creased up to a_w 0.41 without further increase at a_w 0.32, but decreased at a_w 0.23. This action may again be due to the increasing viscosity of these solutions at low water activities.

Addition of glycerol at a_w 0.23 and 0.32 increased the browning rate first (plasticizing effect of glycerol) but decreased it again with higher proportions of glycerol (diluting effect of glycerol and water, inhibitory action of water by the laws of mass action; Figures 8, 9, 10).

Comparing the high and low viscosity Elvanol reaction mixtures (Figures 9 and 10) we see that the samples with the high viscosity Elvanol showed only about 50% of the browning of the low viscosity Elvanol samples. Furthermore, the browning rate in the Elvanol reaction mixtures was higher than in those without Elvanol, due perhaps to the water-binding properties of Elvanol overcoming even the influence of higher viscosities. The browning maximum in most of the Elvanol samples was shifted towards higher glycerol concentrations, indicating that more glycerol was necessary to attain the optimal plasticizing effect than in samples without Elvanol.

In the high viscosity Elvanol reaction mixture, (Figure 9) the differences of the extinction values at different water activities and different amounts of glycerol are smaller than in the samples without Elvanol (Figure 8) and with low viscosity Elvanol (Figure 10). This smaller difference indicates that in high viscosity systems the influence of viscosity is predominant over a wider range of water activities, thus diminishing the influence of water activity and water content.

CONCLUSIONS

Lowering the water content of a sugar–amino acid system increased the browning rate, except in systems in which reactant mobility became limited in high viscosity solutions of low water activities. Partial restoration of the mobility through the plasticizing effect of glycerol increases the browning rate at low moisture contents.

Browning rate in a sugar–amino system is not simply related to water activity. Optimum browning conditions are determined by the amount of water and state of water binding in a distinct system, and by the mobility of reactants in the system. The maximum browning depends on the extent to which these conflicting influences affect the reaction.

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Viscosimetric Studies of Alkaline Degradation of Ovomucin

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Thinning or decrease in viscosity of the gel-like thick portion of the white of chicken eggs is produced by exposure of the high molecular weight glycoprotein ovomucin to alkaline pH. Studies of the rate of change of viscosity of solutions of egg white and of ovomucin as a function of pH, temperature, concentration of hydroxide ion, and concentration of ovomucin indicate that the reaction producing thinning is first-order in hydroxide ion activity and first-order in ovomucin concentration. The activation energy is approximately 7 kcal/mol. The rate of thinning appears to have no relation to the lysozyme concentration.

The thinning of the white of chicken eggs held in storage is a well-known phenomenon (for a review, see Romanoff and Romanoff, 1949). Thinning is a decrease in the viscosity of the thick, gel-like portion of the white of the egg which contains the high molecular weight glycoprotein ovomucin (Almquist and Lorenz, 1932; McNally, 1933; Almquist *et al.*, 1934). Ovomucin, a virus-hemagglutination inhibitor (Lanni and Beard, 1948) of high intrinsic viscosity, has been characterized by Lanni *et al.* (1949), Sharp *et al.* (1950, 1951), and by Donovan *et al.* (1970). A review of the earlier work on isolation and characterization has been given by Warner (1954).

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Balls and Hoover (1940) showed that the amount of ovomucin precipitated from fresh eggs was the same as that precipitated from naturally-thinned eggs; that is, that thinning did not change the amount of ovomucin present. They precipitated ovomucin from fresh eggs and allowed it to be digested by trypsin. The precipitability properties of the proteolytically digested ovomucin were markedly different from those of ovomucin either from fresh eggs or from eggs which had thinned naturally. Thus, although Neumann and Sela (1960) and Hasegawa (1961) have reported proteolytic activity in egg white, thinning does not appear to be produced by proteolytic enzymes.

