Proc. Indian Acad. Sci. (Chem. Sci.), Vol. 102, No. 5, October 1990, pp. 693–696.

Raman spectroscopic study of the environment of tryptophan residues in bovine α-lactalbumin

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Abstract. α-Lactalbumin is an important protein essential for lactose synthesis. The conformation, position and state of different amino acid side chains and the structural changes in environment are important parameters which affect the functional properties. In this paper, the use of Raman bands for the study of the environment of tryptophan residues is presented and discussed.

Keywords. Raman spectra; environment of Trp residues; bovine α-lactalbumin.

1. Introduction

α-Lactalbumin is a small globular protein that acts as a specifier protein essential for lactose synthesis. It is a calcium metalloprotein that possesses an additional binding site for Zn^{2+} ions. Although the stabilizing role of the Ca^{2+} binding was observed by different techniques, the precise effects on the conformation and its impact on the biological processes are still unclear. Besides peptide backbone conformation, the position and the state of the different amino acid side chains are very important physical parameters in the study of the structure and the function of a protein. After our investigation on the tyrosine Raman doublet in α-lactalbumin (Van Dael *et al* 1987), we examine in this work the tryptophan residues and their dependence on specific environmental and structural factors. As pointed out recently by Miura *et al* (1988), two Raman bands (around 880 cm^{1-} and 1360 cm^{1-}) have been found useful in the study of the Trp environment.

2. Results

2.1 *The Trp band at 880 cm^{-1}*

According to normal coordinate analysis, the 880 cm^{-1} band is a mixed mode of the benzene 12-like vibration and N_1H motion. The frequency of this band reflects the strength of the H-bonding at the NH site of the indole ring of Trp (Miura *et al* 1988). The lower the frequency, the stronger is the H-bonding. Within the precision of our experiments ($\pm$ 1 cm^{-1}), no obvious shift of the 880 cm^{-1} band could be detected

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upon the addition of Ca^{2+} to the apo-form of α-lactalbumin. The H-bonding at the NH site is therefore not noticeably affected by the Ca content.

Recently a calcium-induced conformational change was reported (Heremans and Heremans 1988) by observing the intensity ratio of the 880 cm^{-1} band versus the 1450 cm^{-1} band of the CH_2-scissoring mode. Regarding our results obtained on various samples at different temperatures, we doubt that the intensity ratio h_{880}/h_{1450} is a good parameter to monitor the Ca^{2+} influence. The spread of the data depending on sample preparation and temperature makes it difficult to relate this intensity ratio to the Ca^{2+} content in an unambiguous way.

2.2 *The 1360–1340 cm^{-1} Trp doublet*

The intensity of the 1360 cm^{-1} band is mostly interpreted as a contribution of buried Trp residues and indole rings accessible to the solvent are not expected to contribute to that feature (Yu 1974). Miura *et al* (1988) however speculate that the intensity of the 1360 cm^{-1} band is more generally a marker of the hydrophobicity of the environment. Not only buried Trp but also exposed Trp on the molecular surface but surrounded by aliphatic side chains could contribute to this band intensity.

Two methods were used to calculate the relative intensity of the two peaks in the Trp doublet. In the first method (used in figure 1) the peak height of each peak is determined against the adjacent minimum in the protein spectrum at 1375 cm^{-1}. The second method (used in figure 2) takes an extrapolation of a linear baseline from 1375 cm^{-1} to 1480 cm^{-1} as reference. Both these methods give comparable results (table 1).

At low temperature (6°C) where the protein is folded, the Trp environment is not changed by Ca^{2+} addition. Only when the molar ratio > 1, a small increase in hydrophobicity of the environment is observed. At higher temperatures (21–31°C), R remains unchanged for the samples with molar ratio 0·8 and 1·2. For these samples the thermal unfolding has not yet started and the hydrophobic Trp environment is still intact.

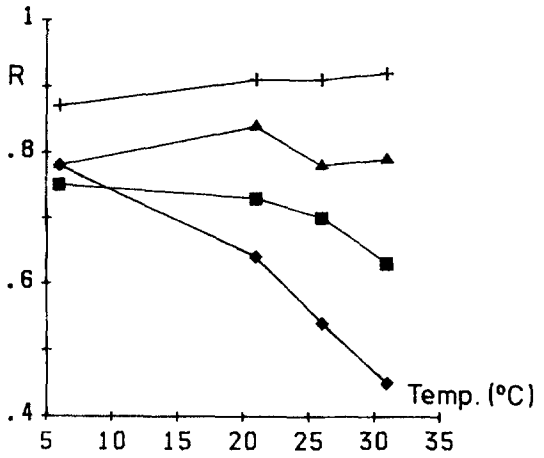

Figure 1. Temperature dependence of $R = h_{1360}/h_{1340}$ in bovine α-lactalbumin without Ca^{2+} (◆), with Ca^{2+} molar ratios 0·4 (■), 0·8 (▲) and 1·2 (+).

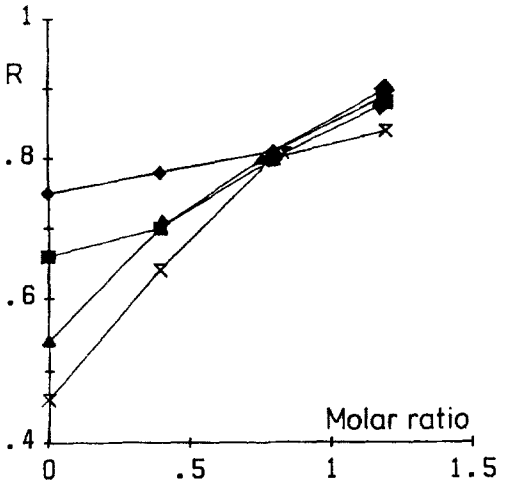

Figure 2. Calcium content dependence of the Trp doublet in bovine α-lactalbumin at $T = 6$(◆), 21 (■), 26 (▲) and 31°C (×).

Table 1. $R = h_{1360}/h_{1340}$ as a function of Ca^{2+} content (molar ratio) at different temperatures. The results obtained by both methods of peak height determination are given.

Molar ratio	Temperature (°C)			
	6	21	26	31
Apo	0·78	0·64	0·54	0·45
	0·75	0·66	0·54	0·46
0·4	0·75	0·73	0·70	0·63
	0·78	0·70	0·70	0·64
0·8	0·78	0·84	0·78	0·79
	0·81	0·80	0·81	0·80
1·2	0·87	0·91	0·91	0·92
	0·89	0·88	0·90	0·84

The 0·4 Ca and the apo sample on the other hand progressively unfold (figures 1 and 2). In this process, Trp is transferred to a more hydrophilic environment.

3. Conclusion

Instead of the intensity of the 880 cm^{-1} band, the Trp doublet at 1340–1360 cm^{-1} can be used as an internal probe for Ca-induced conformational change. Our experiments clearly show an increase of the 1360 cm^{-1} band intensity as a function of Ca^{2+} concentration. The occupation of the Ca^{2+} site thus triggers a conformational reorganization whereby the mean hydrophobicity of the Trp environment has increased.

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