

Infrared Investigation of the Secondary Structure of β -Lactoglobulins

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SUMMARY

β -Lactoglobulin has been examined by infrared spectroscopy in the region of absorption of the amide I and amide II bands and compared with myoglobin and α_s -casein. The results indicate a prevalence of β conformation in the native structure of this protein.

Infrared spectroscopy in the region of absorption of amide I and amide II bands has been used extensively to investigate the conformations of various synthetic polypeptides as well as fibrous proteins in oriented and unoriented films (1-3). As a result, rather detailed assignments of amide I and amide II frequencies to various conformations have been established (4-6).

In view of the rather anomalous optical rotatory dispersion properties of β -lactoglobulin (7-10), it seemed of interest to examine this protein by infrared spectroscopy as well as to establish, if possible, the nature of the conformation responsible for the position and magnitude of the observed Cotton effects.

Because of possible complications caused by solvent absorption and by solvent-solute interactions, β -lactoglobulin A was first examined as a dry film. Subsequent spectra were obtained in D_2O solution (H_2O absorbs very strongly in the region of interest) and compared with the solid state spectrum. To prepare the film, a solution of β -A (10 mg per ml) at its isoelectric point in 0.01 M NaCl was deposited on a crystal of calcium fluoride and evaporated to dryness at room temperature in a vacuum desiccator over silica gel. Solutions of 20 mg per ml at pD 1.0, 7.5, and 11.5 were prepared by dissolving lyophilized protein in D_2O (0.01 M NaCl) and titrating to the final pD with dilute DCl or NaOD. The pD was measured on a Beckman model G pH meter¹ with a glass electrode. Any uncertainty in the final value of pD thus obtained was neglected, since the purpose was merely to examine protein solutions in various states of ionization and denaturation.² Two native samples (one with ionized carboxyls, the other with protonated carboxyls) and one denatured sample (β -lactoglobulin is known to become irreversibly denatured above pH 9.0 and to be stable below that pH value) were examined. For comparison, a myoglobin solution at pD 6.6 (0.1 M NaCl, predominantly α -helix) (12) and an α_s -casein solution at pD 9.0 (0.03 M NaCl, essentially randomly folded) (13) were also examined. The spectra were obtained on a Beckman IR7 spectrophotometer.¹ The reference cell was filled with pure D_2O . Demountable liquid sampling cells with Teflon

spacers (Barnes Engineering Company,¹ type FT) and approximately 0.05-mm path length was employed. The wide pD range of the samples precluded the use of precision cells with amalgamated metal spacers. Because of the uncertainty of the path length of the employed cells, no reliable intensity estimations were possible.

The spectrum of the β -A film is shown in Fig. 1. The amide I band (primarily C=O stretching) is characterized by a sharp maximum at 1632 cm^{-1} and by pronounced skewness on the high frequency side, with weak but reproducible shoulders close to 1650 and 1685 cm^{-1} . The amide II band centers at 1530 cm^{-1} . According to the assignments given by Krimm (6), (see also Miyazawa and Blout (4, 5)) strong bands at 1632 and 1530 cm^{-1} are characteristic of the pleated sheet structure (β structure) of polypeptides and proteins. The observed weak bands (shoulders) close to 1650 and 1685 cm^{-1} could be associated with a small amount of α -helical structure and with anti-parallel chain pleated sheets, respectively (6, 14). (In the amide II region, weak bands at 1516 and 1546 cm^{-1} are compatible with the presence of a small amount of α -helical structure, but the β structure itself gives rise to weak bands at almost the same frequencies.)

Fig. 1 thus leads to the conclusion that in a dry film state the peptide chain carbonyl groups of β -lactoglobulin are to a high degree in the β conformation, with a smaller amount probably existing in α -helical conformation.