Hoover (1940) suggested that a reducing agent naturally present in egg white caused thinning, and *in vitro* showed a large decrease in viscosity when reducing agents were added to solutions of ovomucin. These experiments were confirmed

by MacDonnell *et al.* (1951). However, both Hoover (1940) and Ducay *et al.* (1960) failed to show significant amounts of reducing agents present in egg white. Since egg white separated from the egg under sterile conditions thins upon storage *in vitro* (Balls and Swenson, 1934; Feeney *et al.*, 1951), the thinning of egg white is not caused by substances in other parts of the egg, such as the shell membrane, yolk, or yolk membrane. Failure to find reducing agents in egg white does not eliminate reduction of the disulfide bonds of ovomucin as the cause of thinning, since only small amounts of reducing agents would be required over an extended period. However, it suggests that thinning might take place by other means.

Carbon dioxide is lost through the shell of eggs held in storage and the pH of the egg white becomes more alkaline, often attaining a final pH of 9.5 to 9.7 (Sharp and Powell, 1931). Thinning takes place concurrently. Addition of carbon dioxide to the air outside the egg prevents this rise in pH and also appears to prevent thinning (Sharp, 1929). Previous communications from this laboratory (Donovan, 1967; Donovan and White, 1971) have demonstrated that the disulfide bonds of ovomucoid (a protein with an amino acid composition similar to ovomucin) were hydrolyzed by hydroxide ion. The results presented here show that addition of hydroxide ion thins egg white and isolated ovomucin. These experiments were carried out above pH 9.5 to reduce the time necessary to observe a significant amount of thinning.

MATERIALS AND METHODS

Eggs. Eggs laid by Kimber 137 hens in the morning were broken out the afternoon of the same day for experiments or preparations of ovomucin. The thick white of eggs from Kimber 137 hens is highly gelatinous, as measured by Haugh unit (Haugh, 1937) scores (USDA, 1970). Unless otherwise specified, "egg white" refers to all the white of the egg (chalazae removed), gently blended to homogeneity with a Waring blendor.

Separation of Thick Egg White. This procedure can be used for eggs which have thick whites that remain essentially intact when the white and yolk are separated. Add 600 ml of 0.1 *M* KCl to 400 ml of fresh egg white. Stir gently, allow the thick whites to settle, and decant the supernatant liquid. Repeat until the color of the supernatant liquid is clear. The relative volume of thick white obtained, approximately 50% of the total egg white, is comparable to that obtained by Balls and Hoover (1940), who used the Holst–Almquist (1931) screen for separation of thick white.

Preparation of Ovomucin. The following procedure appears to be the best obtained after 4 years of experimentation with many different techniques. Blend egg white lightly using a blendor. Avoid producing foam, and remove any produced in this or subsequent steps. Add an equal volume of 1 *M* KCl. Stir with a magnetic stirrer until homogeneous. Test for homogeneity by adding a small volume of this egg white solution to 50 to 100 volumes of 0.1 *M* KCl. The egg white solution should disperse cleanly, giving a nearly clear solution without small translucent or transparent lumps. If this dilution is lumpy, the preparation will not be successful. Add 0.2 *M* acetic acid slowly, with stirring, to adjust the pH of the 0.5 *M* KCl solution of egg white to 4.90 ± 0.05. The solution clouds but does not precipitate. Slowly add 8 volumes of distilled water with constant stirring. A finely divided, flocculent precipitate should settle in 4 to 5 hr. It is preferable to let settling take place in the cold room. At the end of 5 hr, the supernatant liquid remains cloudy and will not clear for 24 hr. The suspended material, as shown by starch gel electrophoresis, is a small amount of ovomucin which can be discarded, if desired. Carefully siphon off and discard the supernatant liquid from the precipitate. Do not centrifuge the precipitate or it will become rubbery. The precipitate can be washed by carefully resuspending it in 0.1 *M* KCl and allowing it to settle again, or by adding an equal volume of 1 *M* KCl, then diluting to a salt concentration of 0.1 *M*, and letting the precipitate settle as before. These washing procedures remove most of the egg white proteins which do not bind to ovomucin. If extensive washing is carried out, care should be taken to maintain the pH near 4.9. Although some lysozyme is removed in this preparation procedure, the final precipitate is not free of lysozyme. The precipitate is stored at 4°C as a slurry containing a minimal amount of liquid. Ordinarily this material was used within 3 days. Addition of 4 *M* KCl to make the slurry 2 *M* in KCl made the slurry more translucent, allowed storage for a longer time without apparent deterioration, and allowed aliquots to be taken with a pipette with reasonable precision (2 to 3% reproducibility). Solution of ovomucin was accomplished by raising the pH.

