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SHORT ARTICLE

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The Effect of Four Imidazolium Ionic Liquids on Hen Egg White Lysozyme Solubility

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ABSTRACT: Research shows some potential applications for ionic liquids (ILs) with biomaterials. In this work, the ILs 1-butyl-3-methylimidazolium tetrafluoroborate ([C_4mim]BF_4), 1-butyl-3-methylimidazolium chloride ([C_4mim]Cl), 1-butyl-3-methylimidazolium bromide ([C_4mim]Br), and 1,3-dimethylimidazolium iodine ([dmim]I) were employed to investigate their effects on the solubility of lysozyme in aqueous solutions at pH 4.5. The results demonstrate that lysozyme solubility increases with the addition concentration of [C_4mim]BF_4 and [C_4mimCl] and is nearly invariable with the increase of the [C_4mim]Br concentration. Interestingly, the solubility slowly decreases with the increase of [dmim]I concentration. At constant added IL concentration, the solubility increases with increasing temperature. The effect of ILs on solubility can be attributed to changing interaction among lysozyme molecules. The effect of ILs employed in this work may provide crucial insight into the preparation of high-quality crystals and the development of new crystal forms as well as the design optimization of protein crystallization.

INTRODUCTION

The crystallization of macromolecules and their analysis by X-ray diffraction play an essential role in important areas of modern molecular biology and biotechnology, particularly in the genetic engineering of proteins and rational drug design.[1] However, protein crystallization is not well-understood, and crystals are typically obtained using trial and error strategies because of the many possibilities involved. The space of crystallization conditions' screening is currently expanded by new methodologies and techniques of growing crystals for the determination of the three-dimensional structure of proteins.[2] Ionic strength, temperature, pH, type of salt, and additives are just some of the factors that might influence the outcome of the crystallization experiment.[2−5] One of the major hindrances facing scientists and engineers is the lack of thermodynamic, kinetic, and material property data required for the growth of large, high-quality crystals of biological macromolecules.[1,4,6] Protein solubility data are fundamental to crystal growth studies. Solubility is a measure indicating that all forces maintaining protein molecules in the solution are at a thermodynamic equilibrium state with the crystalline phase. In addition, understanding the behavior of protein solubility is not only important for defining the phase-diagram domain in which crystallization may occur, but it is also a crucial parameter for the characterization of supersaturation conditions, which is essential for controlling the quality and size of protein crystals, and the competition between protein crystallization and amorphous aggregation.[7,8]

Ionic liquids (ILs), as a distinct materials, are attracting more and more attention because of their unique physical and chemical properties and low melting points, as they can exhibit intrinsically useful characteristics such as a wide liquid range, a negligible vapor pressure, a large electrochemical window, and a high electric conductivity.[9−11] ILs are considered as an alternative to volatile organic solvents in chemical processing and extraction and have numerous potential applications in many other fields. For example, these compounds have been applied as solvents for organic reactions[12] and liquid−liquid extraction.[13] Recently, some investigations using ILs for protein crystallization have been reported. Pusey et al.[14] used ethyl ammonium nitrate as a precipitating agent for lysozyme crystallization, and good crystals for diffraction studies were obtained. Pusey et al. have developed this idea by exploring crystallization behaviors of four model proteins using three ILs. Judge et al.[15] have investigated the use of ILs as precipitating agents and additives for protein crystallization for six model proteins. ILs bring modifications in crystal morphology and significantly increase the crystal size in some cases. Crystals grown in ILs as precipitating agents or as additives give an X-ray diffraction resolution similar to or better than that obtained without ILs. Some improvements of morphology and the crystal diffraction quality are observed. However, the data obtained do not indicate a clear mechanism for how the ILs affect the crystallization process, and an essential factor for crystallization, the effect of ILs on the solubility of protein, was not assessed.

The crystallization of the hen egg white lysozyme has been studied for some years since it is an ideal model system for the crystallization of proteins in general.[16−19] In the present study, the effect of four soluble ILs, that is, 1-butyl-3-methylimidazolium tetrafluoroborate ([C_4mim]BF_4), 1-butyl-3-methylimidazolium chloride ([C_4mim]Cl), 1-butyl-3-methylimidazolium bromide ([C_4mim]Br), and 1,3-dimethylimidazolium iodine ([dmim]I), on the solubility of the lysozyme are investigated, aiming to gain insights into the underlying mechanisms of ILs interacting with protein crystallization, to provide valuable thermodynamic data of the model protein, and to present ILs as a potentially useful additives for crystal growth of other proteins.

