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Molecular Conformation of Egg-White Lysozyme and Bovine α-Lactalbumin in Solution*

W. R. Krigbaum and F. R. Kügler

ABSTRACT: Small angle diffraction measurements are reported for hens egg-white lysozyme and bovine α-lactalbumin. The amino acid sequences of these two enzymes exhibit considerable homology, which has led to the suggestion that they may have similar tertiary structures. Lysozyme has a radius of gyration, R, of 14.3 Å, and its equivalent scattering body is a prolate ellipsoid having dimensions 28 × 28 × 50 Å, while α-lactalbumin has R = 16.7 Å, and its equivalent ellipsoid is oblate with dimensions 22 × 44 × 57 Å. We therefore conclude that lysozyme and α-lactalbumin have quite different molecular conformations in solution. The observed R value for lysozyme is quite close to that calculated from the crystallographic coordinates assuming the molecule to be in a vacuum, R = 13.8 Å. This observation, in conjunction with the good agreement between the scattering curves and the electron pair radial distribution functions observed for the molecule in solution and calculated from the crystallographic coordinates, indicates that lysozyme undergoes very little conformational change on dissolution.

Lysozymes form a class of widely distributed enzymes found in a number of organs, tissues, and secretions of vertebrates, as well as in bacteria, phages, and plants. Members of this class may differ considerably in molecular weight, but all exhibit a common capability to rapidly lyse bacterial cell walls by their action as muramidases. Comparison of the amino acid sequence of T4 phage lysozyme (Tsugita and Inoye, 1968) and hens egg-white lysozyme (Jollès *et al.*, 1963; Canfield, 1963) revealed no common primary structure, although there was some compositional similarity in terms of the relative numbers of basic, acidic, and hydrophobic side chains. In the following we shall refer to hens egg-white lysozyme as lysozyme.

Lactose synthetase catalyzes the reaction:

$$\text{UDP-galactose} + \text{glucose} \longrightarrow \text{lactose} + \text{UDP}$$

The soluble enzyme from milk may be separated into two proteins, A and B, both of which are required for lactose synthesis. Ebner, Brodbeck, and coworkers (1966, 1967) have identified the B protein as α-lactalbumin.

Although α-lactalbumin and the lysozymes perform different functions, some evidence for structural similarity of bovine or guinea pig lactalbumin and hens egg-white lysozyme has been provided by Brew and Campbell (1967) and Yasunobu and Wilcox (1958). This inference was confirmed when Brew *et al.* (1967) determined the amino acid sequence of bovine α-lactalbumin. The latter workers observed that over 40 of the residues in α-lactalbumin were identical with the corresponding residues in egg-white lysozyme, and that an additional 27 residues could be classified as conservative replacements (Smith and Margoliash, 1962). Further, each of the two molecules has four disulfide bonds formed by the same half-cystinyl residues. Thus approximately 40% of the residues of α-lactalbumin and lysozyme are identical (Hill *et al.*, 1968), as compared to 44% for the α and β chains of human hemoglobin (Ingram, 1963). In view of the fact that the α- and β-hemoglobin chains have very similar tertiary structures, and that the four disulfide bonds in α-lactalbumin and lysozyme are formed in the same way, there is a possibility that the latter two proteins may have very similar tertiary structures. Browne *et al.* (1969) have investigated the possibility of building an hypothetical molecular model of α-lactalbumin by appropriate alteration of the known structure of lysozyme (Blake *et al.*, 1965). Since α-lactalbumin contains six fewer residues, it was necessary to delete certain residues from the lysozyme structure. A few additional deletions were necessary to achieve maximum homology of the two structures. It was found that the residues to be deleted occurred in loops or at the end of the helical regions, so that it was possible to build a speculative molecular model of α-lactalbumin which retained essentially all of the features of the secondary and tertiary structures of lysozyme.

Evidence that lysozyme and α-lactalbumin have similar conformations in solution was provided by Aune (1968), who found that these two proteins have indistinguishable optical rotatory dispersion curves between 206 and 233 mμ,

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and between 320 and 500 mμ. However, this is essentially a measure of short range interactions. More direct information concerning the conformation of molecules in solution is provided by hydrodynamic or diffraction measurements. Of these two procedures, only diffraction permits independent evaluation of size and shape parameters. The study of coiling polymers by light scattering is an accepted procedure. For the study of particles in the size range of interest here, one must utilize X-ray wavelengths, but the theoretical basis of the analysis is, of course, quite similar.

Although the three-dimensional structure of lysozyme in the crystalline state is known, we found it desirable to perform diffraction studies of both molecules in solution. In this way we obtain a direct comparison of experimentally determined parameters for α-lactalbumin and lysozyme in solution, and also we are able to compare, by calculations based upon the atomic coordinates from the crystal structure (Blake *et al.*, 1965), the molecular conformation of lysozyme in solution and in the crystalline state.

