Solubility of Ovalbumin in Ammonium Sulfate Solutions

Russell A. Judge, Michael R. Johns, and Edward T. White*

Department of Chemical Engineering, The University of Queensland, Brisbane 4072, Australia

The solubilities of the protein ovalbumin in aqueous ammonium sulfate solutions ranging from 18 to 30 g ammonium sulfate/100 g water at 30 °C were determined for pH values in the range 4.58−5.4. A correlation of the data is presented which is estimated to predict solubilities within ±5%.

Introduction

Although vital to protein crystal growth studies, protein solubility data have only been published for a small number of proteins. These include concanavalin A (Mikol and Giege, 1989), porcine pancreatic α-amylase isoenzymes (Boistelle et al., 1992), canavalin (DeMattei and Feigelson, 1991), lysozyme (Howard et al., 1988; Cacioppo and Pusey, 1991), glucose isomerase (Chayen et al., 1988), rennin (Bunn et al., 1971), and ovalbumin (Sorensen and Hoyrup, 1915−17; Green, 1931).

Ovalbumin is soluble in water and is usually recovered from solution by crystallization using high concentrations of ammonium sulfate. The crystallization kinetics of ovalbumin in such solutions have been studied (Judge 1995; Judge et al., 1995, 1996), and this paper reports the solubility of ovalbumin in ammonium sulfate solutions.

Ovalbumin is the major protein in egg white. It has a molecular weight of 45 000 and consists of a single chain of 385 amino acid residues (Nisbet et al., 1981). Ovalbumin contains a single disulfide bond (interconnecting two parts of the chain) and a glycosylation (mainly mannose) site (Stein et al., 1991). Three different phosphate forms of ovalbumin occur in egg white, containing either two, one, or zero phosphate groups per molecule. Typically, in egg white, the ratios of each are about 85:12:3, respectively. These forms are not separated by repeated crystallization (Kitabatake et al., 1988). The isoelectric point for the mixed natural egg white ovalbumin is pI = 4.58 (Warner, 1954). In ammonium sulfate solutions ovalbumin forms needlelike crystals (Sorensen and Hoyrup, 1915−17; Judge et al., 1995).

Experimental Section

The solubility of natural ovalbumin at 30 °C was determined as a function of ammonium sulfate concentration and pH. The results are compared with earlier results of Sorensen and Hoyrup (1915−17) which also included the effect of temperature (0−29 °C).

The ovalbumin used in this study was recovered from egg white by crystallization (Judge et al., 1995), following the method of Sorensen and Hoyrup (1915−17). Solubilities were determined by adding a considerable excess of the purified ovalbumin crystals to ammonium sulfate solutions of known concentrations. The pH was adjusted using either diluted aqueous ammonia or sulfuric acid. By choice of starting conditions, equilibrium was approached by both crystallization and dissolution. The results by the two methods were found to be in good agreement. The slurries were kept in sealed Schott bottles, each stirred with a magnetic stirrer and maintained at 30 °C in a water bath. The pH was checked throughout the runs, but adjustment was seldom necessary. Every 2−3 days a sample of solution was taken for ovalbumin concentration analysis using UV spectrophotometry at a wavelength of 280 nm with A(1%, 1 cm) = 7.0 (Stoscheck, 1990) (i.e. for calculation purposes protein absorbances are expressed in terms of an absorbance coefficient, in that for a 1 cm path length at 280 nm, a 1% ovalbumin solution has an absorbance of 7). The runs were continued until a steady solution concentration was obtained (usually 10−15 days). At the end of a run, the purity of the protein was checked using HPLC and SDS-PAGE (sodium dodecyl sulfate−polyacrylamide gel electrophoresis) analysis (Judge et al., 1995). Deterioration was detected for a small number of the tests, and those data were discarded. The solubility results from this study are given in Table 1.

Table 1. Ovalbumin Solubility, C_O, in Ammonium Sulfate Solutions (C_A), at 30 °C