For some preparations, eggs were carefully washed, sterilized, and broken out under aseptic conditions. Unless the ovomucin preparation was used for an extended length of time (which was not the case in the experiments described below), this precaution was not necessary. However, occasional samples of egg white or ovomucin were examined for viable microorganisms by spread-plating 0.2 ml on Trypticase Soy Agar plates. After incubation at 28°C for 2 to 3 days, normally only two to three colonies were observed. Absence of microbial contamination is an important aspect of these experiments, since degradation of protein could easily result from proteolytic enzymes produced by bacteria.

Viscosity Measurements. Specific kinematic viscosities (ν_{sp}) of solutions were measured: $\nu_{sp} = (t'/t'_0) - 1$, where t' is the flow time of the solution and t'_0 that of the solvent at the same temperature. Viscosities of solutions of ovomucin and egg white were measured with Cannon–Fenske capillary viscometers, usually No. 100, which have a flow time for water of about 60 sec at 25°C. The viscosity bath was controlled to ±0.01°C. No kinetic energy corrections or corrections for shear rate were made. The initial rate of decrease in viscosity was generally obtained by extrapolation of the *rate of change* of viscosity to zero time. The rate of change in viscosity at a given time was calculated for the time interval between successive viscosity measurements. Britton–Robinson buffers were used in one experiment in which temperature dependence of rate of change of viscosity was measured. The temperature dependence of the pH of these buffers (Britton and Robinson, 1931) is given by Britton and Welford (1937).

Other experimental procedures are given in Davis *et al.* (1969), Garibaldi *et al.* (1968), and Donovan *et al.* (1970). The ovomucin used in most of the experiments contained lysozyme. The amount of lysozyme was determined from absorption measurements at 280 and 290 mμ, as described previously (Donovan *et al.*, 1970). Concentrations stated are ovomucin concentrations, not total concentration of protein. Several control experiments (see below) were carried out to establish that the presence of lysozyme has essentially no effect on those experiments in which lysozyme was present.

RESULTS

Thinning of Egg White. Viscosimetric measurements were carried out on egg white diluted 1:1 with 0.1 *M* KCl (approximately the same ionic strength as egg white) for convenience. Figure 1 shows that the specific kinematic viscosity

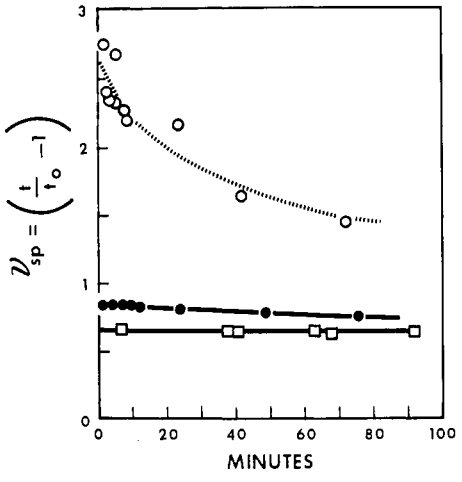

Figure 1. Time dependence of the change in kinematic viscosity at 20°C. Thick egg white, pH 10.6, ○; thin egg white, pH 10.7, ●; blended egg white after removal of ovomucin by high-speed centrifugation, □. All samples diluted 1:1 with 0.1 *M* KCl for measurements

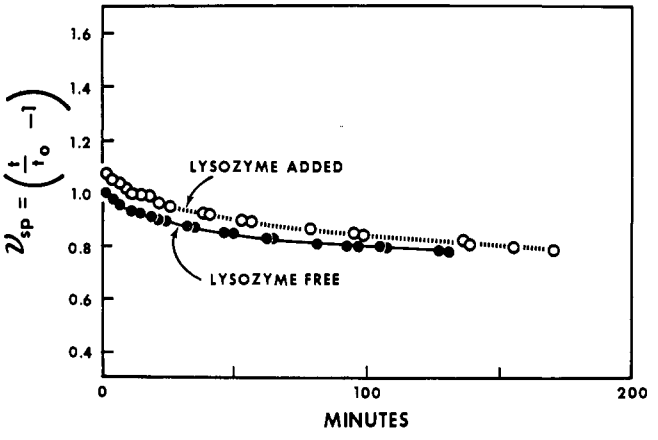

Figure 2. Time dependence of the change in kinematic viscosity of 0.2% solutions of ovomucin in 0.1 *M* KCl, pH 10.45 at 20°C, in the presence and absence of an equal weight concentration of lysozyme