TABLE I: Radius of Gyration, Molecular Weight, and Volume for Egg-White Lysozyme and Bovine α-Lactalbumin.

10^2c (g/ml)	R (Å)	10^{-3} M	10^{-3} V (Å^3)
		Lysozyme	
2.24	13.82	13.2	18.9
1.518	13.95	13.25	19.2
0.741	14.04	14.37	19.5
0.413	14.25	13.95	19.6
0	(14.3)	(14.45)	(19.8)
		α-Lactalbumin	
1.83	15.5	13.25	22.6
0.814	16.25	14.75	24.8
0.413	16.37	14.6	25.5
0.204	16.7	15.7	26.3
0	(16.7)	(15.5)	(26.5)

Experimental Section

Materials and Apparatus. Samples of hens egg-white lysozyme and bovine α-lactalbumin were obtained through the courtesy of Dr. Robert L. Hill. Light scattering and sedimentation studies (Bruzzesi *et al.*, 1965) have shown that lysozyme tends to associate at pH values above 4.5. Our measurements were therefore performed on solutions in 0.15 M NaCl at pH 3.8. Charlwood (1957), using a magnetic float method, has obtained for the partial specific volume of lysozyme $\bar{v}$ = 0.7138. Earlier pycnometric results gave $\bar{v}$ = 0.722 (Wetter and Deutsch, 1951). The value calculated by the method of Cohn and Edsall (1943) is $\bar{v}$ = 0.716, and this has been used for the molecular weight determination. Four solutions were investigated having concentrations in the range 0.413 to 2.24 g/100 ml. The concentrations were determined spectrophotometrically, taking for the specific extinction coefficient $E^{1\%}_{1\text{ cm}}$ 26.0 (Kanarek, 1963).

Solutions of α-lactalbumin were prepared in 0.1 M NaCl at pH 7.0, and these were dialyzed against solvent for 60 hr. Concentrations of the four solutions investigated, which ranged from 0.204 to 1.83 g/100 ml, were determined spectrophotometrically, taking for the specific extinction coefficient at 280 mμ $E^{1\%}_{1\text{ cm}}$ 20.5 (K. Brew and R. L. Hill, private communication 1969). The value for the partial specific volume, $\bar{v}$ = 0.735, reported by Gordon and Semmett (1953) agrees with that calculated from the known sequence by the method of Cohn and Edsall (1943).

Diffraction measurements were performed using a medium resolution Kratky camera with copper radiation and a proportional counter. The distance from the sample to the registration plane was a = 21.0 cm, and the widths of the entrance and detector slits were 120 and 360 μ, respectively; these parameters correspond to a resolution of approximately 600 Å. The intensities were placed on an absolute scale using as a secondary standard a Lupolene platelet of known scattering power (Pilz and Kratky, 1967). Slit desmearing was performed using a modification of the program developed by Heine and Roppert (1962), and by Heine (1963), which accounts for an incident beam of finite height.

Radius of Gyration and Molecular Weight. According to the treatment of Guinier (1939), the intensity scattered at very small angles by a collection of N identical particles may be adequately represented by the relation

$$I(h) = Nn^2 i_e e^{-R^2h^2/3} \tag{1}$$

where n is the number of electrons per particle, i_e is the Thomson scattering factor for one electron at zero angle, and R is the radius of gyration. The reciprocal lattice vector, **h**, has the magnitude $(4\pi/\lambda)\sin\theta$, where λ is the wavelength of the radiation and θ is the Bragg angle. Upon taking the logarithm of eq 1, one obtains

$$\ln I(h) = \ln I_0 - h^2R^2/3 \tag{2}$$

which indicates that R^2 can be evaluated from the initial slope of the plot ln $I(h)$ *vs.* h^2, which is termed the Guinier plot. It should be pointed out that this evaluation is quite independent of any assumption concerning the particle shape. If absolute intensities are measured, the molecular weight can be evaluated from the antilog of the intercept of this plot, I_0:

$$M = 2.1 \times 10^3 \frac{I_0a^2}{P_0Dc(\Delta z)^2} \tag{3}$$

Here P_0 is the intensity of the primary beam per centimeter of length in the plane of registration, D is the sample thickness in cm, and c its concentration in g/ml. The electron density difference in moles/g, Δz, is given by $(z - \bar{v}d_2)$, where z is the number of moles of electrons per gram of solvent and d_2 is electron density of the solute in moles/ml. The magnitude of $(\Delta z)^2$, and hence of M, is quite sensitive to uncertainties in $\bar{v}$. Errors in the concentration arising from uncertainty in the specific extinction coefficient will also affect the value of M. Interparticle interference effects decrease the scattering at the smallest angles, so that extrapolation to zero concentration is necessary for the evaluation of both R and M.

