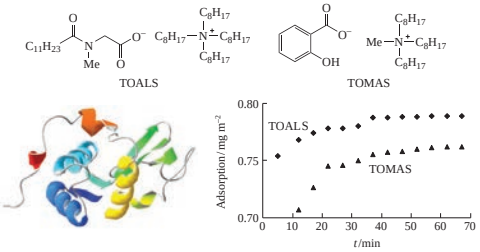
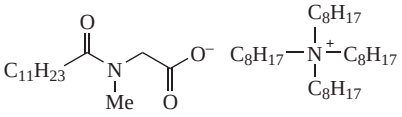
Techniques for the determination of protein concentrations in the bulk of ionic fluids have been developed using tritium-labeled proteins and adsorption at the water–ionic liquid interface with the aid of a sessile drop method.

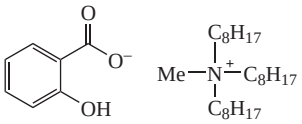


The broad and symmetrical electrochemical windows of ionic liquids provide wide prospects for their use in supercapacitors as electrolyte components.[1] Such materials are widely used as solvents and reagents in organic synthesis[2–7] and extractants.[8–11] The applicability of ionic liquids to protein extraction was considered.[12,13] The interface between water and an ionic liquid is of great interest.[14] The aim of this study was to determine the amounts of a protein in the bulk of ionic fluid using a tritium labeled tracer and in the adsorption layer at the water–ionic liquid interface. The protein amount at the interface was calculated from the interfacial tension isotherms according to the model suggested by Fainerman and co-authors.[15–17]

In this study, we used hen-egg white lysozyme (MP Biomedicals).† The ionic liquids were tetraoctylammonium laurylsarcosinate (TOALS) and trioctylmethylammonium salicylate (TOMAS).



TOALS



TOMAS

To determine the distribution coefficients (D) of lysozyme between the aqueous phase and ionic liquid, lysozyme was labeled with tritium according to a procedure described previously.[18,‡]

Table 1 The distribution coefficients (D) of lysozyme in the aqueous solution–ionic liquid systems and lysozyme equilibrium adsorption (Γ) at the liquid–liquid interface.

System	D	Γ (0.1 mg dm^{-3})/ mg m^2	Γ (3.3 mg dm^{-3})/ mg m^2
Water–TOMAS	0.07±0.02	0.74±0.1	0.79±0.2
Water–TOALS	0.14±0.02	0.60±0.3	0.76±0.1
PBS–TOMAS	0.021±0.005	0.67±0.2	0.76±0.1
PBS–TOALS	0.12±0.01	0.70±0.1	0.74±0.1

In the system containing TOMAS, the equilibration time was four days, whereas D for TOALS linearly increased for seven days. To accelerate the process, a Vortex was used. Table 1 summarizes the equilibrium values of D, which were independent of the ionic strength for the systems containing TOALS or decreased with the ionic strength for TOMAS. The presence of a long hydrocarbon fragment in TOALS probably results in higher values of D. However, the distribution coefficients were not so high to recommend free ionic liquids for lysozyme extraction, but they can be used as additives to improve the efficiency of extraction because the distribution coefficients are higher than those for octane, octanol and *p*-xylene.[19]

To obtain interfacial tension isotherms, we used a sessile drop method. A drop of lysozyme solution (0.1 or 3.3 g dm^{-3}) was squeezed on the bottom of a quartz cuvette that contained 2 cm^3 of TOALS or TOMAS. The drop was photographed with a horizontal microscope equipped with a DCM-130 digital video camera. The surface tension was determined from the shape of the sessile drops using the numerical integration of the Young–Laplace equation and the approximation of the resulting droplet profile to the experimental profile according to a published procedure.[20]

The interfacial tensions were 13.1±0.2 and 8.9±0.2 mN m^{-1} (n = 3, P = 0.95) for TOMAS and TOALS, respectively. In the PBS–ionic liquid system, the interfacial tensions were 7.5±0.2 and 5.4±0.3 mN m^{-1} (n = 3, P = 0.95) for TOMAS and TOALS, respectively. The aromatic group in TOMAS causes the looser packing of molecules at the interface to result in higher interfacial tensions.[21] In the case of a 0.1 g dm^{-3} lysozyme solution, the

† A phosphate buffered saline (PBS) was prepared using Na_2HPO_4 (0.008 M) and K_2HPO_4 (0.002 M). The ionic strength (0.16 M) was adjusted by adding NaCl. To avoid bacterial growth, NaN_3 (0.008 M) was added to the buffer solution.

‡ [^{3}H]Lysozyme (1 cm^3) with a concentration from 0.1 to 1 g dm^{-3} was stirred with TOALS or TOMAS (1 cm^3). The radioactivity of both phases was measured using a RackBeta 1215 liquid scintillation spectrometer (LKB, Finland) after phase separation. Spontaneous distribution was also investigated.

