mass spectral peaks supports the spectroscopic evidence that the various Y bases have identical fluorescent chromophores.

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On the Conformation of Lysozyme and α-Lactalbumin in Solution*

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ABSTRACT: Lysozyme and α-lactalbumin have highly homologous primary sequences but different biological functions. W. R. Krigbaum and F. R. Kügler (1970, *Biochemistry 9*, 1216) have recently reported small-angle X-ray-scattering studies from both proteins in aqueous solution, and conclude that lysozyme and α-lactalbumin have quite different conformations in solution. They also present evidence for the presence of α-lactalbumin dimer in solution. We demonstrate that all of the observed differences in small-angle X-ray scattering from α-lactalbumin and lysozyme in solution can be rigorously accounted for by such dimerization. Thus the experiments of Krigbaum and Kügler strongly suggest that these two proteins have quite similar conformations in solution.

Lysozymes cause cell wall lysis of gram-positive bacteria by a mechanism which is now fairly well understood (Phillips, 1966), while α-lactalbumin has been implicated in the lactose synthetase system (Ebner *et al.*, 1966). The primary sequences of hen egg white lysozyme and bovine α-lactalbumin have been shown to be strikingly similar, with regard to both residue identity (49 residues out of 123–129) and the positions of the disulfide bridges (Brew *et al.*, 1967, 1970). This homology is somewhat surprising in view of the differences in function, but does provide grounds for anticipating homology in tertiary

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structure. With only minor structural modifications, Browne *et al.* (1969) were able to fit the α-lactalbumin sequence to the lysozyme main chain conformation, determined crystallographically by Blake *et al.* (1965).

To determine experimentally whether lysozyme and α-lactalbumin have similar conformations in solution, Krigbaum and Kügler (1970) performed small-angle X-ray-scattering measurements on both proteins in aqueous solution. α-Lactalbumin was studied at neutral pH, while lysozyme was studied at acid pH to minimize dimerization, which becomes serious above pH 4.5. For lysozyme in 0.15 M NaCl, pH 3.8, they obtained 14.3 ± 0.3 Å for the radius of gyration (R_g) and 14,500 ± 800 for the molecular weight (M). For α-lactalbumin in 0.1 M NaCl (pH 7.0), they obtained R_g = 16.7 ± 0.4 Å and M = 15,500 ± 800. From these results and from the shape of the scattering curves at higher angles, they concluded that lysozyme and α-lactalbumin have quite different conformations in solution. Their data for lysozyme are in excellent agreement with the radius of gyration and radial distribution function calculated from the crystallographic structure (Blake *et al.*, 1965) and with the sequence molecular weight (Canfield, 1963).

In the case of α-lactalbumin, the molecular weight obtained by Krigbaum and Kügler (1970) is high compared to the value obtained by sequence analysis (M = 14,200; Brew *et al.*, 1970). Previous determinations of the molecular weight of α-lactalbumin in solution at neutral pH also showed this behavior. For example, Gordon and Semmett (1953) obtained values of M = 15,100 by sedimentation and diffusion, and M = 16,500 by light scattering. Wetlaufer (1961) obtained M = 15,300 by osmometry. Krigbaum and Kügler attribute these high values to the presence of a small proportion of dimer in solution. In this paper we examine the effect of such dimerization on the interpretation of the small-angle X-ray-scattering results.

For a polydisperse system containing n_1 polymer molecules of molecular weight M_1, and radius of gyration R_1, n_2 molecules of weight M_2 and radius R_2, and so forth, the Guinier plot yields a weight-average molecular weight (M_w), defined by

$$M_w = \frac{\Sigma n_i M_i^2}{\Sigma n_i M_i}$$

However, the calculated radius of gyration is the z average, defined by

$$\langle R^2 \rangle_z = \frac{\Sigma n_i M_i^2 R_i^2}{\Sigma n_i M_i^2}$$

In cases of severe polydispersity, the Guinier plot may show some curvature. We wish to emphasize that, for small degrees of polydispersity where no curvature is observable, the experimental value of $\langle R^2 \rangle_z^{1/2}$ is *still* the z average, and is thus exquisitely sensitive to the presence of small amounts of aggregates. This treatment of polydispersity (Tanford, 1961) is universally recognized for light scattering and applies equally well to small-angle X-ray scattering.