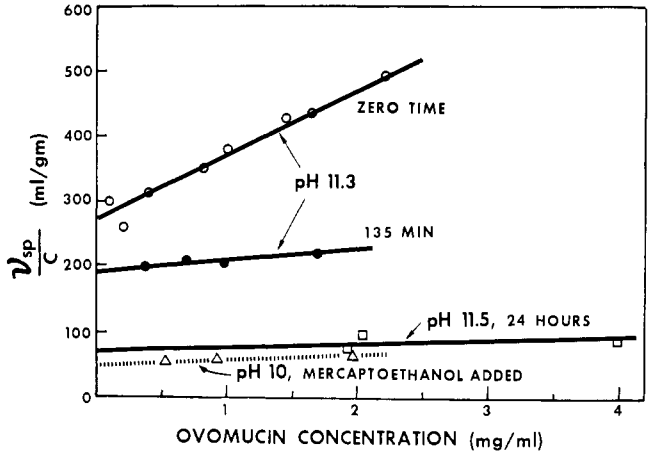

Figure 3. Concentration dependence of reduced kinematic viscosity of ovomucin at zero time of exposure to alkali, after 135 min at pH 11.3 and after 24 hr at pH 11.5, compared with that of ovomucin reduced with 0.02 *M* mercaptoethanol. All measurements in 0.1 *M* KCl, 25°C

of blended thick egg white appears to be three times as great as that of thin egg white, and decreases markedly with time at alkaline pH. Thin egg white, however, shows only a slight decrease in viscosity with time. The markedly higher viscosity of thick egg white parallels its higher ovomucin content, as determined both by Balls and Hoover (1940) and by McNally (1933).

When ovomucin was removed from blended egg white by high-speed centrifugation (Garibaldi *et al.*, 1968), little change in viscosity with time was observed (Figure 1). A reaction between ovomucin and lysozyme has been proposed as an explanation for the thinning of egg white (Hawthorne, 1950). Accordingly, the change in viscosity of lysozyme-free ovomucin (Donovan *et al.*, 1970) was determined, as well as that of an identical sample of ovomucin to which an amount of lysozyme equivalent to that present in egg white was added (Figure 2). In the absence of lysozyme, the usual decrease in viscosity of ovomucin was observed. When lysozyme was added the viscosity of the solution was slightly greater, since the protein concentration of the solution was approximately doubled (lysozyme and ovomucin each comprise approximately 4% of the total protein of the egg white). However, the rate of decrease in viscosity was unaffected by the presence of lysozyme.

Thinning of Ovomucin. The dependence of the reduced kinematic viscosity of ovomucin upon concentration is shown as a function of time at alkaline pH in Figure 3. Since the reduced viscosities are so large, the difference between the intrinsic kinematic viscosity $[\nu]$ and the intrinsic viscosity $[\eta]$ is negligible (Tanford, 1955). Both the reduced viscosity and its concentration dependence decrease markedly with time. After 24 hr at pH 11.5, the intrinsic viscosity is approximately that obtained upon reduction of the disulfide bonds with mercaptoethanol, or by dissolution of the ovomucin in 6 *M* guanidine hydrochloride plus mercaptoethanol. In the latter solvent, the viscosity average molecular weight of ovomucin has been demonstrated to be of the order of 1.6×10^5 (Donovan *et al.*, 1970), whereas the molecular weight of native ovomucin has been estimated to be of the order of 8×10^6 (Lanni *et al.*, 1949). The change in intrinsic viscosity upon reduction with mercaptoethanol does not appear to be accompanied by any change in conformation of the polypeptide chains of ovomucin, since the optical rotatory dispersion (313–578 mμ wavelength range) of the mercaptoethanol-reduced ovomucin is identical to that of the native ovomucin (Donovan *et al.*, 1970). When ovomucin is hydrolyzed by alkali so that its intrinsic viscosity is reduced to that of the mercaptoethanol-reduced ovomucin, there is no observable alteration of the optical rotatory dispersion.

