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The Contribution of Charge Fluctuation at the Second Virial Coefficients of Human Serum Albumin

JØRGEN F. HANSEN*

Department of Blood Fractionations, Statens Seruminstitut, DK-2300 Copenhagen S, Denmark

The second virial coefficients of human serum albumin at zero charge in 0.01 to 0.1 M sodium acetate have been obtained by osmotic pressure measurements, and found to be considerably smaller than expected from the contribution of the non-electrostatic excluded volume. The discrepancy can, however, be explained when the effect of charge fluctuation is taken into consideration.

The second virial coefficient of serum albumin in salt solution has been considered as the sum of the non-electrostatic excluded volume and the Donnan term.[1,2] However, it has not been possible to explain the low value of the second virial coefficient measured at zero charge.[2]

In this work measurements of the second virial coefficient at zero charge in 0.01 – 0.1 M sodium acetate has been compared with values obtained by theoretical expressions. In these expressions account has been taken of the non-electrostatic excluded volume, and of the effect of the charge fluctuation from the protein molecules. The charge fluctuation is due to local fluctuations in the binding of ions – especially hydrogen ions – to the protein molecules. It is known that in isoionic salt-free solutions the charge fluctuation gives a considerable contribution to the osmotic pressure.[2–5] However, in salt solutions the contribution has until now been considered negligible compared to the non-electrostatic excluded volume.

EXPERIMENTAL

Material. Human serum albumin from Statens Seruminstitut, Copenhagen, Denmark. Lot Asf 700527, fractionated according to Cohn's 6th method. The contents of dimer was 5 %, determined by polyacrylaminelectrophoresis.

Measurements of the osmotic pressure. The osmometer was the type described by Tybjærg-Hansen,[6] thermostated to 25.0 ± 0.1°C. Sartorius membrane type SM 11536 was used. The membrane was completely tight to serum albumin. pH was measured on

* Present address: Steno Hospital and Nordisk Insulinlaboratorium, Research Laboratory, Ved Stadion 2, DK-2820 Gentofte, Denmark.

a Radiometer Titrator TTT 1e, calibrated with a Radiometer standard buffer S 1201 (pH 6.5 at 20.0°C) and S 1221 (pH 4.65 at 20.0°C).

Conductivity was measured on a Philips Conductivity Meter Type PR 9501 with Celle PR 9513, calibrated with 0.100 M KCl (Merck *p.a.*).

Deionization was performed on a mixed bed ion-exchanger Amberlite IR 120 and IRA 400. Column: 3.5 × 10 cm. Flow rate 1 ml/min.

Serum albumin concentrations were determined spectrophotometrically, E_{280} (1 %, 1 cm): 5.5 ± 1 %. This value was determined by the Kjeldahl method. The content of nitrogen in human serum albumin is taken as 16 %.

RESULTS

Measurement of charge fluctuation of human serum albumin. The measurement of the osmotic pressure of human serum albumin in isoionic salt-free solutions is shown in Table 1.

Table 1. Osmotic pressure of human serum albumin in isoionic salt-free solution. 25.0°C.

c g/ml	$10^{-2}(\pi/c)$ measured values cm_{H_2O} ml/g	pH	Corrections excluded volume cm_{H_2O} ml/g × 10^{-2}	Corrections progressive ionisation cm_{H_2O} ml/g × 10^{-2}	$10^{-2}(\pi/c)$ corrected values cm_{H_2O} ml/g
0.0228	2.80	5.05	0.24	0.02	2.54
0.0149	3.02	5.08	0.16	0.03	2.83
0.0113	3.12	5.10	0.12	0.04	2.96
0.0074	3.31	5.12	0.08	0.05	3.18
0.0045	3.45	5.14	0.05	0.07	3.33
0.0030	3.51	5.15	0.03	0.10	3.38
0.0020	3.75	5.17	0.02	0.14	3.59

After correction of the measured osmotic pressure for the non-electrostatic excluded volume and the progressive ionisation, Timasheff *et al.*[5] have shown that in isoionic salt-free solution the osmotic pressure divided by the serum albumin concentration can be expressed by the following equation:

$$\frac{\pi}{c} = \frac{RT}{\langle M_n \rangle}\left(1 - \frac{\pi N \varepsilon^3 (\bar{Z}^2)^{3/2}}{3(DkT)^{3/2}\langle M_n \rangle^{1/2}} c^{1/2}\right) \quad (1)$$

π, the osmotic pressure;
c, protein concentration in g/ml;
R, the gas constant;
T, the absolute temperature;
$\langle M_n \rangle$, the number average molecular weight;
N, Avogadro's number;
ε, the charge of the hydrogen ion;
$(\bar{Z}^2)^{1/2}$, the charge fluctuation of the protein molecules;
D, the dielectric constant of water;
k, the constant of Boltzmann.