The extreme sensitivity of the z-average radius of gyration to the presence of small amounts of dimer is illustrated by the small-angle X-ray-scattering experiments of Beeman (1967) on myoglobin. Following preliminary measurements which gave radii of gyration greater than 18 Å, he used gel permeation chromatography to resolve a monomer and a dimer component. The myoglobin monomer was found to have R_g = 16 Å, in close agreement with crystallographic results (Watson, 1967), while the dimer, which constituted somewhat more than 5% of the total material, had R_g = 28 Å. Furthermore, the Guinier plot for the monomer–dimer mixture showed no curvature.

TABLE I: Calculated Radii of Gyration for α-Lactalbumin Solution Containing 5 mole % Dimer.

R (Dimer)/R (Monomer)		Calcd z-Av Radius of Gyration Assuming R (Monomer) = 14.3 Å as Obsd for Lysozyme (Å)	Calcd Value of R (Monomer) to Fit Obsd z-Av Radius of Gyration for α-Lactalbumin (Å)
Model	Value		
Prolate ellipsoids end to end	2.02	17.7	13.5
Observed for myoglobin	1.75	16.7	14.3
Spheres	1.63	16.2	14.8
Prolate ellipsoids side by side	1.40	15.4	15.5

We estimate that about 5 mole % of dimer was present in the α-lactalbumin experiments of Krigbaum and Kügler by comparing their weight-average molecular weight to the amino acid sequence molecular weight. To compute $\langle R^2 \rangle_z^{1/2}$ for this monomer–dimer mixture, some estimate of R_g for the dimer is required. If the monomer is represented as a solid sphere and the dimer as two such spheres in contact, R_g (dimer) = 1.63R_g (monomer). The prolate ellipsoid which best fits the small-angle X-ray data for lysozyme has axial ratios 1:1:1.8. For two such ellipsoids placed symmetrically side by side, R_g (dimer) = 1.40R_g (monomer). If they are placed end to end, R_g (dimer) = 2.02R_g (monomer). For all other configurations of two such ellipsoids in contact, R_g (dimer) falls between 1.40 and 2.02R_g (monomer). We note that, in the case of myoglobin, R_g (dimer) = 1.75R_g (monomer) was observed.

Table I shows the z-average radius of gyration $\langle R^2 \rangle_z^{1/2}$ computed for 5 mole % dimer when R_g (dimer)/R_g (monomer) assume values between 1.40 and 2.02. The third column shows the value of $\langle R^2 \rangle_z^{1/2}$ obtained by assuming that lysozyme and α-lactalbumin monomer have the same radius of gyration, 14.3 Å. If R_g (dimer)/R_g (monomer) = 1.75, as observed for myoglobin, the computed $\langle R^2 \rangle_z^{1/2}$ = 16.7 Å as observed for α-lactalbumin. In the fourth column, we assume that $\langle R^2 \rangle_z^{1/2}$ = 16.7 Å and deduce R_g for α-lactalbumin monomer. We see that even for the extreme case R_g (dimer)/R_g (monomer) = 1.40, R_g for α-lactalbumin agrees with the value found for lysozyme to within 1.2 Å.

We have shown that for a range of reasonable values for R_g (dimer)/R_g (monomer), the observed $\langle R^2 \rangle_z^{1/2}$ and M_w for α-lactalbumin imply that the radii of gyration of α-lactalbumin monomer and lysozyme must be quite similar. Krigbaum and Kügler calculate that 5 mole % of α-lactalbumin dimer would result in a sharp upturn in the small-angle region of the Guinier plot, and that the slope outside this region would give the monomer radius of gyration. Since no such sharp upturn is observed, they conclude that no more than 2 mole % of dimer can be present.

