Similarity in Backbone Conformation of Egg White Lysozyme and Bovine α Lactalbumin

by

Martin J. Kronman
Pioneering Research Laboratory
U.S. Army Natick Laboratories*
Natick, Mass.

Received September 26, 1968

Brew et al. (1967) having determined virtually the entire amino acid sequence of bovine α lactalbumin, conclude that this milk protein and egg white lysozyme share a high degree of sequence homology and hence should also exhibit great similarity in the manner in which their peptide backbones are folded. Model building has indeed shown that it is possible to fold the α lactalbumin sequence to the known conformation (Blake et al. 1967) of the lysozyme backbone with reasonable positioning of the side chains (quoted in Brew et al. 1968). Although the sequence homology and the model building "experiments" cited above indicate that a similarity is a reasonable hypothesis, no direct evidence has yet been presented for it being a reality.

Optical rotation dispersion (ORD) measurements should be useful in this regard; a comparison of the Moffitt-Yang parameter, b_0, however does not provide convincing support for similarity in conformation, i.e. the value for α lactalbumin is of the order of -200 to -250° (Kronman et al. 1965, Kronman et al. 1966) while that for lysozyme is approximately -150° (see for example Tomimatsu and Gaffield, 1965). This difference may be the consequence of side chain optical activity which have been found for both α lactalbumin (Kronman et al., 1966, Kronman, unpublished experiments) and lysozyme (Glazer and Simmons, 1965). Aune (1968) has shown that

*Present address: Department of Biochemistry, Upstate Medical Center, State University of New York at Syracuse, New York 13210

the ORD properties of lysozyme and α lactalbumin show rather close correspondence in the wavelength range 210 to 250 mμ, the region associated with conformation sensitive peptide cotton effects. Uncertainty still remains, however, as to the magnitude of rotational contributions from optically active side chain transitions lying outside of this wavelength region.

In order to obtain less ambiguous information concerning similarities and differences in the conformation of the two proteins, we have carried out circular dichroism (CD) measurements over the wavelength range 185 to 300 mu. This technique has the advantage of yielding relatively narrow positive or negative bands which characterize individual optically active transitions, rather than the broad envelopes observed in ORD which include contributions of varying magnitudes from all optically active transitions.

Circular Dichroism Measurements

CD measurements were made with the Cary model 60 spectropolarimeter-circular dichroism apparatus using 1 and 10 mm cells. Protein concentrations were chosen to yield optical densities no greater than 1.0 at any wavelength. No concentration dependence of ellipticity was noted over the limited concentration range which could be used. The solvents were 0.15M KCl (adjusted to pH 7 with KOH) or pH 7.5 005M TRIS. Because of strong absorption of light by these substances below 200 mμ, solutions of 0.001 M K_2SO_4 adjusted to pH 7 with sulfuric were used in this range. No dependence of the ellipticity on electrolyte type was noted. In general, scans of the CD spectra were made at the slowest possible speed using the synchronous motor and a time constant of 10 seconds.

Results

The near ultraviolet CD spectra of native α lactalbumin and lysozyme exhibit marked differences. The latter protein has a positive band system centered at about 289 mμ and a negative band somewhat below 260 mμ, in good agreement with the observations of Ikeda et al. (1967) and of Beychok (1965).

The former authors suggest that some fine structure may be present in the positive band; our spectra show three distinct ellipticity bands at 282, 289, and 295 mμ.

The CD spectrum of native α lactalbumin, by contrast with that of lysozyme appears to be entirely negative and is centered at about 270 mμ; the amplitudes are nearly three times as great as that of lysozyme. The spectrum is exceedingly complex, being composed of perhaps as many as six bands. This complexity, which is quite reproducible in repetitive scans, precludes any facile resolution into individual bands and makes it difficult to determine if there might be underlying positive components to the spectrum. This complex near-ultraviolet CD spectrum is in accord with the ORD properties of the protein (Kronman et al. 1965; Kronman et al. 1966; Kronman, M.J., unpublished experiments).

Without concerning ourselves in the present publication with the details of the near ultraviolet CD spectra of α lactalbumin and lysozyme, we can state that their features are determined by side chain transitions, e.g. of tyrosyl, tryptophyl or cystine resides. It seems likely, therefore, that the differences observed in Moffitt-Yang parameters (see above) for lysozyme and α lactalbumin may reflect side chain optical activity. We shall consider such side chain CD bands elsewhere (M.J. Kronman, In Preparation).

