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Specific capture of target proteins by oriented antibodies bound to tyrosinase-immobilized Protein A on a polyallylamine affinity membrane surface

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Abstract

In this study, affinity membranes for the specific capture of a target protein to its antibody were fabricated in a three-step process: (1) coating of an amine-functional polymer layer (polyallylamine), (2) immobilization of Protein A by tyrosinase-catalyzed reaction to the amine groups and (3) binding of antibody, through its Fc tail, to the Protein A. The IgG binding isotherm was measured and compared to a membrane onto which Protein A was immobilized through amine-glutaraldehyde chemistry, as a control. The normalized amount of IgG bound, at monolayer saturation, was 30 times higher than that for the control, suggesting the Protein A was more accessible. The affinity constant was 100 times higher, indicating that the site-directed immobilization of Protein A through a remote tyrosine residue results in a more desirable orientation for antibody binding. Affinity membranes for protein capture were created by attaching either anti-BSA (bovine serum albumin) or anti-GFP (green fluorescent protein) to the Protein A. Dot blots were used as a function of concentration to assess the ability of each affinity membrane to capture its specific protein antigen in a layered stack arrangement. The membranes based on tyrosinase-immobilized Protein A were able to selectively bind their respective targets at much lower concentrations than the controls. The membranes could also reproduce blotted shapes, an important attribute for the analysis of proteins from tissue sections.

Keywords: Affinity membrane; Biotechnology; Protein; Antibody; Surface modification

1. Introduction

Current approaches to simultaneously identifying large numbers of proteins from cell lysates include antibody microarrays and reagent coated microspheres such as xMAP® (Luminex Corp.) [1–4]. However, for clinical purposes, such as the diagnosis of disease, a smaller number of protein biomarkers may be of interest and a system capable of simultaneous, high-throughput analysis of small samples from multiple patients in a single measurement is desired. Although antibody microarrays and xMAP® microspheres are very efficient methods for analyzing a large number of proteins, they can only be used to analyze protein samples from one tissue section or blood sample at a time. Another disadvantage of these techniques, particularly for tissue samples, is their inability to retain morphological information related to specific regions of protein biomarker expression.

Layered expression scanning (LES), or multimembrane blotting, was recently developed for the high-throughput proteomic profiling of physiological fluids or tissue sections from multiple samples [5,6]. LES uses a layered stack of track-etched membranes in contrast to the highly porous microporous membranes traditionally used in blotting. In the original LES concept, upon transfer through the stack, each membrane is exposed to an antibody that binds to one particular protein of interest. Track-etched membranes are used to prevent image distortion due to lateral diffusion, thus producing "carbon copies" of each sample from a single transfer. Minimal lateral diffusion is particularly important for maintaining morphological features in tissue sections. With appropriate internal standards, the ability to produce "carbon copies" allows for replication from a single transfer, minimizing false positives/negatives. However, as a result of nonspecific binding, proteins can be depleted as they pass through the membrane stack. This can significantly reduce the

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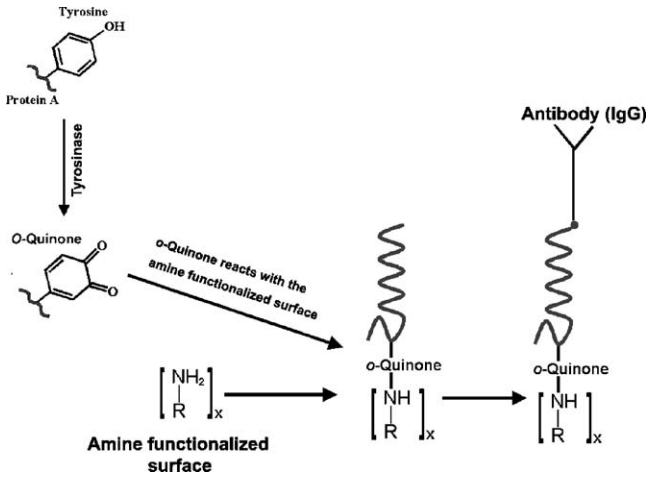

Fig. 1. Immobilization of Protein A through tyrosinase-catalyzed reaction.

sensitivity of the multimembrane approach and, as a result, only a limited number of proteins can be analyzed. Therefore, there is a need to develop an affinity membrane capable of the selective capture of one specific protein. A highly selective affinity membrane will result in reduced protein depletion and increase the sensitivity of multimembrane blotting for proteomic profiling.

