THE EFFECT OF SALTS ON THE IONISATION OF GELATIN

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The internal salt, or "zwitter ion" theory of the structure of amino acids, has received increasing support of recent years. In the case of proteins, however, the data are less certain. According to the classical theory, gelatin is neutral at the isoelectric point by virtue of being either completely non-ionised or else containing a minimum number of dissociating groups, while the zwitter ion structure explains its behavior at this point on the basis of its possessing equal and maximal numbers of positive and negative charges. Such a molecule, with charges distributed over the surface, should be as capable of combining with a neutral salt as with an acid or base, and any such protein-salt compounds, since they involve at least one strong electrolyte, would be expected to be dissociated. Thus:

$$\overset{-+}{\text{protein}} + \overset{+-}{\text{NaCl}} = \overset{+\ -\ +}{\text{Na protein}} + \overset{-}{\text{Cl}}$$

$$\overset{-+}{\text{protein}} + \overset{+-}{\text{NaCl}} = \overset{-+\ -}{\text{protein Cl}} + \overset{+}{\text{Na}}$$

On the other hand, it is extremely difficult to postulate a mechanism whereby the neutral molecule of the classical theory could combine with neutral salt to produce a highly ionised compound, and even in the case of a molecule which bears a very small residual number of charges, any such combination could only be of a very slight order. The existence in considerable amounts of such compounds with neutral salts would therefore be indirect evidence for the zwitter ion structure of protein molecules.

The purpose of this paper is to draw attention to the fact that much of the data already published, particularly by Loeb and his co-workers, contain implicit evidence that the addition of neutral salts to

gelatin solutions increases the ionisation of the gelatin, *i.e.*, that the gelatin therefore combines with neutral salts to produce highly dissociated complexes.

Now if a gelatin solution be contained within a membrane permeable to electrolytes which is immersed in acid, it follows from the Donnan membrane relationship that the concentration of diffusible ions within the membrane is different from that outside it.

In the case of gelatin-HCl solution, on the acid side of pH 4.7, the H-ion concentration is less within the membrane than without, and the Cl-ion concentration is hence greater within the membrane, since

$$[H]_{in}\,[Cl]_{in} = [H]_{out}\,[Cl]_{out}$$

There is therefore an excess of the Cl-ions within the membrane, that is an excess of negative ions, and some of the gelatin must therefore be positively ionised to maintain electrostatic balance. If the H-ion activities are measured, the concentrations of all ions, including that of ionised gelatin, can be calculated. In the presence of known concentrations of salts the conditions are the same, and if the gelatin solution be at the same pH as when the salts are absent, then the comparison of the amounts of gelatin ionised in the two cases would give the effect of the addition of salt on the ionisation of gelatin.

Data on this point are readily available in the literature. The measurements at pH 3.8 are taken from Loeb (1) and Loeb and Kunitz (2). These determinations refer to the solutions at the beginning of the experiment, and in each case it has been necessary to assume that the pH of the outer solution has remained unchanged throughout. This is unavoidable since, not final pH measurements, but only membrane potentials, are given. Now the establishment of a membrane potential is merely the necessary consequence of a difference in concentration of H^+ or Cl^- ions, from whatever cause, since:

$$\text{E.M.F.} = 59(pH_y - pH_x)$$

The agreement between the membrane potential observed and that calculated from the pH difference does not therefore possess any special significance, since it does not of itself confirm the Donnan relationship, but only checks the accuracy of the measurements. Even

if measurements of final pH within and without had been available, however, the correction to be made for the probable slight change in the pH of the outer solution would have been unimportant, since it is merely a difference of pH that is involved. Furthermore the volume of the outer solution was much greater than that of the inner and the pH change was therefore probably slight.

TABLE I

Gelatin Chloride and Sodium Chloride. (1) and (2). 1 per cent Gelatin = 9 Milliequivalents Per Litre

In presence of sodium chloride; outer pH = 3.8								In absence of sodium chloride; $\gamma_H = 0.99$				
Concentration of salt	$-\log_{10}$ molality	γ_H	Membrane potential millivolts	C_{H^+} internal milli-equivalents per litre	C_{Cl^-} internal	C_{Na^+} internal	$C_{gelatin\ ions}^{+}$	Membrane potential millivolts	C_{H^+} external	C_{Cl^-} internal	$C_{gelatin\ ions}^{+}$	Ratio
M:8	0.9	0.84	0	0.189	125.0	125.0	0	32.5	0.581	2.11	1.95	
M:16	1.2	0.87	0.2	0.181	63.20	62.00	1.02	32.5	0.577	2.10	1.94	0.52
M:32	1.5	0.90	1.0	0.172	32.16	30.53	1.46	32.5	0.567	2.05	1.90	0.77
M:50	1.7	0.90	2.0	0.164	21.62	18.66	2.80	33.0	0.542	1.97	1.82	1.54
M:100	2.0	0.92	3.5	0.152	11.54	8.81	2.58	33.5	0.518	1.90	1.76	1.47
M:200	2.3	0.95	6.5	0.131	6.57	3.93	2.51	33.5	0.462	1.70	1.57	1.60
M:400	2.6	0.97	11.0	0.107	4.11	1.62	2.38	33.0	0.384	1.40	1.30	1.83
M:500	2.7	0.97	13.0	0.099	3.61	1.20	2.31	33.0	0.350	1.26	1.16	1.99
M:1000	3.0	0.98	18.0	0.082	2.34	0.497	1.76	33.0	0.280	0.99	0.914	1.93
M:2000	3.3	0.98	24.0	0.063	1.67	0.197	1.41	33.0	0.231	0.846	0.783	1.80
M:4000	3.6	0.99	29.0	0.050	1.30	0.079	1.18	32.5	0.182	0.656	0.606	1.93
M:8000	3.9	0.99	31.0	0.048	0.955	0.037	0.870	32.5	0.172	0.621	0.573	1.52
M:16000	4.2	1.0	33.0	0.044	0.811	0.017	0.749	32.0	0.162	0.596	0.552	1.36

