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Title of Thesis: Preparation of activated polymer surfaces for covalent binding of ligands and their applications in diagnostics

Abstract

Among the different method of immobilization, covalent binding is preferred as it gives stable binding with extended active life of biomolecules. Most of the reported methods of covalent immobilization are tedious, time-consuming and involve harsh chemicals. We have developed a rapid and simple method for activation of polymer surface. Polymers are activated by a heterobifunctional linker 1-fluoro-2-nitro-4-azidobenzene. The photolinker possess thermoreactive flouro group and photoreactive azido group; they are used to immobilize biomolecules in a step wise thermochemical and photochemical reactions or vice versa.

We have developed rapid procedure for immobilization of proteinaceous ligands like enzymes, antigens or antibodies using photochemically activated solid surface and employing non-conventional methods such as high temperature and ultrasound waves. Thus, anti-MBL autoantibody was detected in rheumatoid arthritis patients by heat-mediated enzyme linked immunosorbent assay (HELISA) technique on photoactivated surface in 2 h 45 min which was comparable to that of conventional ELISA, carried out in 18 h. In another break-through development we have invented efficient sonication-mediated enzyme-linked immunosorbent assay (SELISA) technique which has drastically reduced the time of ELISA to 40 minutes.

Recently carbohydrate microarrays are used for glycomic research including carbohydrate-cell interaction study, carbohydrate- protein interaction, glycome sequencing, carbohydrate binding affinity of SARS and HIV viruses, enzymatic modifications and detection of pathogens. The presentation of carbohydrates in an immobilized format facilitates many of such studies. Herein, we demonstrate a versatile method for covalent immobilization of unmodified mono-, and poly- saccharides onto photoreactive cellulose membrane and polystyrene microtiter plate. The carbohydrates immobilized on both supports are capable of binding specific lectins. In the present method, carbohydrate immobilization is simple and eliminates the time consuming and laborious pre-treatment of carbohydrates.

Lastly, we have developed the matrix for chromatography which with important for purification of important biomolecules including immunodiagnostic reagents such as antigen or antibody. As a model system we have used the matrix for affinity purification of Con A. During this study, we have speeded up an important step in affinity chromatography that was elution of pure biomolecule using ultrasound waves.