JOURNAL OF CHEMICAL & ENGINEERING DATA

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Solubility of a Lysozyme in Polyelectrolyte Aqueous Solutions

Jie Lu,*[,†] Xiao-Lin Jiang,[†] Zhen Li,[†] Sohrab Rohani,[‡] and Chi-Bun Ching[§]

[†]School of Chemical and Material Engineering, Jiangnan University, Wuxi 214122, China

[‡]Department of Chemical and Biochemical Engineering, The University of Western Ontario, London, Ontario N6A 5B9, Canada

[§]School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637722, Singapore

ABSTRACT: The solubility of the hen egg-white lysozyme (HEWL) in aqueous solutions at pH 4.5 at various polyelectrolyte concentrations was first measured by a spectroscopical method from (278.15 to 298.15) K under atmospheric pressure. The employed polyelectrolytes were cationic polyacrylamide (CPAM), poly(ethylene imine) (PEI), poly(dimethyldiallylammonium chloride) (PDDA), poly(sodium styrene sulfonate) (PSS), and poly(vinylsulfonic acid, sodium salt) (PVS). The enthalpy and entropy of dissolution have been calculated, and the sol−gel transition has been discussed as well.

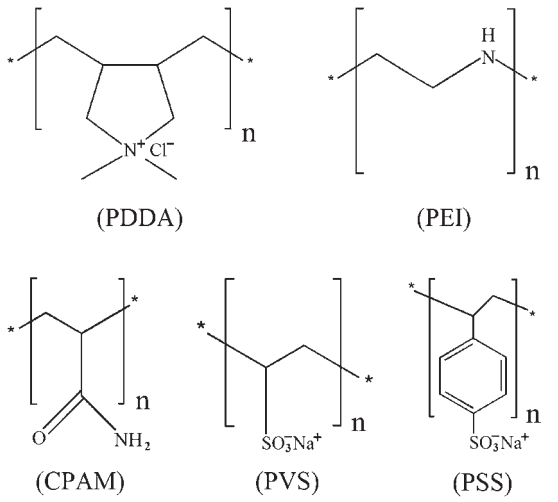
INTRODUCTION

In the context of their separation, purification, and application, it is necessary to obtain information about the solution phase behavior of proteins under various conditions.[1] Recently, physics has shown that the interactions between protein molecules govern their distribution in solution[2] and that the protein solution generally possesses a peculiar phase diagram, that is, a liquid−liquid phase separation and a sol−gel transition.[3] To date, the mechanism of the sol−gel transition is not yet well-understood.[4]

In the past decade, layer-by-layer (LBL) assembly has been particularly considered as a way to construct well-defined spatial architectures with tailored structures and compositions, due to its simplicity and mildness, and it can be controlled at the molecular level with good precision.[5,6] LBL assembly can be conducted by taking advantage of the electrostatic force of attraction, hydrogen bonding, biological affinity, and so forth.[7] The materials employed include synthetic polymers, peptides, proteins, DNA, and polysaccharides.[8] When using polyelectrolytes (PE) and proteins having opposite charges as the anionic and cationic species of the LBL assembly based on electrostatic adsorption, it has been found that proteins embedded in the polyelectrolyte matrix display an enhanced stability against the nanoenvironment,[9] which is of the highest interest for targeted and time-dependent drug release and delivery, biosensors, bioelectronics, and biofuel cells.[10] Calvo et al.[11] reported the LBL film containing glucose oxidase and polyelectrolyte can operate as an electric wire to mediate electron transfer between the enzyme and the electrode. Lvov and co-workers[12] demonstrated that the shrinkage behavior (pore opening and closing) of LBL microcapsules composed of biocompatible and bioresorbable polyelectrolytes can be used for the controlled release of small-molecule antibiotics, protein therapeutics, or plasmid DNA. The release of encapsulated proteins from microcapsules is driven by the concentration difference between the capsule interior and the external medium.[13,14] Fan et al.[15] have applied salting-out and LBL adsorption to develop a new protein delivery system using the nanoaggregates shelled with two oppositely charged polyelectrolytes.