The Guinier plots for egg-white lysozyme and bovine α-lactalbumin are shown in Figures 1 and 2, respectively. For

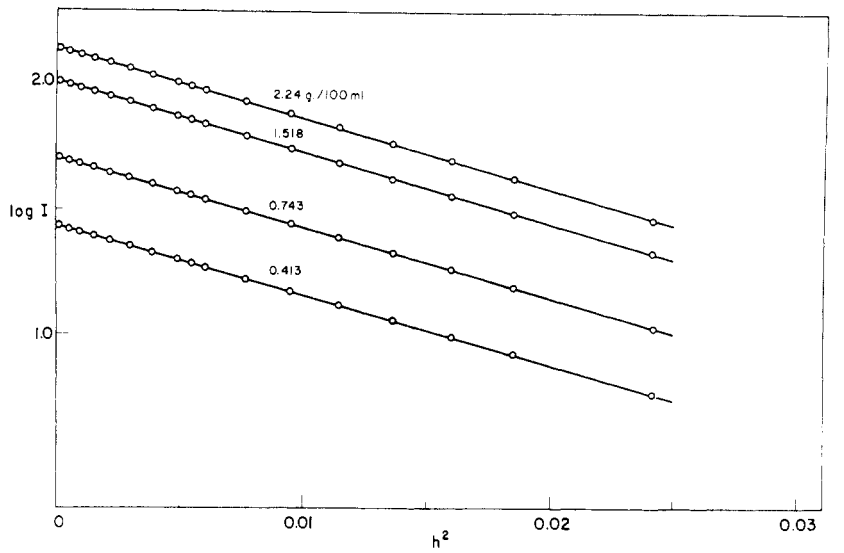

FIGURE 1: Guinier plots for four concentrations of lysozyme in 0.15 M NaCl at pH 3.8.

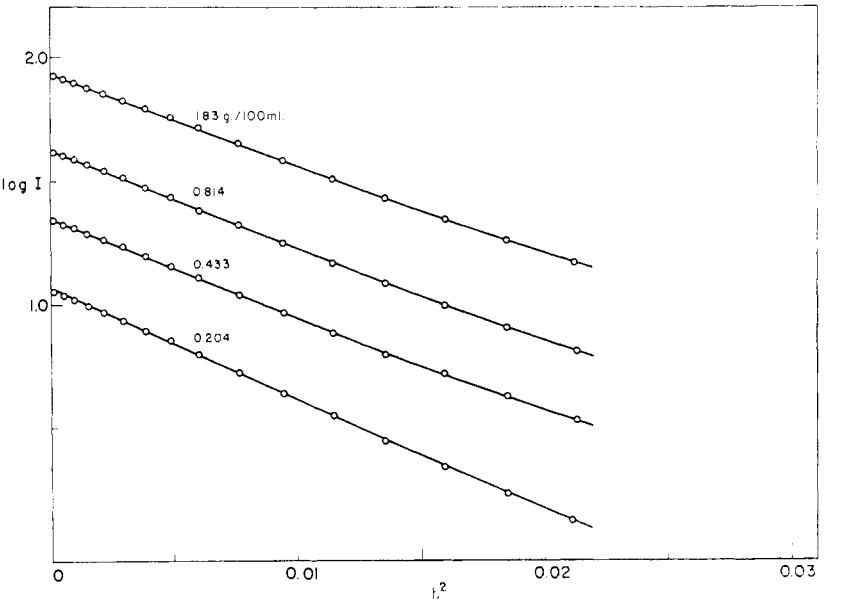

FIGURE 2: Guinier plots for α-lactalbumin in 0.1 M NaCl at pH 7.0.

all concentrations a linear region is found which includes the smallest observed angles. Values of R and M deduced from these plots are given in columns 2 and 3 of Table I. For lysozyme we obtain $R = 14.3 \pm 0.3$ Å and $M = 14{,}500 \pm 700$. Early small angle results by Ritland *et al.* (1950) gave $R = 16.0$ Å and $M = 18{,}000$, while more recent measurements by Luzzati *et al.* (1961) yielded $R = 15.2$ Å and $M = 14{,}200 \pm 200$. The molecular weight calculated from the amino acid sequence of egg-white lysozyme is 14,300. Our results and those of Luzzati and coworkers both agree quite well with the expected molecular weight; however, our value for R is smaller than theirs by somewhat more than would be anticipated from the experimental errors.

