Relaxation of Water Protons in Highly Concentrated Aqueous Protein Systems Studied by $^1$H NMR Spectroscopy

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In this paper we present proton spin-lattice ($T_1$) and spin-spin ($T_2$) relaxation times measured vs. concentration, temperature, pulse interval ($\tau_{CPMG}$) as well as $^1$H NMR spectral measurements in a wide range of concentrations of bovine serum albumin (BSA) solutions. The anomalous relaxation behaviour of the water protons, similar to that observed in mammalian lenses, was found in the two most concentrated solutions (44% and 46%). The functional dependence of the spin-spin relaxation time vs. $\tau_{CPMG}$ pulse interval and the values of the motional activation parameters obtained from the temperature dependencies of spin-lattice relaxation times suggest that the water molecule mobility is reduced in these systems. The slow exchange process on the $T_2$ time scale is proposed to explain the obtained data. The proton spectral measurements support the hypothesis of a slow exchange mechanism in the highest concentrated solutions. From the analysis of the shape of the proton spectra the mean exchange times between bound and bulk water proton groups ($\tau_{ex}$) have been estimated for the range of the highest concentrations (30%–46%). The obtained values are of the order of milliseconds assuring that the slow exchange condition is fulfilled in the most concentrated samples.