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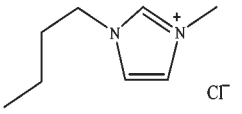
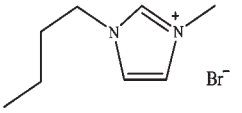
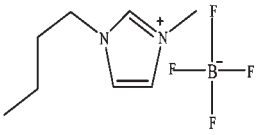
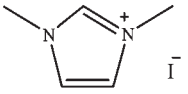
Table 1. Structures and Molecular Weight of ILs Used

ILs	Abbreviation	Structure	Molecular weight
1-butyl-3-methylimidazolium chloride	$[C_4mim]Cl$		174.7
1-butyl-3-methylimidazolium bromide	$[C_4mim]Br$		219.1
1-butyl-3-methylimidazolium tetrafluoroborate	$[C_4mim]BF_4$		226.0
1,3-dimethylimidazolium iodine	[dmim]I		223.9

EXPERIMENTAL PROCEDURES

Reagents. Hen egg white lysozyme (more than 99 % mass fraction purity, according to the supplier) was purchased from Genview (USA) and used without further purification. Distilled, deionized water was used in all of the experiments. Analytical grade anhydrous sodium acetate, glacial acetic acid, and sodium chloride were purchased from Tianjin Kewei Co. of China. The ILs (more than 99 % mass fraction purity) were purchased from Shanghai Cheng Jie Chemical Co., Ltd., of China. The structure of the ILs is shown in Table 1.

Measurement of Solubility. Sodium acetate buffer at pH 4.5 was prepared with deionized water. Protein stock solutions were prepared by dissolving protein powder in buffers at pH 4.5 and then filtered through 0.22 μm filters (Millex-VV) for further experiments. Precipitant solutions were prepared by dissolving the required amount of sodium chloride together with ILs in buffers. The pH of solutions was measured by a digital pH meter (Mettler Toledo 320) and adjusted to 4.5 by the addition of small volumes of NaOH or HCl solution. Solubility data were obtained by controlling the temperature of the protein powder at 25 °C and the respective solvent system in a suspension under agitation for a period of time long enough to reach equilibrium (monitored periodically by UV absorption at 280 nm) when the protein concentration in solution was held constant. Protein concentration was measured by measuring the absorbance at 280 nm with UV spectroscopy (Shimadzu UV-2550), according to the methodology described by Gehle.[20] By repeating the above procedure by changing the IL types and amounts of IL addition at different temperatures, whole sets of solubility data can be obtained.

RESULTS AND DISCUSSION

Lysozyme solubility, x, as a function of salt NaCl molality, m, at 25 °C and pH 4.5 are shown in Figure 1. As expected, x decreases as m increases. It can be observed that the solubility curve sharply declines for $m < 0.8$ mol·kg^{-1} but becomes flat after $m > 0.8$ mol·kg^{-1}. Those data obtained from this work are well-consistent with solubility data previously reported on the same systems in the literature,[2] which ensures the reliability of the measurement.

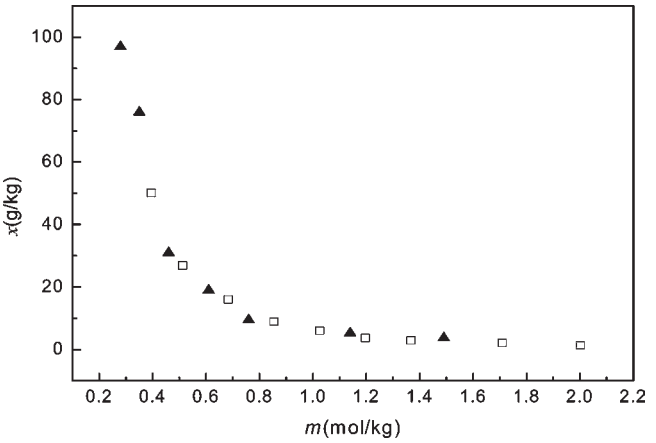


Figure 1. Lysozyme solubility at different NaCl concentrations. □, from this work; ▲, from the work of Annunziata et al.[2]

The solubilities of the lysozyme at IL addition concentrations (in sodium acetate solution with 0.85 mol·kg^{-1} NaCl at 25.0 °C, pH = 4.50) are illustrated in Figure 2 and Table 2. x represents the mass fraction solubility of lysozyme, and x_1 represents the mass fraction of ILs in solution. Neglecting the probable effect of impurities and NaCl, it is suggested that different ILs have different influences on the lysozyme solubility. As shown in Figure 2, the solubility of the lysozyme increases with the increase of addition concentration of $[C_4mim]BF_4$ and $[C_4mimCl]$ but is nearly flat with the increase of addition concentration of $[C_4mim]Br$. Interestingly, the solubility of lysozyme slightly decreases with the increase of addition concentration of [dmim]I. The four ILs used in this study, that is, $[C_4mim]BF_4$, $[C_4mim]Cl$, $[C_4mim]Br$, and [dmim]I, all have a imidazole

