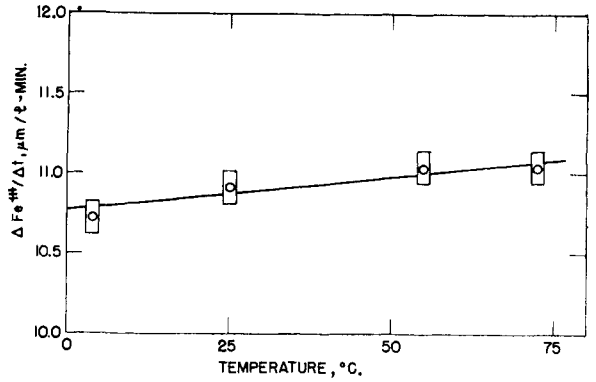

Fig. 1.—Variation of rate of oxidation of ferrous ion with temperature. Rate given as micromoles per liter per minute.

or smaller by a factor of 5 and 10, respectively, than that found by Hardwick and Dewhurst. At 72°, the ferrous oxidation–time curve did not go through the origin. Evidently chloride ion cannot completely protect the reaction from traces of organic matter at this temperature, and the point plotted is the least mean square slope of the line not assuming the origin. If the origin is assumed, this point is raised by 3%.

Some results for solutions without chloride ion are given in Table I. It is to be noted that the ratio of the amount of iron oxidized to the time decreases with time at 50° while the effect is much smaller at 25°. This effect might be expected if small amounts of organic impurities are present. The temperature coefficient of the apparent initial rates is of the order of that obtained by Dewhurst and Hardwick. The least mean square oxidation rate at 50° is 11.1 ± 0.1 while it is 11.0 ± 0.1 at 25°. These values would still represent a maximum rate of oxidation, as the non-linearity of the curve is not considered. However, these values are in good agreement with those obtained in the presence of chloride.

TABLE I

RATIO OF IRON OXIDIZED TO TIME

Irradiation, min.	25°	50°
5	11.3, 11.2	11.8
10	11.2, 11.0	11.7
15	11.3, 11.2	
20	11.0, 11.0	11.4
25	11.0	11.2
30		11.2, 11.3

The temperature dependence, if any, may be due to (a) a change in radiation intensity due to a change in geometry of the source and shielding with temperature although it is hard to see how this could produce an observable effect, or (b) a variation with temperature in the net amount of water decomposition produced by radiation in 0.8 *N* sulfuric acid solutions, or (c) an impurity effect. The amount of impurity necessary to produce this effect is of the order of a few micromoles per liter and thus quite difficult to guard against. In any event, the coefficient is small and need not be considered in comparing results from laboratory to laboratory.

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Changes in the Intrinsic Viscosity and Optical Rotation of Bovine Plasma Albumin Associated with Acid Binding[1]

BY JEN TSI YANG AND JOSEPH F. FOSTER

RECEIVED NOVEMBER 2, 1953

Structural changes in bovine plasma albumin in acid solution have been investigated by measurement of intrinsic viscosities and of specific rotations (extrapolated to zero concentration) over the *p*H range 1.3 to 7.0, both with and without added salt. Below *p*H 4 both $-[\alpha]_0$ and $[\eta]_0$ rise to a maximum at *p*H 2.2–2.7 followed by a decrease at lower *p*H. The height of the maximum is markedly depressed by added electrolyte. The viscosity behavior is in all respects analogous to that familiar in the case of synthetic polymeric electrolytes and can be interpreted on the basis of a swelling due to coulombic repulsion. Extrapolation of the data to zero ionic strength yields a monotonic sigmoid curve when either $-[\alpha]_0$ or $[\eta]_0$ is plotted *vs.* *p*H the curve being, however, much steeper than the titration curve. The total increase in $[\eta]_0$ (hydrodynamic volume) is over 10-fold, the increase in $-[\alpha]_0$ approximately 50%. The parallel shift in $-[\alpha]_0$ and $[\eta]_0$ is suggestive of an all-or-none rather than a gradual stepwise expansion of the molecule. The swelling must be essentially isotropic in view of the almost complete absence of streaming birefringence. The reaction is immeasurably fast and completely reversible as judged by (1) the return of both $-[\alpha]_0$ and $[\eta]_0$ to the original values when the *p*H is returned to the isoelectric point, (2) the fact that the results are independent of the sequence in which the reagents, acid and salt, are added and (3) the observation that regenerated protein gives an all-or-none reaction with sodium dodecylbenzenesulfonate which is indistinguishable from that of the native protein.

The titration behavior of proteins with acids and bases has been the object of much investigation. To a first approximation the results in most cases are in agreement with those expected on the basis of the amino acid content, suggesting that the dissociable groups are located on the surface of the protein molecule and are thus readily available for reaction with protons in the surrounding medium. Recently, however, it has become clear that at least in certain cases the situation is not so simple. Steinhardt and Zaiser[2] have shown that there is associated with the acid titration of carbonylhemoglobin

(1) Journal Paper Number J-2396 of the Iowa Agricultural Experiment Station, Ames, Iowa. Proj. 1223. This research was carried out under contract Nonr-803 (00) of the Office of Naval Research. Presented before the Division of Biological Chemistry of the American Chemical Society, September, 1953.

(2) J. Steinhardt and E. M. Zaiser, THIS JOURNAL, **73**, 5568 (1951); **75**, 1599 (1953); *J. Biol. Chem.*, **190**, 197 (1951).

