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FTIR Examination of Thermal Denaturation and Gel-Formation in Whey Proteins

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ABSTRACT

Second derivative Fourier-transform infrared [DR2-FTIR] spectra of β -lactoglobulin [β LG], serum albumin [BSA], and α -lactalbumin [α LA], three proteins found in bovine whey, are markedly different before and after thermal denaturation. In no case, however, do the heat-treated proteins unfold as completely as does alkaline-denatured β LG [1]. The spectra also suggest that, for β LG and BSA, formation of intermolecularly hydrogen-bonded β -strands precedes the onset of heat-induced gelation.

1. INTRODUCTION

Taking the 2nd derivative of an FTIR spectrum can often resolve badly overlapping band components, such as those typically observed in the Amide I region (1700 - 1610 cm^{-1}) of proteins.¹⁻⁵ Because these Amide I components are associated with different classes of secondary structure, DR2-FTIR spectra can offer new, detailed insights into the conformational changes that accompany thermal denaturation in proteins. Clark et al.⁶ first used dispersive IR spectroscopy to probe the effects of heat treatment on protein folding. More recently, Casal et al.⁵ used Fourier deconvolution and curve fitting to study the effect of heat and pH on the FTIR spectra of β LG. In this note we will discuss our DR2-FTIR results on β LG, BSA, and α LA, and compare their properties with those of whole casein.

2. EXPERIMENTAL

β LG, BSA, and α LA were obtained from Sigma Chemical Co.¹⁻³ Whole casein was kindly provided by H. M. Farrell, Jr., of USDA-ARS-ERRC.^{2,3} The proteins were dissolved (3.0-3.5% w/v) in a 0.05M phosphate buffer prepared in D_2O (at pD 6.2 or 7.8). FTIR spectra (2 cm^{-1} resolution, 0.44 s/scan) were collected at ambient temperature on Nicolet 740 FTIR system, equipped with a water-cooled Globar source, a broad-range MCT detector, as 4096 co-added double-sided interferograms. To denature the proteins thermally, the 75 μm pathlength CaF_2 IR cell containing the protein solution was heated at the selected temperature for 1 hr, and then allowed to cool to room temperature prior to collecting the spectrum.

3. RESULTS AND DISCUSSION

Every globular protein has a characteristic temperature, above which the protein begins to undergo changes in conformation. The nature and extent of these changes vary from protein to protein and with the temperature imposed. For example, the DR2-FTIR spectrum of native β LG at pD 6.2 and 30 $^\circ\text{C}$ exhibits no fewer than seven amide I component bands (Fig. 1).¹⁻⁵ These components result from the α -helices, β -strands, turns, and disordered structures which characterize the conformation of the peptide chain.¹⁻⁵ By contrast, the DR2-FTIR spectrum of the same protein

solution after heating to 80 °C (Fig. 1) is drastically altered. (Heating to as low as 60 or 70 °C also leads to irreversible, if less extensive, structural changes.) At 80 °C, a very broad, unresolved, asymmetric feature is flanked by sharp, intense peaks at 1614 and 1684 cm^{-1} . An amide I component below 1620 cm^{-1} has not been observed in the spectra of typical, native globular proteins.¹⁻³ Such a frequency decrease may be associated with strong, new hydrogen-bonded β -strands. That these features appear prior to gelation of the protein solution further suggests that the hydrogen-bonds are intermolecular.⁶ A similar unusual pair of bands is a common feature of proteins in the presence of simple alcohols, conditions which also promote gel formation.^{4,7,8} A similar pair of bands was noted in the spectrum of poly-L-lysine under conditions in which this polypeptide is presumed to be in the β -form (heating to 35 °C at pH 11),⁷ and in the spectra of thermally-induced gels of a variety of proteins.⁶ On the other hand, alkaline-denatured β LG,¹ whose peptide backbone has largely unfolded into disordered structures, displays a spectrum radically different from those of both native β LG and of the thermally denatured species.^{1,5}

Another protein common to the whey fraction of milk, as well as to serum, is BSA. The native protein is reported to be 40-50% α -helical and about 30% β -sheet³ (in contrast to β LG which has less than 10% α -helix and about 50% β -structure).^{2,3} Nonetheless, despite the marked difference in the native conformations of these two proteins (Figs. 1-2), when BSA at pD 7.8 is heated above its denaturation temperature (ca. 75 °C), it too exhibits a new low-frequency β -component at 1613 cm^{-1} , paired with a weaker, sharp band at 1684 cm^{-1} (Fig. 2). With time, after thermal denaturation, BSA also gels. (The presence of the band at 1651 cm^{-1} , while greatly diminished in intensity from the native state, indicates that not all of the α -helices have uncoiled.)

