The Osmotic Pressure of Concentrated Protein Solutions: Effect of Concentration and pH in Saline Solutions of Bovine Serum Albumin

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Received April 17, 1980; accepted July 18, 1980

Osmotic pressure measurements are reported as a function of bovine serum albumin (BSA) concentration in 0.15 *M* sodium chloride at pH 4.5, 5.4, and 7.4. The measured values increased markedly with increasing BSA concentration and with increasing pH (and therefore increasing macroion charge). At a concentration of 450 g/liter solution and a pH of 7.4, osmotic pressure was nearly five atmospheres, which is more than four times the value measured at the same concentration and a pH of 4.5 and about 30 times the value expected for an ideal solution. A semiempirical analytical expression was developed which gave good agreement between prediction and the experimental data of this and other studies. The data were also compared to the prediction of a three-term virial equation wherein the second and third virial coefficients were calculated by using McMillan–Mayer solution theory. The expression for the potential of mean force was obtained by comparing various contributions to the potential energy of interaction. The terms for electrostatic repulsion and dispersion attraction are the same as those used in the DLVO theory of colloid stability. The predicted curves are of the correct order of magnitude and follow the correct qualitative trend with pH, but they fail to display the strong pH-dependence of the data. The factors responsible for this deficiency are assessed and opportunities for developing a more realistic potential function are identified.

INTRODUCTION

When a protein solution is ultrafiltered by a membrane, a region of increased concentration of the retained solute develops near the membrane surface. The concentration at the surface can approach, or even attain, the solubility limit for the protein, and the driving force for hydraulic flow is reduced by the increased osmotic pressure difference across the membrane. This phenomenon of concentration polarization can thus greatly reduce the hydraulic flux as compared to that attainable with pure water. In order to obtain a fundamental understanding of protein ultrafiltration, data are required for the transport and osmotic pressure properties of these concentrated solutions.

In the past osmotic pressure measurements of protein solutions have generally been confined to the dilute range and have been taken primarily for the purpose of obtaining molecular weight and conformational data (1–3). In only a few instances (e.g., 4–8) have measurements been made up to moderate concentrations, nor are existing theoretical models of highly nonideal solution behavior suitable for à priori prediction at high concentration. The traditional approach is the Donnan membrane equilibrium model. Within this context, the exact multicomponent chemical potential treatment of Scatchard (4, 5) simply correlates data within the range for which it is available. The same is true for semiquantita-

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0021-9797/81/020548-19$02.00/0

tive interpretation of the osmotic virial coefficients of protein solutions in terms of excluded volume and attractive interaction effects (7). The most promising approach is the McMillan–Mayer solution theory (9) from which osmotic virial coefficients can be estimated in a manner analogous to those for the pressure of an imperfect gas. Hill (10, 11, 12) has applied this theory to charged colloid particles which exhibit double-layer repulsion, but no comparison with experimental data has heretofore been attempted.

In this paper we report osmotic pressure measurements for solutions of bovine serum albumin (BSA) at concentrations ranging from 84 up to 475 g/liter solution, in 0.15 *M* sodium chloride at pH 4.5, 5.4, and 7.4. The measurements were made with a static membrane osmometer built to withstand the several atmospheres of pressure generated by these solutions. The data are fit by a semi-empirical correlation suggested by Donnan theory that also gives good agreement with data from other studies. Lastly, the contributions to the potential energy of interaction between albumin molecules in solution are evaluated using physical properties available in the literature, and the resulting expression for the potential of mean force is used with the McMillan–Mayer theory to predict second and third osmotic virial coefficients. The poor agreement that results between predicted and measured osmotic pressure reflects the inadequacy of a three-term virial expansion at the higher protein concentrations examined, and it highlights the need for a better description of the potential of mean force than is currently available to describe the strong pH-dependence of the data.

MATERIALS AND METHODS

Albumin solution. Albumin solutions were prepared by mixing BSA crystals (Pentex grade recrystallized Cohn Fraction IV, cat. no. 81-001, Miles Laboratories, Kanakkee, Illinois) with 0.15 *M* NaCl made from distilled water and analytical grade NaCl. All prepartions included sodium azide (ca. 10 mg/liter) as an antibacterial agent. For concentrations above about 300 g/liter solution, BSA crystals and saline were added to 50-ml centrifuge tubes which were agitated by vigorous vortexing motion.

