554c Unfolding Kinetics of Beta Lactoglobulin on Silica Nanoparticle Surface

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The evolution of fluorescence spectra of aromatic residues of adsorbed beta lactoglobulin molecules on surface of silica nanoparticle of 90 nm diameter when excited at 280 nm was measured. The intensity of the spectra increased with time because of exposure of more aromatic residues as a result of unfolding of the protein molecule. The intensity of fluorescence spectra was measured for native beta lactoglobulin and protein with different extents of denaturation(with guanidium chloride) which were used as reference in the conversion of fluorescence intensity to extent of unfolding. Unfolding of beta lactoglobulin was also characterized by H2O and D2O exchange kinetics as monitored by the evolution of amide II' (1400 to 1500 nm) and amide I (1600 to 1700 nm) peak areas using FTIR. The rate and the extent of D2O exchange were higher for more unfolded protein. Interestingly, the extent of unfolding was more at lower surface concentrations, lower ionic strengths and near pI. The rate as well as the extents of unfolding were found to be smaller at higher surface concentrations possibly due to steric hinderence of neighboring adsorbed molecules on the surface. Since pH 5 is closer to pI of beta lactoglobulin, the adsorbed protein molecule experienced smaller electrostatic interactions with neighboring molecules thus allowing more unfolding. At pH 7, however, the net charge of beta lactoglobulin is -15. As a result, the adsorbed molecules experienced stronger electrostatic repulsion which hindered unfolding. The difference between unfolding at two pH values was found to be more pronounced at lower surface concentrations. Because of shielding of charges at higher ionic strength, the electrostatic repulsion between neighboring adsorbed molecules was suppressed thus enabling more unfolding. Interestingly, the difference in extent of unfolding was significant even at lower surface concentrations.