The results obtained for α-lactalbumin are $R = 16.7 \pm 0.4$ Å and $M = 15{,}500 \pm 800$. We are not aware of other low angle measurements which can be compared with these. An early sedimentation study by Svedberg and Pedersen (1937) gave $M = 17{,}500$, based on $\bar{v} = 0.751$, while a more recent sedimentation and diffusion study (Gordon and Semmett, 1953) yielded 15,100. The same authors reported a value of 16,500 by light scattering, and Wetlaufer (1961) found $M = 15{,}300$ by osmometry. The molecular weight corresponding to the known amino acid composition is 14,200. The fact that all of the solution measurements yield too large a molecular weight may be an indication of a tendency for α-lactalbumin to dimerize; however, the amount of dimer in our solutions must have been very slight since there was no observed upturn in the inner portion of the

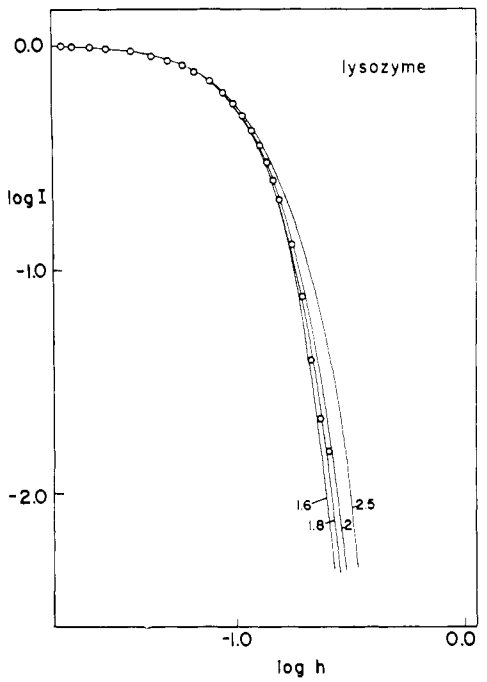

FIGURE 3: Lysozyme scattering curve in the shape region (○) compared with those calculated for prolate ellipsoids of revolution having the axial ratios indicated.

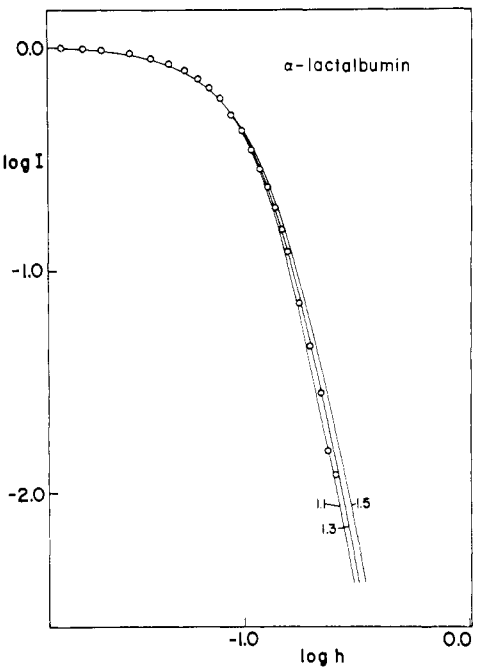

FIGURE 4: Shape scattering curve for α-lactalbumin (○) compared with those for oblate ellipsoids having axial ratios 0.5:1:c for indicated c values.

Guinier plot. This point will be considered further in the Discussion section.

Other Parameters. Knowledge of the scattering curve over a wide range of angles permits application of the theory of scattering by two phase systems developed by Debye and Bueche (1949) and Porod (1951). The invariant, Q, given as a function of the slit smeared intensity by

$$\tilde{Q} = \int_0^\infty \tilde{I}(h)h \, dh \tag{4}$$

depends upon the total volume of the dispersed phase, but not upon the particle size distribution. The volume per molecule, V, can be evaluated from the relation:

$$V = 4\pi^2 I_0/\tilde{Q} \tag{5}$$

where I_0 is the desmeared intensity corresponding to the intercept of the Guinier plot. Porod (1951) has demonstrated theoretically that the outer part of the (smeared) scattering curve varies as h^{-3}, and this relation may be used in evaluating the integral appearing in eq 4 for the range of large h. The molecular volumes determined in this way for lysozyme and α-lactalbumin are 19,700 and 26,500 Å, respectively, as shown in column 4 of Table I. From the partial specific volume and the observed molecular weight one obtains the volume of the dry protein. The difference between the experimental volume and the dry volume yields an estimate of the water content of the protein. Values obtained in this way are 0.10 g of H_2O/g of lysozyme and 0.29 g of H_2O/g of α-lactalbumin. Luzzati *et al.* (1961) reported 0.17 to 0.18 g of H_2O/g of lysozyme.

The intermediate portion of the scattering curve (beyond the linear Guinier region) contains implicit information concerning the shape of the scattering particle. Particle scattering curves have been calculated for some regular shapes (Mittelbach and Porod, 1961, 1962), and one attempts to select a shape and axial ratio which best matches all of the experimental observations. The experimental data, plotted as log $I(h)$ *vs.* log h, are compared with the theoretical normalized intensity function, $\Phi(h)$, plotted as log $\Phi(h)$ *vs.* log hR. The dimensions of the "equivalent scattering body" must, of course, be interpreted with some caution, since one is representing the scattering from the actual molecule with its irregular electron density distribution by that of a smooth particle of uniform electron density.