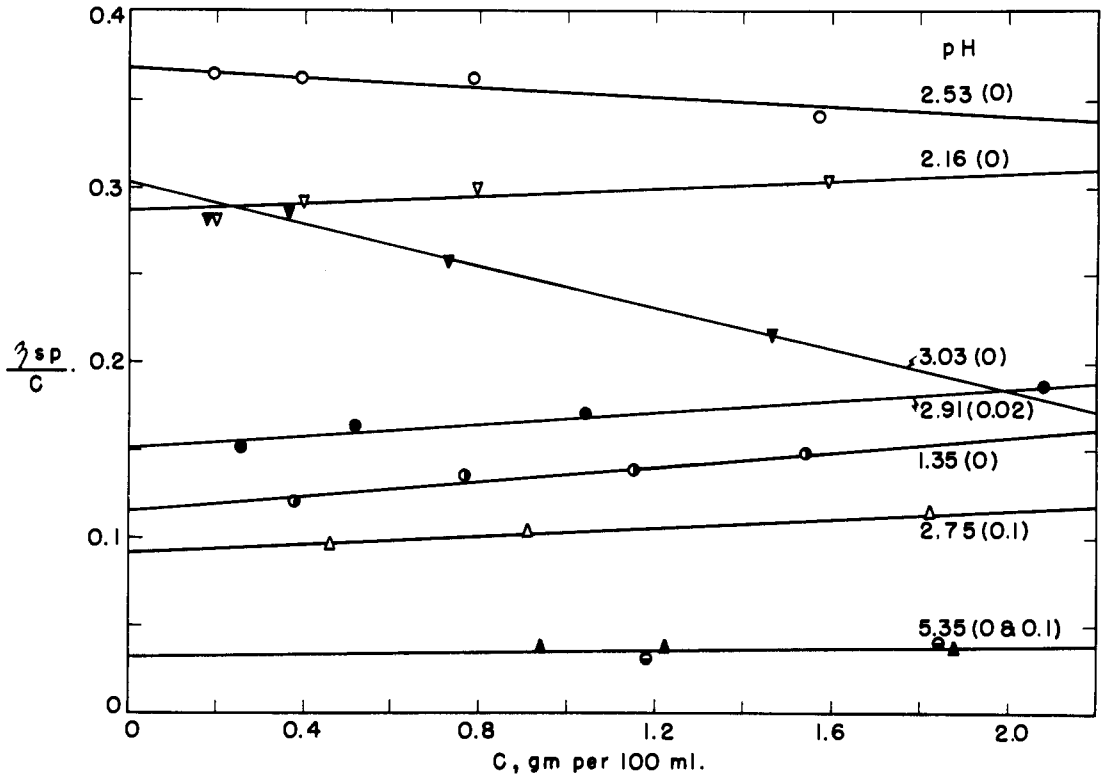

Fig. 1.—Dependence of reduced specific viscosity on concentration for various conditions of *p*H and added NaCl (figures in parentheses).

and ferrihemoglobin a slow reaction which leads to the liberation of a large number of acid binding sites which are not available in the native protein. Tanford,[3] in a detailed analysis of the titration curves of several proteins, has suggested that the acid titration of human and bovine plasma albumins involves a pronounced expansion of the molecule, in contrast to the situation with ovalbumin, β-lactoglobulin and myosin. This change he concluded to be instantaneous and reversible.

On the basis of ion-binding studies Klotz and Ayers[4] have been led to the conclusion that serum albumin undergoes structural changes with *p*H on the alkaline side of the isoelectric point.

Further evidence for pronounced structural changes associated with the acid titration of bovine plasma albumin (A) have been brought to light in recent months. Jirgensons[5] observed a marked enhancement of the optical rotation of A at low *p*H, which was confirmed by the present writers and shown to be associated with a pronounced increase in the specific viscosity of the protein.[6] Macheboeuf, *et al.*,[7] have similarly reported pronounced viscosity increases for horse serum albumin, both above and below the isoelectric point, but interpreted their results on the basis of aggregation rather than as an intramolecular structural change.

In order to clarify further the character of the changes responsible for these observations, measurements of intrinsic viscosity and of specific rotation of A have been carried out over the *p*H range 1.3 to 7.0 at various ionic strengths.

(3) C. Tanford, *Proc. Iowa Acad. Sci.*, **59**, 206 (1952).
(4) I. M. Klotz and J. Ayers, *Disc. Faraday Soc.*, **13**, 189 (1953).
(5) B. Jirgensons, *Arch. Biochem. Biophys.*, **39**, 261 (1952).
(6) J. F. Foster and J. T. Yang, THIS JOURNAL, **76**, 1015 (1954).
(7) E. Gavrilesco, E. Barbu and M. Macheboeuf, *Bull. soc. chim. biol.*, **32**, 924 (1950); S. Bjornholm, E. Barbu and M. Macheboeuf, *ibid.*, **34**, 1083 (1952).

Materials and Methods.—Bovine plasma albumin (A) was supplied through the courtesy of Armour and Company. The protein concentration in aqueous solution was determined with a Beckman model DU spectrophotometer using $E_{1\,cm.}^{1\%}$ 6.70 at 279 mμ. Adjustments of *p*H with dilute HCl or NaOH solution were made with a Leeds and Northrup glass-electrode *p*H meter.

Viscosity measurements were carried out in two Ostwald-Fenske type viscometers at 24.9 ± 0.1° using 10-ml. volumes of solution. Flow times for water were 61 and 63 seconds for the two viscometers.

Optical rotations were measured with a Rudolph High Precision Model 80 polarimeter at room temperature (25 ± 3°) using a sodium vapor source. For most measurements 2-dm. tubes were employed, 4-dm. tubes being used for solutions of the lowest concentrations. Several readings were taken with the tubes in each of four positions, and the results averaged.

For the determination of intrinsic viscosities and extrapolated specific optical rotations the protein solutions were diluted with solvent of the same *p*H and salt concentration as the original solution.

Experimental Results

Intrinsic viscosities $[\eta]_0$ were determined in the conventional manner by plotting the reduced viscosity (η_{sp}/c) against concentration and extrapolating to zero concentration. Some of the representative data at different *p*H values are illustrated in Fig. 1. The corresponding values of $[\eta]_0$ are shown in Fig. 2 where the upper curve was carried out in the absence of any added salt, and the center and lower curves in the presence of 0.02 and 0.10 *N* NaCl, respectively.