Albumin crystals were used as received. According to the manufacturer, the final steps before recrystallization were ion exchange, which ideally removed all micro-ions except H^+ and OH^-, followed by addition of NaOH to raise the pH to 5.2. The average chloride ion content was 3 mg/g protein. No special steps were taken to remove bound lipids. Cellulose acetate electrophoresis in this study indicated 100% albumin purity, and acrylamide gel electrophoresis showed 4–7 polymer bands, thereby indicating the presence of some albumin oligomers.

Solution pH measurements (±0.01 pH unit) were made with a saturated KCl glass electrode. Solution adjustment of pH was made by addition of nonbuffered 0.1 *N* NaOH or HCl. Vigorous vortex mixing was employed to ensure that local protein denaturation would not occur during acid or base addition. The solutions were not analyzed for sodium or chloride ion concentrations. Because of the large aliquots of 0.1 *N* NaOH or 0.1 *N* HCl which were added for pH adjustment, and the slight variability of Cl^- content of different lots of albumin crystals, the concentrations of Na^+ and Cl^- following pH adjustment were slightly different from the 0.15 *M* saline initially added. The maximum difference for the most concentrated solution is estimated to be about 0.03 *M*.

All albumin solutions were noncloudy, but occasionally small strands of apparently denatured protein were observed. For this reason, the final step before an experimental run was filtration through a 0.1-μm filter for albumin concentration up to 300 g/liter or a 0.3-μm filter for solutions of higher concentration.

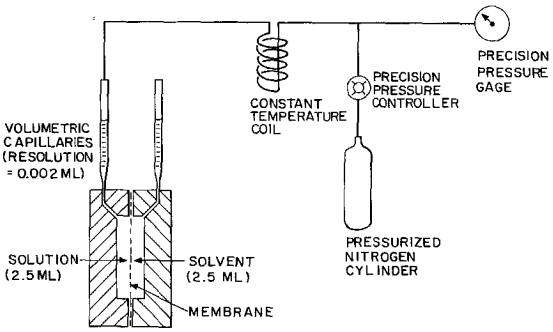

FIG. 1. Schematic diagram of high-pressure membrane osmometer system.

Albumin solutions charged to and discharged from the osmometer were analyzed for pH and for albumin concentration with the biuret method (13). The solution discharged from the solvent chamber was also routinely checked for possible albumin leakage with the biuret method or the Lowry method (14) which is more accurate when protein concentrations are very low. The precision of concentration measurement was ±5%.

Osmotic pressure measurement. The osmotic pressure measurement system is shown schematically in Fig. 1. The osmometer cell consists of two chambers, one for the 0.15 *M* saline solvent and one for the albumin solution. The chambers are separated by a membrane which is impermeable to albumin but permits free passage of water and microions. After the chambers are filled, a volumetric capillary prefilled with the appropriate solution is connected to each chamber. The gas pressure applied to the capillary on the solution chamber is then quickly set to the estimated osmotic pressure and subsequently adjusted in the direction indicated by slight movements in capillary liquid levels. Ultimately, an applied pressure is found for which liquid levels do not change over a period of several hours. This pressure is taken to be the solution colloid osmotic pressure. Applied pressure is measured and controlled to within several mm Hg by use of a precision pressure regulation gauge. The resolution of volume flow measurement by the volumetric capillaries is about 0.002 ml. The osmometer cell and gas temperature equilibration coil are immersed in a temperature bath controlled at 25 ± 0.1°C.

