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Site-selective orientated immobilization of antibodies and conjugates for immunodiagnostics development

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ABSTRACT

Immobilized antibody systems are the key to develop efficient diagnostics and separations tools. In the last decade, developments in the field of biomolecular engineering and crosslinker chemistry have greatly influenced the development of this field. With all these new approaches at our disposal, several new immobilization methods have been created to address the main challenges associated with immobilized antibodies. Few of these challenges that we have discussed in this review are mainly associated to the site-specific immobilization, appropriate orientation, and activity retention. We have discussed the effect of antibody immobilization approaches on the parameters on the performance of an immunoassay.

Contents

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1. Introduction

Immobilized biomolecular systems have revolutionized how we analyze biological/biochemical matrices. However, in such complex matrices, capturing an analyte of interest with high specificity and sensitivity is crucial. Therefore, biorecognition elements, such as receptors and antibodies, are required for capturing a specific analyte out of a complex biological sample. Antibody is one such category of biorecognition molecules that specifically binds to their corresponding antigen. This leads to the core of our long-standing interest in the development of immunodiagnostics. In addition to specificity, lower limits of detection and high sensitivity are the key features of an ideal immunoassay and can be achieved by employing antibodies as capture agents. For this, antibodies are immobilized on the surface of a solid support [1]. Thus, a suitable immobilization approach is always sought that preserves maximum antibody functionality by site-directed and oriented molecular presentation. In this review, we are discussing antibody immobilization strategies that provide (i) site-specific capture guided through various tags and functional groups on antibodies and (ii) orientation achieved through pre-capture biomolecules.

1.1. Antibody

An antibody that is the key constituent of an assay is an immunoglobulin (Ig), typically type G (IgG). It has a molecular weight of ~150 kDa with molecular dimensions of approximately $142 \times 85 \times 45$ Å^3 [2], as shown in Fig. 1.

Structurally, an IgG is a homodimeric protein with two identical pairs of heavy and light chains linked by disulfide bonds [4]. It is worth noting that the structural and molecular composition can vary according to class/isotype (*e.g.*, IgA, IgD, IgE, IgG, and IgM) and even subclass (*e.g.*, IgG1, IgG2a and 2b, IgG3 and IgG4) of antibodies [5]. Like any other protein, chemically an antibody possesses carboxyl, amine, hydroxyl, sulfhydryl, alkyl, and aryl functional groups [6]. Researchers, however, have customized antibodies by adding different functional groups via molecular engineering. In addition, several engineered short variants of these full-length antibodies have been developed. Those antibody derivatives include, but are not limited, Fab (antigen binding fragment), single-chain variable fragment (scFv), and single domain antibody (sdAb). A Fab is a part of an IgG, which contains a whole light chain, and the variable region and the first constant region of heavy chain (Fig. 1). ScFv is composed of variable regions of the heavy and light chains of IgGs, with a linker peptide. An sdAb only contains a monomeric variable region without any linker peptide. It is derived from either camelid antibodies (called V_HH fragment) or novel antigen receptors IgNAR (V_{NAR} fragment), both of which lack the light chains in their structure. The antigen binding capacity is completely gained by the V_HH and V_{NAR} fragment.

Antibodies possess highest binding affinity for their corresponding antigen, even if there are additional receptors that may recognize the given antigen. Therefore, antibodies make excellent probes for immunoanalysis. In addition to these intrinsic properties, antibodies must be immobilized on a solid support with intact structure and functional activity. It is well known that immobilization of antibodies results in activity loss. Therefore, it is crucial to achieve comparative functionality of the immobilized antibodies with respect to those in solution. This allows us to make an educated choice of a suitable immobilization strategy. Such strategies must encourage the use of mild chemistries that don't affect the antigen-binding activity and specificity of antibodies along with their compatibility with different surfaces. In addition, these strategies should provide effective orientation to the immobilized antibodies in order to have their antigen-binding sites freely presented to interact with the analyte in the biological matrix.

2. Applications of antibodies

Antibodies in the development of biosensors and other systems have revolutionized the areas of medical diagnosis, therapeutics, and separation and purification sciences. Immobilized antibody systems are regularly being used in food and drug industry, clinical diagnosis, and environmental monitoring in the form of different analytical technologies. A continuous effort to improve the form and factor of immobilized antibody systems has been made since the development of first plate-based immunoassay in 1980 for increasing the application coverage. Microplate-based conventional immunoassays, such as enzyme-linked immunosorbent assay (ELISA) and immuno/histochemistry (IHC which is performed in the plates with either glass slide bottom or glass slides are kept at the well bottom for reaction), constitute the biggest fraction of *in-vitro* analysis. A few of the most important applications of antibodies are summarized in this section.