Tetragonal Chicken Egg White Lysozyme Solubility in Sodium Chloride Solutions

Elizabeth L. Forsythe,[†] Russell A. Judge,[‡] and Marc L. Pusey*[,§]

Universities Space Research Association, Alliance for Microgravity Material Science and Applications, and Biophysics ES76, NASA/Marshall Space Flight Center, Huntsville, Alabama 35812

The solubility of chicken egg white lysozyme crystallized in the tetragonal form was measured in sodium chloride solutions from 1.6 to 30.7 °C, using a miniature column solubility apparatus. Sodium chloride solution concentrations ranged from 1 to 7% (w/v). The solutions were buffered with 0.1 M sodium acetate buffer, with the solubility being measured at pH values in 0.2 pH unit increments in the pH range 4.0−5.4, with data also included at pH 4.5. Lysozyme solubility was found to increase with increasing in temperature and decreasing salt concentration. Solution pH has a varied and unpredictable effect on solubility.

Introduction

Protein solubility data are fundamental to protein crystal growth studies. Despite this, solubility data have only been published for a small number of macromolecules (Judge et al., 1996), including concanavalin A, porcine pancreatic α-amylase isoenzymes, canavalin, glucose isomerase, rennin, and ovalbumin. The protein most often used in many crystallization studies however is chicken egg white lysozyme crystallized in the tetragonal form. A small sample of such studies include crystal nucleation (Judge et al., 1998), crystal growth rate measurement (Forsythe and Pusey, 1994; Vekilov and Rosenberger, 1996), and even microgravity crystallization (Snell et al., 1995; Ries-Kautt et al., 1997). Some solubility data for chicken egg white lysozyme in sodium chloride solutions have been previously reported (Howard et al., 1988; Ataka and Asai, 1988). These authors measured solubility in batch crystallizations, for which the temperature and pH ranges investigated encompassed both the tetragonal and orthorhombic crystal forms.

In this paper we present extensive solubility data for tetragonal chicken egg white lysozyme obtained using a miniature column solubility apparatus, in which the solubility was determined by both crystallization and dissolution. The data cover the tetragonal lysozyme solubility range in greater detail with smaller increments of pH and temperature than previously recorded. It should also be noted that sodium acetate buffer concentration is known to affect lysozyme solubility (Forsythe and Pusey, 1996), and the buffer concentration used in this study is different from that used by previous researchers (Howard et al., 1988; Ataka and Asai, 1988). A third-order polynomial correlation, fit to the data presented in this paper, with an average error of ±7.4%, has previously been published (Cacioppo and Pusey, 1991). This is the first time, however, that the experimental data have been reported.

* Corresponding author. E-mail: marc.pusey@msfc.nasa.gov.
[†] Universities Space Research Association.
[‡] Alliance for Microgravity Material Science and Applications.
[§] Biophysics ES76.

Table 1. Lysozyme Solubility with Temperature at pH 4.0 in 1, 3, 5, and 7% Sodium Chloride Solutions

	solubility			
t/°C	1%	3%	5%	7%
2.4	32	1.0	0.22	0.14
3.0	35	1.0	0.25	0.15
4.0	42	1.1	0.28	0.18
5.6	48	1.3	0.34	0.20
7.0	56	1.6	0.38	0.23
8.4	63	1.8	0.43	0.24
9.6	75	2.0	0.51	0.29
11.2	95	2.5	0.61	0.34
12.6	111	2.9	0.72	0.39
13.7		3.4	0.83	0.45
15.1		3.9	0.95	0.50
16.2		4.4	1.06	0.56
17.1		4.9	1.19	0.62
18.0		5.3	1.30	0.67
19.0		6.2	1.57	0.87
20.5		7.4	1.75	0.92
22.0		9.2	2.12	
23.3		10.8	2.51	
24.2		13.1	2.91	1.24
25.3		14.9	3.21	1.38
26.0		16.8	3.63	1.52
26.7		18.3	3.95	1.72
27.5		20.1	4.26	1.83
28.3		22.8	4.85	2.11
29.1		25.5	5.35	2.35
29.8		27.5	5.77	2.50
30.5		30.7	6.18	2.71