Figure 2 is a detailed view of the osmometer cell. The chambers are formed by sandwiching a membrane between two cylindrical pieces of Plexiglas, each of

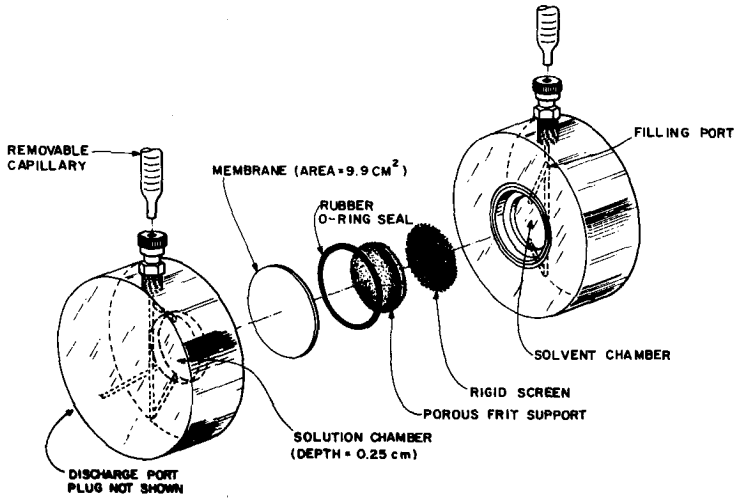

FIG. 2. Exploded view of the membrane osmometer cell.

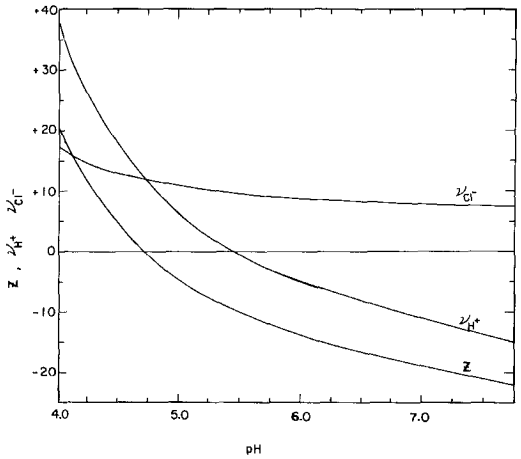

FIG. 3. Bovine serum albumin charge, Z, bound hydrogen ions, ν_{H^+}, and bound chloride ions, ν_{Cl^-} per albumin molecule in 0.15 M NaCl solution as a function of solution pH. Isoelectric pH = 4.72, isoionic pH = 5.46.

which contains a shallow circular depression (0.25 cm deep × 3.56 cm diameter). The membrane is supported on the solution side by a metal screen and a porous frit. One-eighth-in. diameter stainless-steel rods (not shown), equally spaced around the chambers about half-way to the outer perimeter, are used to clamp the unit together. A rubber O-ring impressed on the solvent side of the membrane seals the unit to applied pressures of at least 4500 mm Hg when the two halves are clamped. The cellulosic membranes (Abcor HFA-180 sheet stock) used for all determinations have a rejection coefficient of 0.99+ for albumin and not more than 2×10^{-4} for saline (15). Five membranes were used in the course of about 50 experimental measurements with no detectable differences in results for different membranes.

At the conclusion of each measurement, solvent and solution samples are taken with a syringe and needle via the filling ports. For concentrations of about 400 g/liter or more, rapid sample discharge was attained by removing the plug to the discharge port with the solution under pressure.

To confirm that stable liquid levels in the capillaries are indicative of true thermodynamic equilibrium, two separate determinations were made on identical starting solutions. In one, the initial applied pressure was less than the osmotic pressure of the solution; in the second it was greater. In each case, the applied pressure was adjusted until volume transfer between chambers ceased, and the two osmotic pressure measurements agreed to within about 4%. Additional details are available elsewhere (15).

Albumin valence calculation. For use in the models subsequently employed in this paper, the average net molecular charge of albumin is calculated from its complex equilibria with H^+ and Cl^- ions. In the pH range of our experiments, Na^+ binding is unimportant (16), and the availability of binding sites for H^+ and Cl^- is constant since there are no changes in the protein secondary structure (17). The macroion charge number Z is equal to the difference between the number of bound protons ν_{H^+} and the bound chloride ions ν_{Cl^-} per albumin molecule,

$$Z = \nu_{H^+} - \nu_{Cl^-}. \qquad [1]$$

The isoelectric pH ($Z = 0$) in 0.15 M saline solutions is about 4.72 (18, 19). The average albumin charge number is obtained by combining Tanford's proton binding data from titration measurements in 0.15 M NaCl (17) with the two-site chloride binding model of Scatchard *et al.* (20),

