[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, AND THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

Preparation and Properties of Serum and Plasma Proteins. VII. Osmotic Equilibria in Concentrated Solutions of Serum Albumin[1,2]

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The studies of osmotic equilibria in solutions of serum albumin described in paper II[6] have been extended to much higher concentrations of albumin in 0.15 molal sodium chloride at *p*H 5.4 to 7.2 and 25°. This study was again made with bovine serum albumin, which behaved osmotically the same as the more precious human albumin. The albumin was crystallized by a method slightly different from that used with the preparation previously studied and its osmotic behavior was also slightly different.

Experimental Technique

The measurements of the osmotic pressure were carried out using the techniques that have been described in Paper II.

The protein concentrations in all of the present experiments were determined by micro-Kjeldahl analysis for nitrogen, using a factor of 6.25 to convert grams nitrogen to grams protein. The chloride concentrations in experiments 55–60 were determined by leaching with several portions of hot water the residuum remaining after evaporation of an aliquot of the solution inside the membrane to dryness at 105° and titrating the dissolved chloride with dilute silver nitrate solution. A differential potentiometric end-point was employed. In experiments 63–66 the residuum was digested with nitric acid on the steam-bath and the excess silver was titrated with sodium chloride solution, as in paper II, but using a potentiometric end-point as above. The *p*H was determined on solutions diluted to 6% albumin with 0.15 *M* sodium chloride.

The bovine serum albumin was prepared by the Armour Laboratories,[7] Chicago, using the method of Cohn and Hughes,[8] and was designated as ACB-2. For experiments 55, 58, 65, and 66 the 25% albumin was used as received. For the other experiments the albumin solution was dialyzed against distilled water at 0–5° for four days and then dried from the frozen state. The solutions for the osmotic measurements were made up from this dried material and solid sodium chloride, and 0.1 *M* sodium hydroxide solutions were used for *p*H adjustment when necessary.

Results

The experimental results are presented in Table I. The first column gives the number of the experiment, the second gives the concentration of protein in the inside solution and the third the concentration in the outside solution, columns 4 and 5 give the *p*H, and the valence of the protein calculated from the *p*H and the titration curve of paper II. Columns 6 and 7 give the mean concentration of sodium and chloride ions on the two sides of the membrane. Column 12 gives the measured osmotic pressure.

Column 8 shows the values of $-b_{23} \times 10^4$ calculated from the equation

$$\ln m_3/m_3' = -b_{23}w_2 \qquad (1)$$

There is no evidence that b_{23} changes with albumin concentration or with *p*H. Column 11 shows the difference between the measured value of m_3' and the value calculated from equation 1 with $b_{23} = -4.2 \times 10^{-4}$, with the assumption that m_3 is exact. Our earlier measurements gave an average of -3.6×10^{-4} in this *p*H range.

In paper II we calculated B_2 by the equation

$$P = 0.268w_2(1 + B_2w_2) \qquad (2)$$

and found no evidence that B_2 varies with the concentration, and that it can be represented from $z_2 = 9$ to -17 by the equation

$$B_2 \times 10^5 = 554 - 27.4z_2 \qquad (3)$$

Our present results over a larger concentration range show that B_2 increases with increasing albumin concentration, and can be represented by the equation

$$B_2 \times 10^5 = (417 - 24.5z_2)(1 + 0.002w_2) \qquad (4)$$

Column 13 gives $B \times 10^5$ calculated by equation 2 and column 14 gives the measured osmotic pressure minus that calculated from equation 2 with B determined from equation 4. At $w_2 = 60$, the approximate concentration of most of the previous experiments, our new results give

$$B_2 \times 10^5 = 467 - 27.4z_2 \qquad (5)$$

The factor of z_2, which is certainly very nearly the same as that in the previous experiments, was chosen exactly the same. The constant term is definitely smaller.

Our measurements indicate that the Armour crystallized bovine serum albumin interacts more

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[2] This paper is also given the number III in the series "Physical Chemistry of Protein Solutions."

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[6] G. Scatchard, A. C. Batchelder and A. Brown, THIS JOURNAL, **68**, 2320 (1946).

[7] We welcome this opportunity to thank Dr. Jules Porsche of the Armour Laboratories for this material and for his coöperation in many other ways.

[8] E. J. Cohn and W. L. Hughes, Jr., to be published.