The shape curve for lysozyme is compared with the theoretical curves for several prolate ellipsoids of revolution in Figure 3. The best agreement is obtained with the axial ratio 1:1:1.8 ± 0.1. From the relationship for the radius of gyration in terms of the half-axes a, b, and c,

$$R^2 = (a^2 + b^2 + c^2)/5 \tag{6}$$

and the observed value, R = 14.3 Å, one obtains for the axial lengths of the equivalent ellipsoid 28 × 28 × 50 Å. The volume of this smooth body, 20,400 Å^3, agrees reasonably well with the experimental volume, 19,800 Å^3, deduced from the invariant.

The scattering curve for α-lactalbumin in the shape region is compared in Figure 4 with the theoretical curves for oblate ellipsoids having axial ratios 0.5:1:c. The best agreement with the experimental scattering curve is obtained with c = 1.3 ± 0.1. In order to yield the observed radius of gyration, R = 16.7 Å, the axial dimensions must then

be 22 × 44 × 57 Å. The volume of this smooth particle is 28,000 Å^3, or about 6% larger than the experimental volume, V = 26,500 Å^3.

Conformation of Lysozyme in Solution and in the Crystalline State. Phillips and coworkers have been able to offer a detailed proposal for the mechanism of the enzymatic action of lysozyme from their crystal structure studies of lysozyme (Blake *et al.*, 1967; Blake, 1967) and of complexes of lysozyme with saccharides (Phillips, 1966, 1967). This proposal permits the results of substrate binding studies to be interpreted at the molecular level. The picture which emerges from these studies is so impressive in detail that one must conclude that lysozyme is one of the few enzymes whose mode of action is well understood. The development was based upon the assumption that the molecular structure deduced from crystal studies is the same as that existing in aqueous solution. While the success of this endeavor implies the correctness of the basic postulate, more direct evidence might be desirable. Some information which substantiates the hypothesis is already available. Butler and Rupley (1967) have shown that the association constants of *N*-acetyl-D-glucosamine and its dimer are almost identical for lysozyme in the crystalline state and in solution. Studies of nuclear magnetic resonance line broadening and chemical shifts by Dahlquist and Raftery (1968, 1969) lead to a number of conclusions concerning the mechanism of binding by lysozyme molecules in solution which are in good agreement with those reached from crystal structure studies. Also, tritium exchange experiments with lysozyme and a lysozyme–saccharide complex (Praissman and Rupley, 1968) suggest that the molecular conformation is the same in the two states. Despite this evidence based on short range effects, it is worthwhile to investigate the degree of similarity of lysozyme in solution and in the crystalline state by a measurement sensitive to long range effects. Our small angle study provides such a comparison.

A qualitative indication is provided by the dimensions of the equivalent scattering ellipsoid 28 × 28 × 50 Å, which may be compared with the overall molecular dimensions deduced from the crystal structure, 30 × 30 × 45 Å. A more precise comparison may be obtained from the radius of gyration, R, which may be calculated from the crystallographic atomic coordinates through use of the expression:

$$R^2 = \sum_j f_j[(x_j - x_0)^2 + (y_j - y_0)^2 + (z_j - z_0)^2] \Big/ \sum_j f_j \quad (7)$$

Here f_j is the atomic scattering factor of atom j located at (x_j, y_j, z_j,) while (x_0, y_0, z_0) are the coordinates of the electronic center of gravity. The hydrogen atoms were omitted from this calculation. If the coordinates of any nonhydrogen atom were missing in the list, the corresponding number of electrons was added to the location of the α-carbon atom of that residue. This calculation gave R = 13.8 Å, which is somewhat less than the experimental value, R = 14.3 ± 0.3 Å, obtained from the Guinier plot. The difference, 0.5 Å, is about the same as that observed for myoglobin by comparing the value calculated from the crystal structure, R = 15.5 ± 0.5 Å (Watson, 1967), with those obtained experimentally, R = 16.4 ± 0.4 Å (Krigbaum and Brierre, 1965) and R = 16.0 ± 0.4 Å (Beeman, 1967).

