QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY FOR THE ANALYSIS OF NONCOVALENT COMPLEXES FORMED BETWEEN GLUTATHIONE S-TRANSFERASE AND VARIOUS LIGANDS

Jennifer A. Burgess[1,3], *Lu-Yun Lian*[2] *and Simon, J. Gaskell*[3]

1. Department of Chemistry, University of Wollongong, N.S.W. 2522
2. Department of Biomolecular Sciences, UMIST, Manchester, U.K. M60 1QD
3. Michael Barber Centre for Mass Spectrometry, UMIST, Manchester, M60 1QD

The applicability of mass spectrometry (MS) to the analysis of noncovalent complexes formed between biopolymers and ligands has been a topic of much interest in recent years. A consequence of the gentle conditions of electrospray ionization (ESI) is that noncovalent complexes can be maintained intact through ionisation and transfer into the gas phase. The correlation of mass spectrometric information with the solution behaviour of noncovalent interactions is still questionable and further research into model systems will continue to be important in the development of ESI-MS studies of such complexes.

The quadrupole time-of-flight hybrid mass spectrometer has proven to be a sensitive instrument with high mass accuracy and an extended mass range. Spraying from solutions in which noncovalent complexes are stable yields lower charge states compared with typical protein ESI conditions. As a result it is critical to be able to detect higher mass-to-charge (m/z) ratios (>4000) than allowed when m/z analysis is performed using a quadrupole analyser. The quadrupole time-of-flight mass spectrometer provides highly sensitive MS analysis of high m/z ions but also with the capability for tandem MS of lower m/z species.

Glutathione S-transferases (GSTs) are a group of enzymes important in cellular detoxification. GST dimers catalyse the conjugation of glutathione (GSH) to endogenous and xenobiotic electrophiles1. The GST system is an ideal model for the study of noncovalent complexes because the binding of GST to a number of ligands has been well characterised in solution.

Analogs of GSH were used to study noncovalent interactions with GST. Specifically, S-alkyl derivatives were chosen to minimise any possible covalent binding through the free thiol of GSH. The GST/S-hexylglutathione system was found to be simple and robust and clearly demonstrated protein dimer formation and the ligand-protein interaction. The results revealed that each GST dimer bound two molecules of S-hexylglutathione. This was consistent with data from other methods of determining structural information. This system was therefore chosen for investigation of the complexes formed between the ligand and various mutations of GST. Low energy collision activated decomposition was used to demonstrate the applicability of MS for the determination of relative binding affinities.

(1) R.N. Armstrong, Chem. Res. Toxicol., 10, 2-18, (1997)