Rheo-NMR Studies of Macromolecules

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Abstract

In this thesis, the effects of simple shear flow on macromolecular structure and interactions are investigated in detail via a combination of Nuclear Magnetic Resonance (NMR) spectroscopy and rheology, namely Rheo-NMR. A specially designed NMR couette shear cell and benchtop shear cell, developed in-house, demonstrated that the direct measurement of the above phenomena is possible.

First, to determine whether the shear cells were creating simple shear flow, results were reproduced from literature studies of liquid crystal systems which report shear effects on: Cetyl Trimethyl Ammonium Bromide (CTAB) in deuterium oxide, and Poly(gamma-benzyl-L-glutamate) (PBLG) in m-cresol.

Next, the possible conformational changes to protein structure brought about by shear were investigated by applying shear to Bovine β-lactogobulin (β-Lg). As the protein was sheared, a small, irreversible conformational change was observed by means of one-dimensional and two-dimensional ^{1}H NMR with reasonable reproducibility. However, no observable change was detected by means of light scattering. A large conformational change was observed after shearing a destabilized β-Lg sample containing 10% Trifluoroethanol (TFE) (v/v). From an NMR point of view, the sheared state was similar to the structure of β-Lg containing large amounts of α-helices and, interestingly, similar to the structure of β-Lg containing β-sheet amyloid fibrils. Gel electrophoresis tests suggested that the changes were caused by hydrophobic interactions. Unfortunately, this proved to be difficult to reproduce.

The effect of shear on an inter-macromolecular interaction was investigated by applying shear during an enzyme reaction of pectin methylesterase (PME) on pectin. Experimental method and analysis developments are described in detail. It was

observed that under the conditions studied, shear does not interfere with the de-esterification of pectin with two types of PME, which have different action mechanisms at average shear rates up to 1570 s^{-1}.

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