Achieving high specificity for protein capture requires the immobilization of antibodies on the membrane surface. To achieve high sensitivity, the immobilized antibodies must be properly oriented on the surface [7–9]. Several different methods have been reported in the literature for surface immobilization of antibodies. These techniques include (1) direct spotting [10], (2) covalent attachment using glutaraldehyde chemistry or a variety of other chemistries [7,9,11], (3) immobilization via affinity tags [13,14], (4) biotinylation of capture molecules and their immobilization on streptavidin coated supports [12,15], (5) binding to Protein A or Protein G coated surfaces [16,17], (6) DNA-directed immobilization (DDI) [8], and (7) cutinase- or AGT-fusions [18,19]. Among these techniques, one commonly used to control IgG orientation relies on the ability of IgG to bind, through its Fc tail, to Protein A or Protein G, which has been attached to an amine-functional surface using glutaraldehyde chemistry. Although the use of Protein A or Protein G in this fashion can improve the antigen-binding ability of immobilized antibody, a significant fraction of these Fc-binding proteins are lost during surface immobilization and cannot orient antibodies properly [16,17]. Given that proteins can contain a large number of amino acid residues with pendant amine groups, primarily lysines and arginines, this approach often results in multiple orientations for the immobilized Protein A, and can even crosslink the protein, leading to additional loss of bioactivity.

One solution to improving the amount of active Protein A (and subsequently, active antibody) on a surface is to use a more specific reaction, such as a terminal histidine tag, to link the Protein A to the surface in an effort to control the orientation of the molecule [13]. In this paper, a simple method to immobilize Protein A on an amine-functional polymer surface is reported as a means of controlling the subsequent orientation of an antibody, without the use of affinity tags or fusion constructs. While the technique is applicable to any amine-functional polymer, polyallylamine was used here. The technique is based on the enzymatic reaction of Protein A through its tyrosine residues (Fig. 1). The enzyme used is tyrosinase, which selectively converts tyrosine residues into *o*-quinones, which in turn, are highly reactive towards primary amines [20–28]. As a control membrane against which to compare this technique, Protein A was also immobilized to the amine-functional membrane surface using glutaraldehyde chemistry (Fig. 2). Depending on the source of the sequence [29], Protein A has 5–8 tyrosine residues, only one of which is located in the IgG-binding B domain of the molecule. For comparison, Protein A has over 67–69 lysine, arginine and histidine residues (those with pendant amine groups), 8 of which are in the B domain. Although not all of these groups are accessible for reaction by either method, using the tyrosine residues to immobilize Protein A should lead to significantly fewer orientations on the membrane surface and a higher probability of binding IgG in an orientation that will be optimal for target antigen binding in an LES assay.

Antibody binding isotherms were measured for both types of immobilization methods using fluorescently labeled IgG and compared quantitatively with a Langmuir adsorption model. The ability of these affinity membranes to selectively capture their targets was determined by transferring protein samples through a four-layer stack. Two of the membranes (layers 1 and 3) had anti-BSA (bovine serum albumin) and two (layers 2 and 4) had anti-GFP (green fluorescent protein) on their surfaces. Samples containing BSA, GFP or a mixture of the two were used. BSA

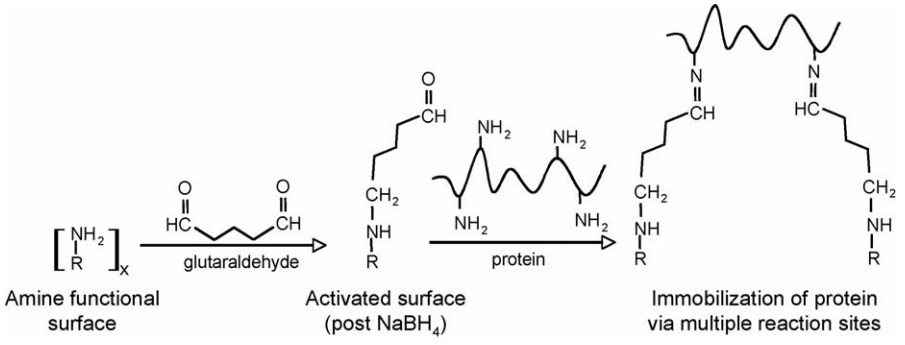

Fig. 2. Immobilization of Protein A through glutaraldehyde reaction.