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*AGGREGATION PHENOMENA IN EGG ALBUMIN SOLUTIONS AS DETERMINED BY LIGHT SCATTERING MEASUREMENTS**

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The various ultracentrifugal measurements reported for the molecular weight of egg albumin are not in complete agreement, as values between 34,500 and 46,000 have been obtained.[1,2] Investigating the behavior of this protein, Sjögren and Svedberg[3] have reported also that, within the pH range 4 to 9, egg albumin is stable and monodisperse.

During the course of extensive studies on the effect of freezing on various proteins and enzymes, it was found, however, that freezing causes, solely because of its physical influence, a change in the state of aggregation of egg albumin, inducing an aggregation at higher concentrations and a disaggregation at lower concentrations of the protein particles in solution.[4] These results were recently confirmed by Neduzhii[5] and others. Furthermore, experiments of Putzeys and Brosteaux,[6] and of Heller and Klevens,[7] extended these results by noting that egg albumin also undergoes aggregation at normal temperatures within the above mentioned pH range of alleged stability.

The causes influencing the state of aggregation of this protein under the above conditions were not yet investigated, and hence we studied the factors governing the aggregation occurring at normal temperature. To study the influence of concentration and time, we adopted the light-scattering method, which is very suitable for the study of aggregation phenomena,[7] the rapidity of the single measurements offering a notable advantage over the determination of particle weights by ultracentrifugation. This is to our knowledge the first attempt of a study of the mechanism of protein aggregation and denaturation by a continuous direct measurement of changes of particle weights.

Regarding the kinetics of aggregation, Oster[8] has pointed out that, in a system of polymerizing or aggregating particles, the light scattering increases with the progress of the reaction. Namely according to Rayleigh,[9]

the intensity of scattered light τ, called also turbidity, and expressed in terms of the extinction coefficient, is proportional to the number N of particles per unit volume, and also to the square of the volume of each particle:

$$\tau = ANV^2 \quad . \quad (1)$$

Although, because of aggregation, the number of particles is decreasing, the mean volume of each particle is increasing in the same proportion. In a polydisperse system the intensity of the scattered light is the sum of the contributions from each of the components of the mixture.

Equation (1) is limited to a system of independent, dielectric, spherical particles of small diameter as compared with the wave-length of the light. Putzeys and Brosteaux[6] have already established the fact that no correction for the turbidity due to depolarization of the scattered light is required in the case of egg albumin, owing to the symmetry of its particles, whereas the radius, $r = 27.2$ Å, of these particles in the hydrated state, as calculated by Bull,[10] is certainly small enough as compared with the applied wave-length ($\lambda_{air} = 4358$ Å).

For calculating the particle weight M from the turbidity measurements, Debye's[11] formula

$$\frac{Hc}{\tau} = \frac{1}{M} + 2BC \quad (2)$$

was applied, where c is the concentration, B[†] is a constant for a determined system, and H is a function of the wave-length and of the refractive indices of the solvent and the solution. For calculating this constant we used the specific refractive increment of egg albumin reported by Barker.[12] For computing the average particle weight at various states of aggregation, it was assumed that the specific refractive increments as well as the coefficient B are not affected by the state of aggregation. For highly aggregated particles, a correction for the asymmetry of the light scattering would probably be necessary; this, however, would only further raise the reported values.

Experimental.—To determine the intensity of the scattered light we modified the photoelectric Tyndallometer of the type described by P. P. Debye.[13] The instrument[‡] was calibrated with a sample of polystyrene, the absolute turbidity of which was determined in Debye's laboratory.[§]

The egg albumin was prepared according to the method of La Rosa,[14] recrystallized twice, and dialyzed against distilled water for 3 days. The preparation of the protein was carried out at 4°C., the resulting 6% solution being stored at this same temperature until use.

For each series of measurements the stock solution was centrifuged, filtered in a specially designed sintered glass filter of fine porosity, then transferred into several rectangular glass cells used in the Tyndallometer and there diluted to the various desired concentrations with distilled water.