We have performed a similar calculation, described in

TABLE II: Computed Slope of Guinier Plot as a Function of h^2.

h^2	R (Å)
0.001	16.54
0.005	15.71
0.01	15.05
0.015	14.69
0.02	14.50
0.03	14.40
0.06	14.30

the Appendix, which indicates that the slope would converge to the monomer slope only at much higher angles than examined experimentally by Krigbaum and Kügler. Furthermore, our calculation shows a gradual change in slope throughout the experimental region, without any abrupt changes at small angles. In fact, the experimental Guinier plot for α-lactalbumin does show a gradual change of slope entirely consistent with our calculation. The experimental Guinier plot for lysozyme, on the other hand, is quite linear.

Krigbaum and Kügler also find differences between the two proteins by considering the shape of the scattering curve at higher angles, and by computing integrals over the entire scattering curves. It is well known (Guinier and Fournet, 1955), however, that the shape of the entire scattering curve is drastically influenced by polydispersity. For a monomer–dimer mixture, the shape of the scattering curve depends on the size and shape of both monomer and dimer. Parameters deduced from such a scattering curve obviously do not represent the size and shape of the monomer alone. On the contrary, information as to the shape of the scattering particles in solution depends on terms of order $\langle R^4 \rangle$ and higher in the series expansion of $(\sin hR)/hR$.

We conclude that all the differences in small-angle X-ray scattering from lysozyme and α-lactalbumin can be explained by the presence of α-lactalbumin dimer.[1] Furthermore, if about 5 mole % of α-lactalbumin dimer is actually present, then with mild assumptions about the geometry of the dimer one can show that the radii of gyration of α-lactalbumin and lysozyme monomers must agree within 1.2 Å. These results strongly suggest that the conformations of these two proteins are similar in aqueous solution.

Appendix

The scattered intensity from N isolated identical particles is $I(h) = I_e(h)NF^2(h)$, where $I_e(h)$ is the scattering for an isolated electron (essentially angle-independent for small-angle scattering) and $F^2(h)$ is the structure factor of a particle.

For N_1 monomer and N_2 dimer particles, $I(h) = I_e(h) \cdot (N_1F_1^2(h) + N_2F_2^2(h))$.

We make use of the law of Guinier, $F^2(h) = n^2 \exp(-h^2 \cdot R^2/3)$, where n is the number of electrons per particle. For ellipsoids representative of lysozyme, the law of Guinier is virtually exact in the experimental range of h as shown by detailed calculations (Guinier and Fournet, 1955) and by the observed linearity of the experimental Guinier plot for lysozyme (Krigbaum and Kügler, 1970).

Since $n_1/n_2 = M_1/M_2$ for particles of the same chemical composition

$$\frac{I(h)}{I(h=0)} = \frac{N_1M_1^2 \exp(-h^2R_1^2/3) + N_2M_2^2 \exp(-h^2R_2^2/3)}{N_1M_1^2 + N_2M_2^2}$$

For α-lactalbumin, with $R_2 = 1.75R_1$, $M_2 = 2M_1$, and 0.047 mole % dimer,

$$\frac{I(h)}{I(h=0)} = \frac{\exp(-h^2R_1^2/3) + 0.21 \exp(-1.02h^2R_1^2)}{1.21}$$

where $R_1 = 14.3$ Å as observed for lysozyme. The radius of gyration is obtained from the slope of $\log[I(h)/I(h=0)]$ *vs.* h^2. Table II gives the radius of gyration computed from the instantaneous slope of the Guinier plot as a function of h^2. The reported experimental data cover the range of h^2 from 0.001 to 0.02, and the experimental radius of gyration is determined from the slope of the Guinier plot in the limit as $h^2 \to 0$.

[1] The presence of a few per cent of dimer is a sufficient but not necessary condition for interpretation of the observed data. Alternatively, one might consider the possibility of aggregation of α-lactalbumin or of the presence of some impurity, but very similar conclusions would still be obtained.

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