Spectra obtained in D_2O solution are presented in Fig. 2. The amide I band of native β -lactoglobulin (pD 1.0 and 7.5) is very similar to the band obtained on the dry film. Strongest absorption is observed at 1632 cm^{-1} (the characteristic β structure frequency) and weaker shoulders around 1650 and 1685 cm^{-1} . In the denatured state (pD 11.5), the amide I band becomes almost symmetrical and displays a single maximum at 1643 cm^{-1} . The band is practically identical with the corresponding band of the randomly folded (13) protein α_s -casein. For further comparison, myoglobin in D_2O solution at pD 6.6 was examined. It gave rise to a single amide I band with a sharp maximum at 1650 cm^{-1} , *i.e.* at the position characteristic of the α -helical structure. (Myoglobin is known to be at least 77% in α -helical conformation (12).) These results indicate that in D_2O solution the β conformation and the α -helical conformation give rise to amide I bands at the same frequencies as in dry films, while randomly folded proteins (α_s -casein and denatured β -lactoglobulin) produce a sharp band centering at 1643 cm^{-1} .

Absorption in the amide II region is more difficult to interpret in D_2O solution. If the solution is basic, COO^- groups absorb between 1550 and 1600 cm^{-1} (15), partially overlapping with the amide II band. Furthermore, since the amide II band is associated to a large degree with NH bending (15), exchange with D_2O results in a shift to considerably lower frequencies. The presence of the amide II band in one sample of native

¹ It is not implied that the United States Department of Agriculture recommends the above mentioned company or its products to the possible exclusion of others in the same business.

² The pD values given in the text are those actually read on a pH meter in D_2O solution. Glasoe and Long (11) suggest that a more correct value of pD is obtained by adding 0.40 to the pH meter reading. The pD values selected for the experiments reported in the present study are all such that a change by 0.40 would not lead to conformational changes in the proteins examined.

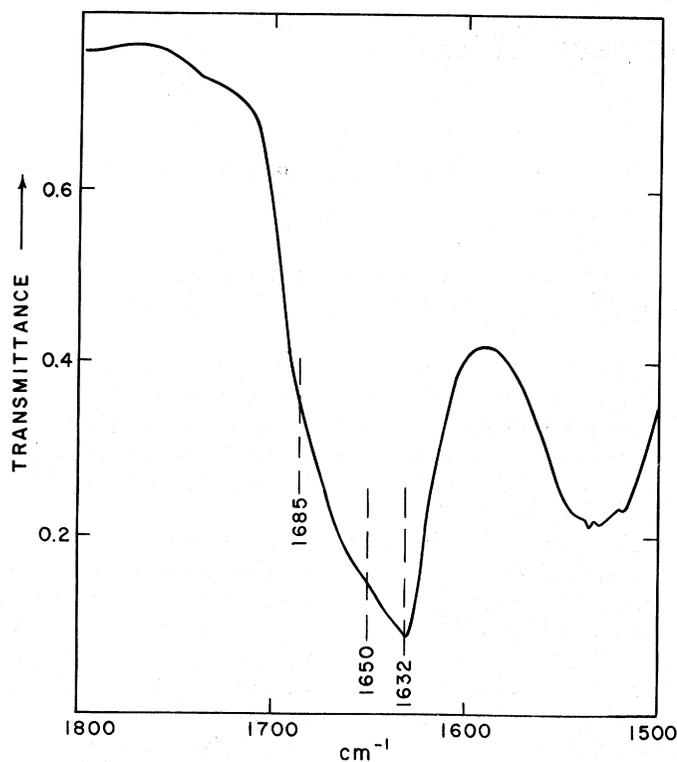


FIG. 1. Infrared spectrum of β -lactoglobulin A as a solid film

β -lactoglobulin (the pD 1 sample which was run immediately after preparation) shows that the native protein must be quite compact, because H-D exchange is slow. No such bands are observed in denatured lactoglobulin or in α_s -casein, which have loose, randomly folded structures. (The absorption slightly above 1700 cm^{-1} at pD 1 is caused by side-chain COOH groups.)