The cells were then placed in a thermostat and at regular intervals the turbidity of the solutions measured, first allowing sufficient time for the solutions to reach the temperature of the bath. The pH of the solution was determined with a Cambridge meter and found to be 4.18–4.20, independent of the dilution of the protein solution (no buffers used), remaining unchanged throughout the time of each experiment.

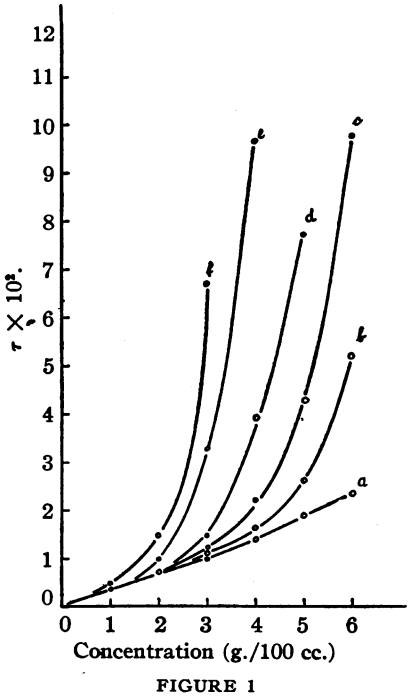

FIGURE 1

Change of turbidity with time as a function of concentration. (*a*) 3 hrs. at 25°C.; (*b*) 20 hrs. at 25°C.; (*c*) 2 hrs. at 35°C.; (*d*) 5 hrs. at 35°C.; (*e*) 13 hrs. at 35°C.; (*f*) 24 hrs. at 35°C.

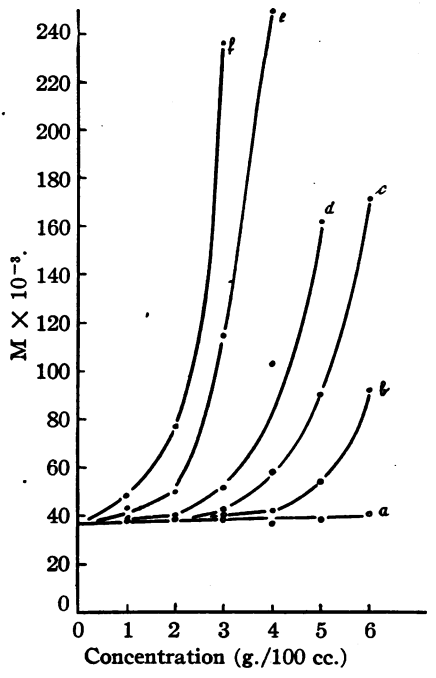

FIGURE 2

Change of average particle weight with time as a function of concentration. For explanation of curves see legend for figure 1.

Results.—Turbidity-concentration curves for the albumin solutions at varying time intervals are shown in figure 1, the concentrations ranging from 1 to 6%. The temperature was initially kept at 25°C., and then raised to 35°C., as indicated in the legend. Due to the fact that the coefficient B of equation (2) was found to be approximately zero, there is a nearly linear relationship between the initial turbidities (curve 1*a*) of the various solutions and their concentrations. The increase in turbidity with time, which is characteristic of the more concentrated solutions, is due to aggregation.

At the temperature of 25°C. only the 5 and 6% solutions show a rapid aggregation, whereas the solutions of lower concentration are relatively stable. As we raised the temperature to 35°C. a rapid aggregation occurred also with the more dilute solutions, namely at 2, 3 and 4%, whereas the turbidity of the 1% solution still remained nearly constant. Thus, a very marked influence of concentration on the rate of aggregation is evident, and may be immediately recognized from the shape of the curves. The aggregation