The first section of Table I gives the salt concentration and observed membrane potentials, the outer solution being at pH 3.8. From the membrane potentials were calculated the H-ion activities within the membrane, and from these the H-ion concentrations by use of Lewis and Randall's figures (3) for the activity coefficients of the H-ions in solutions of various strengths (Column 3), neglecting, of course, the unknown contribution of the gelatin to the ionic strength of the solution. The concentrations of the other ions, and therefore of the

gelatin which must be ionised to produce electrostatic equilibrium, follow. The second section gives the figures for a gelatin solution at the same pH as in the first section, but in equilibrium with HCl only. The last column shows the value of the ratio:

$$\frac{\text{Gelatin ionised in presence of NaCl.}}{\text{Gelatin ionised in absence of NaCl at same pH.}}$$

This ratio is plotted in the curve, the abscissae being the negative logarithms of the molality of the salt solution; this scale is simpler than plotting direct reciprocals of molality and corresponds closely to the pH scale.

The figures show clearly that apart from the depression of ionisation produced by very concentrated salt solutions in acid, the addition of salt to the gelatin-HCl or gelatin-NaOH systems increases the ionisation of the gelatin. The variation of the ionisation ratio in the pH range 3.8 to 4.3 reaches a maximum corresponding to about M/1000 NaCl solution. The curve is, however, the resultant of two effects, (a) of decreasing salt concentration from M/8 to M/16000 and (b) of increasing pH from 3.8 to 4.3, and at constant pH would be slightly flatter.

The effect of salts in depressing the osmotic pressure, viscosity and swelling of gelatin, as shown by Loeb, is to be considered quite apart from this increase in ionisation of the gelatin, which is most simply explained on the formation of complex salts.

The observations of Pauli (4) and (5) on electrolyte-free proteins, show from conductivity and ionic mobility measurements that proteins combine with zinc chloride to produce complex zinc-protein ions, positively charged, and also, under certain conditions, to give complex chlorine-protein ions. Northrop and Kunitz (6) also find that gelatin combines readily with calcium, copper and other salts to produce positively charged particles, and that the combination even close to the isoelectric point, becomes, in high salt concentrations, stoichiometrically equivalent to the combination with hydrogen.

In this connection it is possible to show that gelatin possesses an effective combining power toward metal ions of from 2.5 to 3.6, for the formation of complex ions under the conditions of the experiment. In Table II are given the concentrations, in millimols per 1000 gm.,

for Donnan systems of gelatin with calcium and copper chlorides. Column 1 gives the external concentration of the salt, as given by Northrop and Kunitz, and the second and third columns give the in-

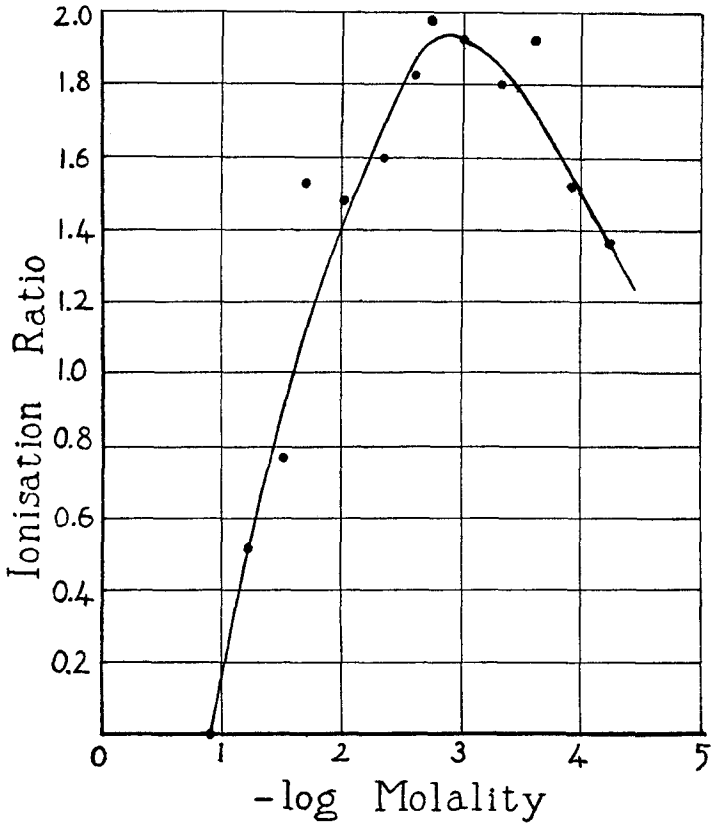

FIG. 1. Ionisation of gelatin produced by adding salt solution, from data of Loeb and Kunitz. The ratio of the amount of gelatin ionized in presence of NaCl to the amount ionized without NaCl but at the same pH is plotted against concentration of NaCl solution.

