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Chemiluminiscnce and tumor markers

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Abstract

This observation revolves around the chemiluminescence biomarkers These are applied for validation methods and screening. Here, was revised the development of the new analytical tool through immunological (antibody) and chemiluminescent methods combination, named chemiluminescence immunoassay (CLIA). In the tumoral process, increased expression of specific antigens is associated in patients with certain tumors. Thus, in our laboratories CLIA has been tested to study different tumoral lesions from glandular tissues, like prostate and thyroid. A sensitive chemiluminescence (CL) imaging immunoassay method for detection of multiple tumor markers with high throughput, easy operation, and low cost was developed. The immunosensor array was prepared by covalently immobilizing capture antibodies on corresponding sensing sites on a silanized disposable glass chip. Gold nanoparticle-based bioconjugates with a high molar ratio of horseradish peroxidase (HRP) to detection antibodies were used for signal amplification. Under a sandwich immunoassay, the CL signals triggered by HRP captured on each sensing cell were collected by a charge-coupled device for simultaneous measurement of biomarkers and combination diagnosis of certain tumors. As a proof of concept, the immunosensor array was applied to detect α-fetoprotein, carcinoma antigen 125, carbohydrate antigen 153, and carcinoembryonic antigen and to screen patients with liver, breast, or ovarian cancers. This method showed wide linear ranges over 5 orders of magnitude and much lower detection limits than previously reported multiplexed immunoassays. The high throughput and acceptable stability, reproducibility, and accuracy showed good applicability of the proposed multiplex CL imaging immunoassay in clinical diagnosis. In conclusion, there is a great opportunity for introducing the adaptive chemiluminescence devices, since clinical diagnostics represents a huge, well-established and important analytical field. Structural requirements for chemiluminescent reactions and the different factors that affect the efficiency of analysis are included in the review. Chemiluminescence application in immunoassay is the new version for this review. Practical considerations are not included in the review since the main interest is to state, through the aforementioned applications, that chemiluminescence has been, is, and will be a versatile tool for pharmaceutical analysis in future years.

Keywords: chemiluminescent reactions, chemiluminescence biomarkers, immunoassay

Introduction

Luminescence is the most conveniently defined as the radiation emitted by a molecule, or an atom, when these species return to the ground state from the exited state. According to the source of excitation, luminescence phenomenon could be classified as photoluminescence (fluorescence and phosphorescence) when the excitation source is energy from absorbed light, chemiluminescence-

energy from chemical reactions and bioluminescence energy from biologically catalyzed reactions. An exited molecule has the same geometry and is in the same environment as it was in ground state. In this situation it either emits a photon from the same vibrational level to which it was exited initially or it undergoes in vibrational level prior to emission of radiation [1,2]. For an isolated molecule in the gas phase, the only way to lose vibrational energy is to emit an infrared photon, which is less probable than undergoing an electronic transition to return to the ground state. Therefore, one tends to see photon emission from higher vibrational levels of exited states in gas phase spectra at low pressure. In a solution, however, thermal relaxation of a vibrationally exited molecule is quite rapid through transfer of excess vibrational energy from the solute molecule to the solvent. In fact, this process is so efficient that all the excess vibrational energy of the exited state is lost, and this process occurs in to sec. This means that before an exited molecule in a solution can emit a photon, it will undergo vibrational relaxation, and therefore photon emission will always occur from the lowest vibrational level of an excited state [3,4]. Once a molecule arrives at the lowest vibrational level of an exited singlet state, it can do a number of things, one of which is to return to the ground state by photon emission. This process is called fluorescence. The lifetime pf an exited singlet state is approximately to sec and therefore the decay time of fluorescence is of the same order of magnitude. The quantum efficiency of fluorescence is defined as the fraction of molecules that will fluoresce. In addition to fluorescence, one also encounters radiationless process where molecules in an excited singlet state may return to the ground state without the emission of a photon, converting all the excitation energy into heat: the process called internal conversion. Generally, internal conversion is an inefficient process and is probably only a small fraction of the total excitation energy in most molecules. Although population of triplet states by direct absorption from the ground state is insignificant, a more efficient process exists for population of triplet states from the lowest excited singlet state in many molecules. This process is referred to as intersystem crossing and is a spin-dependent internal conversion process.