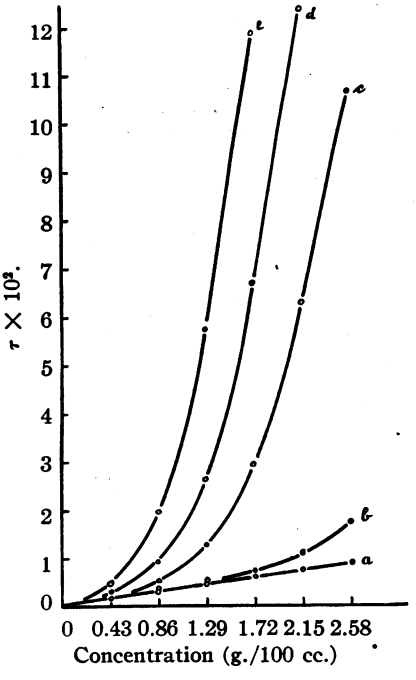

FIGURE 3

Change of turbidity with time as a function of concentration. (*a*) 1 hr. at 20°C.; (*b*) 2 hrs. at 40°C.; (*c*) 14 hrs. at 40°C.; (*d*) 24 hrs. at 40°C.; (*e*) 48 hrs. at 40°C.

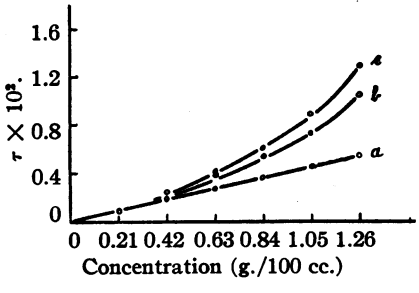

FIGURE 4

Change of turbidity with time as a function of concentration. (*a*) 1 hr. at 25°C.; (*b*) 6 days at 25°C.; (*c*) 1 day at 30°C.

proceeds until a visible cloudiness appears, when the turbidity readings were stopped. This is the reason why the curves 1*d*, *e* and *f* do not show the values for the more concentrated solutions.

The same results in terms of average particle weight of the egg albumin, calculated on the basis of the above-mentioned assumptions, are presented in figure 2. Each curve clearly indicates the relation between the concentra-

tion and aggregation. By extrapolating to infinite dilution, a relatively low value of 37,000 for the molecular weight of egg albumen was obtained, which, however, is within the range of the values recorded in the literature.

In figures 3 and 4 is shown the influence of concentration and time on the turbidity of more dilute solutions: namely, 0.43–2.58% and 0.21–1.26%, respectively. Again an initial linear relationship between turbidity and concentration was found. On standing, the turbidity increases indicating that aggregation has occurred, the rate being of the same over-all depend-

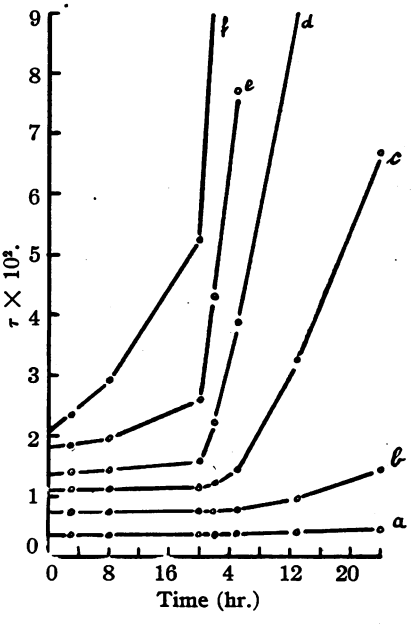

FIGURE 5

Change of turbidity as a function of time. During the first 20 hrs. the temperature was kept at 25°C., then raised to 35°C. (*a*) 1% solution; (*b*) 2% solution; (*c*) 3% solution; (*d*) 4% solution; (*e*) 5% solution; (*f*) 6% solution.

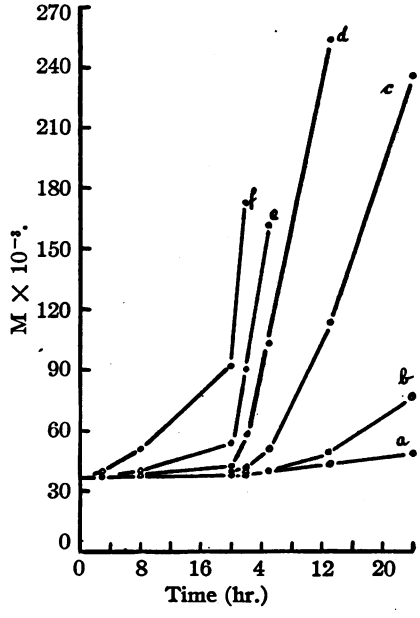

FIGURE 6

Change of average particle weight as a function of time. For explanation of curves see legend for figure 5.

ence on temperature and concentration as above. Presentation of the results in terms of particle weight is omitted, as the curves are similar to those recorded in figure 2.