A second detailed comparison is furnished by the shape of the scattering curve averaged over all possible orientations of the particle. The scattering power, $\overline{F^2(h)} = I(h)/i_e$, may be calculated for the particle in a vacuum using the relation of Debye (1925):

$$\overline{F^2(h)} = \sum_j \sum_k F_j F_k (\sin hr_{jk})/hr_{jk} \quad (8)$$

where F_j is the structure factor for the jth scattering element and r_{jk} is the distance between elements j and k. Since this calculation can be rather lengthy, we have constructed an array of r_{jk} values containing as entries the numbers zero, and 1.0 to 45.0 in steps of 0.1. A parallel array contained the corresponding values of r_{jk}^2. When the square of an interelement distance was calculated, it was paired with one of the entries in the r_{jk}^2 table by rounding up. The number of tabulated occurrences in that category was increased by one, and the F_jF_k product was stored in a fourth array. Even with this programming device, the computer time required to treat 961 points was rather long. For the sake of comparison we have therefore performed the calculation in three levels of approximation. In the first case each residue was represented by one scattering element located at the α-carbon atom (129 points). For the second approximation each residue was replaced by two weighted scattering elements. One of these (representing the glycyl portion) was located at the α-carbon, and the other (representing the side chain) was located at an atom at the extremity of the side chain (244 points). Finally, the calculation was repeated using all the known atomic positions (961 points). The results appear in Figure 5. Comparison of the dashed and full curves indicates that 244 points furnish a very satisfactory approximation. These two curves diverge at log h = −0.58 (h = 0.26 Å^{-1}). For smaller h values there is good agreement between these two theoretical scattering curves and the experimental data for lysozyme in solution (circles).

The foregoing calculations involve the assumption that the shape of the scattering curve is independent of the medium surrounding the particle. We have attempted to assess the effect of this assumption in two ways. In the first of these the structure amplitudes were replaced by the magnitudes of the electron density differences, *e.g.*:

$$\Delta F_j = |F_j - \rho_1 V_j| \quad (9)$$

where ρ_1 is the electron density of the solvent (electrons/Å^3) and V_j is the volume of the jth scattering element in Å^3. The dashed curve in Figure 6 shows the result of this calculation for 961 points, while the full curve represents the scattering for the molecule in a vacuum (eq 8). A more rigorous treatment of the effect of solvent on the scattering curve has been given by Hyman and Vaughan (1967). They obtained the following expression for the "internal" contribution to the scattering power per particle:

$$\overline{F^2(h)} = \langle F^2 \rangle - \rho_1 \langle F\Phi^* \rangle - \rho_1 \langle F^*\Phi \rangle + \rho_1^2 \langle |\Phi|^2 \rangle \quad (10)$$

where

$$\langle F^2 \rangle = \sum_j \sum_k f_j f_k (\sin hr_{jk})/hr_{jk}$$

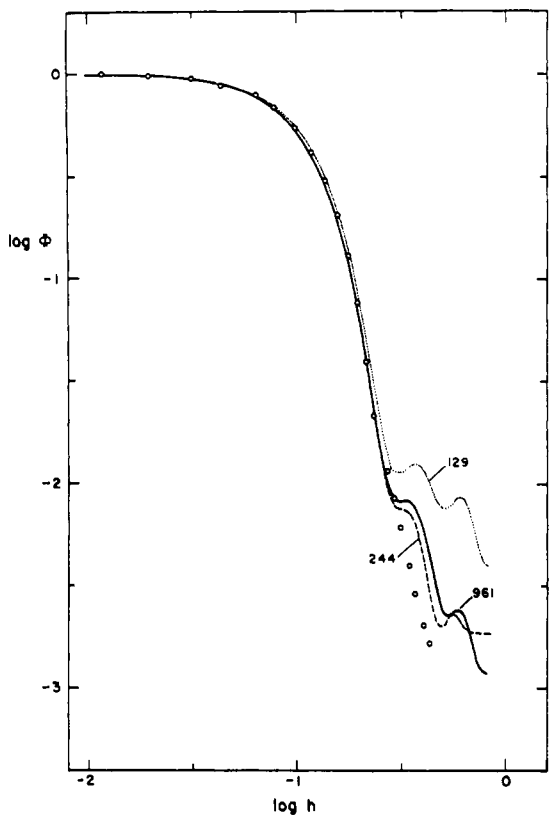

FIGURE 5: Lysozyme scattering curve (○) compared with those calculated (eq 8) using crystallographic coordinates for the indicated number of points per molecule.