TABLE I

No.	w_2	w_2'	pH	$-z_2$	m_3	m_3'	$-b_{23}$ $\times 10^4$	$-\beta_{33}$	$-\beta_{23}$	$m_3' -$ m_3' calcd.	P	B $\times 10^5$	$P -$ P calcd.
59	284.0	0.0	5.39	0.1	0.1564	0.1486	1.80	1.075	22.8	0.0098	219.1	662	0.5
64	104.5	.1	5.41	.3	.1544	.1479	4.12	1.088	52.3	.0001	42.42	493	− .6
56	59.82	.09	5.42	.4	.1504	.1467	4.16	1.116	52.7	.0000	20.64	483	.0
63	156.3	.0	5.42	.4	.1564	.1460	4.40	1.075	56.7	− .0005	77.77	548	− .9
66	140.4	.1	6.30	7.2	.1661	.1482	8.11	1.016	101.6	− .0084	78.0	765	+ .2
58	285.5	.0	6.31	7.3	.1815	.1603	3.76	0.932	46.1	− .0007	282.6	943	+1.0
65	216.7	.2	6.33	7.4	.1765	.1621	3.93	0.958	48.3	.0009	165.5	854	−0.4
55	63.92	.32	6.39	7.8	.1572	.1531	4.16	1.070	52.2	.0000	23.77	614	− .9
60	184.5	1.8	7.05	12.2	.1711	.1538	5.83	0.987	70.4	− .0047	138.7	984	− .2
57	69.16	0.07	7.23	13.5	.1553	.1506	4.46	1.083	54.4	− .0003	30.18	910	+ .7

strongly with sodium chloride than the Harvard Medical School preparation 456 by an amount which might indicate interaction with about one more chloride ion per albumin molecule. It appears to have the same molecular weight but its osmotic pressure at $w_2 = 60$ is less by 0.92 mm.

Discussion

The concentrations studied in this paper cover such a range, that it is desirable to extend the equations used in the previous paper. The general methods used there lead to complications, but the restriction to a special case of a three component system and the use of certain approximations makes the integration much simpler. Equation 1 of paper I[9] is

$$\frac{\partial \mu_K}{\partial m_2} + \Sigma_J \frac{dm_J}{dm_2}\frac{\partial \mu_K}{\partial m_J} = \frac{dp'}{dm_2}\frac{\partial \mu_K'}{dp'} + \Sigma_J \frac{dm_J'}{dm_2}\frac{\partial \mu_K'}{\partial m_J'} \qquad (6)$$

We will consider the special case in which each dm_J/dm_2 is zero. Then

$$\frac{\partial \mu_K}{\partial m_2} = -\overline{V}_K'\frac{dP}{dm_2} + \frac{dm_K'}{dm_2}\frac{\partial \mu_K'}{\partial m_K'} \qquad (7)$$

Substitution of the definitions of potentials[9] gives

$$\Sigma_i \frac{\nu_{2i}\nu_{Ki}}{(\nu_{Ki}m_K + \nu_{2i}m_2)} + \beta_{2K} = \frac{\overline{V}_K'\dfrac{dP}{dm_2}}{RT} + \frac{d\ln m_K'}{dm_2}(\nu_K + \beta_{KK}m_K') \qquad (8)$$

To integrate, we will assume that the first term on the right is negligibly small since we have seen in paper I that it is less than the experimental error. We will also assume that the factor in parentheses in the second term is independent of m_2. For sodium chloride it varies only from 1.86 to 1.83 as m_K' varies from 0.05 to 0.2 M, so the variation due to changes in m_2 may well be ignored.[10] We will also assume that β_{2K} may be represented by $\beta^0_{2K} + \beta^0_{22K}m_2 + \beta^0_{222K}m_2^2/2 + \ldots$ Then

$$-(\nu_K + \beta_{KK}m_K)\ln\frac{m_K}{m_K'} - \Sigma_i\nu_{Ki}\ln\left(1 + \frac{\nu_{2i}m_2}{\nu_{Ki}m_K}\right) = \beta^0_{2K}m_2 + \beta^0_{22K}m_2^2/2 + \beta^0_{222K}m_2^3/6 + \ldots \qquad (9)$$

For sodium chloride, with the definition of ν_{2i} of paper I[9]

$$-(2 + \beta_{33}m_3')\ln\frac{m_3}{m_3'} - \ln\left(1 - \frac{z_2^2m_2^2}{4m_3^2}\right) = \beta^0_{23}m_2 + \beta^0_{223}m_2^2/2 + \beta^0_{2223}m_2^3/6 + \ldots \qquad (10)$$

Equation 10 differs from the combination of 21 and 2 of paper II[6] by the term $-\ln(1 - z_2^2m_2^2/4m_3^2)$ and the higher terms in the expansion of β_{23}. The largest value of $(z_2^2m_2^2/4m_3^2)$ studied here is less than 0.003, which corresponds to an error of 0.15% in m_3'. Therefore, the constancy of b_{23} is equivalent to constancy in β_{23}, and terms higher than β^0_{23} may be neglected. The values of $-\beta^0_{23}$ calculated from equation 10 are given in column 10 of Table I, and the values of $-\beta_{33}$ used in the calculations are given in column 9.