The main objective of this work is to measure the solubility of lysozyme in aqueous solutions at various temperatures and concentrations of polyelectrolytes. The polyelectrolytes employed include cationic polyelectrolytes [poly(ethylene imine), PEI; poly(dimethyldiallylammonium chloride), PDDA; cationic polyacrylamide, CPAM] and anionic polyelectrolytes [poly(sodium styrene sulfonate), PSS; poly(vinylsulfonic acid, sodium salt), PVS], as shown in Scheme 1.[16] These results will contribute to a better understanding of the thermodynamic behavior of globular proteins in polyelectrolyte solutions.

Scheme 1. Structures of Polyelectrolytes Used



EXPERIMENTAL SECTION

Table 1 reports the specifications and suppliers of the chemicals used in this work. All chemicals were used without further purification. The 0.22 mol·kg^{-1} sodium acetate buffer was prepared with ultrafiltered, deionized water (Millipore, Billerica, MA),

Special Issue: Kenneth N. Marsh Festschrift

Received: July 26, 2011
Accepted: September 8, 2011
Published: September 20, 2011

dx.doi.org/10.1021/je200789w | *J. Chem. Eng. Data* 2011, 56, 4808–4812

and the pH was measured by a Mettler Toledo 320 digital meter (Mettler Toledo, Columbus, OH) and slightly adjusted to pH 4.5 by the addition of sodium hydroxide or ethanoic acid solution. Sodium azide, at a concentration of $7.7 \cdot 10^{-3}$ mol·kg^{-1}, was added to the buffer solution as an antimicrobial agent. Polyelectrolyte solutions with desired concentrations were prepared by dissolving known amounts of polyelectrolytes in the buffer.

A jacketed glass vessel of 30 mL was used to measure the solubility of protein under various temperatures and polyelectrolyte concentrations. A Teflon-coated magnetic stirring bar ensured proper mixing in the vessel. The temperature was controlled by a circulator (PolyScience, Niles, IL). First, excess amounts of lysozyme were added to the polyelectrolyte solution and stirred for 12 h. Then the agitation was stopped, and the suspension was allowed to further settle. An aliquot of supernatant was periodically withdrawn and filtered using Millex-VV 0.22 μm filters (Millipore, Billerica, MA). The solid−liquid equilibrium state was regarded to be reached when the refractive index of the supernatant remained unchanged, which was conducted on a DR 301-95 refractive meter (Krüss, Hamburg, Germany). Finally, the supernatant in equilibrium with a macroscopically observable solid was diluted with the buffer, and its concentration was obtained through measuring the absorbance at 280 nm by a UV-2550 spectrophotometer (Shimadzu, Tokyo, Japan) at room temperature. The extinction coefficient of lysozyme obtained through calibration experiments is $2.4 \cdot 10^3$ cm^{-1} in the mass fraction range of $(0 \text{ to } 2) \cdot 10^{-4}$. The calibration curve was determined in the buffer at room temperature. All experiments were replicated three times. The data reported in this work are the average of the replicates.

Table 1. Purities and Suppliers of Chemical Samples

chemical name	supplier	mass fraction purity	molecular weight
lysozyme	Sigma-Aldrich	≥ 0.98[f]	14300
sodium azide	Sigma-Aldrich	≥ 0.99[g]	65.01
sodium acetate	Sigma-Aldrich	≥ 0.99[g]	82.03
ethanoic acid	Sigma-Aldrich	≥ 0.99[g]	60.05
sodium hydroxide	Sigma-Aldrich	≥ 0.99[g]	40.00
CPAM[a]	Fuji Kasei	≥ 0.90[h]	$6 \cdot 10^6 \sim 12 \cdot 10^6$
PSS[b]	Fuji Kasei	≥ 0.90[h]	10600
PDDA[c]	Fuji Kasei	0.20[i]	$1 \cdot 10^5 \sim 2 \cdot 10^5$
PEI[d]	Fuji Kasei	0.46[i]	$7.5 \cdot 10^5$
PVS[e]	Fuji Kasei	0.25[i]	5000

[a] CPAM = cationic polyacrylamide. [b] PSS = poly(sodium styrene sulfonate). [c] PDDA = poly(dimethyldiallylammonium chloride). [d] PEI = poly(ethylene imine). [e] PVS = poly(vinylsulfonic acid, sodium salt). [f] Sodium dodecyl sulfate polyacrylamide gel electrophoresis. [g] Gas chromatography. [h] Gel permeation chromatography. [i] Aqueous solution.