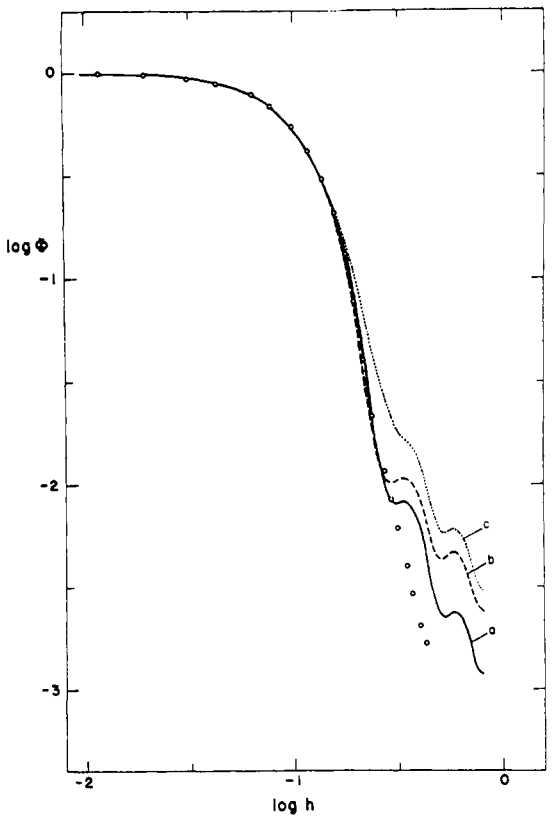

FIGURE 6: A test of the effect of the medium on the theoretical scattering curve for lysozyme: (——) eq 8, (---) eq 9, (· · · ·) eq 11.

$$F = \sum_j f_j \exp(i\mathbf{h}\cdot\mathbf{r}_j)$$

$$\Phi = \int_{\text{molecule}} \exp(i\mathbf{h}\cdot\mathbf{r}_j)\, dr_j$$

For the special case in which the volume occupied by a solute molecule can be approximated by a sphere of radius r_0, eq 10 simplifies to

$$\overline{F^2(h)} = \langle F^2\rangle - 2\rho_1\Phi\langle F\rangle + \rho_1{}^2\Phi^2 \qquad (11)$$

where the following equalities now hold:

$$\langle F\rangle = \sum_j f_j\, (\sin hr_j)/hr_j$$

$$\Phi = (4\pi/h^3)(\sin hr_0 - hr_0 \cos hr_0)$$

The scattering curve calculated according to eq 11 with 961 points is represented by dotted curve c in Figure 6. Evidently lysozyme, with an axial ratio of 1.8:1, departs too severely from spherical shape for eq 11 to apply. The dashed curve b, calculated using eq 9, differs only slightly from full curve a in the region $h < 0.26$ Å^{-1}, and both of these agree quite well with the experimental data (circles) within this region. The use of ΔF_j values in eq 8 results in a slightly larger apparent radius of gyration, 14.1 Å, for the crystallographic molecule in an aqueous medium. The theoretical curves exhibit two maxima in the outer region, corresponding to distances of about 10.7 and 18 Å. Our experimental measurements were performed on solutions of rather low concentration, and no attempt was made to optimize the instrumental parameters for study of the outer portion of the scattering curve. This may explain why the data exhibit no indication of the maximum which appears in the theoretical curves near log $h = -0.47$, and which corresponds to the 18 Å distance.

A final comparison of the conformation of the lysozyme molecule in the crystalline state and in solution is obtained by taking the Fourier transform of the respective scattering curves. We define a radial probability function, $P(r)$, in the following way:

$$P(r) = (2/\pi)\int_0^\infty hr\,(\overline{F^2(h)}/n_e{}^2)\sin hr\, dh \qquad (12)$$

where n_e is the number of excess electrons in the region containing the molecule. If we treat the case of the scattering by the molecule in a vacuum, then n_e is simply n, the number of electrons per molecule. The quantity $(\overline{F^2(h)}/n_e{}^2)$ is the scattering power of the molecule normalized to unity at $h = 0$. Thus $n_e{}^2P(r)dr$ gives the number of pairs of electrons having separations between r and $r + dr$. The open circles in Figure 7 represent the $P(r)$ function calculated according to eq 12 from the experimental scattering curve measured for lysozyme in solution. The full curve marked a was ob-

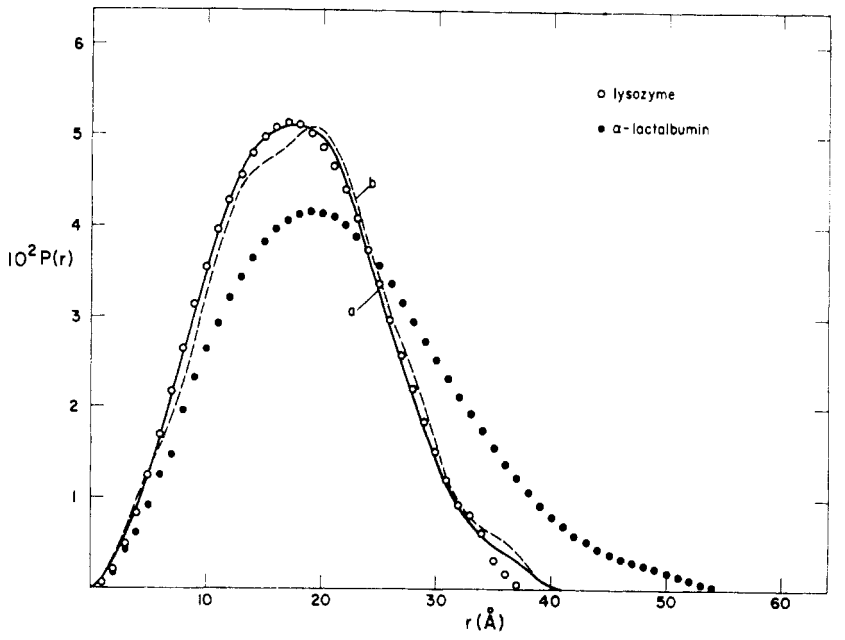

FIGURE 7: Electron pair radial distance function $P(r)$ for lysozyme (○) and α-lactalbumin (●). Theoretical curves a and b were calculated from crystallographic coordinates of lysozyme using eq 8 and 9, respectively.