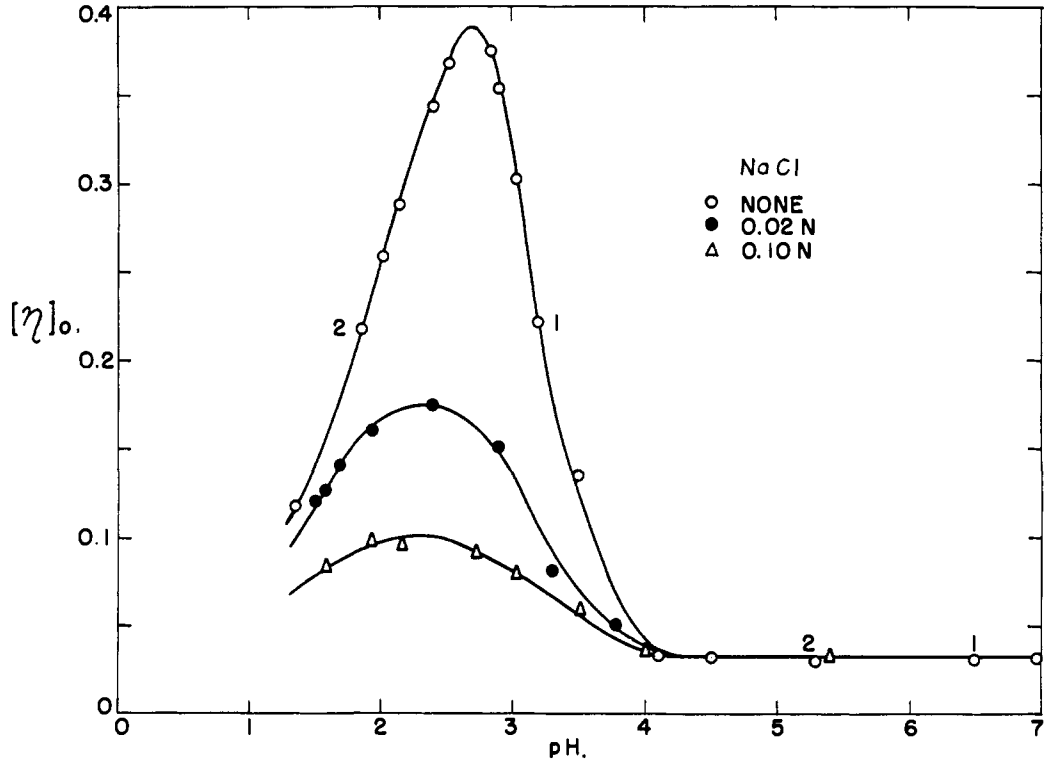

Fig. 2.—Dependence of intrinsic viscosity on *p*H with and without added NaCl as shown.

The dependence of specific rotation on concentration is shown in Fig. 3. The limiting values of specific rotation ($-[\alpha]_0$) in the presence of 0, 0.02 and 0.10 *N* NaCl are plotted as a function of *p*H in Fig. 4.

It was highly desirable to eliminate, in some

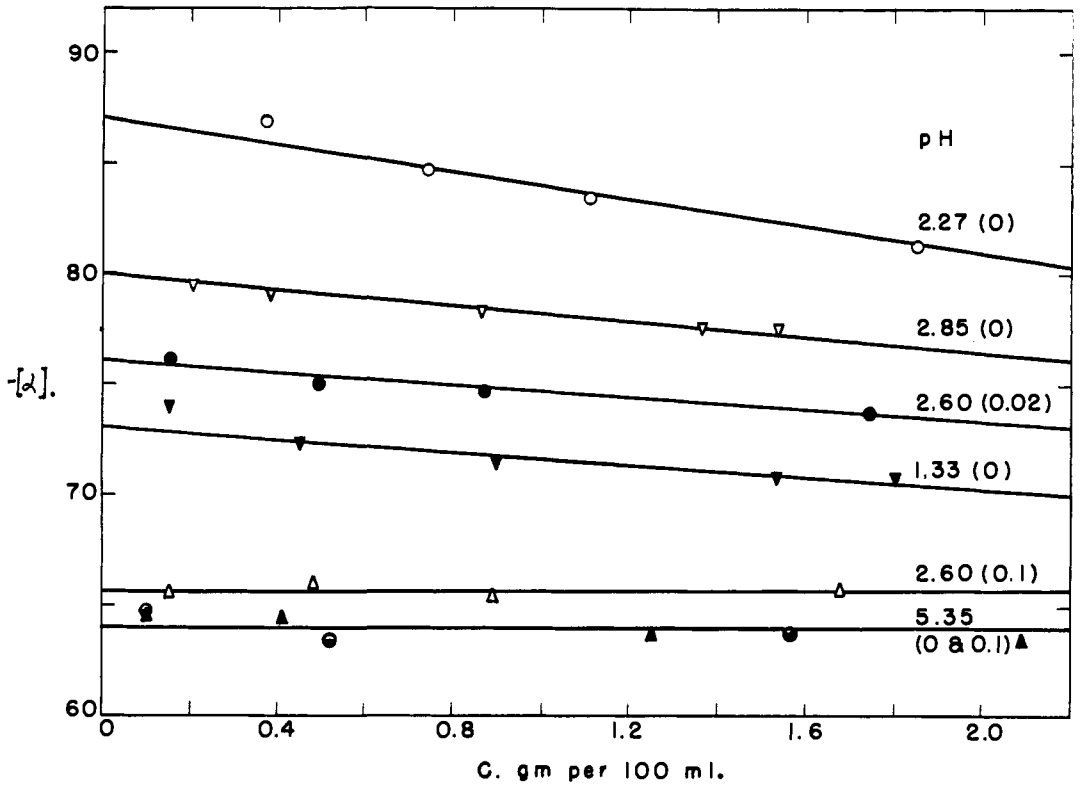

Fig. 3.—Dependence of specific rotation on concentration for various conditions of *p*H and added NaCl (figures in parentheses).