A molecule in excited triplet state may not always use intersystem crossing to return to the ground state. It could loss energy by emission of a photon: the process called phosphorescence. A triplet-singlet transition is much less probable than a singlet-singlet transition. The lifetime of the excited triplet state can be up to 10 sec, in comparison with 10^{-5} s to 10^{-9} s of average lifetime of an excited singlet state. Emission from triplet-singlet transition can continue after initial irradiation [19, 20].

Review

Immunoassays based on chemiluminescence have substantially greater sensitivity and dynamic range than those based on earlier-generation detection techniques. Efficient light emission with low background is coupled with the high sensitivity and broad range of the photomultiplier detector. For every photon of light striking the surface of the photomultiplier, there is a 10^6 fold electronic amplification of the signal. Photomultipliers have very low background noise and inherent dynamic ranges of 5 to 6 orders of magnitude[15].

Chemiluminescence enzyme immunoassay (CLEIA), which integrates the advantages of immunoassay and chemiluminescence determination such as high specificity and throughput, rapidity and convenience in operation and relatively simple and inexpensive instrumentation[16, 17].

The chemiluminescence imaging have also been extensively applied for the evaluation of the spatial distribution of a given target molecule, chemical or biochemical process on macro or microsamples associated with traditional methods, immunohistochemistry (IMH), in situ hybridization (ISH), enzyme or chemical reactions are used for the localization of antigens, gene sequences, enzymes or metabolites in cells and tissue sections[2].

Even though a wide range of different bio- and chemiluminescent systems have been applied in conventional chemiluminescence assays, only a small number of luminescent systems proved suitable for imaging applications. The main requirement, which is particularly crucial in

imaging microscopy, is the localization of the luminescent signal in close proximity to the site where the luminescent reaction takes place[6].Efficient light emission with low background is coupled with the high sensitivity and broad range of the photomultiplier detector. For every photon of light striking the surface of the photomultiplier, there is a 10^6 fold electronic amplification of the signal. Photomultipliers have very low background noise and inherent dynamic ranges of 5 to 6 orders of magnitude[15].

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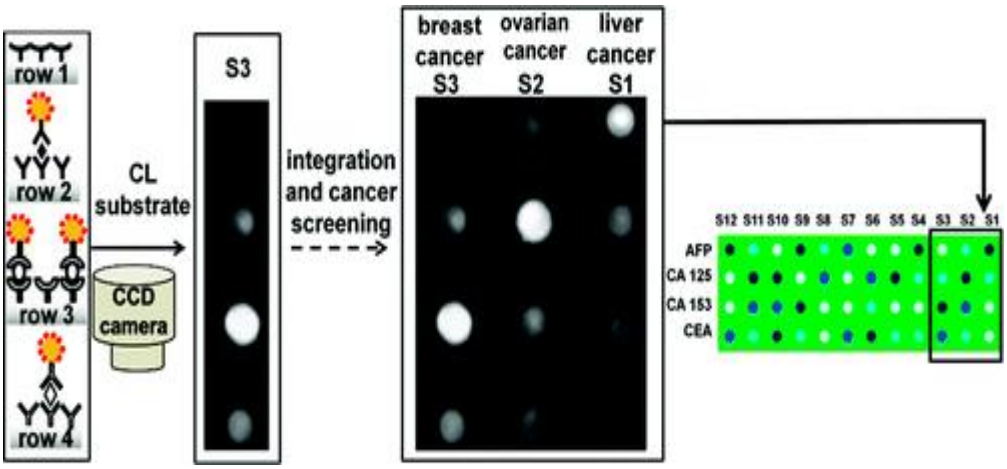
For example, bio- and chemiluminescence imaging microscopy a target molecule is often detected through its binding to a biospecific probe labeled with an enzyme that catalyzes a chemiluminescent reaction. Accurate localization of the target, down to the micrometer scale, requires that the light emission take place close to enzyme label[17].IMH is based on the use of highly specific antibodies, able to bind to an endogenous and/or tumoral antigen.

Application in clinical Diagnosis

Despite years of research and hundreds of reports on tumor markers in oncology, the number of markers that have emerged as clinically useful is pitifully small. Often, initially reported studies of a marker show great promise, but subsequent studies on the same or related markers yield inconsistent conclusions or stand in direct contradiction to the promising results[25].