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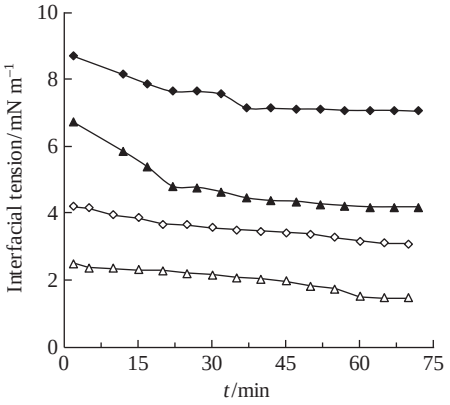

Figure 1 Time dependence of the interfacial tension at the aqueous phase–ionic liquid interfaces (◆, ◇ TOMAS; ▲, △ TOALS) for lysozyme solutions (3.3 g dm^{-3}); (◆, ▲) water and (◇, △) PBS.

interfacial tensions were 9.1±0.5 and 7.8±0.5 mN m^{-1} (water solution) or 5.4±0.1 and 2.5±0.1 mN m^{-1} (PBS solution) for TOMAS and TOALS, respectively. The equilibrium was reached in 30 min and kept constant in 24 h. Figure 1 shows changes in the interfacial tension of the aqueous solution–ionic liquid systems containing lysozyme. Such curves are typical of protein adsorption at aqueous–air and liquid–liquid interfaces for free proteins and protein–surfactant mixtures.[22,23] The interfacial tensions in the systems containing PBS were significantly lower than those in water due to the electrostatic mechanism of lysozyme adsorption at the interface.

The interfacial isotherms were described by the Fainerman model. The protein molecules were considered in a number of states of different molar areas, varying from a maximum value (ω_{max}) at very low surface coverage (low surface pressure) to a minimum value (ω_{min}) at high surface coverage (high surface pressure). The molar areas of two neighboring conformations differ from each other by the molar area increment ω_0 equal to the molar area of the solvent. Thus, the surface aria of the *i*th state was determined as follows:

$$\omega_i = \omega_{min} + (i-1)\omega_0. \quad (1)$$

The surface pressure is determined according to:

$$-\frac{\pi\omega_0}{RT} = \ln(1-\theta_p) + \theta_p\left(1-\frac{\omega_0}{\omega}\right) + a_p\theta_p^2. \quad (2)$$

Here, π is the surface pressure; R is the gas constant; T is temperature; and a_p is the intermolecular interaction parameter.

Thus, the total adsorption and the surface coverage are described by the following equations:

$$\Gamma = \sum_{i=1}^{n}\Gamma_i, \quad (3)$$

$$\theta_p = \omega\Gamma = \sum_{i=1}^{n}\omega_i\Gamma_i. \quad (4)$$

The equation for the adsorption isotherm for each state *i* of the adsorbed protein molecule is the following:

$$b_p c_p = \frac{\omega\Gamma_i}{(1-\theta_p)^{\omega_i/\omega}}\exp\left(-2a_p\frac{\omega_i}{\omega}\theta_p\right). \quad (5)$$

Here, c_p is the protein bulk concentration, and b_p is the equilibrium adsorption constant for the protein.

The following parameters for lysozyme were used: ω_{min} = = 7.72×10^6 m^2 mol^{-1}, ω_{max} = 2.55×10^7 m^2 mol^{-1} and ω_0 = = 4.95×10^5 m^2 mol^{-1} (water).[24] The intermolecular interaction parameters and equilibrium adsorption constants are summarized in Table 2.

Table 2 Parameter of intermolecular interaction and the equilibrium adsorption constant for lysozyme aqueous solution–ionic liquid systems.

System	a_p	b_p/dm^3 mol^{-1}	b_{p2}[a]/dm^3 mol^{-1}
Water–TOMAS	0.46	1.6×10^7	3.75×10^3
Water–TOALS	0.42	5.9×10^6	3.75×10^3
PBS–TOMAS	0.51	5.6×10^6	3.75×10^3
PBS–TOALS	0.42	7.6×10^6	3.75×10^3

[a] b_{p2} is the constant of the formation of the second layer.

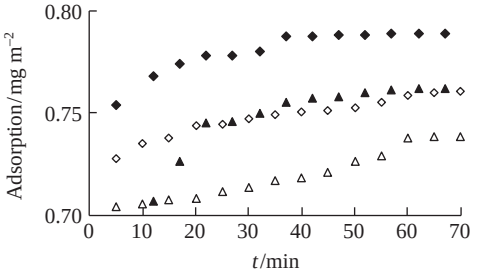

Figure 2 Time dependence of lysozyme adsorption at the aqueous phase–ionic liquid interfaces (◆, ◇ TOMAS; ▲, △ TOALS) for lysozyme solutions (3.3 g dm^{-3}); (◆, ▲) water and (◇, △) PBS.

In most cases, the values of b_p were lower than the equilibrium adsorption constant for the water–air system.[25] Note that the total charge of globular lysozyme changed from positive to negative when NaCl was added (PBS containing systems).[26] The increase in the ionic strength results in the formation of a layer of counterions.[27] Figure 2 shows the kinetics of lysozyme adsorption. Our results revealed that, in the system with TOMAS, the repulsion between protein molecules and ionic liquids increased with the ionic strength (Table 1). The effect was not significant in the system with TOALS.

Thus, for the first time, we described the behavior of lysozyme in the aqueous solution–ionic liquid systems using radionuclide and tensiometry methods. The protein adsorption at the aqueous–ionic liquid interface can be calculated from the isotherms of interfacial tension. In the systems containing TOMAS, both the distribution coefficient and the adsorption decreased with raising ionic strength. For the systems containing TOALS, the increase in the ionic strength also decreased the protein adsorption, but it did not influence a distribution ratio.

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