The corrections shown in Table 1 are calculated by the following equations:

The non-electrostatic volume:

$$\frac{RT}{\langle M_n \rangle} 4\bar{v}_{sp} c \tag{2}$$

Serum albumin is here supposed to be an impermeable sphere. $\bar{v}_{sp}$ is the partial specific volume of serum albumin, taken to be 0.735 ml/g.[7]

The progressive ionisation, including the Donnan term, is:

$$\frac{RT}{\langle M_n \rangle} 2\langle M_n \rangle S(\sqrt{1 + ([H^+/2S)^2} - 1)\frac{1}{c} \tag{3}$$

S, salt concentration in the non-protein solution of the osmometer.

Eqn. 3 is derived from the conditions of electroneutrality, and equal salt activity on both sides of the membrane.

$[H^+]$ is calculated from the pH values of Table 1.

S could not be measured directly. The value used has been calculated from the conductivity of water deionised on the same column as the serum albumin. The conductivity was 1.3×10^{-6} mho. Using the equivalent conductance of NaCl, S is 1×10^{-5} mol/l. This value is in agreement with the one used by Timasheff *et al.*[5]

$\langle M_n \rangle = 70\,000$ g/mol is taken from Table 2.

The corrected π values are obtained by subtracting the corrections from the measured values.

In Fig. 1 the corrected osmotic pressures are plotted as a function of the square root of the serum albumin concentration. The shown straight line is calculated according to the method of least squares.

$\langle M_n \rangle$ is found to 64 000 g/mol. A charge fluctuation of 4.07 is calculated from the slope and eqn. 1. The values determined by Timasheff *et al.*[5] are from 3.5 to 4.0, depending on the preparation of serum albumin.

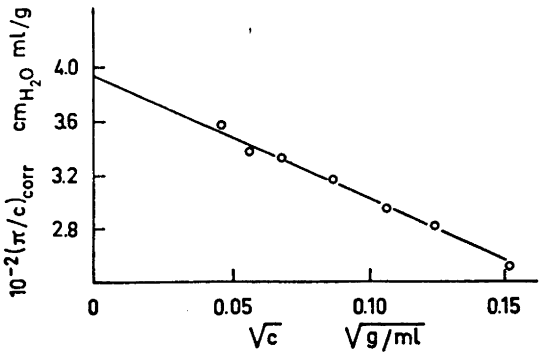

Fig. 1. The corrected osmotic pressures of human serum albumin in isoionic salt-free solutions, plotted as function of the square root of protein concentration. 25.0°C.

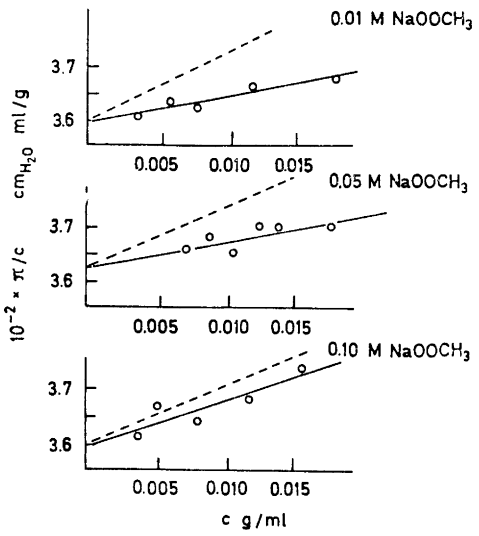

Fig. 2. Osmotic pressure of human serum albumin at zero charge in solutions of sodium acetate. 25.0°C. The dotted lines represent $\Gamma_2 = 3$ ml/g.

The second virial coefficient measured in sodium acetate solutions at zero charge. The concentration-dependence of the osmotic pressure in dilute solution of serum albumin can be expressed by the equation:

$$\frac{\pi}{c} = \frac{RT}{\langle M_n \rangle}(1 + \Gamma_2 c) \tag{4}$$

Γ_2, the second virial coefficient.

Fig. 2 shows measurements of osmotic pressure of human serum albumin in sodium acetate solutions at zero charge.

As π/c depends linearly on c, eqn. (4) can be used to calculate Γ_2.

The straight lines shown in Fig. 2 are calculated according to the least squares method.

Γ_2^{m} in Table 2 is calculated from the slope of the straight lines.

Table 2. Parameters derived from osmotic pressure measurements of human serum albumin in sodium acetate at zero charge.