Experimental Section

As detailed experimental methods for the preparation of the protein and the use of the miniature column solubility apparatus have been previously published (Cacioppo and Pusey, 1991; Pusey and Munson, 1991; Cacioppo et al., 1991; Pusey and Gernert, 1988), only a brief outline of the method is presented here. Chicken egg white lysozyme (grade III) was purchased from Sigma Chemical Co. (St. Louis, MO). It was dissolved in sodium acetate buffer (pH 4.0), dialyzed against the same, and recrystallized by dialysis against sodium acetate buffer pH 4.0 containing 5% (w/v) sodium chloride. The crystals were harvested and redissolved by exhaustive dialysis against sodium acetate buffer at the pH set for the solubility experiments. Lysozyme prepared in this way has a purity

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Table 2. Lysozyme Solubility with Temperature at pH 4.0 in 2, 2.5, 3.5, 4, and 4.5% Sodium Chloride Solutions

	solubility				
t/°C	2%	2.5%	3.5%	4%	4.5%
4.0	3.1	1.6	0.61	0.40	0.29
6.8	4.7	2.3	0.91	0.63	0.44
8.4	6.3	3.0	1.26	0.91	0.56
10.0	7.5	3.6	1.41	0.95	0.63
10.8	8.8	3.8	1.56	1.03	0.69
11.9	10.0	4.5	1.69	1.17	0.84
12.5	10.1	4.5	1.77	1.19	0.79
13.0	10.1	4.5	1.74	1.20	0.78
13.7	10.7	4.8	1.86	1.27	0.83
14.4	11.6	5.1	2.01	1.38	0.90
15.0	12.6	5.6	2.17	1.47	0.98
15.5	13.5	5.9	2.26	1.54	1.02
16.6	15.5	6.6	2.55	1.74	1.17
17.3	16.5	7.1	2.75	1.88	1.27
18.0	18.1	7.7	2.94	2.05	1.38
18.7	19.6	8.5	3.21	2.22	1.50
19.4	22.0	9.1	3.45	2.38	1.63
20.0	24.1	10.0	3.74	2.57	1.75
20.7	26.9	11.1	3.93	2.70	1.89
22.0	33.6	13.1	4.59	3.03	2.07
22.5	34.8	13.9	4.77	3.13	2.15
23.1	37.3	14.5	5.05	3.35	2.28
23.9	42.7	16.1	5.45	3.77	2.45
24.5	45.3	17.1	5.74	4.03	2.71
25.0	47.9	17.8	6.09	4.45	3.08
25.6	50.8	18.9	6.50	4.74	3.23
26.3		20.8	6.98	5.38	3.58
27.1		23.7	7.78	6.06	3.98
27.7		26.4	8.62	6.55	4.38
28.3		29.4	9.43	6.96	4.46
29.0		32.0	10.33	7.61	5.03
29.9		36.1	11.90	8.70	5.65
30.7		40.3	13.14	9.98	6.38

Table 3. Lysozyme Solubility with Temperature at pH 4.2 in 2, 3, 4, 5, and 7% Sodium Chloride Solutions

	solubility				
t/°C	2%	3%	4%	5%	7%
1.6	4.6	1.5	0.69	0.53	0.33
3.6	4.8	1.4	0.71	0.59	0.32
6.5	5.6	1.7	0.89	0.73	0.39
10.3	8.1	2.5	1.28	1.00	0.58
12.0	13.6	3.9	1.82	1.51	0.82
14.2	12.9	3.8	1.80	1.33	0.79
16.0	23.6	5.9	2.90	2.12	1.08
18.4	22.6	6.1	2.86	1.77	1.09
20.0	36.7	8.9	4.24	3.01	1.46
22.1	37.6	9.6	4.17	2.74	1.50
23.2	54.0	12.9	6.83	3.77	1.79
24.9	77.3	14.0	6.93	3.76	1.94

with respect to other protein species of 98.8%, as determined by SDS−PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) analysis with Coomassie blue staining using a PHASTSYSTEM with PHASTGEL gradient 8−25% gels (Pharmacia LKB Biotechnology, Piscataway, NJ). Only one other protein species was present, a 28 kDa impurity, a lysozyme dimer (Back, 1984; Thomas et al., 1996) that is commonly present in commercial lysozyme preparations and is readily incorporated into lysozyme crystals (Skouri et al., 1995).