RESULTS AND DISCUSSION

Solubility. The solubility of the lysozyme at different temperatures in the presence of various polyelectrolytes is listed in Tables 2 to 6. Like most globular proteins, the lysozyme possesses the character of normal solubility, which generally increases with temperature in all polyelectrolyte solutions. By means of linear regression for the van't Hoff relation $\ln S = -(\Delta H^{diss}/RT) + (\Delta S^{diss}/R)$ using the solubility data, we obtained the dissolution enthalpies ΔH^{diss} and dissolution entropies ΔS^{diss}, as shown in Tables 2 to 6.

Many works have demonstrated that the solubility of a biomolecule in aqueous solutions is a result of different potentials like electrostatic, van der Waals, hydrophobic, hydration, and so forth existing in the system.[17] At a pH 4.5 and temperature of

Table 2. Mass Fraction Solubility (x), Dissolution Enthalpy (ΔH^{diss}), and Dissolution Entropy (ΔS^{diss}) of the Lysozyme in Aqueous Solutions with Different CPAM Concentrations (m_1)[a]

$10^5\, m_1$	$10^3\, x$					ΔH^{diss}	ΔS^{diss}
mol·kg^{-1}	T/K = 278.15	T/K = 283.15	T/K = 288.15	T/K = 293.15	T/K = 298.15	kJ·mol^{-1}	J·mol^{-1}·K^{-1}
1.1	149.3	171.1	198.1	224.7	260.0	19.1	110.1
2.2	120.9	137.3	156.5	176.9	202.0	17.6	103.3
3.3	101.0	114.0	128.3	143.5	161.2	16.1	96.1
4.4	87.4	96.7	106.8	118.0	130.7	13.8	86.9
5.5	77.1	84.6	92.3	98.7	106.5	11.0	75.9
6.6	70.0	75.9	81.1	86.1	91.1	9.0	67.8

[a] The average molecular weight was taken as $9 \cdot 10^6$.

Table 3. Mass Fraction Solubility (x), Dissolution Enthalpy (ΔH^{diss}), and Dissolution Entropy (ΔS^{diss}) of the Lysozyme in Aqueous Solutions with Different PDDA Concentrations (m_2)[a]

$10^4\, m_2$	$10^3\, x$					ΔH^{diss}	ΔS^{diss}
mol·kg^{-1}	T/K = 278.15	T/K = 283.15	T/K = 288.15	T/K = 293.15	T/K = 298.15	kJ·mol^{-1}	J·mol^{-1}·K^{-1}
1.3	20.6	32.3	49.8	75.8	113.7	58.9	236.9
2.0	14.5	22.6	34.6	52.4	78.1	58.0	230.9
2.7	10.3	15.8	23.8	35.5	52.3	56.0	220.7
3.3	7.4	11.1	16.4	24.0	34.6	53.2	207.8
4.0	5.2	7.8	11.5	16.6	23.8	52.4	202.1
4.7	4.4	6.5	9.5	13.7	19.6	51.5	197.4

[a] The average molecular weight was taken as $1.5 \cdot 10^5$.

dx.doi.org/10.1021/je200789w |J. Chem. Eng. Data 2011, 56, 4808–4812

Table 4. Mass Fraction Solubility (x), Dissolution Enthalpy (ΔH^{diss}), and Dissolution Entropy (ΔS^{diss}) of the Lysozyme in Aqueous Solutions with Different PEI Concentrations (m_3)

$10^5\ m_3$	$10^3\ x$					ΔH^{diss}	ΔS^{diss}
mol·kg^{-1}	T/K = 278.15	T/K = 283.15	T/K = 288.15	T/K = 293.15	T/K = 298.15	kJ·mol^{-1}	J·mol^{-1}·K^{-1}
1.3	109.4	143.0	185.0	237.4	302.0	35.0	164.9
2.7	23.1	30.1	38.8	49.6	62.9	34.5	150.2
4.0	8.3	10.7	13.7	17.4	21.8	33.3	137.5
5.3	4.8	6.1	7.7	9.7	12.1	31.9	127.7
6.7	3.1	4.0	5.0	6.2	7.6	30.8	120.2
8.0	2.2	2.8	3.4	4.1	5.0	27.9	107.0

Table 5. Mass Fraction Solubility (x), Dissolution Enthalpy (ΔH^{diss}), and Dissolution Entropy (ΔS^{diss}) of the Lysozyme in Aqueous Solutions with Different PSS Concentrations (m_4)