The dependence of the logarithm of the initial rate of change in viscosity of ovomucin solutions upon ovomucin concentration at constant pH is shown in Figure 4. The line of unit slope drawn through the experimental points indicates that the rate of change of viscosity is first order in ovomucin concentration. The dependence of the rate of thinning of ovomucin solutions upon pH is shown in Figure 5. Although the scatter of the points in this figure reflects the deficiencies of a viscosimetric approach to the study of the thinning reaction, the least squares line of slope 0.8 ± 0.1 indicates that the rate of the reaction is approximately first-order in hydroxide ion activity. Similar experiments with egg white show a linear relation between the logarithm of the initial rate of change of viscosity and pH.

The rate of change of viscosity is a function of temperature (Figure 6). The natural logarithm of the initial rate of change in viscosity divided by hydroxide ion activity (equivalent to a second order rate constant) was plotted against reciprocal

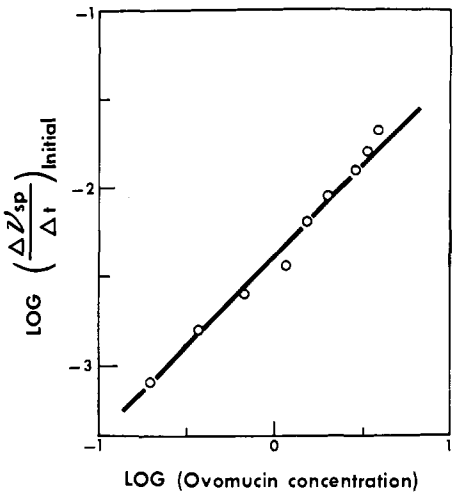

Figure 4. Dependence of the logarithm of the initial rate of change in specific kinematic viscosity upon the concentration of ovomucin (mg/ml) in 0.1 *M* KCl, pH 11.4, at 25°C. The line drawn through the points has a slope of unity

temperature (Figure 7). Data for buffered and unbuffered solutions of freshly prepared ovomucin obtained by different experimenters about 1 year apart agreed within experimental error. The calculated activation energy is 6.8 ± 0.5 Kcal mol^{-1}.

The time dependence of the intrinsic viscosity during the first 10 min of the thinning reaction (Figure 8) was used to obtain an estimate of the rate constant. These calculations are outlined below.

DISCUSSION

The high viscosity of the thick portion of egg white has long been attributed to the presence of ovomucin, a polydisperse glycoprotein of high molecular weight. Since removal of ovomucin reduces the viscosity and eliminates the viscosity decrease with time in alkaline solution (Figure 1), it is apparent that ovomucin is intimately involved in the thinning process. Lysozyme, although strongly bound by ovomucin (Hawthorne, 1950; Brooks and Hale, 1959, 1961; Garibaldi *et al.*, 1968), does not appear to participate in thinning (Figure 2), contrary to the suggestions of Hawthorne (1950), Cotterill and Winter (1955), and Brooks and Hale (1961).

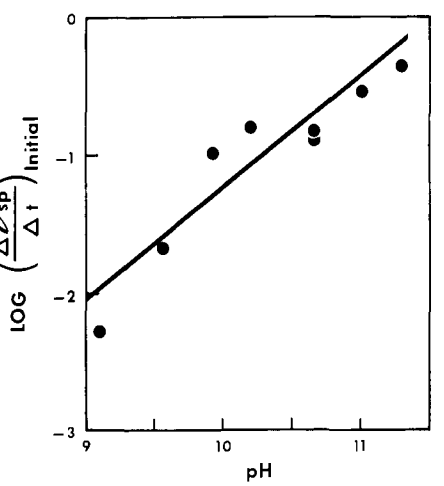

Figure 5. Dependence of the logarithm of the initial rate of change in specific kinematic viscosity of ovomucin upon pH in 0.1 *M* KCl, 25°C