C_A/(g/100 g of water)	pH	C_O/(g/100 g of water)	comments[a]
18.10	4.75	10.87	C HS
19.30	4.70	8.73	C HS
21.00	4.86	8.16	D HS
21.95	4.85	4.87	C HS
21.97	4.88	5.20	C HS
23.20	5.20	9.84	C HS
23.58	4.90	1.93	D
23.93	5.10	3.51	C HS
24.00	4.71	1.00	C HS
24.00	4.90	1.70	D
24.00	5.20	7.00	D
24.00	5.40	13.58	C HS
24.20	4.70	1.00	C HS
24.20	4.92	1.22	C HS
24.20	5.13	3.18	C HS
24.25	5.32	7.42	C HS
24.50	4.91	1.17	C HS
24.70	4.65	0.78	C H
24.70	4.75	0.90	C HS
25.70	4.57	0.39	C HS
25.82	5.10	1.44	C HS
26.00	5.10	1.08	D HS
26.00	5.20	1.41	D
26.00	5.20	1.73	D HS
26.51	4.90	0.52	D
27.01	4.93	0.48	C HS
27.11	4.90	0.45	C HS
27.60	4.90	0.39	C HS
28.00	5.20	0.64	D
28.00	5.35	1.66	C H

[a] The letters C and D represent values by crystallization or dissolution; H and S refer to ovalbumin purity checks by HPLC and SDS-PAGE.

* To whom correspondence should be addressed. Email: tedw@cheque.uq.edu.au.

0021-9568/96/1741-0422$12.00/0

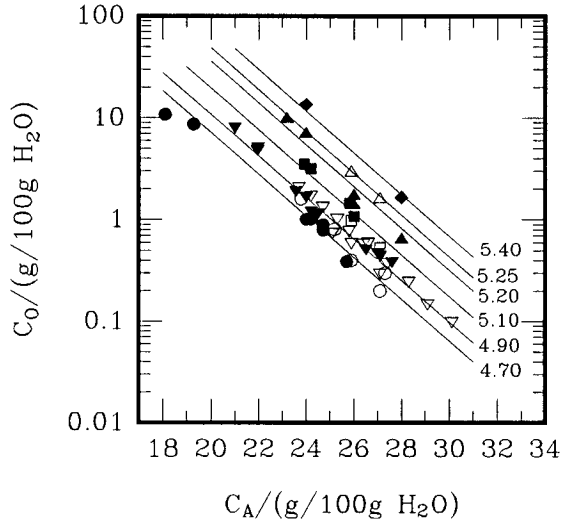

Figure 1. Effect of ammonium sulfate concentration, C_A, on ovalbumin solubility, C_O: Sorensen and Hoyrup (1915−17), 18 °C, pH (○) 4.70, (▽) 4.85, (□) 5.00, (△) 5.25; this work, 30 °C, pH (●) 4.70, (▼) 4.90, (■) 5.10, (▲) 5.20, (◆) 5.40.

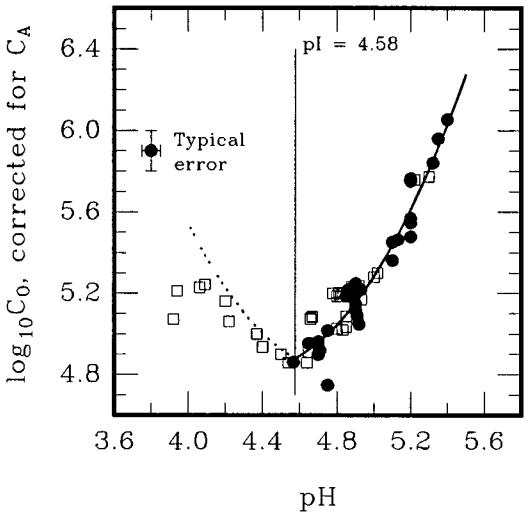

Figure 2. Effect of pH on ovalbumin solubility, C_O: (□) Sorensen and Hoyrup (1915−17), 18 °C; (●) this work, 30 °C.

Table 2. Confidence intervals (95%) on parameters in correlation

term	coefficient	95% confidence interval
constant[a]	5.06	±0.16
t/°C	−0.006	±0.002
C_A/(g/100 g of H_2O)	−0.205	±0.010
(pH − 4.58)	0.5	±0.4
(pH − 4.58)^2	1.1	±0.5

[a] Evaluated after selecting coefficients for other terms.

All concentrations in this work are expressed as the mass of the species per 100 g of water, i.e. ovalbumin, C_O, and ammonium sulfate, C_A, as g/100 g of water.

Results

At a given pH and temperature, the ovalbumin solubility falls exponentially as the ammonium sulfate concentration is increased (Figure 1), as suggested by Green (1931). The data from this work at 30 °C and the data of Sorensen and Hoyrup (1915−17) at 18 °C are shown, and parallel lines result for each pH and temperature. Adjusted for the effect of ammonium sulfate concentration, the results can be plotted against pH (Figure 2). For pH values above the isoelectric point (pH > 4.58), the results can be fitted by a quadratic curve. The present solubility values are consistent with the earlier values but extend the data to lower ammonium sulfate concentrations and higher pH. If the effect of pH is now removed, these results and the earlier results of Sorensen and Hoyrup (1915−17) can be plotted against temperature (Figure 3). There is a slight, but definite, linear trend.