The proteins, mainly antibodies, are extensively used as diagnostic tools in a wide array of different analyses. Antibody-based immunoassays are the most commonly used type of diagnostic assay and still one of the fastest growing technologies for investigation of biomolecules[26].Light-emitting chemical reactions (chemiluminescence - CL) and biological reactions (bioluminescence BL) have a diverse range of analytical applications but relatively few have been adopted by routine clinical laboratories[2].



For example, the principle of CL has been employed in the field of Obstetrics and Gynecology for the early detection of cervical cancer and pre-cancer[6].Tumor growth and metastases, as well as drug efficacy, have been monitored in living animals by injecting a mouse with luminescence recombinant tumor cells and imaging the produced light[27]. Alternatively, primary tumors and unknown metastases can be revealed in vivo by using engineered light-emitting cells as probes for tumor location[28].The concentration of carcinoembryonic antigen (CEA) in serum obtained from different carcinosis patients was detected by using the method of double antibody sandwich CL immunoassay and the results obtained by this method are fairly well agreeable to those obtained by a consecrated detection method (RIA)[29].Zhuang and co-workers (2004)[29] synthesized a new biacridine compound, 10,10-dimethyl-3, 3-disulfo-9, 9-biacridine (DMDSBA) as a CL label and established a sandwich CLIA method for the determination of carcino-embryonic Ag (CEA) in human serum for detection of tumoral diseases.

A chemiluminescence enzyme immunoassay (CLEIA) based on alkaline phosphatase ALP-labeling has been proposed for AFP detection. But ALP-labeling methodology shows two weak points when compared with HRP-labeling methods, which will bring high background and unavoidably, unproportionate or false positive results in the clinical usage[30].Zhang and co-workers (2009)[31] pretreating the magnetic particles with fluorescein isothiocyanate (FITC) labeled anti-AFP monoclonal antibody (FITC-McAb), a one step CLEIA based on magnetic particles was developed for AFP with high simplicity and sensitivity, as well as wide linear range. The proposed magnetic particle based CLEIA was used to evaluate AFP in human sera samples and a good correlation was obtainedwhen comparing the results with that from a commercial electrochemiluminescence immunoassay kit..The α-fetoprotein (AFP) is the most widely used tumor marker through CL for the diagnosis of Hepatocellular carcinoma (HCC)[32,33].

Despite the prostate cancer (PCa) has become a most widespread and stubborn disease and a major cause of death in the old age male population nowadays[34]. Zheng and co-workers (2008)[24] development sensitive chemiluminescence immunosensor was developed for the detection of PSA. A sandwich assay format was established by using a monoclonal antibody pair acting as the capture probe and detecting probe, respectively.

Most of the current PSA detection methods are usually based on immunoassays. The more established approaches include enzyme-linked immunosorbent assays (ELISA)[35], time-resolved immunofluorometric assay[36], surface Plasmon fluorescence immunoassay[37], bioluminescent immunoassay[38], electrochemical[39] and surface-enhanced Raman scattering (SERS)[40].

Lately, several new PSA detection methods employing the nanowire electrodes[41], the nanoparticle-based bio bar code[42], and the microcantilever method[43] are proposed. Although they all have their individual strengths, The high detectability and rapidity of CL techniques, along with the availability of microarray-based analytical devices, allows the development of high throughput screening assays, in which simultaneous, multi-analyte detection is performed on multi samples[17].

Therefore, more and more medical experts and chemists are interested in CLIA. However, the development of CLIA is dependent on the application of the sensitive and selective chemiluminescent probe[29].

Improvements in analytical sensitivity will likely lead to the discovery of new analytes tumours detection. Technical enhancement holds the promise of detecting very low concentrations in serum using nanoparticles as labels and CL detection[45,46].

In conclusion, chemiluminescent immunoassay (CLIA) is a fast and simple method without radioactive pollution, its sensitivity is usually higher than that of fluorescent immunoassay and enzyme immunoassay[29].

Nowadays, the method using nanoparticle, especially metal, as biological labels has attracted considerable interest. As Biological labels, NPs present many advantages[45, 46, 47].

Recently in our laboratory we tested the applications of chemiluminescence immunoassay with galectin-3 acridinum ester conjugated to anti-Galectin3 antibody in prostatic and thyroid tumors and chemiluminescent assays with acridinum ester conjugated with Concanavalin-A in breast lesions.