In figure 5 the turbidity values presented in figure 1 are plotted against the time of observation. The turbidity increases nearly linearly with time when the temperature is kept constant. The strong inflection of the curves is caused by the change of temperature from 25 to 35°C. Similar is the relationship of the increase in particle weight of the egg albumin with time,

presented in figure 6. The same linear dependence of aggregation on time was observed in all the other experiments, particularly in that shown in figure 4, where the very slow aggregation, due to low concentration and low temperature, was measured over a period of 6 days.

Discussion.—The rate of aggregation of dialyzed crystalline egg albumin was found to be particularly dependent upon the temperature and the concentration of the protein solution. However, thus far, no simple mathematical relationship could be found between the concentration and the rate of aggregation. The temperature coefficient Q_{10}, defined as the ratio of the reaction rate constants k at the temperatures $T + 10$ to that at T, i.e., k_{T+10}/k_T may be calculated from the relation deduced by Oster,[8] where the turbidity, τ, at any time, t, can be expressed by the relation

$$\tau_T = AN_0V_0^2(1 + k_Tt) \qquad (3)$$

assuming that the aggregation is a second-order reaction analogous to the linear polymerization. From equation (3), by derivation, we easily obtain

$$Q_{10} = \frac{k_{T'+10}}{k_{T'}} = \frac{(\partial\tau/\partial t)_{T=T'+10}}{(\partial\tau/\partial_t)_{T=T'}} \qquad (4)$$

Whereas for ordinary chemical reactions Q_{10} is 2–3, temperature coefficients higher than 600 have been reported for protein denaturations.[15] The temperature coefficient of our aggregation reaction, while assuming intermediate values between the above expressed limits, was not constant but continued to increase with rise in temperature. Further experiments over a wider temperature range will be reported later.

The difference in the temperature constants of the aggregation reaction just mentioned and the heat denaturation of egg albumin indicates that the two phenomena are not identical. Supporting evidence for this postulation is that our solutions during the aggregation do not show any measurable increase in free sulfhydryl groups, and do not seem to have their ability to crystallize impaired. Both phenomena are characteristic for the heat denaturation. The phenomenon seems also to differ from the denaturation of egg albumin by acids, as this occurs below pH 4, with larger aggregates appearing only below pH 3.[3]

We have, therefore, to consider egg albumin as being a very labile system, upon the state of aggregation of which a small change in environment, such as concentration or temperature, has a profound effect. The influence of other factors, such as pH and ionic strength, and the particle size distribution of the aggregates, will be discussed in future communications.

Summary.—The particle weight of crystalline egg albumin was determined by the light-scattering method. Aggregation phenomena were observed at pH 4.20, i.e., within the usually accepted pH range of stability. Whereas the turbidity increases linearly with time, the rate of this increase,

coinciding with the progress of aggregation, was found to be particularly dependent on the concentration and the temperature of the solution. During the aggregation no increase in free sulfhydryl groups could be detected, and the protein did not seem to have its ability to crystallize impaired.

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** Communication No. 168.

† Edsall[16] has recently discussed the influence of the pH and ionic strength on the coefficient B of the proteins.

‡ Built by Mr. W. H. Baker, Elmhurst, L. I., N. Y.

§ The sample was obtained through the courtesy of Mr. T. J. Deszczynski of the Department of Chemistry, Columbia University, New York, N. Y.

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*UNUSUAL GENE-CONTROLLED COMBINATIONS OF CARBOHYDRATE FERMENTATIONS IN YEAST HYBRIDS**

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The ability of Saccharomyces to ferment different sugars is gene-controlled and we have built up breeding stocks which are differentiated by their abilities to ferment galactose, melibiose, maltose, sucrose, raffinose and alpha methyl glucoside (1944, 1947, 1948). Many combinations of gene-controlled fermentative abilities which have not been hitherto described