For the osmotic pressure we take equation 4 of paper I[9] with some rearrangement, again for a three component system with dm_K/dm_2 equal to zero, to give

$$V_m\frac{dP}{dm_2} = m_2\frac{\partial \mu_2}{\partial m_2} + \frac{dm_K'}{dm_2}\left(\Sigma_J m_J\frac{\partial \mu_J'}{\partial m_K'}\right) \qquad (11)$$

V_m is the volume, under the pressure p', of that amount of a solution of component K at concentration m_K which contains one kilogram of component 1. To eliminate the solvent from the factor in parentheses, we subtract $\Sigma_J m_J'(\partial \mu_J'/\partial m_K')$, which is zero by the Gibbs–Duhem relation, to give

$$V_m\frac{dP}{dm_2} = m_2\frac{\partial \mu_2}{\partial m_2} + \frac{dm_K'}{dm_2}(m_K - m_K')\frac{\partial \mu_K'}{\partial m_2} \qquad (12)$$

With the definitions of potentials used above

$$\frac{V_m}{RT}\frac{dP}{dm_2} = 1 + \Sigma_i\frac{\nu_{2i}^2m_2}{\nu_{Ki}m_K + \nu_{2i}m_2} + \frac{dm_K'}{dm_2}\left(\frac{m_K - m_K'}{m_K'}\right)(\nu_K + \beta_{KK}m_K') + \beta_{22}m_2 \qquad (13)$$

To integrate, we will assume that V_m and $(\nu_K + \beta_{KK}m_K')$ are constant,[10] and that β_{22} may be expressed as $\beta^0_{22} + \beta^0_{222}m_2 + \beta^0_{2222}m_2^2/2 + \ldots.$ Then

$$\frac{PV_m}{RT} = m_2 - \Sigma\nu_{iKi}m_K\ln\left(1 + \frac{\nu_{2i}m_2}{\nu_{Ki}m_K}\right) - (\nu_K + \beta_{KK}m_K')\left[m_K\ln\frac{m_K}{m_K'} - (m_K\ m_K')\right] + \beta^0_{22}m_2^2/2 + \beta^0_{222}m_2^3/3 + \beta^0_{2222}m_2^4/8 + \ldots \qquad (14)$$

If component K is a uni-univalent salt such as sodium chloride, this becomes

$$\frac{PV_m}{RT} = m_2 - m_3\ln\left(1 - \frac{z_2^2m_2^2}{4m_3^2}\right) -$$

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(9) G. Scatchard, THIS JOURNAL, **68**, 2315 (1946).

(10) Each of these assumptions must be justified for each particular case. The use of a more general expression is not very complicated.

$$(2 + \beta_{33}m_3')\left[m_3 \ln \frac{m_3}{m_3'} - (m_3 - m_3')\right] + \beta^0_{22}m_2^2/2 + \beta^0_{222}m_2^3/3 + \beta^0_{2222}m_2^4/8 + \ldots \quad (15)$$

If the second and third terms are large, we should limit the expansion to the following terms. For albumin solutions with sodium chloride, however, these terms are so small that it is convenient to expand the logarithms, to give

$$\frac{PV_{\mathrm{m}}}{RT} = m_2 + \frac{z_2^2m_2^2}{4m_3} + \frac{z_2^4m_2^4}{32m_3^3} + \ldots - (2 + \beta_{33}m_3')\left(\frac{(m_3 - m_3')^2}{2m_3} + \frac{(m_3 - m_3')^3}{3m_3^2}\right) + \beta^0_{22}m_2^2/2 + \beta^0_{222}m_2^3/3 + \beta^0_{2222}m_2^4/8 + \ldots \quad (16)$$