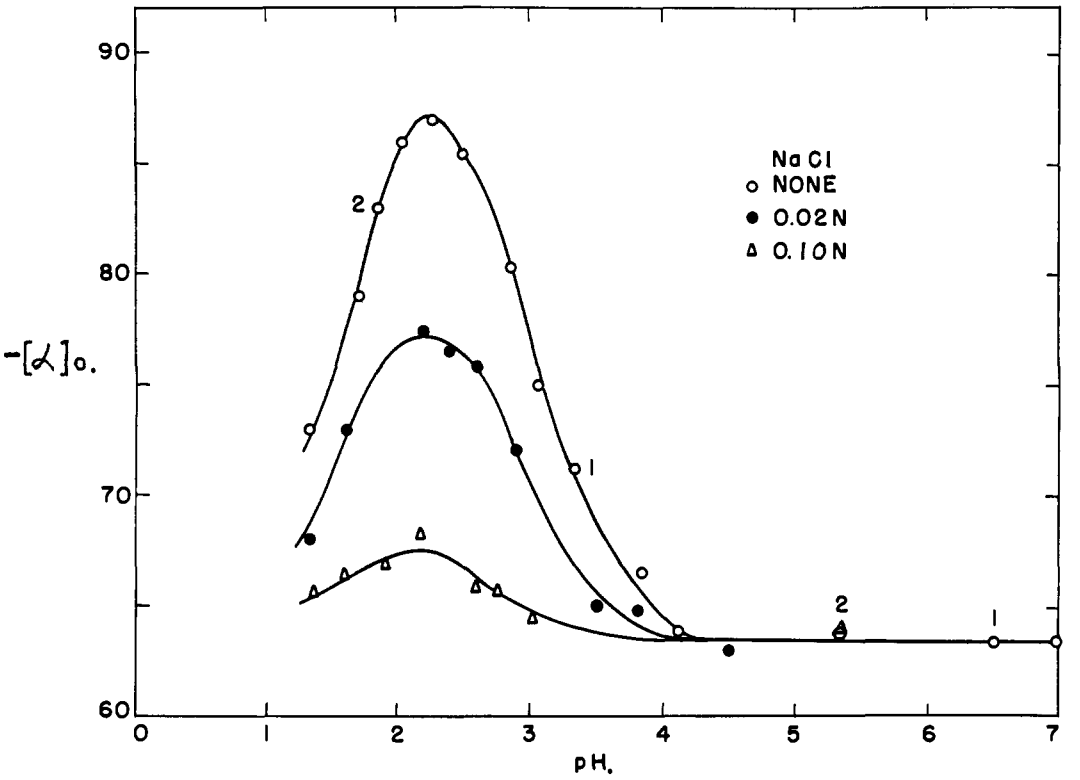

Fig. 4.—Dependence of limiting specific rotation on *p*H with and without NaCl as shown.

manner, ionic strength as a variable. It was found empirically that the results could be expressed, at least over the range investigated, in the forms

$$\frac{1}{[\eta]_0} = \frac{1}{[\eta]_\infty} + A\sqrt{\frac{\Gamma}{2}} \qquad (I)$$

and

$$-\frac{1}{[\alpha]_0} = -\frac{1}{[\alpha]_\infty} + B\sqrt{\frac{\Gamma}{2}} \qquad (II)$$

In Figs. 5 and 6 the viscosity and rotation data, respectively, are plotted according to these relations. Admittedly the data are too limited to fully demonstrate the justification of this procedure but for present purposes it appears to yield reasonable approximations for values extrapolated to zero ionic strength (termed $[\eta]_\infty$ and $-[\alpha]_\infty$).[8]

In Fig. 7, the extrapolated values $-[\alpha]_\infty$ and $[\eta]_\infty$ are plotted as a function of *p*H. The two properties are seen to fit a common sigmoidal curve. For comparison, too, there is plotted the titration curve of human serum albumin, taken from the work of Tanford.[10] This curve represents data obtained at 0.15 ionic strength. Unfortunately, these seem to be the only reliable data available.

Discussion

Some increase in viscosity of a solution of charged colloidal molecules due to increased charge is to be expected on the basis of the electroviscous effect. Unfortunately, no adequate theoretical treatment is at hand for the estimation of the magnitude of this effect. The only equation available, derived independently by Smoluchowski[11a] and Krasny-Ergen,[11b] expresses the effect in terms of the zeta potential and radius of the colloidal molecules, and the conductivity, viscosity and dielectric constant of the solvent. The equation involves all of the assumptions inherent in the Einstein viscosity equation. In a test of this equation with ovalbumin Bull[12] found a maximum increase in viscosity of only about 50% at low *p*H, an effect only 10% or less than predicted by the equation. The authors have verified this increase but have observed that here, as in the present case, there is a small but significant parallel increase in optical rotation so that even this small effect may not be due entirely to electroviscosity.

(8) Pals and Hermans[9] have found, in the case of salts of pectin and carboxymethyl cellulose, that an empirical equation of the form

$$[\eta]_0 = D + \frac{E}{\sqrt{\frac{\Gamma}{2}}}$$

holds reasonably well and provides a method of extrapolation to infinite ionic strength. It is of interest that their relation, and the one used by us, can both be considered as special cases of a general equation of the form

$$[\eta]_0 = D + \frac{1}{\frac{1}{[\eta]_\infty} + A\sqrt{\frac{\Gamma}{2}}}$$

at high and at low ionic strengths, respectively. Thus at high ionic strength, $1/[\eta]_\infty$ becomes negligible in the denominator of the last term, yielding the equation of Pals and Hermans. At low ionic strength *D*, which has the significance of $[\eta]_0$ at infinite ionic strength, would become negligible as compared to that last term, yielding the empirical equation I upon taking the reciprocal of both sides.

(9) D. T. F. Pals and J. J. Hermans, *Rec. trav. chim.*, **71**, 433 (1952).