$$\nu_{Cl^-} = \frac{n_1 k_1 [Cl]\gamma \exp(2wZ)}{1 + k_1[Cl]\gamma \exp(2wZ)} + \frac{n_2 k_2 [Cl]\gamma \exp(2wZ)}{1 + k_2[Cl]\gamma \exp(2wZ)} \qquad [2]$$

where $n_1 = 10$, $k_1 = 44\ M^{-1}$, $n_2 = 30$, $k_2 = 1.1\ M^{-1}$, and [Cl] is the free chloride ion concentration in solution, 0.15 M. The parameters γ and w are calculated for our conditions to be 0.78 and 0.026, respectively (15). For a given pH, ν_{H^+} is found from Tanford's titration data as shown in Fig. 3,

and iterative calculation is then used to solve Eqs. [1] and [2] simultaneously for the values of ν_{Cl^-} and Z. These results are also shown in Fig. 3.

The isoionic pH ($\nu_{H^+} = 0$ by definition), measured following addition of BSA (50 g/liter) to 0.15 *M* NaCl, ranged from 5.22 to 5.55 pH for the various lots of albumin used in this work. These values are in good agreement with 5.46 pH as given by Tanford (17).

EXPERIMENTAL RESULTS

The albumin concentration C_p and pH measured in the solution discharged from the osmometer, the calculated albumin charge number, and the measured osmotic pressure are tabulated in Table I. The discharge concentration varied from the initial concentration by ±10% at most, and the pH of the discharged solution was never significantly different (±0.05 pH units) from its initial value. The albumin concentration in the solvent chamber discharge was usually 1 to 3 g/liter. These low concentrations did not contribute significant corrections to the reported osmotic pressures. From tests for thermodynamic equilibrium, the precision of osmotic pressure measurements was estimated to be within ±5%.

Reduced osmotic pressure π/C_p is plotted in Fig. 4 as a function of albumin concentration. The data at the lowest concentration for each pH are consistent with extrapolation to a value for the intercept, RT/M_p = 0.270 mm Hg/g/liter solution, which corresponds to the molecular weight of 69,000 first determined by Scatchard (5). This value is higher than that for monomeric albumin determined by amino acid sequencing, 66,100 (21), and it could result from the presence of about 5% dimers or higher oligomers. The osmotic pressure exhibits a strong dependence on albumin concentration and solution pH. At all values of pH, the slope at low concentration, and consequently the second virial coefficient, is positive. The nonlinear increase in π/C_p with increasing C_p indicates that the third term of the virial expansion makes a significant contribution to the osmotic pressure at all but the lowest concentrations studied. At the highest concentration examined, the osmotic pressure (about 5 atm) of the pH 7.4 solution is about five times larger than that of the pH 4.5 solution and about 30 times larger than the value predicted for an ideal solution by the van't Hoff equation.

In order to describe these results by an analytical expression, we fit the albumin osmotic pressure data to the following semi-

TABLE I

Osmotic Pressure of Bovine Serum Albumin Solutions

Albumin concentration, C_p (g/liter solution)	Solution pH	Albumin charge, Z	Osmotic pressure, π (mm Hg)
84	7.35	−20.2	48
91	7.37	−20.3	59
211	7.40	−20.4	332
211	7.46	−20.6	334
289	7.48	−20.7	844
325	7.34	−20.2	996
325	7.38	−20.3	996
354	7.40	−20.4	1423
357	7.50	−20.8	1638
413	7.44	−20.6	2620
428	7.44	−20.6	2806
448	7.42	−20.5	3640
91	5.41	−9.2	41
130	5.40	−9.1	74
144	5.40	−9.1	90
234	5.44	−9.5	260
240	5.45	−9.6	229
245	5.42	−9.3	269
338	5.40	−9.1	618
395	5.42	−9.3	1005
411	5.44	−9.5	1230
414	5.42	−9.3	1286
430	5.41	−9.2	1370
454	5.45	−9.6	1529
126	4.46	+5.5	47
182	4.54	+3.6	93
278	4.52	+4.1	182
317	4.50	+4.5	228
318	4.50	+4.5	244
343	4.52	+4.1	284
418	4.57	+3.2	716
475	4.50	+4.5	889

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