The CD spectra of native and acid "denatured" α lactalbumin and lysozyme, on the other hand, show great similarity below 240 mμ. (Figure 1). Native α lactalbumin exhibits a flat region from about 223 mμ down to its minimum at 208 mu, while the curve for acid denatured protein is steeper over the same wavelength interval and the 208 mμ minimum is less shallow. The shape of the lysozyme spectrum is intermediate between that observed for native and denatured α lactalbumin and is comparable with those reported by Sarkar and Doty (1966) and by Timasheff et al. (1966). The difference between the CD spectra of native and acid "denatured" α lactalbumin appears to be largely the result of an underlying CD band just below 230 mμ, most likely of negative

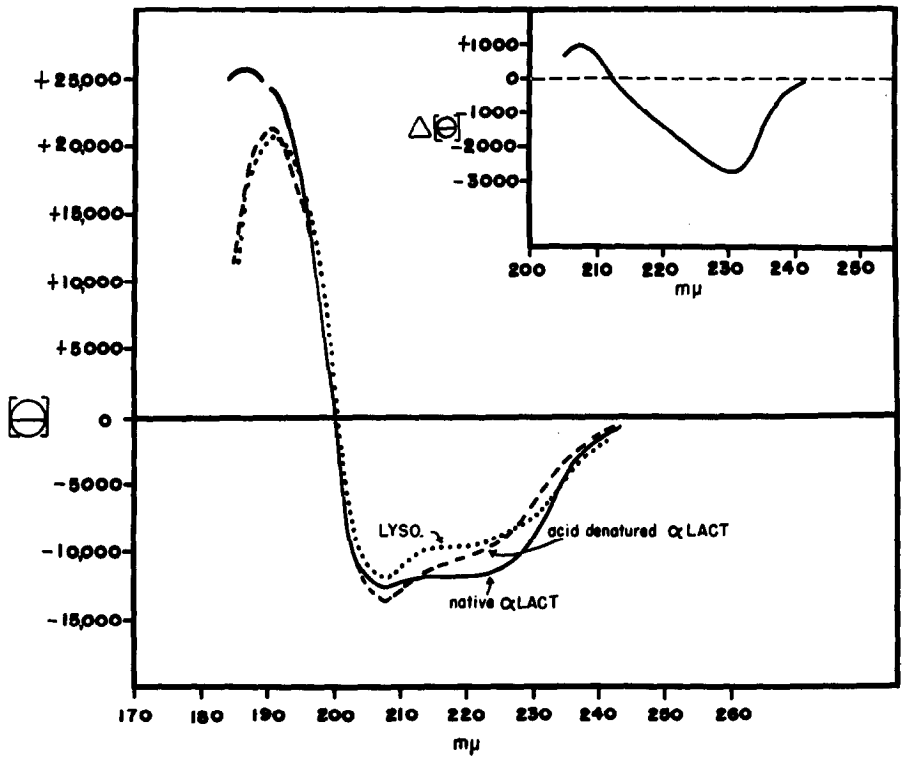

Figure 1. Circular Dichroic Spectra of Lysozyme, Native and Acid "denatured" α Lactalbumin. Solvents as given in text. The "denatured" protein was at pH 2.0. Insert: difference in circular dichroic spectra for native and "denatured" α lactalbumin.

---

sign for the native protein (see insert, Figure 1). We believe that this band, like those at longer wavelengths, arise from side chain optical activity. Although one, in general, anticipates side chain transitions occurring above 250 mμ, there is reason to believe that they may also appear well below this wavelength. (Coleman and Blout, 1968; Simmons and Glazer, 1967). The relative flatness of the lysozyme curve may mean that a similar band underlies this spectrum as well. Parenthetically it should be noted that the so-called "acid denatured" α lactalbumin does not differ greatly in conformation from the native protein, although many of its properties are different (Kronman, 1967).

The CD spectra of native lysozyme and acid "denatured" α lactalbumin show

TABLE 1

Circular Dichroic Properties of Native Lysozyme and α Lactalbumin and Acid Denatured α Lactalbumin