(10) C. Tanford, THIS JOURNAL, **72**, 441 (1950). In a private communication Dr. Tanford informs us that he has as yet unpublished data on bovine serum albumin which are essentially identical with the data on the human protein.

(11) (a) M. V. Smoluchowski, *Kolloid-Z.*, **18**, 194 (1916); (b) W. Krasny-Ergen, *ibid.*, **74**, 172 (1936).

(12) H. B. Bull, *Trans. Faraday Soc.*, **36**, 80 (1940).

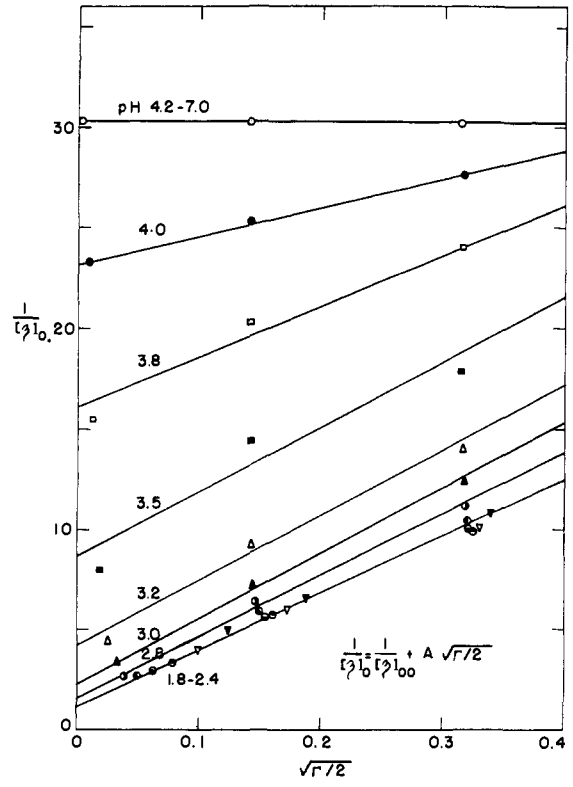

Fig. 5.—Figure illustrating extrapolation of intrinsic viscosity data to zero ionic strength.

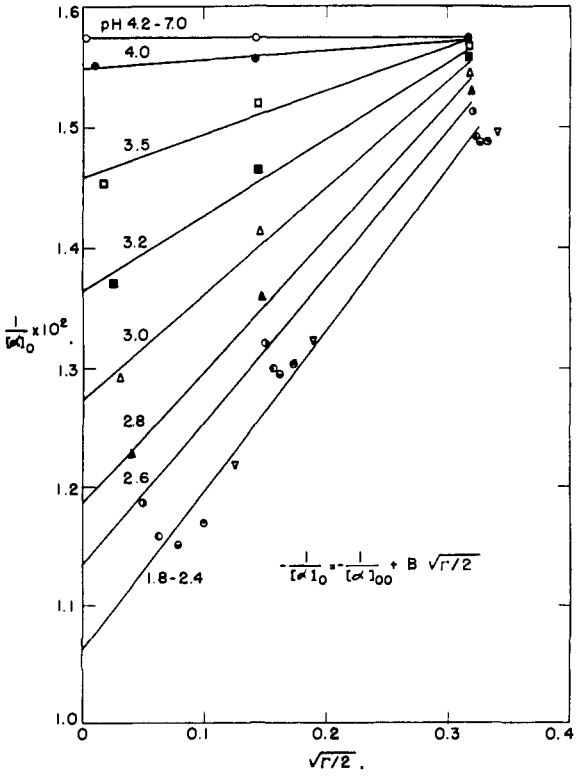

Fig. 6.—Figure illustrating extrapolation of limiting specific rotation data to zero ionic strength.

Thus, while the theory of Smoluchowski and Krasny-Ergen might account for the observed increases it is extremely doubtful if this is significant. There is no apparent reason why the effect, if due to electroviscosity, should be so much greater in the case of A than with ovalbumin at similar values of the zeta potential (electrophoretic mobility). Presumably the electroviscous effect depends essentially on factors external to the surface of the molecule so that there would be little opportunity for differences in structural detail in the two proteins to be manifested. It is, on the other hand, easily possible that such structural factors may result in marked differences in the ease of swelling or unfolding of the molecule. The authors are thus inclined to attribute the viscosity increase to such effects.

More convincing, perhaps, is the observed parallel shift in optical rotation which verifies that some pronounced structural alteration is responsible for the observed viscosity increase.[13]

Similar effects of *p*H on viscosity are now well known in the case of polymeric electrolytes and have been attributed to the swelling effect resulting from coulombic repulsion as the charge is enhanced.[14] Flory[15] prefers to consider the charge zero (due to neutralization by gegenions), in the case where the swollen molecule is very large compared to the magnitude of the double layer thickness, and attribute the swelling to the osmotic force resulting from the Donnan equilibrium existing between the interior and exterior of the swollen molecule. Adopting a similar point of view Scatchard[16] has calculated the magnitude of such forces in the case of A and has suggested that the molecule might expand, at physiological *p*H, to relieve this pressure.[17]

At *p*H values of approximately 2.5 the positive charge of the protein would reach its maximum so that further lowering of the *p*H would serve merely to increase the ionic strength of the environment. This increase, and particularly the increase in concentration of gegenions, would be expected to reduce the repulsion in the normal way and thus reduce expansion. This ionic strength effect is seen also in the depression of the curves at 0.02 and 0.1 *M* NaCl.

The total increase in viscosity observed is of the order of ten-fold suggesting an increase in the effective volume of the molecule by this factor. It should be pointed out that intrinsic viscosities of

(13) The change in rotation cannot be attributed to the change in state of ionization of the dissociable groups of the protein *per se* since, except for the (relatively few) terminal residues, these are far removed from asymmetric centers. The situation is thus quite different from the case of the amino acids where rotation is very sensitive to the charge state.