TABLE II: Comparison of Size and Shape Parameters for α-Lactalbumin and Lysozyme.

Substance	State	R (Å)	V (Å^3)	Overall Dimensions (Å)
α-Lactalbumin	Solution	16.7 ± 0.4	26,500	22 × 44 × 57
Lysozyme	Solution	14.3 ± 0.3	19,800	28 × 28 × 50
Lysozyme	Crystal	13.8[a]		30 × 30 × 45

[a] Calculated for the molecule in a vacuum.

tained from the transform of $\overline{F^2(h)}$ calculated according to eq 8 using the crystallographic coordinates for lysozyme. Dashed curve b was also computed from the crystallographic coordinates, but in this case an attempt was made to account for the aqueous medium through replacement of F by ΔF as given by eq 9. Both of the theoretical curves, a and b, calculated using the crystallographic coordinates agree quite well with the $P(r)$ function obtained from the solution scattering curve (open circles). This indicates that the overall molecular shape, and the distribution of electron density within the molecule, must be quite similar for lysozyme in the crystal and in solution.

The filled circles in Figure 7 represent the $P(r)$ function calculated from the scattering curve of α-lactalbumin. One sees from comparison with the open circles that the distribution of electron density is skewed toward larger distances in the case of α-lactalbumin, as would be expected on going from a prolate to an oblate ellipsoidal shape.

Discussion

The parameters characterizing the molecular conformation of α-lactalbumin and lysozyme are collected in Table II. Despite their amino acid sequence homology, these two enzymes have grossly different sizes and shapes in solution. The equivalent ellipsoid is oblate for α-lactalbumin and prolate for lysozyme. Further, the volumes deduced from the invariant differ by more than 30%. Comparison of the radii of gyration requires some further discussion of the possibility of dimerization in α-lactalbumin. Our molecular weight, 15,500 ± 800, in common with the other values deduced from solution studies of this enzyme, is higher than the value M = 14,200 calculated from the amino acid sequence. We have examined the possible effect of dimerization on the scattering curve by illustrative calculations involving the known lysozyme structure, and which assume that dimerization occurs along the long axis. This assumption will result in dimerization having the largest possible effect on the radius of gyration. Comparison of theoretical curves in the Guinier region reveals that 4 mol % of dimer would be clearly evident by an upturn in the region of the smaller h values covered in our measurements, while the curvature for 2 mol % dimer might be obscured by the scatter of the points associated with normal experimental errors. If this upturn is ignored, then both the slope and intercept will be too small, with the result that the values of R and M will approach those of the monomer, rather than being characteristic of the monomer–dimer mixture. We therefore believe that correction for possible dimerization could not reduce R below 16.0 Å for α-lactalbumin, which differs

from the value deduced for lysozyme, $R = 14.3 \pm 0.3$ Å, by substantially more than the combined experimental errors. The electron pair radial distributions for these two enzymes shown in Figure 7 are also quite different. In spite of the considerable degree of sequence homology, the conformations of α-lactalbumin and lysozyme in solution are quite different. It is barely possible that their conformations will show more resemblance in the crystalline state, but this seems rather unlikely.

On the other hand, our measurements reveal that the tertiary structure of lysozyme in aqueous solution is very similar to that found in the crystalline state. This is demonstrated rather qualitatively by the overall dimensions summarized in Table II, and more precisely by comparison of the values for the radius of gyration found in column three. The R value deduced from the solution studies is only slightly larger than that calculated for the crystallographic molecule in a vacuum, and this small difference may be halved by considering the effect of the aqueous medium. The experimental and theoretical scattering curves appearing in Figures 5, 6, and 7 provide more detailed proof that the shape of the lysozyme molecule in aqueous solution is very similar to that deduced from the crystal structure studies. A close similarity of the molecular structure in solution and in the crystalline state was previously observed for myoglobin as mentioned above, but not for chymotrypsinogen or α-chymotrypsin (Krigbaum and Godwin, 1968). The latter authors have suggested that the degree of conformational change on dissolution may be largest for those molecules having a small amount of α helix. Kotelchuck and Scheraga (1969) have given for the helix contents of myoglobin, lysozyme, and α-chymotrypsin the approximate values 79%, 36%, and 3%, respectively.

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