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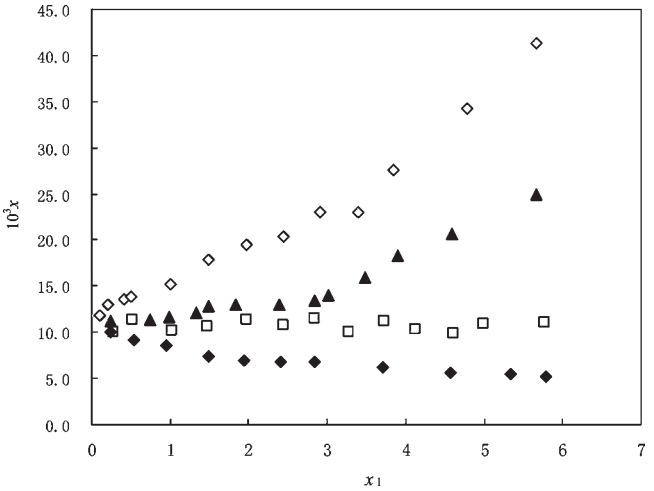

Figure 2. Variation of lysozyme mass fraction solubility (x) with IL mass fraction (x_1) in sodium acetate solution with 0.85 mol·kg^{-1} NaCl at pH 4.50 and 25.0 °C. ◇, [C_4mim]BF_4; ▲, [C_4mim]Cl; □, [C_4mim]Br; ◆, [dmim]I.

Table 2. Variation of Lysozyme Mass Fraction Solubility (x) with IL Mass Fraction (x_1) in Sodium Acetate Solution with 0.85 mol·kg^{-1} NaCl at pH 4.50 and 25.0 °C

$10^2\,x_1$	$10^3\,x$	$10^2\,x_1$	$10^3\,x$	$10^2\,x_1$	$10^3\,x$
[C_4mim]BF_4		[C_4mim]Cl		[C_4mim]Br	
0.100	11.87	0.249	11.17	0.279	10.05
0.200	13.03	0.744	11.32	0.517	11.39
0.408	13.61	0.990	11.64	1.020	10.20
0.498	13.81	1.332	12.06	1.468	10.68
1.000	15.16	1.497	12.86	1.980	11.41
1.498	17.82	1.836	12.93	2.439	10.72
1.970	19.42	2.391	13.04	2.847	11.47
2.439	20.34	2.847	13.40	3.279	10.09
2.913	22.96	3.007	14.08	3.726	11.21
3.391	23.07	3.475	15.97	4.132	10.29
3.846	27.65	3.892	18.35	4.608	9.890
4.780	34.20	4.598	20.65	4.988	10.98
5.669	41.26	5.669	24.95	5.776	11.03
[dmim]I					
0.249	10.02				
0.537	9.201				
0.951	8.629				
1.497	7.445				
1.932	6.956				
2.411	6.754				
2.847	6.773				
3.717	6.134				
4.580	5.668				
5.339	5.472				
5.794	5.183				

group in common but differ in their anion and represent two different parent cations. From the results obtained in this study, it can be concluded that ILs do not always affect the solubility of protein in a similar manner and IL screening would be required for protein crystallization purposes.

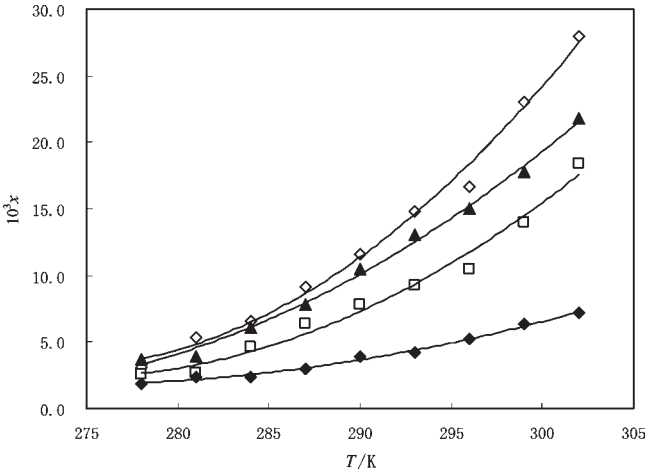

Figure 3. Variation of lysozyme mass fraction solubility (x) with temperature with IL mass fraction x_1 = 0.029 in sodium acetate solution with 0.85 mol·kg^{-1} NaCl at pH 4.50. ◇, [C_4mim]BF_4; ▲, [C_4mim]Cl; □, [C_4mim]Br; ◆, [dmim]I.

