Conformation of Linear Aggregates of Thermally Denatured Ovalbumin

TAKESHI FUKUDA* AND YOSHINOBU TSUJII

Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan

TAIHEI KOSEKI, NAOFUMI KITABATAKE, AND ETSUSHIRO DOI*

Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan

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When thermally denatured under suitable conditions, ovalbumin forms *linear* polymers (aggregates) of varying degrees of polymerization.[1] At temperatures below, say, 30 °C, the polymers remain stable within the time scales of experiments. In a previous paper,[2] we have studied the molecular characteristics of those polymers by means of gel permeation chromatography (GPC), light scattering (LS), and viscometry. The values of the z-average mean-square radius of gyration $\langle s^2\rangle_z$ and intrinsic viscosity $[\eta]$ were appropriately corrected for the polydispersity of each sample and analyzed on the basis of the wormlike cylinder model.[3] The result showed that the ovalbumin linear polymers may be described by a wormlike cylinder with $M_L = 1600$ Å^{-1}, $d = 120$ Å, and $q = 230$ Å, where M_L, d, and q are the molecular weight per unit contour length, diameter, and persistence length, respectively. On the other hand, an electron microscopic observation indicated that d was about 50 Å.[1] The difference between the two estimates of d was much too large to be attributed to experimental errors or artifacts, but we could give no clear interpretation for that. Here we will show that the difference can be understood as evidence showing that the protein aggregates in a helical fashion, a classical but experimentally unverified notion of Pauling.[4]

A main part of the previous result was reorganized in Table I. The weight-average molecular weight M_w and the z-average mean-square radius of gyration $\langle s^2\rangle_z$ were determined by LS. The M_w/M_n ratio, where M_n is the number-average molecular weight, was estimated by a GPC analysis in which a calibration curve was constructed in an iterative manner so as to be consistent with all of the M_w data.[2] The distribution curve estimated by this GPC analysis was reasonably well approximated by the Schulz-Zimm function,[5] and on this basis, the $\langle s^2\rangle_z$ and $[\eta]$ data were corrected for the polydispersity of each sample. For example, the weight-average mean-square radius $\langle s^2\rangle_w$ of a polymer with a Schulz-Zimm distribution is given by

$$\langle s^2\rangle_w = \langle s^2\rangle_z(1+h)^b\Gamma(2+h)/\Gamma(2+b+h) \qquad (1)$$

where $h^{-1} = (M_w/M_n) - 1$, and b is the slope of the log $\langle s^2\rangle$ vs log M curve. To estimate $\langle s^2\rangle_w$, log $\langle s^2\rangle_z$ was plotted against log M_w, and the slope of the curve at each value of M_w was used in eq 1 to obtain a first approximation of $\langle s^2\rangle_w$, the logarithm of which was again plotted against log M_w to obtain a higher order approximation. The weight-average intrinsic viscosity $[\eta]_w$ was estimated by a similar iteration. As the table shows, the correction factors of $\langle s^2\rangle_z$ thus obtained are fairly large, while those of $[\eta]$ are less than 5% in all cases.

As already mentioned, these data, when applied to the theory of the wormlike cylinder model due to Yamakawa et al.,[3] give a diameter value inconsistent with the electron microscopic observation. A possible interpretation of this inconsistency may be that the ratio d/q in this system exceeded the applicability limit of the theory.[3] This problem is resolved by the recent theory of Yoshizaki et al.[6] based on the touched-bead wormlike chain model, which has exceedingly enlarged the applicability limit. Figure 1 shows the results of the analysis based on this theory. The circles in the figure show the experimental data, and the curves were simulated for the three parameters M_L, λ^{-1} (=$2q$), and d (=0.74 × bead diameter)[6] so as to give the best fit to the $\langle s^2\rangle_w$ and $[\eta]_w$ sets of data simultaneously. The optimum sets of parameter values thus obtained are $M_L = 1700$ Å^{-1}, $\lambda^{-1} = 550$ Å, and $d = 90$ Å. This value of d is somewhat smaller than the previous one but still very different from what was observed under the electron microscope. Moreover, a wormlike cylinder characterized by these values of M_L and d indicates that the effective volume v_e occupied by 1 g of the protein in solution is 2.2 mL/g. This value is about 3 times as large as the partial specific volume $\bar{v}$ (about 0.75 mL/g) and seems too large to be explained in terms of bound or free water possibly present within and around the protein molecule. It follows that the ovalbumin linear aggregates cannot be precisely represented by the wormlike cylinder model.