If we compare this with the equation

$$PV_{\mathrm{m}}/RT = m_2\,(1 + B_2w_2 + B^0_{22}w_2^2) \quad (17)$$

and a similar expansion of equation 1 to give

$$b_{23}w_2 = -\left(\frac{m_3 - m_3'}{m_3}\right) - \left(\frac{m_3 - m_3'}{m_3}\right)^2\Big/2 \quad (18)$$

we find that

$$B^0_2\left(\frac{w_2}{m_2}\right) = \frac{z_2^2}{4m_3} - (2 + \beta_{33}m_3')\,\frac{b_{23}^2m_3}{2}\left(\frac{w_2}{m_2}\right)^2 + \frac{\beta^0_{22}}{2} \quad (19)$$

$$B^0_{22}\left(\frac{w_2}{m_2}\right)^2 = +\,(2 + \beta_{33}m_3')\,\frac{b_{23}^3m_3}{6}\left(\frac{w_2}{m_2}\right)^3\frac{\beta^0_{222}}{3} \quad (20)$$

Combining these equations with equation 4, we obtain, for $m_3 = 0.15$

$$\beta^0_{22} = 575 - 34z_2 - 3.33z_2^2 + 230 = 805 - 34z_2 - 3.33z_2^2 \quad (21)$$

$$\beta^0_{222} = 119{,}000 - 7{,}000z_2 + 3400 = 122{,}400 - 7000z_2 \quad (22)$$

The term arising from b_{23} is almost a third of the total at $z_2 = 0$ for β^0_{22}, but only a thirtieth for β^0_{222}, and the Donnan term contributes nothing to β^0_{222}. Therefore β^0_{222} is determined to a good approximation from B^0_{22} alone.

Recalling that β_{23} is nearly inversely proportional to m_3, we may write

$$\beta_2 = \ln \gamma_2 = \ln \gamma_2^0 + \beta^0_{23}m_3 \ln m_3 + \beta^0_{22}m_2 + \beta^0_{222}m_2^2/2 \quad (23)$$

in which $\ln \gamma_2^0$ is a constant depending upon the choice of standard state. With the values of the parameters given above, we obtain for this sample of crystallized bovine serum albumin (ACB-2) in aqueous sodium chloride solutions at 25°

$$\ln \gamma_2 = \ln \gamma_2^0 - 8.0 \ln m_3 + (805 - 34z_2 - 3.33z_2^2)m_2 + (61{,}700 - 3500z_2)m_2^2 \quad (24)$$

This equation should be valid over the ranges of m_2 from 0 to 250, m_3 from 0.1 to 0.2 and z_2 from 0 to −13.

Summary

The osmotic pressure and the distribution of salt across a semipermeable membrane have been measured for crystallized bovine serum albumin up to concentrations of 25% in 0.15 *M* aqueous sodium chloride at *p*H 5.4 to 7.2 and 25°.

Although the molecular weight appears to be the same, the protein–electrolyte and protein–protein interactions were slightly different from those of the sample previously studied.

The equations for osmotic equilibria have been extended to higher approximations.

Analytical expressions have been derived for the measured quantities, for the interactions and for the activity coefficient as a function of the concentration of albumin and of salt.

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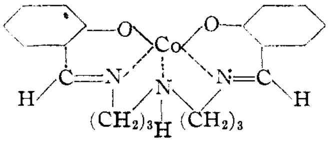
The Oxygen-Carrying Synthetic Chelate Compounds. VI. Equilibrium in Solution

BY O. L. HARLE[1] AND M. CALVIN

In the previous paper of this series[2] the equilibrium between oxygen and the solid chelates was described. These were primarily on compounds of Type I,[3] that is, chelate compounds derived from the Schiff base formed by salicylaldehyde or its derivatives with ethylenediamine. The present paper contains the results of equilibrium measurements on chelate compounds in solution. The chelates used in this study were primarily of Type II[3]; that is, compounds derived from the Schiff base formed by salicylaldehyde or its derivatives with γ,γ'-diaminodipropylamine.



Type II.

The reason for the lack of overlap between the measurements on the solids and those on solutions is primarily the fact that: (1) the pressures over solutions of Type I are very low, a few millimeters or less, so that the vapor pressure of the solvent interferes, making such measurement somewhat more difficult than those on solutions of Type II where the pressure range is relatively high; and (2) the pressures over the solids of Type II are in general above one atmosphere and the

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[1] Abstracted from the thesis submitted in 1944 by O. L. Harle to the Graduate School of the University of California at Berkeley in partial fulfillment of the requirements for the Ph.D. degree.

[2] E. W. Hughes, W. K. Wilmarth and M. Calvin, THIS JOURNAL, **68**, 2273 (1946).

[3] Calvin, Bailes and Wilmarth, *ibid.*, **68**, 2254 (1946).