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Review

State of the art and recent advances in immunoanalytical systems

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Abstract

This article is an overview the state of the art and the recent developments in immunosensors. Homogeneous immunosensors, heterogeneous immunosensors, integrated immunosensors and biochip format immunosensors are presented, based on optical, electrochemical, magnetic or mechanical detection/transduction systems.
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Keywords: Electrochemical; Immuno-chip; Immunosensor; Magnetic; Mechanical; Optical

Contents

1. Introduction

In the last 10 years, immuno-enzymatic methods (ELISA: "Enzyme Linked Immunosorbent Assay") acquired more and more popularity to the detriment of the radio-immunoassay (RIA). The main reasons of this growing interest are ease of use and safety, since radiolabels and radio-labelled molecules are restricting in term of handling, storage and elimination.

Concomitantly to the arising of those classical ELISA tests on microtiter plates, a large number of immunosensor systems were described in the literature. Based on the biosensor technologies, those sensors attempted to bypass the inherent problems of the microtiter plate tests and particularly the time consumption. Most of the developed immunosensors include a sensing layer supporting a particular immobilised antigen or antibody. The solid support used is generally in close contact with a trans-

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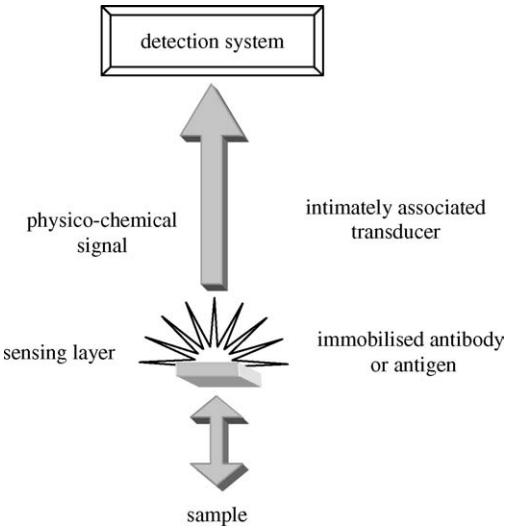

Fig. 1. General representation of the immunosensor functional architecture.

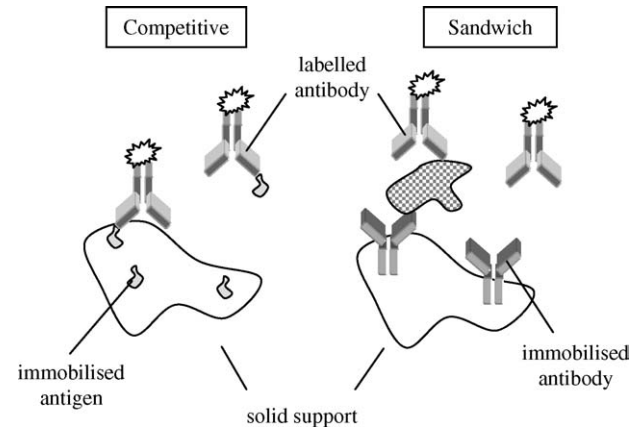

Fig. 2. Schematic representation of the competitive and sandwich type immunotests.

ducer needed for the detection of the formed immune complex (Fig. 1).

The strategy is, then, on the one hand to use the high sensitivity of the detection/transduction systems, in order to minimize the time measurement, and on the other hand to take advantage of the regeneration of the immobilisation support to perform various analysis with a single immunosensor.

The detection systems involved are directly related to the labelling, enzymatic or not, performed on the antigen or on the antibody. For each particular detection type, a specific labelling will be preferred, even if some labels could be used with different detection methods (i.e. the horseradish peroxidase which could be used for fluorescence, chemiluminescence, absorbance and electrochemical measurements). In the present review, the different immunosensors are grouped in categories according to the detection method used.

2. Generalities

Theoretically, all immunochemical techniques could be used to design immunosensors. Nevertheless, because the immunosensors are offered as rapid testing systems, the reaction conditions, and particularly the reagent concentrations (in competitive format) and the incubation times have to be adapted.

Most of the developed immunosensors are based either on competitive or sandwich assay, when applied to the detection of low (herbicides, toxins) and high (proteins, cells) molecular weight molecules, respectively (Fig. 2).

Two approaches could be considered when dealing with competitive immunosensors. A first-one in which immobilised antibodies react with free antigens in competition with labelled antigens. A second-one, using immobilised antigens and labelled antibodies, is generally preferred and prevents all the problems related to antibody immobilisation (loss of affinity, orientation of the immobilised protein).

A few theoretical aspects are presented herein since, compared to immuno-tests on microtiter plate, immunosensors rarely use incubation times long enough to reach the steady state.

In steady state conditions, the amount of labelled antibodies (Ab^*) (or antigens), linked to the immobilised antigens (Ag) (or antibodies) depends only on the affinity constant K (Eq. (1)). Conversely, when the steady state could not be reached because of time or diffusion limitations, the amount of immobilised antigens will have a great influence on the performances of the test.

Most of the competitive immunosensors could, then, be considered as analytical systems, which quantify the amount of "still-free" labelled antibodies after a definite incubation time with the antigen of the sample (Mallat et al., 2001). In those conditions, the larger the amount of immobilised antigens, the faster the reaction will be with the antibodies to be measured and the better the performances of the immunosensor.

Those considerations are useful to understand why immobilisation supports with high specific surface such as porous surfaces, macrometrics membranes (with micron size thickness), and polymeric gels are usually preferred:

$$Ab^* + Ag \underset{k_d}{\overset{k_a}{\rightleftarrows}} Ab^* : Ag, \qquad K = \frac{k_a}{k_d} = \frac{[Ab^* : Ag]}{[Ab^*][Ag]} \tag{1}$$

3. Homogeneous phase immunosensors

Most of the immunosensors described in the literature are based on the separation of the antigen:antibody complexes by immobilising one or the other reagent. Nevertheless, homogeneous phase immunosensors were proposed, which could detect the formation of the immune complex without separation. Those systems are based on the physico-chemical properties of luminescent molecules.

The light emission of a luminescent molecule such as isoluminol, linked to an antigen was shown to be increased following the reaction with a specific antibody (Schroeder et al., 1976; Kohen et al., 1979). Nevertheless, the light emission increasing level highly depends on the antigen structure and on the interaction types involved during the recognition by the antibody

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