J. Surfaœ Sci.Technol., Vol 20, No. 3-4, pp. 179-194, 2004

Accessibility of the Various Regions of ß-Lactoglobulin during Thermal Unfolding and Refolding

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Abstract — Structure of the refolded ß-lactoglobulin has been compared with that of native ß-lac-toglobulin by measuring the accessibility of the various regions of the protein molecule. Site selective fluorescence labelling was employed to introduce fluorophores into various regions of ß-lac-toglobulin and collisional quenching of intrinsic and labelled fluorophores was used to measure the Stern-Volmer quenching constant used here as a measure of the exposure or accessibility of the region. Our data indicate that tryptophan residues of the refolded ß-lactoglobulin relocate from the central region towards the protein surface and this relocation helps to open up the access path to the central cavity. Measurement of kinetics of reaction of the free thiol group with DTNB reveals that in the refolded ß-lactoglobulin most of the free thiols are involved in the interchange reaction with disulphides. Blocking of the thiol group with IAEDANS inhibits this interchange reaction. There is difference in quaternary structure between the native and the thermally refolded ß-lac-toglobulin. On heating and subsequent cooling of ß-lactoglobulin solution, much of the native dimeric form dissociates into monomer and some minor fractions of the high molecular species of ß-lactoglobulin were also observed.