$10^3\ m_4$	$10^3\ x$					ΔH^{diss}	ΔS^{diss}
mol·kg^{-1}	T/K = 278.15	T/K = 283.15	T/K = 288.15	T/K = 293.15	T/K = 298.15	kJ·mol^{-1}	J·mol^{-1}·K^{-1}
2.8	gelation	gelation	gelation	gelation	gelation		
3.3	4.5	5.6	6.9	8.5	10.3	28.6	115.3
3.8	4.9	6.1	7.5	9.2	11.3	28.7	116.4
4.2	5.2	6.6	8.1	10.0	12.3	29.5	119.7
4.7	5.6	7.1	8.7	10.7	13.3	29.7	121.1
5.7	6.1	7.7	9.6	11.9	14.6	30.1	123.2

Table 6. Mass Fraction Solubility (x), Dissolution Enthalpy (ΔH^{diss}), and Dissolution Entropy (ΔS^{diss}) of the Lysozyme in Aqueous Solutions with Different PVS Concentrations (m_5)

$10^3\ m_5$	$10^3\ x$					ΔH^{diss}	ΔS^{diss}
mol·kg^{-1}	T/K = 278.15	T/K = 283.15	T/K = 288.15	T/K = 293.15	T/K = 298.15	kJ·mol^{-1}	J·mol^{-1}·K^{-1}
2.0	0.7	0.9	1.2	1.5	2.0	38.6	135.4
4.0	2.4	3.5	5.0	7.1	9.8	47.9	179.7
6.0	4.6	7.4	11.8	18.6	28.7	63.3	240.2
8.0	7.5	12.9	21.8	36.1	58.7	70.8	271.4
10.0	10.7	19.5	34.6	60.2	103.0	78.0	300.0
12.0	14.5	27.3	50.0	90.0	158.6	82.4	318.5

18 °C, the mass fraction solubility of lysozyme in water was reported as 0.36.[18] As shown in Tables 2 to 6, all polyelectrolytes exhibit a same effect of salting-out on the solubility of lysozyme, to a different tendency and extent.

The isoelectric point of lysozyme is around 10.7.[19] In water the dissolved lysozyme molecules are present in the form of zwitterions $^{-}$(Lys)$^{+}$ and carry net positive charges at pH 4.5. When cationic or anionic polyelectrolytes are added, the hydration of introduced ions shall decrease that of lysozyme zwitterions, which will result in a lower solubility of the lysozyme in polyelectrolyte solutions.[20]

The hydration of cationic polyelectrolytes is dependent upon their types and concentrations. As shown in Tables 2 to 4, the solubility of lysozyme decreases with an increase in the concentration of cationic polyelectrolytes. This can be attributed to an increased hydration of cationic polyelectrolytes when their concentrations are increased. Tables 2 to 4 also show that the hydration ability of PDDA and PEI may be higher than that of

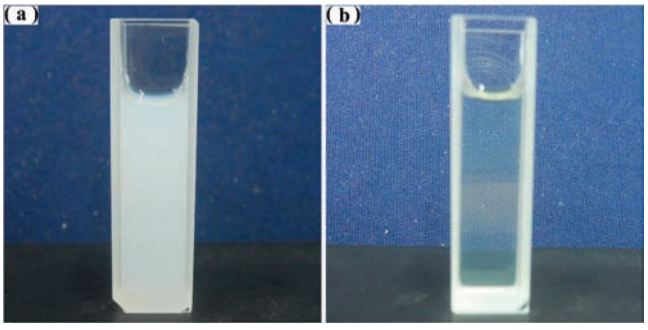


Figure 1. Formation of colloidal particles of lysozyme and PSS in aqueous solution: (a) before centrifugation; (b) after centrifugation.

CPAM. On the other hand, the introduction of excess positive charges from cationic polyelectrolytes shall also increase the electrostatic repulsion between positive charged lysozyme

dx.doi.org/10.1021/je200789w |J. Chem. Eng. Data 2011, 56, 4808–4812

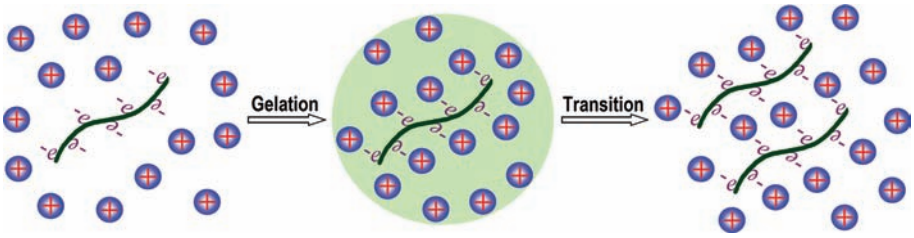


Figure 2. Schematic illustration for the gel−sol transition of the lysozyme in PSS aqueous solution.