Native α LA, a third whey protein, is intermediate in structure (ca. 30% α -helix and 40% β -sheet)^{2,3}. Although the spectrum of this protein at pD 7.8 changes significantly after heat treatment, implying corresponding conformational alteration (Fig. 3), no intense amide I component appears below 1620 cm^{-1} , nor does this protein appear to gel under these conditions.

Caseins are the other principal proteins of milk. They stand in marked distinction to the whey proteins:^{2,3} the DR2-FTIR spectra of caseins display no resolved amide I band components and even after a casein solution is heated at 95°C for more than 2 hr, no change is obvious in their spectra nor do the heated solutions show visible evidence of gel formation. These facts are consistent with the assumption that caseins are easily hydrated proteins whose structure is largely aperiodic (both turns and disordered structures).^{2,3} Apparently these macromolecules are much less susceptible to irreversible, heat-induced changes in secondary structures than are the more regularly structured globular proteins.

4. CONCLUSIONS

Comparison of the DR2-FTIR spectra of β LG, BSA, and α LA before and after heat treatment discloses striking changes but reveals that under such conditions none of the three totally unfolds into a completely disordered state (Figs. 1-3). Although native β LG and BSA have rather different secondary structures, heating either of these proteins for 1 hr at 80 °C or above induces new, intermolecularly hydrogen-bonded β -strands (as evidenced by the appearance of new bands near 1614 and 1684 cm^{-1}) and an increase in the proportion of disordered structure^{5,6} (Figs. 1-2). At the concentrations used in this study (3-3.5 % w/v), heat-treated solutions of both proteins will ultimately gel. By contrast, although the DR2-FTIR spectrum of a thermally denatured α LA solution is clearly different than that of the native protein (Fig. 3), there is evidence for the formation of but few

intermolecular β -sheets (only weak amide I bands are observed at 1613 and 1684 cm^{-1}). Nor does α LA gel under such conditions.

ACKNOWLEDGMENT

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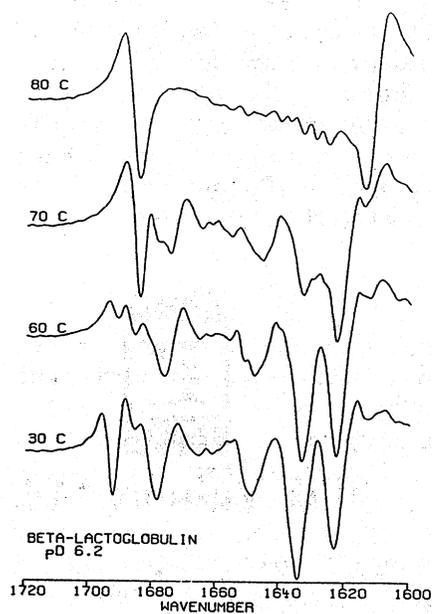


FIG. 1

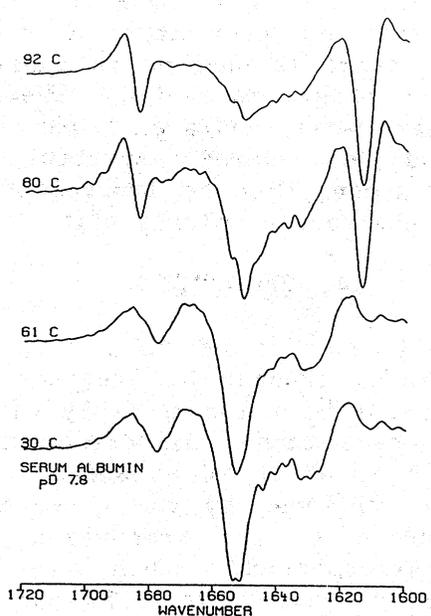


FIG. 2

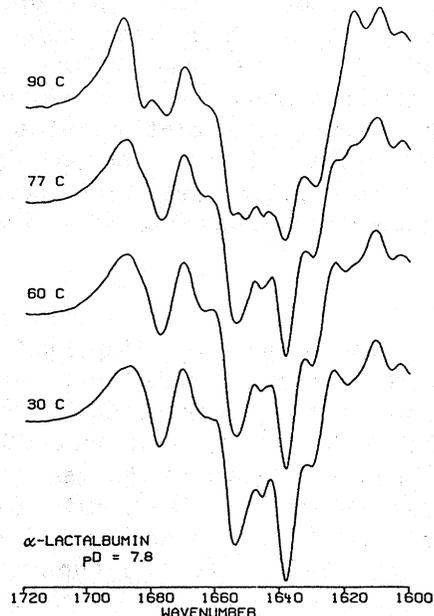


FIG. 3