ternal concentrations of chloride and metal ions calculated from the membrane potential. Just as in Table I, there should be an excess of negative ions, and this is given by a (Column 6). Now the amounts of copper found, $[Cu]_2$, are in excess of the theoretical by a consider-

able amount, $[Cu]_3$, which, since it is not subject to the Donnan relationship, cannot be present in the normal ionic form. It can only be present in association with the gelatin that has to be positively

TABLE II

*Gelatin and Copper Chloride, pH 5.0***

C_{Cu} external	$C_{\bar{Cl}}$ internal	C_{Cu}^{++} internal calculated $= [Cu]_1$	C_{Cu}^{++} internal found $= [Cu]_2$	Excess Cu^{++} $= [Cu]_3$	$\frac{C_{Cl}}{2} - [Cu]_1 = a$	Effective combining power $= \frac{[Cu]_3}{a} \times 2$
8.8	19.6	7.1	10.7	3.6	2.7	2.67
8.05	21.97	4.32	13.15	8.83	6.66	2.65
187.9	391.5	173.1	204.6	31.5	22.7	2.77
178.0	360.0	174.0	184.5	10.5	6.0	3.50
192.5	389.3	188.4	199.5	11.1	6.3	3.52
485.0	976.0	479.0	495.5	16.5	9.0	3.66
Copper Chloride + Deaminized Gelatin, pH 4.1†						
191.8	387.1	188.4	194.1	5.7	4.7*	2.46
291.2	584.7	287.9	294.0	6.1	4.1*	2.97
383.9	771.4	380.4	386.5	6.1	4.9*	2.49
500.0	1014.0	487.0	515.0	28.0	19.6*	2.86
Gelatin + Calcium Chloride, pH 5.0‡						
C_{Ca} external	$C_{\bar{Cl}}$ internal	C_{Ca}^{++} internal calculated $= [Ca]_1$	C_{Ca}^{++} internal found $= [Ca]_2$	Excess Ca^{++} $= [Ca]_3$	$\frac{C_{Cl}}{2} - [Ca]_1 = a$	Effective combining power $\frac{[Ca]_3}{a} \times 2$
295.0	594.0	291.0	299.0	8.0	6.0	2.67
494.0	992.0	490.0	501.0	11.0	6.0	3.67
744.5	1495.0	739.0	751.0	12.0	8.5	2.82
984.0	1976.0	976.6	994.5	17.9	11.4	3.16

* Values corrected for concentration of H^+ ion.
** See Table I, Northrop and Kunitz (6).
† See Table II (6).
‡ See Table VII (6).

ionised to neutralise the excess of negative ions. In the first case, the excess of divalent negative ions is 2.7 millimols, which must therefore be neutralised by 2.7 millimols divalent positive ions. The excess of

copper over the theoretical is 3.6 millimols, and these must therefore be absorbed into the formation of 2.7 millimols divalent positive Cu-gelatin ions, so that the gelatin must be acting as though $\frac{3.6}{2.7} \times$ 2-valent. This effective valency or combining power of the gelatin for complex ion formation is given in the last column, and is seen to increase slowly with increasing concentration of salt, from a value of 2.67 upwards. Calcium chloride yields similar results; deaminised gelatin shows a lower combining power. In this last case correction has to be made for the H-ion concentration; at pH 5.0 this is negligible, at pH 4.1 it reduces the value of a by 0.4.

As in the previous tables, the figures represent small differences between large values, and the agreement is therefore very satisfactory. The formation of ionisable compounds by the addition of salts goes far to explain the solubility of the globulins in neutral salt solutions, since the idea, so often put forward, that the ions are soluble and the non-ionised protein is not, now receives a definite mechanism for the production of ions by salt. As Vickery and Osborne (7) point out, this fact has been somewhat of a mystery until now.

Furthermore, the formation of complex ions, while difficult to explain on the classical theories of protein structure, falls readily into line with the zwitter ion conception and is in fact an expected corollary therefrom.

SUMMARY

The effect of the addition of sodium chloride to gelatin solutions is shown from the Donnan relationship to increase the ionisation of the gelatin, the increase produced in acid solutions reaching a maximum at about 1/1000 molar salt concentration. This effect is attributed to the formation of complex ions.

From the similar action of calcium and copper chlorides the effective combining power of gelatin for complex positive ion formation is deduced. The bearing of complex ion formation on the zwitter-ionic structure and solubility phenomena of proteins is pointed out.

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