Correlation Between HPLC and Force-Spectroscopy in the Determination of Microcystins in Real Samples

Bueno, C. C., Cardoso, F.P., Etchegaray, A.

Faculdade de Química, CEATEC, PUC-Campinas, Campinas, Brazil.

Microcystins are a group of toxic nonribosomal peptides produced by specific cyanobacterial species that form blooms in eutrophicated water. Several human fatalities have been reported after cronical intoxication with microcystin LR (MLR), the most toxic form of microcystins. Thus, the availability of a simple and direct detection method is essential for actions to avoid human exposure. Very sensitive and reliable methods are available, such as HPLC and enzyme-linked immunosorbent assays (ELISA). We have recently shown an application of molecular recognition using immune-atomic force microscope (AFM), in which standard MLR and its specific antibody were coupled to AFM for the successful detection of microcystin. The aim of this work is to show a correlation on the results of real sample analysis based on antibody/antigen-binding experiments and HPLC. A real sample was collected from an eutrophicated lake. After concentration and confirmatory analysis for the presence of microcystins by ELISA and HPLC, specific ligand-binding experiments were performed with anti-microcystin antibodies. For instance, UV spectroscopy experiments in comparison with standard MLR, demonstrate similar spectral changes observed upon formation of the specific antibody-antigen complex. A more sensitive response was shown by molecular recognition. Stronger uncoupling forces were observed on the real sample in comparison with MLR, suggesting the presence of microcystin RR, which cross-react with the anti-microcystin antibodies. HPLC analysis also suggests the presence of microcystin variants. The very sensitive methods that can be developed using specific antibody-antigen interactions, allowed the identification of MLR and additional variants in real samples, suggesting its application in biosensor development.

Acknowledgements: Fapesp and Fapic-Reitoria (PUC-Campinas)
Key words: nonribosomal peptides, microcystins, UV spectroscopy, force spectroscopy.