When ovomucin is exposed to alkali, its intrinsic viscosity falls to a value close to that measured when its disulfide bonds are split by excess of reducing agent, and the concentration dependence of the reduced viscosity is greatly diminished (Figure 3). This observation is consistent with the hypothesis that thinning of egg white may be produced by the alkaline cleavage of the disulfide bonds of ovomucin, since polypeptide chains of the same molecular weight and same conformation, as indicated by optical rotatory dispersion, should be produced regardless of the manner in which the disulfide bonds are cleaved. However, in the absence of further evidence, splitting of other types of bonds is not excluded. The reduction in molecular weight of ovomucin which occurs upon splitting of disulfide bonds is substantial. Lanni *et al.* (1949) estimate a molecular weight from sedimentation and viscosity in the neighborhood of 8×10^6. The polypeptide chains obtained by complete reduction of disulfide bonds to thiol have a viscosity average molecular weight of about 1.6×10^5 (Donovan *et al.*, 1970). Thus, a decrease in molecular weight, probably of a factor of about 50, occurs when ovomucin is thinned by reduction, and, most likely, by reaction with hydroxide ion also.

The thinning of ovomucin can be represented by an empirical equation for the rate-limiting step

$$\text{rate} = k_2 P^m [OH^-]^n \quad (1)$$

Here, k_2 is the intrinsic second-order rate constant for the reaction, P the ovomucin concentration, m the order of the reaction with respect to ovomucin, and n the order of the reaction with respect to hydroxide ion activity. Equation 1 can be written in logarithmic form, in terms of pH, as

$$\log (\text{rate}) = \log k_2 + m \log P - n\text{p}K_w + n\text{pH} \quad (2)$$

The slope of a plot of the logarithm of the rate *vs.* pH should equal n, the order of the reaction with respect to hydroxide ion. The slope of the least squares line of Figure 5 is 0.8 ± 0.1. The rate-limiting reaction for the hydrolysis of ovomucin, as measured by viscosity change, is thus first-order with respect to hydroxide ion.

The rate constant for degradation of a linear polymer can be obtained from the initial rate of change of a physical property which is a measure of a molecular weight average (Tan-

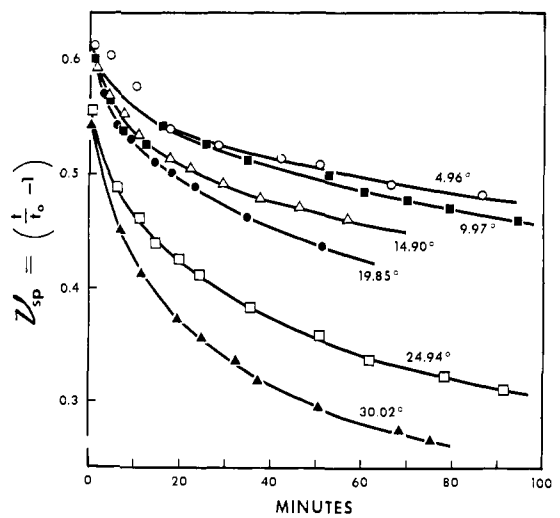

Figure 6. Change in specific kinematic viscosity of ovomucin as a function of time at several temperatures. Ovomucin concentration 1.5 mg/ml in 0.3 ionic strength Britton–Robinson buffer, pH 11.3 at 20°C

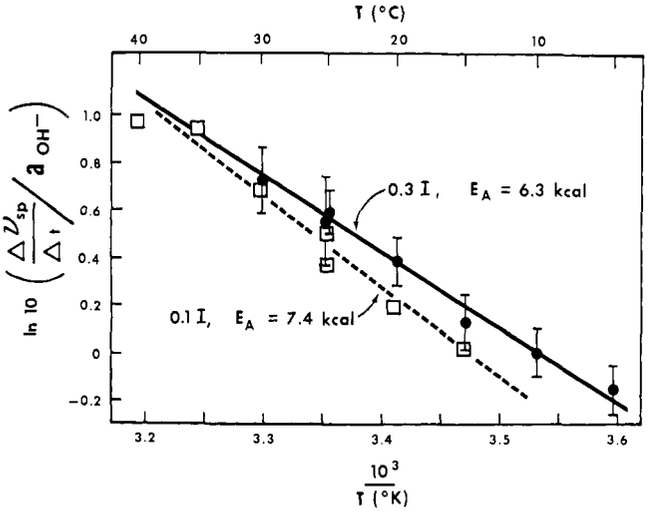

Figure 7. Arrhenius plot for alkaline degradation of ovomucin in 0.3 ionic strength Britton–Robinson buffer, ●, and in 0.1 *M* KCl, □. Bars give estimated errors