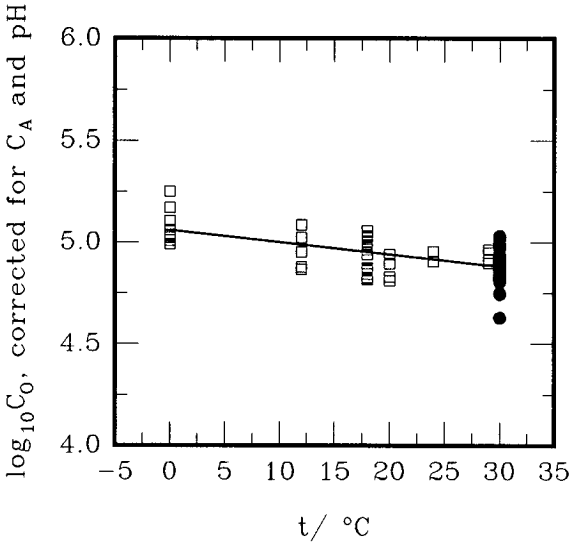

Figure 3. Effect of temperature on ovalbumin solubility, C_O: (□) Sorensen and Hoyrup (1915−17); (●) this work.

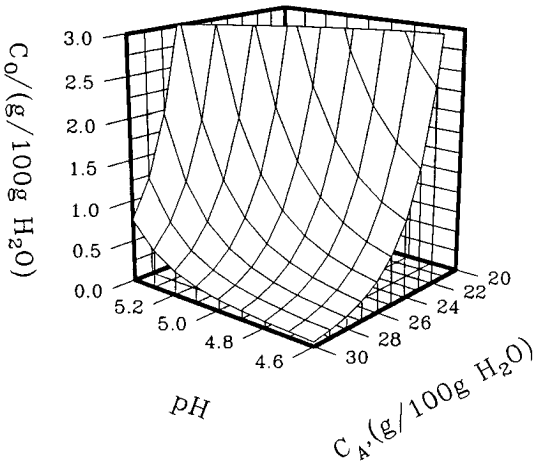

Figure 4. Three-dimensional plot generated using correlation (eq 1) for ovalbumin solubility, C_O, at 30 °C as a function of ammonium sulfate concentration, C_A, and pH.

Correlation

Using the form of correlation suggested by these plots, best estimates of the coefficients were obtained by fitting against the present and previous experimental data (80 points). The resulting correlation as a function of ammonium sulfate concentration (18 ≤ C_A, g/100 g of water ≤ 30), pH (4.58 ≤ pH ≤ 5.4), and temperature (0 ≤ t, °C ≤ 30) is

$$\log_{10}(C_O) = 5.06 - 0.006t - 0.205C_A + 0.5(\text{pH} - 4.58) + 1.1(\text{pH} - 4.58)^2 \quad (1)$$

where C_O is the ovalbumin solubility concentration in g/100 g of water, t is temperature in °C, and C_A is the ammonium sulfate concentration in g/100 g of water. The 95% confidence intervals on the coefficients in the correlation

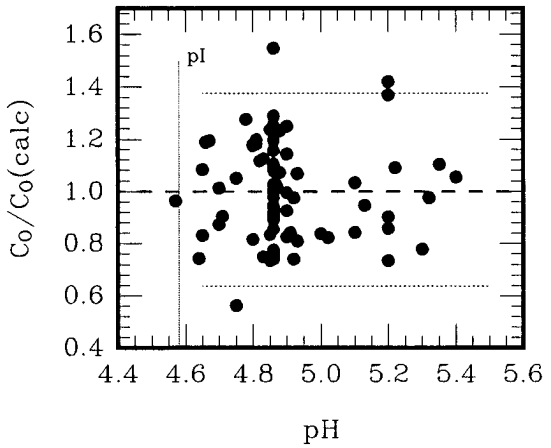

Figure 5. Ratio of ovalbumin solubility, C_O, to that calculated from eq 1, C_O(calc). The lines enclosing 95% of the data within a factor of 1.5 are shown.

are given in Table 2. This equation was used to draw the lines shown in Figures 1−3.

A three-dimensional plot of ovalbumin solubility as a function of C_A and pH at 30 °C is shown as Figure 4.