Lysozyme solubility was obtained using a miniature column technique developed for the rapid determination of protein solubility diagrams (Pusey and Munson, 1991). The technique uses small columns (1.0, 0.4, or 0.1 mL) packed with microcrystals, through which lysozyme solution at a set pH and sodium chloride concentration is passed. Microcrystals for the columns were generated by dialysis of prepared lysozyme solution against 5% (w/v) sodium chloride in sodium acetate buffer. The crystalline beds provide high crystal surface area and small interstitial solution volumes to increase the rate at which equilibrium

Table 4. Lysozyme Solubility with Temperature at pH 4.4 in 2, 3, 4, 5, and 7% Sodium Chloride Solutions

	solubility				
t/°C	2%	3%	4%	5%	7%
2.1	3.2	1.3	1.07	0.63	0.36
3.4	3.7	1.3	0.84	0.53	0.37
4.2	3.7	1.5	1.09	0.59	0.40
5.1	5.1	1.5	1.24	0.68	0.41
6.5	4.5	1.5	0.83	0.60	0.43
6.9	4.7	1.7	1.24	0.83	0.44
7.7	5.1	1.6	0.93	0.65	0.48
8.9	5.9	2.0	1.26	0.99	0.52
10.6	6.7	2.2	1.22	0.84	0.58
11.5	7.7	2.6	1.52	1.01	0.57
12.2	8.1	2.6	1.40	0.96	0.65
12.9	9.1	3.0	1.49	0.95	0.60
13.6	9.5	3.0	1.57	1.08	0.73
15.7	13.9	4.2	2.07	1.25	0.77
16.6	13.1	4.0	2.08	1.39	0.88
17.3	16.6	4.9	2.35	1.46	0.88
18.3	16.2	4.9	2.51	1.65	1.01
18.9	20.7	5.9	2.91	1.77	1.06
19.7	19.6	5.7	2.80	1.78	1.15
20.5	24.4	7.2	3.74	2.30	1.25
21.1	23.2	6.8	3.32	2.14	1.28
22.6	31.4	9.0	5.37	2.82	1.51

Table 5. Lysozyme Solubility with Temperature at pH 4.5 in 2, 3, 4, 5, and 7% Sodium Chloride Solutions

	solubility				
t/°C	2%	3%	4%	5%	7%
1.8	2.4	1.0	0.70	0.45	0.29
2.9	2.7	1.1	0.73	0.49	0.32
4.1	2.9	1.2	0.77	0.52	0.33
5.2	3.1	1.2	0.80	0.58	0.36
6.5	4.0	1.4	0.89	0.63	0.41
7.9	4.1	1.6	0.99	0.70	0.44
9.1	5.7	2.1	1.25	0.86	0.54
10.4	6.3	2.4	1.33	0.95	0.58
11.8	7.0	2.6	1.46	1.06	0.65
13.3	8.2	3.0	1.64	1.17	0.72
14.7	9.2	3.4	1.83	1.30	0.78
15.4	10.6	4.0	2.04	1.40	0.82
16.0	10.5	3.9	2.08	1.48	0.87
16.5	12.7	4.6	2.32	1.60	0.92
17.2	12.0	4.4	2.36	1.65	0.96
17.9	14.9	5.1	2.61	1.77	1.00
18.7	13.8	5.0	2.70	1.84	1.05
19.2	16.9	5.9	3.02	2.02	1.15
20.0	16.4	5.9	3.10	2.13	1.20
20.5	19.9	6.8	3.43	2.30	1.28
21.4	19.6	7.1	3.59	2.43	1.37
22.7	23.7	8.3	4.16	2.79	1.56

is approached. The true solubility value is bracketed by running columns in pairs, one approaching solubility by crystallization and the other by dissolution. Temperature was maintained within ±0.1 °C using a water bath. Lysozyme solution concentrations were measured by UV spectroscopy using A(1%, 1 cm, 281.5 nm) = 26.4 (Aune and Tanford, 1969).