(14) (a) R. M. Fuoss and U. P. Strauss, *J. Polymer Sci.*, **3**, 602 (1948); (b) J. J. Hermans and J. T. G. Overbeek, *Rec. trav. chim.*, **67**, 761 (1948); (c) W. Kuhn, O. Kunzle and A. Katchalsky, *Helv. Chim. Acta*, **31**, 1994 (1948).

(15) P. J. Flory, *J. Chem. Phys.*, **21**, 162 (1953).

(16) G. Scatchard, *Am. Scientist*, **40**, 61 (1952).

(17) It would be extremely difficult, if not impossible, to distinguish between the two possible effects and for purposes of the present discussion it is not at all necessary. It does seem probable that in the case of the relatively small molecule under consideration the degree of neutralization of the charge would be relatively low and the more correct point of view to adopt would be that expansion arises from charge repulsion.

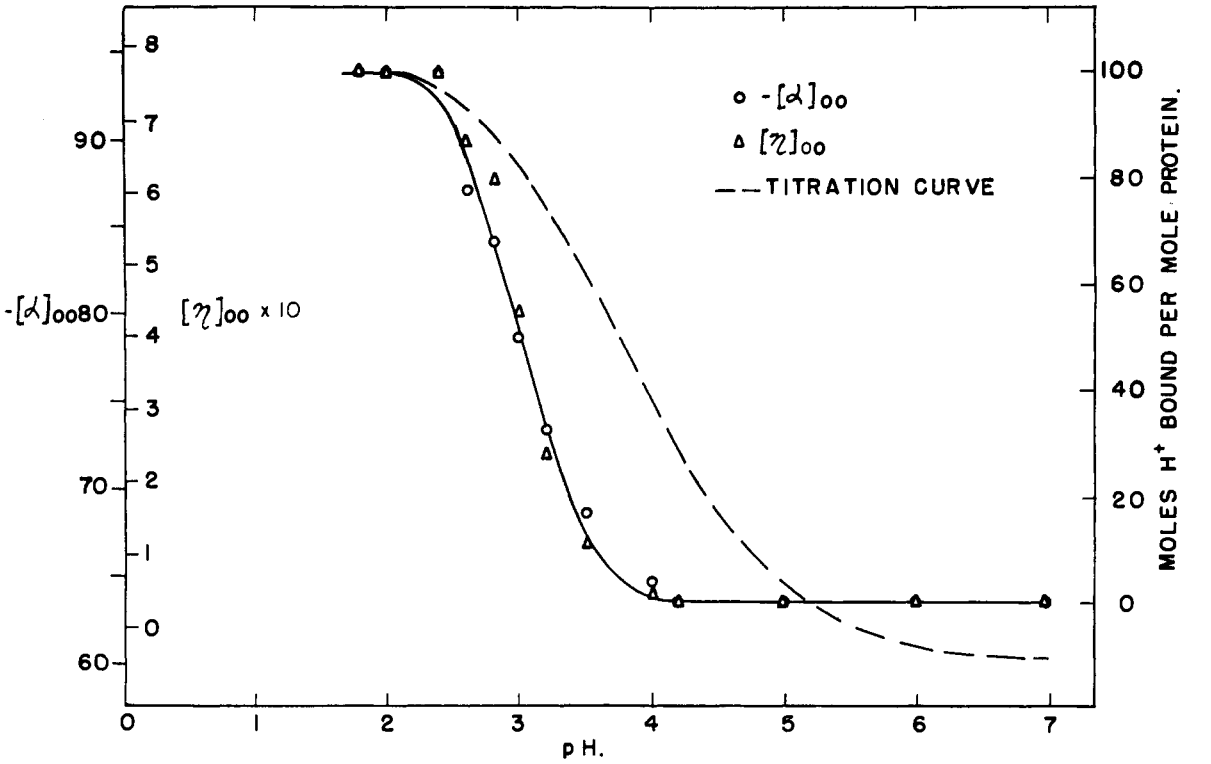

Fig. 7.—Dependence of specific rotation and intrinsic viscosity at zero ionic strength on *p*H. For comparison there is given the titration curve, according to Tanford,[10] of human serum albumin at 0.15 ionic strength.

the order ten times even this maximal value have been obtained in the case of synthetic polymeric electrolytes, suggesting that the swelling of A is somewhat inhibited.

The slopes of the η_{sp}/c *vs.* c plots are also in general accord with observations on polymeric electrolytes. Thus at high ionic strength or at low molecular charge (above *p*H 4) the slopes are zero or slightly positive as is found for uncharged polymers. At high charge and low ionic strength there is observed a definite negative slope which can probably be attributed to the increased swelling resulting from the decreased gegenion concentration as the solute concentration is decreased. In the case of the usual polyelectrolyte, however, such plots at low ionic strength characteristically exhibit a curvature which is not seen here. Perhaps this, too, is a consequence of the more limited swelling in the protein case.

It is most interesting that the optical rotation shows a concentration effect which is in complete accord with this picture of an increased swelling with decreasing ionic strength. Near the isoelectric point and at high ionic strength no dependence of $-[\alpha]$ on concentration was found. With increasing charge and decreasing ionic strength the slope is increasingly negative (Fig. 3). No evidence for a maximum in the $-[\alpha]$ *vs.* c curves, observed by Jirgensons,[18] was found.

The maxima in the curves of both $[\eta]_0$ and $-[\alpha]_0$ *vs.* *p*H fall at 2.2 except in the case of the viscosity experiments with no salt added. It seems probable that this last curve is shifted to the right slightly by virtue of the electroviscous effect which is doubtless appreciable under these conditions.

While this proposed expansion is qualitatively in accord with the previously mentioned picture developed by Tanford,[3] certain discrepancies appear on closer inspection. In the first place the magnitude of the effect appears to be much less than would be required to account for the decrease in the electrostatic free energy term which he observed. His titration data were obtained at ionic strengths of 0.15; on the basis of our results it would appear that swelling would be very minor under such conditions. A complicating factor is the possible change in effective dielectric constant which would result from swelling and imbibition of water. Thus the electrostatic term might change by a much larger factor than that due to the increase in molecular radius alone. The other apparent discrepancy lies in the fact that his calculated electrostatic interaction terms indicated swelling to begin just below the isoelectric point (near *p*H 5) whereas we observed no increase in the viscosity or rotation above *p*H 4.

