The Change of Molecular Weight of Egg Albumin in Aqueous Solutions

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Heller and Klevens⁽¹⁾ have recently measured the molecular weight of egg albumin in aqueous solutions by the light transmission method, and obtained a value of 47,000 immediately after the preparation of the sample at 4°C. With the lapse of time after the preparation, however, the value obtained gradually increased, namely 64,000 after 24 hours and 81,000 after 72 hours. This phenomenon was considered to be due to the occurence of aggregation, but the details of the process were not relevantly worked out by these authors. The purpose of this study is to elucidate the nature of the process, with special reference to the effect of temperature upon the phenomenon in question.

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(1) W. Heller and H. B. Klevens, *Phys. Rev.*, **67**, 61 (1945). W. Heller, ibid., **68**, 5 (1945).

The solution used was a 0.3% aquous solution of egg albumin, which was prepared by dissolving egg albumin (preparation of Takeda Pure Chemicals LTD.) in distilled water. The relative value of the molecular weight of egg albumin in aqueous solution was measured by determining the turbidities of solutions, using a Pulfrich photometer provided with a special device for measuring the scattered light. The change of the molecular weight was observed at several intervals of time in the aqueous solution of egg albumin, which was immersed in the water baths regulated at 30°C., 40°C. and 50°C., respectively.

The results obtained are shown in Fig. 1, where the abscissa is the time in hours and the ordinate is relative value W of the weight average molecular weight, assuming the value immediately after the preparation of the solution as unity. As will be seen from this figure, the value of W increased rapidly within a few hours and reached the maximum value in four hours. In one experiment observation was continued over night, but from the fifth hour on, the value W was found to remain practically unchanged. To one of the samples (200 cc.), in which W had already reached its maximum value at 30°C., 5 cc. of 10 N urea solution were added, whereby no change of W could be observed. The rise of temperature caused both the increase of the initial velocity and the increase of the maximum value of W.

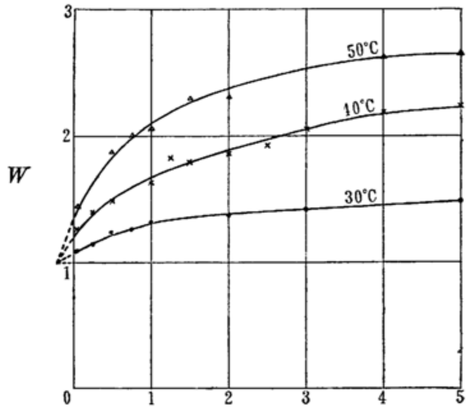

Fig. 1.

As was suggested by Heller and Klevens, the observed increase of molecular weight may be due to the process of aggregation. Let us assume that the aggregation takes place in the manner of the following polymerization reaction.

$$M_1+M_1\xrightarrow{k}M,\ M_1+M_2\xrightarrow{k}M_3,\ M_1+M_3\xrightarrow{k}M_4,\ldots\ldots$$

$$M_2+M_2\xrightarrow{k}M_4,\ M_2+M_3\xrightarrow{k}M_5,\ldots\ldots\ldots\ldots\ldots$$

$$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

$$M_i+M_i\xrightarrow{k}M_{2i},\ldots\ldots M_i+M_j\xrightarrow{k}M_{i+j},\ \ldots\ldots$$

$$\ldots\ldots\ldots\ldots\ldots$$

where M_1, M_2...... indicate monomer, dimer and so on, i and j being assumed to be any integer. The velocity constant for each step of aggregating reactions is assumed to be the same. For such a type of multistep reactions, a theory had already been develoded by Flory,⁽²⁾ according to whom, the number of moles of i-mers, N_i, which has been formed at the time t, is given by the following equation;

$$N_i=N_0p^{i-1}(1-p)^2 \tag{1}$$

in which

$$p=\frac{kt}{2+kt}.$$

N_0: Total number of moles of the monomer at the beginning. It has already been shown by Doty, Zimm and Mark⁽³⁾ that whereas osmotic pressure measurements yield a number average molecular weight, turbidity measurements give a weight average. Therefore, the value W which we have observed is the relative value of weight average molecular weight M to the molecular weight of the monomer M_0. Hence,

$$\begin{aligned}W&=M/M_0\\&=\frac{1}{M_0}\cdot\frac{\sum\limits_i (iM_0)^2Ni}{\sum\limits_i iM_0Ni}\\&=\frac{\sum i^2Ni}{\sum iNi}\\&=\frac{\sum i^2p^{i-1}}{\sum ip^{i-1}}\end{aligned} \tag{3}$$

Using the relations

$$\sum_i ix^{i-1}=(1-x)^{-2} \tag{4}$$

$$\sum_i i^2x^{i-1}=(1-x)^{-3}(1+x), \tag{5}$$

equation (3) becomes

$$W=\frac{1+p}{1-p}$$

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(2) P. J. Flory, *J. Am. Chem. Soc.*, **58**, 1877 (1936); **59**, 241 (1937); **61**, 1518, 3334 (1939); **62**, 1057, 1561, 2255, 2261 (1940); **63**, 3083, 3091, 3096 (1941).

(3) P. M. Doty, B. H. Zimm and H. Mark, *J. Chem. Phys.*, **13**, 159 (1945).

$$=1+kt. \qquad (6)$$

This equation indicates that the initial velocity of the increase of W is k. The value k for each temperature can be evaluated from the data of our experiments. Based on the temperature dependency of k, the activation energy of the reaction was calculated to be 7 kcal./mole.

If we further assume that each reaction step has the same equilibrium constant (K), then the following relation must hold for the number fractions $m_i=N_i/N_0$;

$$K=\frac{m_{i+j}}{m_i\cdot m_j}.$$

This equation must hold for any choice of i and j, but the equations thus obtained are not necessarily independent of each other. Actually, the relation ($i=2$, $j=2$) can be derived from the equations for ($i=1$, $j=1$), ($i=1$, $j=2$) and ($i=1$, $j=3$). As will be seen from this example, the independent relationship will be the following;

$$m_iK=x^i, \qquad (7)$$

in which

$$x = Km_1.$$

Using equations (4), (5) and (7), the relative value of weight average molecular weight at equilibrium may be expressed by

$$W=\frac{1}{M_0}\cdot\frac{\sum\limits_i (iM_0)^2Ni}{\sum\limits_i iM_0Ni}$$

$$=\frac{\sum i^2mi}{\sum imi}$$

$$=\frac{\sum i^2x^i}{\sum ix^i}$$

$$=\frac{1+x}{1-x}. \qquad (8)$$

For obtaining the relation between K and W, we shall use the following relation;

$$\sum_i im_i=1, \qquad (9)$$

which must be true according to the definition of m. Substituting equation (7) in (9), we have

$$K=\frac{x}{(1-x)^2}. \qquad (10)$$

Since we have equation (8) relating x to W, we can express K in terms of W at equilibrium; that is

$$K=\frac{1}{4}(W^2-1). \qquad (11)$$

Using this equation, we evaluated K at each temperature, and calculated the change of heat content from the temperature dependency of K, which amounted to 7 kcal./mole. If we extrapolate our result of analysis to the case of lower temperatures, the value of W at equilibrium is calculated to be 1.04 at 0°C., which shows that almost no egg albumin molecules will aggregate at 0°C. Whether or not this conclusion is valid, it remains to be checked by further investigations.

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