Protein	$(\theta) \times 10^{-3}$				
	230 mμ	220 mμ	208-209 mμ	191 mμ	186-189 mμ
Native Lysozyme	$-7.80 \pm 0.8_5$	$-9.40 \pm 0.9_1$	$-11.6 \pm 0.8_6$	$20.8 \pm 2._3$	$16.6 \pm 3._8$
Native α Lactalbumin	$-9.10 \pm 0.5_8$	$-11.8 \pm 0.5_7$	$-12.6 \pm 0.6_0$	$24.2 \pm 4._8$	$25.4 \pm 3._7$
Acid Denatured α Lactalbumin	$-6.43 \pm 0.5_8$	$-10.5 \pm 0.6_3$	$-13.3 \pm 0.5_0$	$21.4 \pm 4._5$	$17.6 \pm 5._4$

a. The average deviations shown correspond to replicate independent experiments. A minimum of three such experiments were made for each protein. The signals to noise ratios were typically: 250:1, 230 mμ; 200:1, 220 mμ; 100:1, 208-209 mμ; 10:1, 191 mμ; 5:1, 186-189 mμ.

rather close correspondence from 208 mμ down to the lower wavelength limits of the measurements. Native α lactalbumin exhibits in addition to the positive 191 mμ band an additional one below 190 mμ. While the absolute value of the ellipticity is not too reproducible in this wavelength region (see Table 1), repetitive scans consistently show this feature for the native protein and demonstrate its absence for lysozyme and the acid "denatured" protein. We believe that this low wavelength band may also reflect side chain optical activity

As we have indicated above, the relatively small differences in shapes of the CD curves can be explained rather well in terms of differences in optical activity associated with side chains. The ellipticities, however, in general are virtually within experimental error (Table 1). The wavelength region, 191 to 230 mμ should provide a good deal of information concerning backbone configuration, since it includes primarily dichroic contributions from transitions involving the peptide bond, e.g. alpha helix, antiparallel beta and "unordered" conformations (see Timasheff et al. 1966 for a recent discussion). The relatively close correspondence of ellipticities of lysozyme and α lactalbumin in this wavelength region (Figure 1, Table 1), therefore, speaks strongly for a close similarity in the manner in which the peptide backbones of these two proteins are folded.

The x-ray crystallographic structure of lysozyme (Blake et al. 1967) reveals about 30% helix, 10% antiparallel beta and about 60% "unordered" structure. The CD spectrum of lysozyme is consistent with such a conformational distribution. By implication α lactalbumin should thus have a comparable distribution of conformations.

Caution, however, should be exercised in formulating detailed interpretations of CD data in terms of conformation, because of significant gaps in our current knowledge of the dichroic properties of model conformations, e.g. helical structures such as those in lysozyme which have bent hydrogen bonds (Nemethy et al. 1967). Thus, while we cannot say at present that the

α lactalbumin backbone conformation corresponds in all detail to that of lysozyme, it is probable that no gross differences exist between them. The details of the conformation of α lactalbumin, as well as the ultimate proof of the similarity of the two proteins most clearly await the determination of the x-ray crystallographic structure.

References

1. Aune, K., Ph.D. Dissertation, Duke University, (1968)
2. Beychok, S., Proc. Nat'l Acad. Sci. U. S., 53, 999 (1965)
3. Blake, C. C. F., Mair, G. A., North, A. C. T., Phillips, D. C. and Sarma, V. R., Proc. Roy. Soc. (London) B167, 365 (1967)
4. Brew, K., Vanaman, T. C. and Hill, R. L., J. Biol. Chem. 242, 3747 (1967)
5. Brew, K., Vanaman, T. C. and Hill, R. L., Proc. Nat'l Acad. Sci., U.S. 59, 491 (1968)
6. Coleman, D. L. and Blout, E. R., J. Am. Chem. Soc. 90, 2404 (1968)
7. Ikeda, K., Hamaguchi, K., Imanishi, M. and Amano, T., J. Biochem. (Tokyo) 62, 315 (1967)
8. Kronman, M. J., Biochem. et Biophys. Acta. 133, 19 (1967)
9. Kronman, M. J., Blum, R. and Holmes, L. G., Biochem. and Biophys. Res. Comm. 19, 227 (1965)
10. Kronman, M. J., Blum, R. and Holmes, L. G., Biochemistry 5, 1970 (1966)
11. Nemethy, G., Phillips, D. C., Leach, S. J. and Scheraga, H. A., Nature 214, 363 (1967)
12. Sarkar, P. K., and Doty, P., Proc. Nat'l Acad. Sci., U. S. 55, 981 (1966)
13. Simmons, N. S., and Glazer, A. N., J. Am. Chem. Soc. 89, 5040 (1967)
14. Timasheff, S. N., Susi, H., Townend, R., Stevens, L., Gorbunoff, M. and Kumosinski, T. F. in "Conformation of Biopolymers", G. N. Ramachondran, Ed., Academic Press. N. Y. (1966)
15. Tomimatsu, Y. and Gaffield, W., Biopolymers 3, 509 (1965)