In all cases the reaction has been found to be immeasurably fast. No change in either viscosity or rotation with time has been observed.[19] Numerous attempts have been made to observe streaming birefringence in these solutions but without success. Both the rapid character of the change and the absence of orientability lend support to the idea that

(18) B. Jirgensons, *Arch. Biochem. Biophys.*, **41**, 333 (1952).

(19) There is a slow development of turbidity, at low *p*H, in the albumin solutions but this has been traced to the precipitation of a trace impurity, probably fatty acid, which can be removed by high speed centrifugation with no measurable loss in nitrogen.

the change is essentially a swelling of the molecule rather than an unfolding.[20]

The ready reversibility of the reaction, too, seems in better accord with the picture of a swelling which does not involve a drastic rearrangement of the polypeptide chains. This reversibility is demonstrated in a number of ways. The order of addition of HCl and NaCl was found to be immaterial. At constant *p*H, addition of NaCl immediately depressed both $[\eta]$ and $-[\alpha]$. Both properties could be restored to the original isoelectric values either by neutralizing the acid with NaOH (points "1," Figs. 2 and 4) or by dialyzing out the acid (points "2," Figs. 2 and 4). In addition it was found that the regenerated protein yields an "all-or-none" reaction with dodecylbenzenesulfonate, as revealed by electrophoretic analysis, indistinguishable from that given by the original protein, whereas heat denatured A does not possess this property.[22]

The results of Macheboeuf, *et al.*,[7] on horse serum albumin are strikingly similar to our own viscosity results and there would seem to be little doubt that the two proteins behave very similarly in this regard. Their explanation, in terms of end-to-end aggregation seems clearly to be inapplicable, at least in the case of the bovine protein. Preliminary light scattering studies yield no evidence for aggregation in these systems at low *p*H.[23] Furthermore, no evidence is found for dissociation of the molecule, a result which is of interest in connection with published results of Weber[24] on the depolarization behavior of fluorescent conjugates of this protein. Weber observed a marked drop in the polarization in precisely the *p*H range in which we observe the viscosity and rotational increase and attributed the result to dissociation of the molecule. It would appear that this explanation is incorrect and that rather the increase in depolarization must result from an increase in intramolecular freedom rather than in an increase in the rotary diffusion constant of the molecule as a whole.

Perhaps the most surprising and significant feature of the results is the closely parallel character of the shifts observed in the two properties. If the viscosity increase, with increasing charge, were due to a gradual swelling of the molecule as is generally assumed for synthetic polyelectrolytes it would seem most surprising that there should be such a simple correlation. On the other hand, if it is assumed that the swelling is of an all-or-none character, so that a molecule exists at a given time in one or the other form, the correlation would be expected due to the additivity which should exist, to a first approximation, in the two properties. We thus favor the concept that a definite transition is involved, and that the effects of *p*H and ionic strength are merely to alter the equilibrium constant for the transition.

It is interesting to inquire as to the possible character of this transition. Unfortunately, both viscosity and particularly optical activity are such complex properties that nothing more than speculation is possible at present. However, attention should be called to the most interesting work of Robinson and Bott[25] who have demonstrated an excellent correlation between the specific rotation of films of a synthetic polypeptide (γ-methyl-L-glutamate–DL-phenylalanine copolymer) and the relative percentage of α- and β-forms. This suggests the very interesting possibility that the swelling of the protein is due to an $\alpha \rightarrow \beta$ transition; if true the optical rotation method would be a most convenient tool for following such transitions.

As was mentioned above, the authors have carried out similar studies on ovalbumin. In that case only very minor changes in rotation and viscosity were detected at low *p*H indicating that this protein is not as subject as is A to this type of reversible alteration. It would be of interest to study a variety of other proteins in this regard. In particular it seems possible that the changes in carbonylhemoglobin and ferrihemoglobin observed by Steinhardt and Zaiser[2] might be associated with similar changes in viscosity and optical rotation.

While ovalbumin does not demonstrate the reversible swelling exhibited by A it does apparently unfold, probably irreversibly, upon either heating,[26] or treating with cationic detergents[27] or urea.[28] It is tempting to suggest that in a general way protein denaturation can be represented by a series of reactions which might be abbreviated as[29]

$$\text{native protein} \rightleftharpoons \text{expanded protein} \longrightarrow \text{unfolded protein} \longrightarrow \text{aggregated protein}$$

The first (reversible) step is the one under consideration in this report and may involve an α–β transition or some similar configurational change. It probably represents a balance between coulombic repulsion forces and attractive forces such as intra-chain hydrogen bonds and van der Waals forces. Thus the position of this equilibrium will be markedly dependent on *p*H, ionic strength and the presence of hydrogen bonding agents such as urea. In the case of A it is suggested that this equilibrium can be shifted to the right relatively easily, and that further the protein does not tend to

(20) According to the hydrodynamic theory developed by Scheraga and Mandelkern[21] the value of their δ function, which is proportional to the product of $[\eta]_0$ times the reduced rotary diffusion constant $(\eta\theta/T)$, should decrease if there is a pronounced unfolding of the molecule (leading to an increase in the axial ratio of the effective hydrodynamic ellipsoid). The complete absence of streaming birefringence suggests that $\eta\theta/T$ remains above 5, possibly 10 as compared to a probable value of the order of 30–50 for the native protein. The decrease in $\eta\theta/T$ thus is no greater than, and probably less than, the increase in $[\eta]_0$ so that if anything the value of δ must increase. A weak point in this argument lies in the fact that glycerol was added for the attempted flow birefringence measurements, since orientation could not be expected in water even if considerable unfolding did take place. It is possible that glycerol caused a refolding of the molecule, although this seems extremely doubtful. Due to the obvious importance of electrostatic forces it seems probable that if anything the effect should be enhanced in the glycerol-rich medium.

