

A COMPREHENSIVE INVESTIGATION OF THE INTERRELATIONSHIP BETWEEN FLUORESCENCE AND UV-DIFFERENCE SPECTROSCOPY OF DENATURATION OF OVALBUMIN BY UREA AND β -BME

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ABSTRACT

The structural thermodynamic and functional aspects of ovalbumin of chicken egg, unfolding induced by urea and β ME(β -mercaptoethanol) has been studied at pH 7.0. Ovalbumin belongs to the Serpin class of protein. We have shown that the transition from native to denatured induced by urea and β ME passes through essential unfolding of the protein. The phenomenon of denaturation of ovalbumin has been studied in terms in λ_{\max} , fluorescence intensity, change in Gibbs free energy at zero denaturant concentration, ΔG_D (H_2O) using the LEM (Linear Extrapolation Method). The fluorescence intensity (specially tryptophan fluorescence intensity) should be a minimum (10%) decrease on addition of 1M urea and maximum (97.7%) decrease on addition of 1N β ME and 9M urea mixture in ovalbumin. Intensity quenching due to environmental change (or substantial conformational change) The chemical deaturation leading to exposure of tyrosine residues was studied with UV-difference spectroscopy as function of concentration of urea and β ME. The study showed that ovalbumin was highly denatured in presence of urea and β ME. The UV-difference spectra were evaluated to calculate Gibbs free energy change, $\Delta G_D(H_2O)$, using the linear extrapolation method, which reflects the stability difference between native and denatured species The study should that ovalbumin was highly denatured in presence of urea and β ME due to disruption of hydrogen bonds as well as intra- and interchain disulfide bonds indicating the flexibility of ovalbumin increase on addition of β ME, so it becomes susceptible to digestion.

KEYWORDS: Interrelationship, Fluorescence, UV-Difference Spectroscopy, Denaturation, Ovalbumin, Urea and β -BME