	Measured values					Calculated values			
I	pH	$\bar{Z}$	$\langle M_n \rangle$ g/mol	Γ_2^{m} ml/g	Γ_2^{ms}[a] ml/g	Term of excluded volume ml/g	Donnan term ml/g	Term of charge fluctuation ml/g	Γ_2^{c} ml/g
0.10	4.75	0	70 100	2.4	2.0	3.0	0	−1.0	2.0
0.05	4.76	0	69 700	1.1	1.6	3.0	0	−2.1	0.9
0.01	4.96	0	70 300	1.3	0.9	3.0	0	−5.0	−2.0

[a] Γ_2^{ms}, values calculated with $\langle M_n \rangle = 70\,000$ g/ml.

The molecular weight is obtained from the intercept on the ordinate axis.

The isoelectric points shown in Table 2 are obtained from measurements on bovine serum albumin in sodium acetate.[6] These isoelectric pH values are supposed to be applicable for human serum albumin, as Tanford *et al.*[9] could find no differences in the number and distribution of the acid and the basic groups of the two serum albumins.

Γ_2^m is below 3 ml/g. Statistical analyses show that Γ_2 measured in 0.05 and 0.01 M sodium acetate are different from $\Gamma_2 = 3$ ml/g at 99 % level, and that Γ_2 measured in 0.1 M is different at 75 % level. 3 ml/g is the values expected at zero charge, when only the non-electrostatic excluded volume is considered.[2]

Γ_2^m is in accordance with the measurements of Scatchard *et al.*,[10] see also Tanford.[2] In addition they agree with the measurements of Timasheff *et al.*[5] as they also find decreasing values with decreasing ionic strength.

The equation for the osmotic pressure of serum albumin in salt solution at zero charge is, when the term of charge fluctuation is taken into account:[11,12]

$$\frac{\pi}{c} = \frac{RT}{\langle M_n \rangle}\left(1 + 4\bar{v}_{sp}c - \frac{\pi N \varepsilon^3 (\bar{Z}^2)^2 \sqrt{1000}}{4\sqrt{2}\,(DkT)^{3/2}\sqrt{I}\,\langle M_n \rangle\,(1 + a\varkappa)^2}c\right) \quad (5)$$

a is the Debye-Hückel parameter.

$$\varkappa^2 = \frac{4\pi N \varepsilon^2}{DkT}\left(\frac{2I}{1000} + \frac{\bar{Z}^2 c}{\langle M_n \rangle}\right) \quad (6)$$

I, the ionic strength of the salt.

The first term in eqn. (6) expresses the contribution of the salt ions to the screening of the electric potential between the protein molecules. The second term expresses the contribution of the protein molecules. The Γ_2^c shown in Table 2 is obtained from eqns. (5, 6) by use of the following values: $\bar{v}_{sp} = 0.735$ ml/g;[7] $a\varkappa$, the values calculated for pure salt solutions, are applied. This means that screening of protein molecules is neglected.

The values for $1/(1 + a\varkappa)$ are:

in 0.10 M salt 0.56
0.05 M salt 0.77
0.01 M salt 0.82

$\langle M_n \rangle = 70\,000$ g/mol.

$(\bar{Z}^2) = 16.5$. This value is the one obtained from measurements of osmotic pressure in isoionic salt-free solution. The use of this value is supposed to be a good approximation in sodium acetate solutions. The reason is that acetate ions are weakly bound to serum albumin.[13]

DISCUSSION

The non-electrostatic excluded volume in Table 2 is determined assuming that serum albumin is an impermeable sphere. However, serum albumin is shown to be an ellipsoid with axial ratio of about 4.[2] This means that the non-electrostatic excluded volume must exceed 3 ml/g. If this is the case the

discrepancy between the experimental Γ_2 and the values calculated from the non-electrostatic excluded volume may be even greater than previously assumed.

In the isoionic salt-free solution a molecular weight of 64 000 g/mol is obtained. This is less than the 70 000 g/mol measured in sodium acetate solutions. Furthermore from Table 2 it follows that at low ionic strength the Γ_2^c is smaller than the Γ_2^m. These two discrepancies are at least partly caused by the approximate value of S in the correction for progressive ionisation. If S is 0.2×10^{-5} mol/l a molecular weight of 69 000 g/mol and a charge fluctuation of 3.85 are obtained. If these values are used in the calculations of Table 2, the term for the charge fluctuation will increase with approximately 20 % and the differences between Γ_2^m and Γ_2^c become less pronounced.

To obtain a better accordance between the measured and calculated values of second virial coefficients, more accurate determinations of the ionic strength and the Debye-Hückel parameters will be necessary.

CONCLUSION

By including the term of charge fluctuations at least a qualitative agreement between the measured and calculated values of the second virial coefficients in salt solutions has been obtained. It is concluded that the charge fluctuation gives a non-vanishing contribution to the second virial coefficient in salt solutions.

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