(21) H. A. Scheraga and L. Mandelkern, THIS JOURNAL, **75**, 179 (1953).

(22) J. T. Yang and J. F. Foster, *ibid.*, **75**, 5560 (1953).

(23) G. F. Hanna, unpublished experiments in this Laboratory.

(24) G. Weber, *Biochem. J.*, **51**, 155 (1952).

(25) C. Robinson and M. J. Bott, *Nature*, **168** (1951). One of us (JFF) is indebted to Dr. Robinson for calling his attention to this publication and to him and also Dr. Bamford of Courtaulds, Ltd., for a most interesting and helpful discussion of these points.

(26) J. F. Foster and E. G. Samsa, THIS JOURNAL, **73**, 3187 (1951).

(27) G. F. Hanna and J. F. Foster, *J. Phys. Chem.*, **57**, 614 (1953).

(28) J. F. Foster and E. G. Samsa, THIS JOURNAL, **73**, 5388 (1951).

(29) In a private communication Dr. W. Kauzmann has suggested a similar mechanism on the basis of his very interesting studies of urea denaturation of these same proteins.

undergo the second (irreversible) change at room temperature. This latter fact may result from the large content of disulfide linkages in this protein, approximately 16 per molecule.[30] Thus it can be imagined that these restrain the altered polypeptide chains so that actual unfolding is prevented, thus facilitating the return of the chains to their native configuration when the force of repulsion is released. Ovalbumin, on the other hand, appears to undergo the initial transition much less readily than A; furthermore, possibly due to its very low content of disulfide linkages, perhaps one per molecule,[31] the expanded molecule is not stable but goes on, irreversibly, to the unfolded form. Clearly many other possibilities exist and this mechanism is highly speculative. It would appear to deserve further experimental test, however, and such studies are in progress in this Laboratory.

NOTE ADDED DECEMBER 7, 1953.—The very interesting studies of Kauzmann and co-workers on the effect of urea and of guanidine salts on the viscosity and optical rotation of serum albumin and other proteihs have now appeared in print.[32] These studies represent a marked advance in our understanding of denaturation and in the case of A appear to be rather closely related to our own. Thus it appears probable that the marked effects they observe in urea below 40° represent the same change in configuration which takes place in acid. This conclusion is based on (1) the similar shifts in viscosity and rotation in the two cases (2) the reversibility in both cases and (3) the fact that unpublished studies in this Laboratory by flow birefringence indicate that there is no unfolding of A under the conditions they employ.

Another very interesting communication by Gutfreund and Sturtevant[33] concerning a reversible reaction of A in acid should be mentioned. These workers have observed a heat uptake, upon reducing the *p*H of A solutions from 4.5 to 3.4, which is measurably slow (half-time approximately 2.5 minutes) and which is almost certainly a reflection of the same process covered in the present paper. The half-time is compatible with our conclusion that the reaction is immeasurably fast since in our studies measurements were not made earlier than about twenty minutes after mixing.

(30) J. T. Edsall, *Adv. Protein Chem.*, **3**, 464 (1947).
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[CONTRIBUTION FROM THE GENERAL ELECTRIC RESEARCH LABORATORY]

The Autoxidation of 2-Nitropropane in Basic Solution[1]

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2-Nitropropane is oxidized by air to acetone and nitrite ion in an autocatalytic manner in the presence of an excess of aqueous base at room temperature. The reaction is catalyzed by the addition of ferric and other ions and completely inhibited by the addition of arsenic trioxide and other compounds. The observed autocatalysis, catalysis, inhibition and dependence of rate on the concentration of hydroxide ion has been explained on the basis of a free radical mechanism proceeding by an ion–radical chain and involving an intermediate hydroperoxide. An essential feature of the proposed oxidation chain is a one-electron transfer between a peroxy radical and the anion of 2-nitropropane to produce a peroxy anion and a free alkyl radical. It has been postulated that the hydroperoxide formed in this manner can either dissociate to alkoxy and hydroxyl radicals (autocatalysis), or react with the anion of 2-nitropropane *via* a S_N2 displacement on oxygen to produce the observed products of the reaction.

Introduction

The autoxidation of carbanions or organometallic compounds possessing considerable ionic character has received extensive investigation. It has been found that in ether solution triphenylmethylsodium reacts with oxygen to give mainly triphenylcarbinol with the formation of traces of triphenylmethyl peroxide but without the formation of isolable amounts of triphenylmethyl hydroperoxide.[2] Aliphatic Grignard reagents are known to react very readily with oxygen, with the formation of the corresponding alcohol as a major product,[3] except for triphenylmethylmagnesium bromide which has been reported to yield 57% of triphenylmethyl peroxide.[4] Small amounts of peroxidic materials have been detected in the air oxidations of cyclohexyl- and ethylmagnesium halides at low temperatures,[5a] and it has been postulated that the oxidations proceed *via* the formation of peroxidic intermediates.[5] Recently, it has been found that the slow addition of a dilute ethereal solution of an aliphatic Grignard reagent to a saturated ethereal solution of oxygen results in the formation of the hydroperoxide as the major product.[6] Phenylmagnesium bromide, when oxidized in ethyl ether, produces benzene, phenol, biphenyl and α-phenylethanol.[3a,5b,7] The formation of the latter compound suggests that the reaction involves free radicals which may attack the ethyl ether to produce acetaldehyde. It has been found that the yield of phenol can be improved by replacing the ethyl ether by benzene[8] or by phenetole,[7] or by coöxidizing an aromatic and aliphatic Grignard reagent.[3b,8] The reaction of a large number of organolithium compounds with oxygen has been investigated and found to yield the same products as the oxidations of the corresponding Grignard reagents.[9]

The autoxidations of triphenylmethide ion, Grignard reagents and organolithium compounds

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