ford, 1961). At the beginning of a degradation reaction, the number of links broken is a linear function of time, t, and the weight average degree of polymerization, $\bar{x}_w$, is given by

$$1/\bar{x}_w = 1/(\bar{x}_w)_0 + kt/2 \quad (3)$$

where the subscript 0 refers to the initial degree of polymerization, and k is the pseudo-first-order rate constant for the bimolecular reaction. Although the viscosity average degree of polymerization $\bar{x}_v$ is closer to the weight average than to the number average when there is the usual sort of polydispersity in the molecular weight of the polymer molecules (Flory, 1953), the exact relation depends on the molecular weight distribution. For the following the average degree of polymerization will be left unsubscripted for simplicity, but eq 3 will be assumed to be a sufficiently good approximation for use with viscosity measurements.

For polymers like ovomucin, which have large intrinsic viscosities, the difference between specific viscosity, $\eta_{sp} = (\rho t'/\rho_0 t'_0) - 1$, and specific kinematic viscosity, $\nu_{sp} = (t'/t'_0) - 1$, is negligible, since the polymer contributes little to the density of the solution (Tanford, 1955). Here, ρ is density, t' is flow time, and the subscript 0 refers to solvent values. For large intrinsic viscosities, the Mark–Houwink equation can accordingly be written

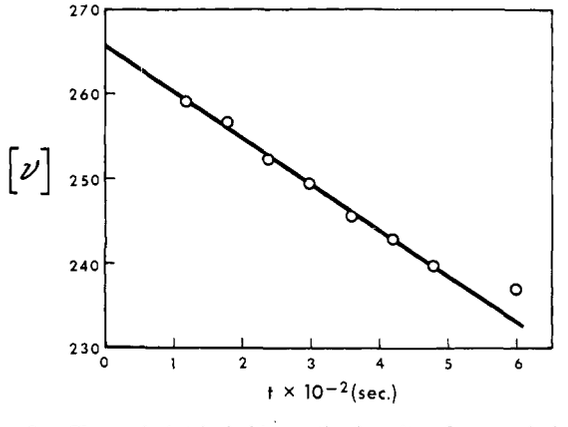

Figure 8. Change in intrinsic kinematic viscosity of ovomucin in the first 10 min of exposure to pH 11.4, at 25 °C, in 0.1 *M* KCl

$$[\eta] = KM^a \simeq [\nu] \quad (4)$$

and the Huggins Equation, for weight concentration, c, of polymer, can be approximated by

$$\nu_{sp}/c = [\nu] + k'[\nu]^2 c \quad (5)$$

In what follows, M is the polymer molecular weight, $\bar{M}$ the appropriate average molecular weight, and M_0 the monomer molecular weight. Combining eqs 4 and 5 with the definition of specific kinematic viscosity

$$(t'/t'_0) - 1 = KM^a c + k'[KM^a]^2 c^2 \quad (6)$$

Since

$$\bar{M}(t) = M_0 \bar{x}(t) \quad (7)$$

(Tanford, 1961), there results

$$(t'/t'_0) - 1 = K[\bar{x}(t)]^a cM_0^a + k'\{K[\bar{x}(t)]^a M_0^a\}^2 c^2 \quad (8)$$

Since in any one rate experiment, the concentration of polymer, c, is constant

$$d(\nu_{sp})/dt = KM_0^a c\, d[\bar{x}(t)]^a/dt + \text{term in } c^2 \quad (9)$$