The described experiments do not constitute structure determinations in the strict sense of the word. Observed frequencies of absorption bands of samples with unknown configuration (in particular the frequency of the amide I band) were compared with corresponding frequencies of samples with known configuration under similar experimental conditions, with well established characteristic frequencies for dry protein films (6), and with frequencies predicted by calculations (4-6). On this basis, the presented results indicate that β -lactoglobulin A has a tightly folded secondary structure, the most prominent feature of which is probably a pleated sheet or β structure. While some α -helix also appears to be present, the existence of still other conformations cannot be excluded. (Weak but reproducible shoulders were observed at 1637, 1665, and 1672 cm^{-1} .) The reported frequencies for the compact α -helical and β structures apply to nondeuterated proteins in D_2O solution, *i.e.* before deuterium exchange has taken place in the peptide groups. These values are very close to latest tabulations (6). The amide I frequency of the random structures is lower than reported values for random nondeuterated proteins (6), because of deuterium exchange which causes a slight shift of the amide I band to lower frequency (16). Spectra of other genetic variants of β -lactoglobulin (β -B and β -C) were also examined. They were practically identical with the data reported here for the A variant, suggesting that the secondary structures are very similar. It appears from the described experiments that infrared investigation of the amide I band in D_2O solution is useful for obtain-

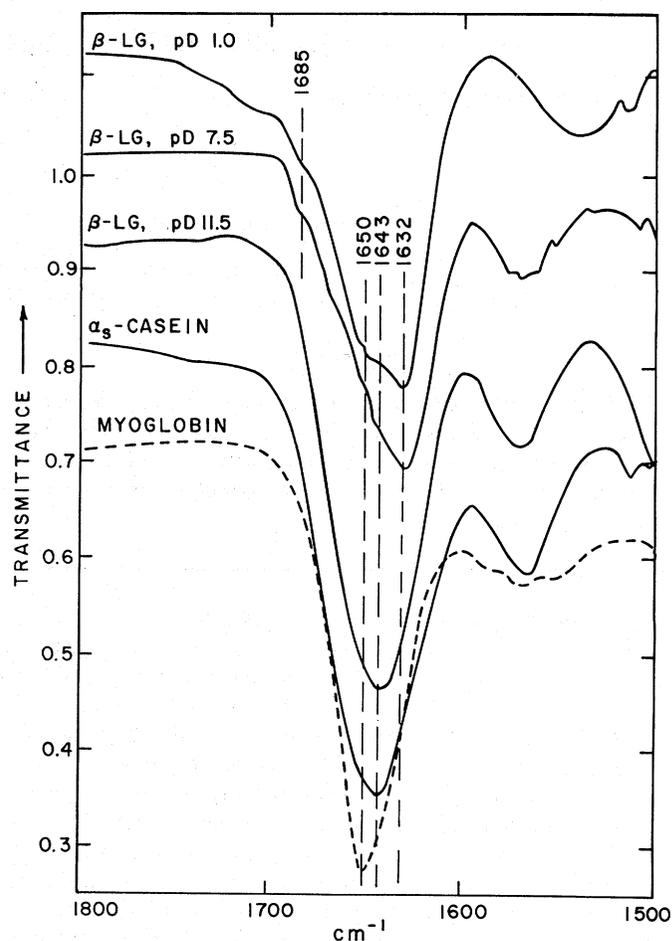


FIG. 2. Infrared spectra of native and denatured β -lactoglobulin (LG) A, native myoglobin, and α_s -casein in D_2O solution. The pD values are those read on a pH meter in D_2O solution. Consecutive spectra are linearly displaced by 0.1 scale unit. Concentration, 20 mg per ml; path length, approximately 0.05 mm. (The observed intensities are approximate because of uncertainties in path length. Peak absorptivity values for the amide I band are in the range 3 to 4 liters per g cm, if absorptivity at 1800 cm^{-1} is taken as reference.)

ing information regarding the secondary structure of globular proteins. An examination of a variety of such proteins is presently in progress in our laboratory.

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