Table 3. Variation of Lysozyme Mass Fraction Solubility (x) with Temperature with IL Mass Fraction x_1 = 0.029 in Sodium Acetate Solution with 0.85 mol·kg^{-1} NaCl at pH 4.50

T/K	$10^3\,x$	T/K	$10^3\,x$	T/K	$10^3\,x$
[C_4mim]BF_4		[C_4mim]Cl		[C_4mim]Br	
278.0	3.123	278.0	3.724	278.0	2.575
281.0	5.329	281.0	3.944	281.0	2.647
284.0	6.587	284.0	6.047	284.0	4.653
287.0	9.174	287.0	7.814	287.0	6.357
290.0	11.60	290.0	10.44	290.0	7.832
293.0	14.77	293.0	13.02	293.0	9.271
296.0	16.67	296.0	14.96	296.0	10.47
299.0	23.03	299.0	17.79	299.0	13.98
302.0	27.99	302.0	21.78	302.0	18.44
[dmim]I					
278.0	1.897				
281.0	2.319				
284.0	2.373				
287.0	2.976				
290.0	3.881				
293.0	4.219				
296.0	5.209				
299.0	6.333				
302.0	7.168				

Some works[4,14] have already indicated that the effect of additives on macromolecule solubility strongly correlates to the strength of molecule interactions. The result from this work, that is, increasing lysozyme solubility with the [C_4mim]BF_4 and [C_4mim] Cl concentration increase, indicates that either repulsive interactions are induced or attractive interactions are reduced among lysozyme molecules. Interestingly, the solubility decrease of lysozyme after adding IL [dmim]I shows that either attractive interactions are enhanced or hydrophobic sites with a salt are promoted to form, while with the presence of [C_4mim]Br, the effect of [C_4mim]Br on lysozyme solubility is negligible, indicating that the minimum effect on molecular

Table 4. Fitting Parameters of the Solubility Data of the Lysozyme in the Presence of an IL with x_1 = 0.029 Using the Equation $x = A + BT + CT^2$ Where x Is the Mass Fraction Solubility, T Is Temperature, and A, B, and C Are Parameters

IL	A	$B \cdot 10^3$	$C \cdot 10^3$
$[C_4mim]BF_4$	3.034	−9.300	29.40
$[C_4mim]Cl$	1.867	206.1	16.30
$[C_4mim]Br$	2.338	−37.90	19.40
[dmim]I	1.742	2.400	6.500

interactions is introduced. The effect of ILs on lysozyme solubility may provide a new, important method to study the role of ILs in protein crystallization from the thermodynamics point of view, which may lead to the improvement of macromolecule crystallization.

To determine the effect of temperature on solubility, the variation of lysozyme solubility with ILs, x_1 = 0.029 and sodium acetate solution with 0.85 mol·kg^{-1} NaCl at pH 4.50, with temperature is studied, and the measured results are shown in Figure 3 and Table 3. All data in Figure 3 are fitted to a second-order polynomial, and the corresponding fitting parameters are listed in Table 4. The solubility in all IL solutions increases with increasing temperature, and the solubility magnitude of the lysozyme at different ILs is in the order of $[C_4mim]BF_4$ > $[C_4mim]Cl$ > $[C_4mim]Br$ >[dmim]I.

Limited numbers of ILs have been chosen to investigate how ILs affect lysozyme crystallization behavior in this study. The future work will focus on the correlation between the rate of nucleation or growth and the effect of ILs on those rates. Solution structure and specific interactions in the presence of ILs are also addressed.

CONCLUSIONS

The effect of four soluble ILs on lysozyme solubility was investigated in this work. The solubility increases with increasing IL concentration of $[C_4mim]BF_4$ and $[C_4mimCl]$. For $[C_4mim]Br$, there is almost no change in solubility with an increase of IL concentration, while the solubility of lysozyme slowly decreases with the increase of addition concentration of [dmim]I. At a certain IL addition concentration, the solubility of the lysozyme increases with increasing temperature. Some probable mechanisms of the effect of ILs on lysozyme crystallization are proposed: that ILs can influence the interactions between lysozyme molecules, change solubility, and thus affect nucleation in crystallization. Those findings can contribute to a better understanding of the effect of ILs involved in macromolecular crystallization, which may be useful for optimizing crystallization and providing a rational design of new conditions with ILs.

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