When rigid particles like globular proteins aggregate hand-in-hand in a linear chain, aggregation will occur at *specific* regions of the molecule. (The thermal denatur-

Table I
Characteristics of Linear Aggregates Obtained by Heating Ovalbumin Solution (5 mg/mL in 20 mmol/dm³ of Potassium Phosphate Buffer, pH 7.0, Heated at 80 °C)

heating time, min	$10^{-6}M_w$	$\langle s^2\rangle_z^{1/2}$, Å	$\langle s^2\rangle_w^{1/2}$, Å	$[\eta]$, mL/g	$[\eta]_w$, mL/g	M_w/M_n
native	0.048			4.5		
3	0.74			13.1	13.4	1.54
5	1.37	250	177			1.81
10	1.73	300	224	26.6	27.6	1.68
30	2.81	412	306	37.0	38.7	1.97
60	3.64	485	355	46.0	48.2	2.08
120	5.00	600	426	54.8	57.7	2.46
360	7.24	782	547	73.7	77.8	2.71
1440	8.85	880	621	90.4	95.5	2.63

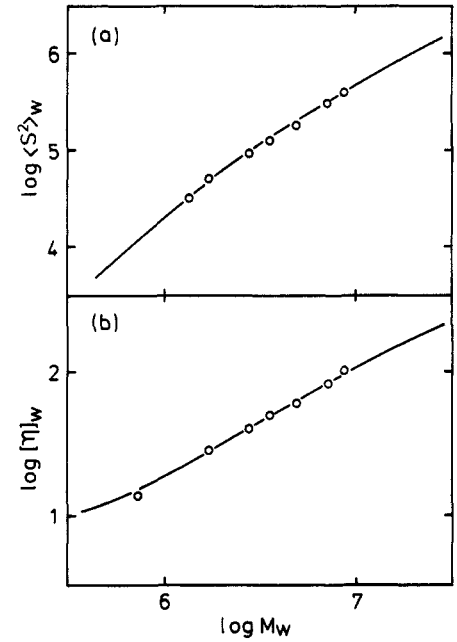

Figure 1. (a) log $\langle s^2\rangle_w$ and (b) log $[\eta]_w$ plotted against log M_w. The solid curves represent the touched-bead wormlike chain model with $M_L = 1700$ Å, $\lambda^{-1} = 550$ Å, and $d = 90$ Å ($d = 0.74$ × bead diameter[6]).

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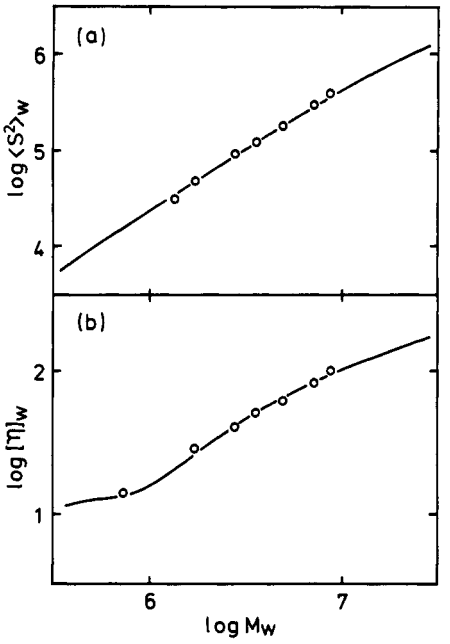

Figure 2. (a) log $\langle s^2 \rangle_w$ and (b) log $[\eta]_w$ plotted against log M_w. The solid curves represent the touched-bead helical wormlike chain model with M_L = 1100 Å, λ^{-1} = 800 Å, d = 60 Å, $\lambda^{-1}\kappa_0$ = 7, and $\lambda^{-1}\tau_0$ = 5 (d = 0.74 × bead diameter[6]).

ation of ovalbumin was observed to cause only a minor structural change involving a conformational transition of a very limited portion of the helix to the coil.[1] Accordingly, the conformation of the thermally denatured ovalbumin is considered not to differ very much from that of the native molecule, but perhaps certain aggregative regions buried within the molecule before denaturation may come out on the molecular surface after denaturation.[1]) If aggregation occurs at specific regions of a particle, it will be a rather rare occasion that the particles should aggregate in a straight line. It is more likely that they aggregate in a helical form, as was pointed out by Pauling a long time ago.[4]

Figure 2 shows the results of the analysis based on the touched-bead helical wormlike chain model.[6] This model includes, on top of M_L, λ, and d, two parameters κ_0 and τ_0 to characterize a helix. In view of possible errors in the experimental data, particularly those associated with the sample polydispersity and its correction, results of least-square curve-fitting calculations which are carried out without imposing any restrictions on these five parameters may be of questionable reliability. For this reason, we have imposed the restriction that 40 Å ≤ d ≤ 60 Å, in order to see if this model gives results consistent with the electron microscopic image. The optimum sets of values obtained under this restriction are M_L = 1100 Å, λ^{-1} = 800 Å, d = 60 Å, $\lambda^{-1}\kappa_0$ = 7, and $\lambda^{-1}\tau_0$ = 5. As the figure shows, the experimental data are well reproduced by this model too.

These values of d and M_L indicate that v_e is 1.5 mL/g, a value which sounds more reasonable than that estimated for the nonhelical chain. Naturally this value of d itself is consistent with the microscopic image, while that estimated for the nonhelical chain was very inconsistent with it. Furthermore, there is the already-noted reason to believe that linear aggregates of proteins have a tendency to form helices. Thus, the ovalbumin linear aggregate is highly likely to be in a helical form. The diameter and pitch of the characteristic helix corresponding to the above parameter values are 150 and 340 Å, respectively.

References and Notes

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