molecules and water molecules hydrated with cationic polyelectrolytes. With regard to anionic polyelectrolytes, in addition to the ionic hydration which will greatly decrease the solubility, the introduction of net negative charges shall increase the electrostatic attraction between positive charged lysozyme molecules and water molecules hydrated with anionic polyelectrolytes, leading the solubility to increase with the concentration of the anionic polyelectrolytes. In general, the effect of polyelectrolytes on the solubility of lysozyme depends on both the specific nature and the concentration of polyelectrolytes.[21,22]

Dissolution Enthalpy. Dissolution enthalpy ΔH^{diss} can be regarded as a reflection of the nature of intermolecular contacts and hydration. The larger ΔH^{diss} corresponds to the larger interfacial energy between crystal and solution, leading to more difficulty in the crystallization of the solute from the solution.[23] As expected, the dissolution enthalpy ΔH^{diss} of lysozyme is positive, showing that the dissolution of lysozyme in polyelectrolyte aqueous solutions is an endothermic process. In addition, $\Delta G^{diss} = \Delta H^{diss} - T \cdot \Delta S^{diss}$ are found to be negative, demonstrating that the dissolution of lysozyme in polyelectrolyte aqueous solutions is spontaneous. On the other hand, ΔH^{diss} was decreased with a decrease in the anionic polyelectrolyte concentration and with an increase in the cationic polyelectrolyte concentration, indicating that both anionic polyelectrolytes with low concentrations and cationic polyelectrolytes with high concentrations can promote nucleation when they are utilized as the precipitants for protein crystallization at pH values less than its isoelectric point.

Gelation. As shown in Table 5 and Figure 1, when the lysozyme powders were added into the PSS solutions at concentrations less than 3.0 mol·kg^{-1}, gelation took place. After centrifugation, those large gel-like particles settled down, and microgels can still be observed.

As shown in Figure 2, when the lysozyme was added to the solution of strong polyelectrolyte PSS at low concentration, the lysozyme and PSS will pack closely and formed a series of complexes because of strong electrostatic attraction, and they became denser as the PSS concentration increased (in the case that the amount of lysozyme was enough for the complexation). During centrifugation, the big complexes can be settled down, whereas the small ones cannot. When the PSS concentration reached a critical value (e.g., 3.3 mol·kg^{-1} in this work), the lysozyme molecules shall be detached from the microgels to the vicinity of newcomers of PSS, followed by the phase transition from gel to solution.[24] Being quite different from PSS, PVS is a kind of weak polyelectrolyte and is much less negatively charged. When the lysozyme was dissolved in the PVS aqueous solution at pH 4.5, lysozyme molecules with positive charges and PVS molecules with less negative charges will not form the mirogels.

CONCLUSIONS

We have presented experimental results on the solubility of lysozyme in aqueous solutions of CPAM, PEI, PDDA, PSS, and PVS over a temperature range from (278.15 to 298.15) K. The solubility generally increases with the temperature. All polyelectrolytes show a predominant effect of salting-out on the solubility to a different tendency and extent. At pH 4.5, the solubility of the lysozyme will be further decreased with the addition of cationic polyelectrolytes but increased with the addition of anionic polyelectrolytes, which can be attributed to the results of electrostatic and hydration interactions. Furthermore, a strong anionic polyelectrolyte like PSS and net positively charged lysozyme will form colloidal particles at low concentrations of PSS. These results can contribute to a better understanding of the thermodynamic behavior of charged proteins in polyelectrolyte solutions. Further work will focus on the clarification of sol−gel transitions which are frequently found in most protein solutions.

AUTHOR INFORMATION

Corresponding Author

*E-mail: dr.lujie@gmail.com. Fax: 86-510-8591 7763.

Funding Sources

This work was supported by the National Natural Science Foundation of China (Nos. 21176102 and 21176215), the Scientific Research Foundation for Returned Chinese Scholars, and the Fundamental Research Funds for the Central Universities (No. JUSRP30904).

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