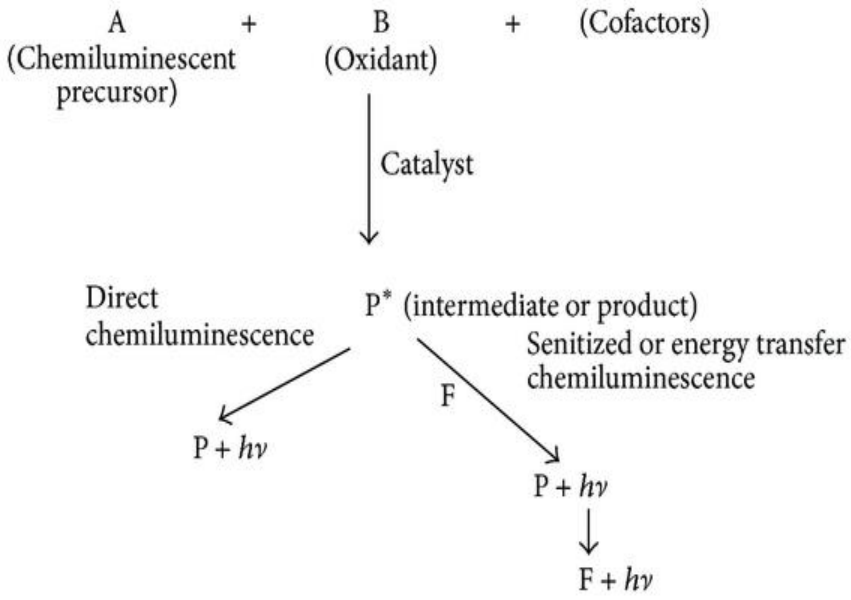


Figure 2.

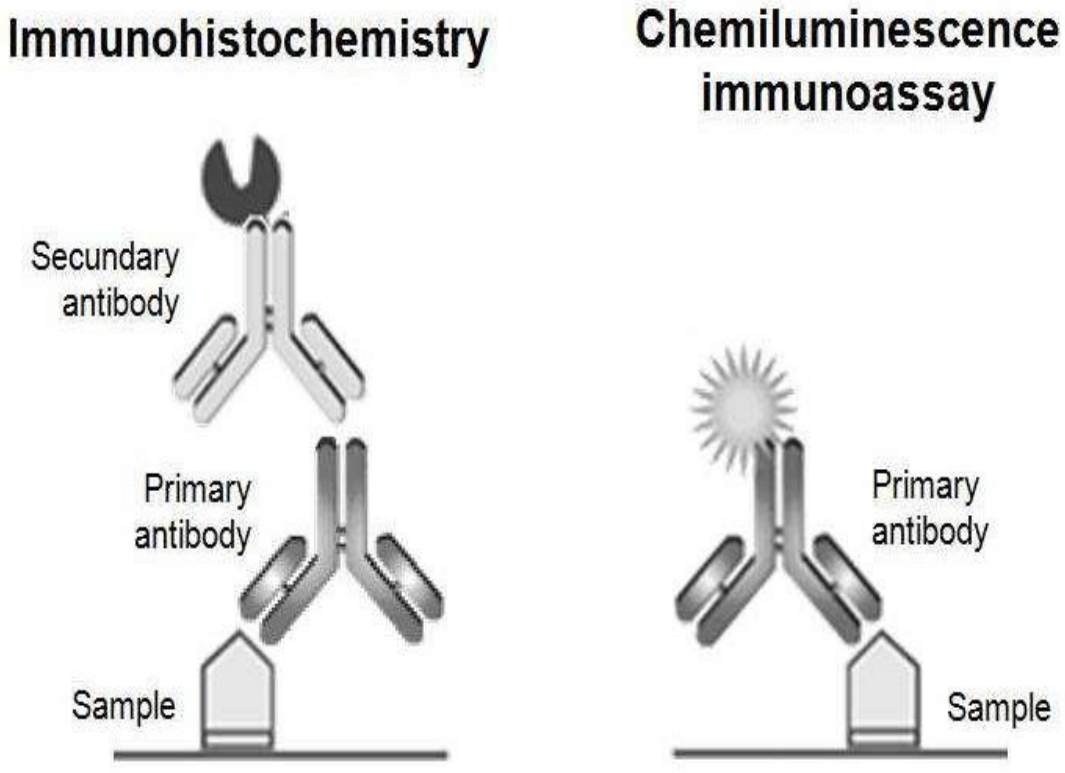


Figure 3.

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