At low polymer concentrations, the term in c^2 can be neglected. An experimental justification for this will be given below. Using eq 3, the derivative on the right-hand side of eq 6 becomes

$$d[\bar{x}(t)]^a/dt = -\tfrac{1}{2} ak(\bar{x}_0)^{a+1} (1 + \tfrac{1}{2} \bar{x}_0 kt)^{-(a+1)} \quad (10)$$

For $\bar{x}_0\, kt \ll 2$, in the initial stages of the degradation reaction,

$$[d(\nu_{sp})/dt]_{initial} \simeq -\tfrac{1}{2} akKM_0^a (\bar{x}_0)^{a+1} c = (\text{constant})\ kc \quad (11)$$

Thus, the initial rate of change of specific kinematic viscosity is proportional to the pseudo-first-order rate constant of the degradation reaction, multiplied by the concentration of polymer present. If the logarithm of both sides of eq 11 is taken, there results

$$\log(d\nu_{sp}/dt)_{initial} \simeq \log(\text{constant}) + \log k + \log c \quad (12)$$

In Figure 4, the slope of the plot of logarithm of initial change in viscosity as a function of logarithm of ovomucin concentration is unity from 0.2 mg/ml to about 5 mg/ml, with some suggestion of a larger slope at higher concentration. The data of Figure 4 thus not only represent a justification for neglect of the term in c^2 in eq 9, but also serve to demonstrate that, as indicated by eq 11, at constant ovomucin concentration, the initial change in viscosity is a measure of the rate constant of the reaction. Accordingly, Figure 5 shows that, at constant ovomucin concentration, the pseudo-first-order rate constant is directly proportional to hydroxide ion concentration.

An approximate value of the pseudo-first-order rate constant can be obtained from measurements of intrinsic viscosity obtained at the beginning of the degradation reaction. By combination of eq 3, 4, and 7, and using a series expansion

$$[\nu] \simeq K[M_0(\bar{x})_0]^a\{1 - akt(\bar{x})_0/2 + a(a+1)[kt(\bar{x})_0/2]^2/2! - \ldots\} \quad (13)$$

For a plot of $[\nu]$ *vs.* t, the ratio (slope)/(intercept) equals $-ak(\bar{x})_0/2$ approximately. Given the initial average degree of polymerization, the pseudo-first-order constant, k, can be obtained. When a is assumed equal to 0.7, and $(\bar{x})_0$ equal to 50, the first-order rate constant k was found to be 1.2 ×

10^{-5} sec^{-1} (Figure 8). The second-order rate constant, k_2, calculated from this experiment at 25°C and pH 11.4, is accordingly 5×10^{-3} l. mol^{-1} sec^{-1}. A total variation in this calculated rate constant of a factor of 5 is obtained by assuming combinations of a between 0.5 and 1.0 and of $(\bar{x})_0$ between 40 and 100. Accordingly, it appears that the rate constant for degradation of ovomucin at pH 11.4 is about the same as that observed for the alkaline hydrolysis of the disulfide bonds of ovomucoid (Donovan, 1967; Donovan and White, 1971).

The near equality in rates of the thinning reaction and rate of alkaline hydrolysis of disulfide bonds in proteins suggests that alkaline hydrolysis of disulfide bonds of ovomucin is responsible for the thinning of egg white of eggs held in storage. Rate constants for loss of cystine from ovomucin, as determined by amino acid analysis, and of the increase in absorption near 240 mμ when ovomucin is held at pH 11.3 (Donovan and Mapes, 1971) are approximately equal to the rate constant calculated above for the thinning measured viscosimetrically. However, it may not be legitimate to extrapolate the results of these experiments carried out above pH 11 to as low a pH as 9.5, *e.g.*, it is possible that the thinning which takes place at lower pH occurs by a different mechanism. There is the additional complication that the activation energy observed in these experiments, 7 Kcal/mol, is significantly less than the 19 Kcal/mol observed for alkaline hydrolysis of disulfide bonds in ovomucoid and aliphatic disulfides (Donovan and White, 1971). Since the rate of the thinning reaction cannot be determined accurately by viscosimetric means at the lower pH values, experiments are now being carried out to determine the chemical composition and molecular weight of ovomucin prepared from fresh eggs and from eggs which have naturally thinned.

ACKNOWLEDGMENT

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