On the Hydrogen-Deuterium Exchange in Proteins and Nucleic Acids Monitored by Vibrational Spectroscopy

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A way of probing the structure and dynamics of proteins and nucleic acids is to monitor hydrogen-deuterium exchange [1, 2]. It is well known that groups exposed to the solvent exchange fastest, and the hydrogens of a structured region exchange more slowly compared with the hydrogens of an unstructured part. This is due to hydrogen bonding, low solvent accessibility, and steric blocking. A protected group can be regarded as "closed" to exchange.

In order go get insights into the structure and interactions between these types of molecules, we have developed an infrared and Raman probe of exchangeable hydrogenic sites in these biomolecules. The method is based upon the measurement in real time of the decay of band intensities associated with specific molecular groups, as these become progressively exchanged by deuterium in heavy water environment. The isotopic exchange measured by infrared spectroscopy has been carried out using a conventional liquid cell containing a thick front plate to fix two cylinders containing heavy water buffer. Two dialysis membranes having an appropriate molecular weight cutoff are placed on the cell filling holes of the front plate. The Raman measurements of the isotopic exchange were carried out using a single fibre of hollow microdialysis tubing with 216 μm diameter and variable molecular weight cutoff, the fibre being maintained within the Raman cell. This cell was either a glass capillary tube of 1 mm diameter or a quartz cell of 40 μl capacity. A constant flow of 4-6 ml/h, which is sufficient to maintain constant D_2O effluent concentration at the tubing boundary, was accomplished with a syringe injection pump. We have optimised the said accessories by changing their geometrical characteristics and reached measurements of exchange rates as fast as 0.4 min^{-1} and 1.5 min^{-1} by infrared and Raman spectroscopy respectively. Applications of these exchange probes to nucleic acid and protein molecules and subsequent treatment of the spectra by principal component analysis and two-dimensional correlation spectroscopy demonstrate the usefulness of this methodology for investigating the structure and dynamics of biological macromolecules.

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