DETECTION OF VIRUS LIKE PARTICLES BY LASER INDUCED FLUORESCENCE CAPILLARY ELECTROPHORESIS

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Virus-like particles (VLP) are multi-protein structures that mimic the organization and conformation of a native virus but lack its genome, potentially yielding vaccine candidates which are safer and cheaper than the ones prepared from authentic viruses.

Recombinant technology and particularly, the insect cell/baculovirus expressing system is normally used for the production of these particles [1].

The VLP used in this work are composed of three structural proteins (VP2, VP6 and VP7) from rotavirus, distributed in three layers as illustrated in figure 1. Sixty dimers of VP2 comprise the inner layer. The middle shell is formed by 260 trimers of VP6 while the outer layer is composed of 780 monomers of the glycosylated protein VP7.

The optimization of the production and purification processes is an important aspect for obtaining well formed particles with the correct stoichiometry and therefore, the development of tools for the control of the biotechnological process and the final product are of paramount importance. Usually, control tools such as immunoaffinity techniques like Elisa and Western-Blot, or others

like electronic microscopy and SDS-Page are used for this purpose. However, each of these methodologies lacks one or more of the following characteristics: sensitivity, selectivity, quantitation ability or response speed and therefore the development of separative methods such as chromatography or capillary electrophoresis are desirable. Such methods should allow the detection of very small quantities of VLPs and distinguish between well formed particles and unbound proteins in solution.

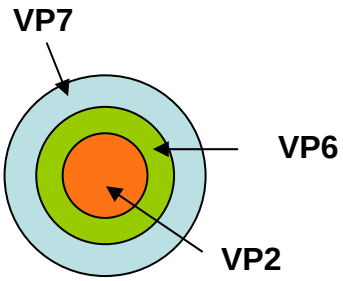

Figure 1: Protein assembly in rotavirus like particles

Capillary electrophoresis (CE) with laser induced fluorescence (CE-LIF) has been proved as one of the most sensitive techniques for protein analysis, allowing detection at the attomole level. It has also been successfully used for the detection of intact microorganisms such as bacteria and viruses [2].

In this poster we describe preliminary work in the development of a capillary electrophoresis method for the detection of intact VLP. Particles have been successfully labelled with a fluorescence marker after a pre-concentration step and detected, after cleanup by size exclusion chromatography, using a homemade CE apparatus equipped with a laser induced fluorescence detector. A non sieving SDS containing borate buffer has been used, but on-going work involves the application of capillary gel electrophoresis methods for detection of unbound VPs.

[1] Journal of Biotechnology, 120(1), 2005, 72-82

[2] Analytical Chemistry 76(14), 2004, 4175-4181