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Lanthanide polymer labels for multiplexed determination of biomarkers in human serum samples by means of size exclusion chromatography-inductively coupled plasma mass spectrometry

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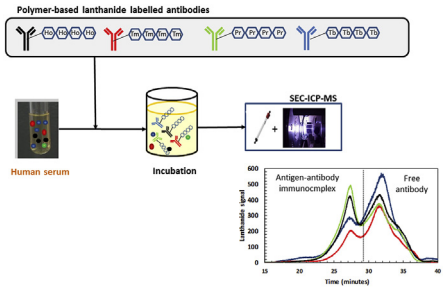
HIGHLIGHTS

- Polymer lanthanide labelling is used for the first time in homogenous immunoassay.
- The labelling strategy proposed allows multiplex biomarker analysis with SEC-ICPMS.
- Sensitivity and LoDs are improved regarding previously reported DOTA-labelling.

GRAPHICAL ABSTRACT



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ABSTRACT

Lanthanide polymer-labelled antibodies were investigated to improve the analytical figures of merit of homogeneous immunoassays with inductively coupled plasma mass spectrometry (ICP-MS) detection for multiplexed biomarker analysis in human serum samples. Specific monoclonal antibodies against four cancer biomarkers (CEA, sErbB2, CA 15.3 and CA 125) were labelled with different polymer-based lanthanide group to increase the number of metal labels per binding site. After the immunoreaction of the biomarkers with the specific antibodies, antigen-antibody complexes were separated by size-exclusion chromatography followed by ICP-MS detection. The polymer label could be loaded with 30-times more atoms of the lanthanide that the lanthanide-DOTA complex traditionally used for this purpose elsewhere [1] which resulted in a 10-fold improvement in both sensitivity and detection limits. Analytical figures of merit obtained with the lanthanide polymer labelling strategy make the detection of the biomarkers feasible below the threshold reference values used in clinical analysis. This labelling method was successfully validated by analyzing a control human serum spiked with the four biomarkers at three different concentration levels. For all the biomarkers studied, the recovery values ranged from 95% to 110% whereas inter-assay and intra-assay precision were lower than 8%. Results obtained with this approach were equivalent to those obtained by heterogenous-based immunoassays based on the detection by

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electro-chemiluminescence or ELISA. However, the method developed offers better analytical figures of merit using a smaller amount of sample.

1. Introduction

Recently, a number of immunoassays based on the use of metal-labelled antibodies and the determination of antibody-antigen complexes by inductively coupled plasma mass spectrometry (ICP-MS) have been proposed for the determination of biomolecules, and, in particular, proteins [2,3]. The ICP-MS quantification offers several advantages over the conventional detection techniques employed in immunoassays (colorimetry, fluorimetry, etc.), such as, e.g., (i) specificity to heteroatom detection; (ii) compound-independent detection sensitivity; (iii) high elemental sensitivity and dynamic range; (iv) limited sample treatment; (v) stability of the reagents against time, temperature and light (the isotopic masses do not change, bleach or degrade); (vi) reduction of non-specific background; (vii) independence of analytical response from incubation or storage times and (viii) multiplexed detection [3,4].

In general, the immunoassay procedures employed with ICP-MS detection has been carried out in heterogenous phase in different type of formats (e.g. sandwich-based, Western blot, etc.). Antibodies are usually labelled by either metal nanoparticles [5,6] or lanthanides [7,8]. The advantage of elemental nanoparticles is the possibility of the introduction of a significant number of atoms per conjugate which allows the amplification of the analytical response. This advantage is set off by the high affinity of nanoparticles to surface of labware and/or ICP-MS sample introduction system, increasing wash-in and wash-out times, and by the difficulty to synthetize nanoparticles of uniform size. Lanthanides are introduced as DOTA or DTPA chelates due to its extraordinary thermodynamic stability [7]. The similar chemical properties make lanthanides well suited for multiplex assays: different antibodies can be easily and specifically labelled with different lanthanides in the same experimental workflow.