Results

In the following tables sodium chloride concentration, C_{Nacl}, is given as a weight/volume percent, that is, grams of sodium chloride/100 mL of solution. Lysozyme concentration is given as mg of lysozyme/mL of solution. The presented solubility data are the average of the experimental data measured by crystallization and dissolution at the same conditions. The estimated error associated with the presented solubility data varies with sodium chloride concentration. At 1% sodium chloride concentration the error is ±1 mg/mL, at 2 and 2.5% it is ±0.5 mg/mL, at 3% it is ±0.2 mg/mL, and at 3.5−7% the error is ±0.1 mg/mL or better. This is thought to be due to faster equilibration

Table 6. Lysozyme Solubility with Temperature at pH 4.6 in 2, 3, 4, 5, and 7% Sodium Chloride Solutions

	solubility				
t/°C	2%	3%	4%	5%	7%
1.7	3.6	1.1	0.61	0.48	0.35
2.3	3.9	1.6	0.66	0.46	0.35
3.2	3.8	1.5	0.68	0.49	0.37
4.1	4.0	1.7	0.68	0.49	0.37
4.5	4.0	1.3	0.74	0.55	0.39
5.2	4.3	1.6	0.77	0.54	0.41
5.9	4.7	2.0	0.80	0.57	0.42
6.4	5.9	2.1	0.94	0.64	0.47
7.0	5.1	2.2	0.88	0.60	0.45
7.5	5.6	1.9	1.11	0.77	0.51
8.0	5.6	2.4	0.95	0.66	0.47
8.4	6.0	2.0	1.10	0.76	0.53
8.9	6.1	2.6	1.02	0.71	0.51
9.4	6.8	2.4	1.39	0.92	0.61
10.5	8.8	2.4	1.33	0.91	0.64
10.9	9.8	3.4	1.36	0.86	0.61
11.4	8.4	2.7	1.47	1.00	0.70
13.4	10.7	3.4	1.37	0.93	0.63
14.7	12.3	3.8	1.54	1.05	0.71
15.1	16.7	5.3	1.98	1.24	0.87
16.0	14.7	4.5	1.80	1.19	0.80
17.4	19.5	5.9	2.26	1.45	0.96
18.2	19.9	5.8	2.30	1.53	1.00
20.0	28.2	8.6	3.06	1.96	1.24
22.3	33.8	10.2	3.68	2.38	1.49
23.6	37.4	11.5	4.22	2.71	1.68

Table 7. Lysozyme Solubility with Temperature at pH 4.8 in 2, 3, 4, 5, and 7% Sodium Chloride Solutions

	solubility				
t/°C	2%	3%	4%	5%	7%
2.8	2.8	1.2	0.55	0.39	0.30
4.3	3.1	1.3	0.62	0.44	0.33
5.6	3.4	1.4	0.67	0.48	0.35
7.0	3.8	1.6	0.70	0.46	0.32
8.2	4.2	1.8	0.83	0.58	0.40
9.7	5.0	2.0	0.94	0.65	0.46
11.2	5.8	2.3	1.06	0.74	0.51
12.7	6.8	2.7	1.23	0.82	0.58
15.6	9.3	3.6	1.59	1.07	0.75
17.1	11.0	4.2	1.82	1.23	0.84
19.6	17.8	6.0	2.53	1.65	1.13
21.3	19.5	6.9	2.90	1.90	1.29
22.6	22.1	8.2	3.34	2.22	1.53

