

The transition from the native to the acid-state characterized by multi-spectroscopy approach : study for the holo-form of bovine α -lactalbumin.

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The transition of the holo-form of bovine α -lactalbumin from the native (N) to the pH-generated acidic-state (A-state) was analyzed by probing its tertiary and secondary structure using a concerted spectroscopic approach combining near- and far-UV circular dichroism (CD), electrospray ionization ion mobility mass spectrometry (ESI-IM-MS), vibrational circular dichroism (VCD), and Fourier transform infrared spectroscopy (FTIR) in the attenuated total reflection (ATR) and transmission (TR) modes. The spectroscopic results, which relied on the interaction of an electromagnetic field with different molecular targets, confirmed the decay of extensive rigid side-chain packing interactions during the pH-induced N $\rightarrow$ A-state transition and revealed the targets' dependence on secondary structural changes. Independent analyses of the spectral changes using two methods of multivariate analysis, such as principal component analysis and two-dimensional correlation spectroscopy, revealed small but significant differences in the secondary structure as a result of the all-or-none transition. The cooperativity of the transition was quantitatively described using values corresponding to the mid-point (t_m) and width of the transition (Δt_m). The averages of the two parameters, calculated using the data collected by the different probes, were equal to 3.5 ± 0.2 and 0.6 ± 0.1 (SE), respectively. The variable two-state nature of the cooperative N $\rightarrow$ A-state transition confirmed that the protonation of the side chain carboxyl groups on the Asp and Glu residues and that the release of a Ca^{2+} ion induced structural changes on both the secondary and tertiary levels. The changes have been confirmed by results obtained from the concerted spectroscopic approach.

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