The 95% confidence goodness of fit of the data to the equation is a factor of 1.5 on C_O (±0.16 on $\log_{10}[C_O]$). Treating the scatter as random experimental error, the equation should predict mean values within a 95% error of ±5% on C_O. Residual plots, such as Figure 5, indicated no residual trend with any of the variables. The residuals passed the Anderson−Darling criterion (in MINITAB) and may be assumed to be normally distributed.

Using the correlation, changing the temperature from 0 to 30 °C decreases solubility by 40%, changing pH from 4.58 to 5.40 increases solubility over 10-fold, and increasing the ammonium sulfate concentration from 18 to 30 g/100 g of water decreases the solubility by a factor of about 300.

Literature Cited

Boistelle, R.; Astier, J. P.; Marchis-Mouren, G.; Desseaux, V.; Haser R. Solubility, phase transition, kinetic ripening and growth rates of porcine pancreatic α-amylase isoenzymes. *J. Cryst. Growth* **1992**, *123*, 109−120.

Bunn, C. W.; Moews, P. C.; Baumber, M. E. The crystallography of calf rennin (chymosin). *Proc. R. Soc. London* **1971**, *B178*, 245−258.

Cacioppo, E.; Pusey, M. L. The solubility of the tetragonal form of hen egg white lysozyme from pH 4.0 to 5.4. *J. Cryst. Growth* **1991**, *114*, 286−292.

Chayen, N.; Akins, J.; Campbell-Smith, S.; Blow, D. M. Solubility of glucose isomerase in ammonium sulphate solutions. *J. Cryst. Growth* **1988**, *90*, 112−116.

DeMattei, R. C.; Feigelson, R. S. The solubility dependence of canavalin on pH and temperature. *J. Cryst. Growth* **1991**, *110*, 34−40.

Green, A. A. Studies in physical chemistry of the proteins. *J. Biol. Chem.* **1931**, *93*, 495.

Howard, S. B.; Twigg, P. J.; Baird, J. K.; Meehan E. J. The solubility of hen egg-white lysozyme. *J. Cryst. Growth* **1988**, *90*, 94−104.

Judge, R. A. Investigating the bulk crystallization of proteins. Ph.D. Thesis, University of Queensland, Brisbane, Australia, 1995.

Judge, R. A.; Johns, M. R.; White, E. T. Protein purification by bulk crystallization: The recovery of ovalbumin. *Biotechnol. Bioeng.* **1995**, *48*, 316−323.

Judge, R. A.; Johns, M. R.; White, E. T. Crystal growth kinetics of the protein ovalbumin. Submitted for publication to *AIChE J.* **1996**.

Kitabatake, N.; Ishida, A.; Doi, E. Physicochemical and functional properties of hen ovalbumin dephosphorylated by acid phosphatase. *Agric. Biol. Chem.* **1988**, *52*, 967−973.

Mikol, V.; Giege, R. Phase diagram of a crystalline protein: Determination of the solubility of concanavalin A by a microquantitation assay. *J. Cryst. Growth* **1989**, *97*, 324−332.

Nisbet, A. D.; Saundry, R. H.; Moir, A. J. G.; Fothergill, L. A.; Fothergill, J. E. The complete amino-acid sequence of hen ovalbumin. *Eur. J. Biochem.* **1981**, *115*, 335−345.

Sorensen, S. P. L.; Hoyrup, M. Studies on proteins. *Compt. Rend. Lab. Carlsberg* **1915−17**, *12*, 12−67, 164−212, 213−261.

Stein, P. E.; Leslie, A. G. W.; Finch, J. T.; Carrell, R. W. Crystal structure of uncleaved ovalbumin at 1.95 Å resolution. *J. Mol. Biol.* **1991**, *221*, 941−959.

Stoscheck, C. M. Quantitation of protein. General methods for handling proteins and enzymes. *Methods Enzymol.* **1990**, *182*, 50−68.

Warner, R. C. In *Egg Proteins*; Neurath, H., Bailey, K. Eds.; The Proteins: Chemistry, biological activity, and methods, Vol. II, Part A; Academic Press: New York, 1954; pp 435−485.

Received for review August 17, 1995. Accepted January 16, 1996. Financial support was provided for R.A.J. by the Arthur Joseph Deakin Scholarship and by the Department of Chemical Engineering, The University of Queensland. [⊗]

JE950208D

[⊗] Abstract published in *Advance ACS Abstracts*, March 1, 1996.