A new method for the non-covalent modification of hydrophobic surfaces polystyrene and polysulphone for the immobilization of Streptavidin Horse Radish Peroxidase

[1]Williams W.T., [1]Liebenberg L.,[2]Jacobs E.P., [1]**Swart P.**,[3]Bredenkamp M.

[1]Department Biochemistry, [2]UNESCO Associated Centre for Macromolecules and Materials, [3] Department of Chemistry and Polymer Science, University of Stellenbosch

A new approach to modification of an inert hydrophobic surface is proposed in this project. An enzyme can be immobilized non-covalently to a surface while maintaining its activity. This is accomplished by using a modified surfactant, pluronic® that adsorbs strongly onto hydrophobic surfaces and makes use of the principle of the strong non-covalent interaction of streptavidin and biotin. A cross-linking system can be used to immobilize Horse Radish Peroxidase that has been conjugated to streptavidin onto an inert hydrophobic surface.

Pluronic® F108 a tri-block copolymer was modified to have hydrazine functionality. These reactive termini were biotinylated. The center block of this polymer adsorbs onto hydrophobic surfaces. We coated polysulphone (PSf) tubes and polystyrene that has been coated onto microtiter plates with the biotinylated pluronic. Sites were created for streptavidin to bind to the hydrophobic surface. The surfaces were incubated in a serial dilution of streptavidin conjugated to horseradish peroxidase. When subsequently incubated in substrate 2-2-azinobis(3-ethyl benzthiazoline-6-sulphonic acid) peroxidase activity was observed.

PSf membranes and the polystyrene surface that was coated with biotinylated pluronic bound streptavidin-HRP specifically in a concentration dependent manner. A typical inverted sigmoidal curve (R^2) was observed. The uncoated surfaces showed little retention of the streptavidin-HRP conjugate and the binding that did occur was non-specific and not concentration dependant.

This means that the method confers enzymatic activity to an inert hydrophobic surface. The implication of this is that robust membrane bioreactors can be constructed. These reactors can be regenerated and reused making their maintenance cost effective. An affinity purification system can also be constructed by immobilizing a receptor that can capture valuable affinity ligands.