Table 8. Lysozyme Solubility with Temperature at pH 5.0 in 2, 3, 4, 5, and 7% Sodium Chloride Solutions

	solubility				
t/°C	2%	3%	4%	5%	7%
1.8	2.3	1.1	0.65	0.62	0.66
2.9	2.4	1.2	0.69	0.64	0.70
4.2	3.1	1.6	0.96	0.73	0.65
4.6	3.0	1.6	0.97	0.87	0.70
5.2	2.8	1.4	0.82	0.67	0.74
5.8	3.1	1.7	1.02	0.86	0.71
6.5	3.2	1.7	0.93	0.73	0.82
7.9	3.6	1.8	1.05	0.87	0.92
8.5	4.4	2.2	1.35	1.00	0.93
9.8	5.0	2.5	1.57	1.09	1.07
10.4	5.7	2.6	1.54	1.29	1.29
11.0	5.6	2.7	1.76	1.24	1.23
11.8	6.3	2.8	1.67	1.37	1.38
12.5	6.5	3.1	2.02	1.43	1.49
14.7	7.7	3.6	2.03	1.53	1.56
16.0	8.7	4.1	2.29	1.71	1.65
17.2	9.8	4.7	2.55	1.93	1.79
18.7	11.2	5.3	2.93	2.20	1.90
20.0	13.1	6.2	3.32	2.49	2.06
21.4	15.4	7.2	3.87	2.85	2.19

rates at the higher salt concentrations. The data in Tables 3−10 represent the combination of at least two and sometimes four or more data sets, while Tables 1 and 2 contain only a single data set. As the estimated error, based on the reproducibility of the data, was consistent in Tables 3−10, indicating reliability of the experimental method, the results in Tables 1 and 2 have been included.

Table 9. Lysozyme Solubility with Temperature at pH 5.2 in 2, 3, 4, 5, and 7% Sodium Chloride Solutions

	solubility				
t/°C	2%	3%	4%	5%	7%
3.2	3.2	1.3	1.07	1.05	0.96
4.0	3.3	1.4	1.07	1.06	0.97
5.1	3.6	1.5	1.14	1.16	1.04
6.3	4.0	1.7	1.23	1.26	1.10
7.4	4.6	1.8	1.32	1.35	1.15
8.0	4.9	1.9	1.39	1.37	1.15
9.1	5.3	2.1	1.47	1.39	1.19
10.4	6.0	2.3	1.59	1.50	1.27
11.4	6.9	2.5	1.75	1.79	1.52
12.4	7.3	2.8	1.85	1.71	1.40
13.6	8.3	3.1	2.06	1.86	1.49
15.0	9.6	3.6	2.29	2.01	1.58
16.4	11.0	4.1	2.57	2.20	1.69
17.6	13.4	4.6	3.00	2.59	1.95
18.1	13.3	4.9	3.05	2.50	1.88
22.2	21.4	7.8	4.53	3.37	2.39
23.5	25.8	9.0	5.11	3.90	2.67

Table 10. Lysozyme Solubility with Temperature at pH 5.4 in 2, 3, 4, 5, and 7% Sodium Chloride Solutions

	solubility				
t/°C	2%	3%	4%	5%	7%
2.8	2.0	0.8	0.69	0.58	0.57
4.3	2.3	0.9	0.74	0.62	0.61
5.6	2.5	1.0	0.80	0.66	0.65
7.0	2.8	1.2	0.89	0.74	0.72
8.2	3.2	1.3	0.96	0.78	0.74
9.7	3.6	1.5	1.08	0.85	0.82
11.2	4.1	1.7	1.21	0.95	0.92
12.7	4.7	1.9	1.33	1.03	0.96
14.1	5.5	2.3	1.55	1.23	1.08
15.6	6.4	2.7	1.78	1.40	1.21
17.1	7.4	3.1	2.04	1.59	1.35
18.5	8.7	3.6	2.41	1.86	1.51
19.9	9.9	4.2	2.73	2.08	1.61
21.3	12.2	5.0	3.38	2.54	1.84
22.6	13.9	5.7	3.72	2.80	2.09

Lysozyme solubility data reported as a function of temperature and sodium chloride concentration are given for pH 4.0 in Tables 1 and 2. Tables 3−10 contain data obtained at pH 4.2, 4.4, 4.5, 4.6, 4.8, 5.0, 5.2, and 5.4, respectively. In these tables it can clearly be seen that lysozyme solubility increases with temperature and decreases with increasing sodium chloride concentration. The effect of pH is not clearly seen and varies unpredictably with pH.

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