Despite higher simplicity, homogeneous immunoassays with ICP-MS detection have been scarcely investigated in the literature in comparison with their heterogenous counterparts. Terenghi et al. [9] showed that a mixture of antibodies, each labelled with a different lanthanide, could react with different biomarkers in liquid samples and the antigen-antibody (Ag-Ab) complexes formed could be isolated by size-exclusion chromatography (SEC) and specifically determined by ICP-MS. The main benefits of this approach were: (i) multiplexed capability; (ii) small sample amount consumption; and (iii) virtually no sample preparation. However, signal amplification was limited since only a single lanthanide atom was introduced per binding site of the antibody. Several authors demonstrated that the number of lanthanide atoms per antibody can be increased by using metal-loaded polymers [10–14], which leads to an increase in sensitivity. This labelling strategy was successfully employed for single-cell ICP-MS analysis [11–13]. Waentig et al. [14] compared polymer-based lanthanide labelling with other lanthanide-based labelling strategies for protein quantification in solid phase immunoassays (Western Blot, SDS-PAGE, etc.). These authors noted that this labelling strategy improves significantly sensitivity which result in limits of detection in the low fmol range. However, there has been no attempt so far to investigate the potential of antibodies conjugated with metal-labelled polymers in homogenous assays.

The aim of this work was to evaluate lanthanide polymer labels for multiplexed biomarker analysis by the use of homogeneous immunoassay in which Ag-Ab complex, free metal-labelled antibody and free metal are separated by SEC and quantified with ICP-MS. To this end, monoclonal antibodies against four biomarkers (CEA, sErbB2, CA 15.3 and CA 125) usually present in human serum samples were labelled with a different polymer-based lanthanide moiety. Size-exclusion chromatography was used to isolate the Ag-Ab complexes whereas ICP-MS on-line detection was used for quantification. The method was benchmarked against those using the labelling with DOTA-chelates [9].

2. Experimental

2.1. Regents and materials

Carcinoembryonic antigen (CEA) was obtained from Sigma-Aldrich (St. Quentin-Fallavier, France). The soluble form of human epidermal growth factor receptor 2 (sErbB2) was purchased from antibodies-online (Aachen, Germany). Cancer antigen 15.3 (CA 15.3) was obtained from MyBioSource (San Diego, CA) and CA 125 was from Fitzgerald (MA). Goat polyclonal antimouse immunoglobulin (IgG) antibody (H&L) was purchased from Abcam (Cambridge, UK).

Mouse IgG subclass 1 (IgG_1) antihuman monoclonal antibody (mAb) for α-CEA (clone 1C11) and mouse IgG_1 antihuman mAb for α-CA 125 (clone X325) were purchased from Gene Tex (Irvine, CA). Mouse IgG_1 antihuman mAb for α-sErbB2 (clone 5J297) was obtained from antibodies-online (Aachen, Germany) and mouse IgG_1 antihuman mAb for α-CA 15.3 (clone M002204) was from LifeSpan BioSciences (Seattle, WA). The antibody solutions should not contain additives, such as bovine serum albumin (BSA) or gelatin, because the latter could be labelled as well and cause interferences. Upon reception, mAb were divided into single working aliquots and stored at −20 °C.

MAXPAR™-polymer -Ab labelling kits were obtained from Fluidigm (Les Ulis, France). Human Albumin Albutein™ 20% was purchased from Grifols Biologicals Inc. (Los Angeles, CA). 1,4,7,10 − Tetraazacyclododecane - 1,4,7 - tris(aceticacid) - 10-maleimido - ethylacetamide (DOTA) was obtained from Macrocyclics (Dallas, TX). Tris (2-carboxyethyl) - phosphine hydrochloride (TCEP), Trizma™ base, lanthanide chlorides ($HoCl_3$, $TbCl_3$, $TmCl_3$, $PrCl_3$) with natural isotopic abundance, ammonium acetate (≥98%, for molecular biology), monosodium phosphate, disodium phosphate, ethylenediaminetetraacetic acid disodium salt (EDTA), dimethyl sulfoxide (DMSO), sodium chloride and polyethylene glycol sorbitan monolaurate (Tween 20) were from Aldrich (Schelldorf, Germany). Acetic acid glacial and 69% w w^{-1} nitric acid were purchased from Panreac (Barcelona, Spain). Rare earth 100 μg mL^{-1} Complete Standard was provided by Inorganic Ventures (Lakewood, Colorado) and *DC™ Protein Assay Kit* was from Bio-Rad (Marnes-la-Coquette, France).

Ultrapure water 18 MΩ cm from a Milli-Q water purification system (Millipore, Paris, France) was used throughout the work.

Amicon™ Ultra-0.5 mL centrifugal filters for DNA and protein purification and concentration (Merck Millipore, Cork, Ireland) with different cutoff limits (3, 30 and 50 kDa) were used

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