Trends in Analytical Chemistry 72 (2015) 45–52

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Lanthanide elements as labels for multiplexed and targeted analysis of proteins, DNA and RNA using inductively-coupled plasma mass spectrometry

Thomas C. de Bang *, Søren Husted

Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, DK-1871, Frederiksberg C, Copenhagen, Denmark

ARTICLE INFO

Keywords:
Bioanalytical technique
Hybridization assay
Immunoassay
Inductively-coupled plasma mass spectrometry
Label
Lanthanide
Laser ablation
Multiplexed analysis
Nucleic acid
Protein

ABSTRACT

Among the rare-earth elements, lanthanides constitute unique labels in bioanalytical assays and are detected with high sensitivity by inductively-coupled plasma mass spectrometry (ICP-MS). ICP-MS is a versatile technology that, based on coupling to various bioanalytical techniques, has facilitated multiplexed analysis of both proteins and nucleic acids. In this review, we present different types of bioanalytical applications used to detect these biomolecules. Moreover, we review common conjugation approaches and lanthanide-chelating tags used in these assays. Multiplexed lanthanide immunoassays have developed substantially over the past few years and, in the most recent applications, analysis of more than 30 proteins has been achieved. In parallel, methods using lanthanide-labelled DNA probes combined with ICP-MS detection have emerged and enabled multiplexed analysis of up to 15 different DNA sequences. We anticipate the further development of quantitative lanthanide-hybridization assays of hitherto unseen multiplexed character, with great potential for nucleic-acid research.

Contents

Abbreviations: AuNP, Gold nanoparticle; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; DTPA, Diethylenetriamine pentaacetic acid; DTT, Dithiothreitol; ICP-MS, Inductively-coupled plasma mass spectrometry; LA-ICP-MS, Laser-ablation inductively-coupled plasma mass spectrometry; LC, Liquid chromatography; MC, Mass cytometry; MMP, Magnetic microparticle; MNP, Magnetic nanoparticle; SEC, Size-exclusion chromatography; TCEP, Tris(2-carboxyethyl)phosphine; TRF, Time-resolved fluorescence.

* Corresponding author. Tel.: +45 6 136 5328.
E-mail address: tdb@plen.ku.dk (T.C. de Bang).

http://dx.doi.org/10.1016/j.trac.2015.03.021
0165-9936/

1. Introduction

Analytical methods for targeted analysis of proteins and nucleic acids (DNA and RNA) are essential for validation of results obtained by global and untargeted omics techniques. Moreover, the simultaneous analysis of several target analytes (multiplexed analysis) reduces the inherent bias associated with sequential simplex analysis of a sample, thereby reducing the risk of obtaining false-positive or false-negative results. Consequently, new analytical methods for multiplexed analysis are required to ensure precise and accurate results.

Antibody-based assays (immunoassays), such as Western blotting and enzyme-linked immunosorbent assays (ELISA), constitute the gold standard for protein validation. Targeted analyses of nucleic acids are based on sequence-specific DNA oligonucleotides (DNA probes), which can be used directly in hybridization assays or as primers in amplification reactions. The most common detection methods employed in immunoassays and hybridization assays facilitate only simplex analysis, due to the chemical nature of the labels used. These labels typically produce uniform signals that are difficult to discriminate as for example chemiluminescence, fluorescence, colored products or radioactivity.

However, the use of fluorescent labels with different emission lines has enabled multiplexed analysis, although the development of highly multiplexed assays is hampered by the bandwidth and thus the potential overlap of the emission lines. Inductively-coupled plasma mass spectrometry (ICP-MS) has in recent years gained increased attention as a complementary technique for targeted analysis of proteins and nucleic acids. In particular, methods combining lanthanide labelling of antibodies or DNA probes has enabled sensitive and highly multiplexed assays with more than 30 proteins and 15 DNA targets, respectively. In this review, we give an overview of the methodology and the many exciting applications of lanthanide-based multiplexed ICP-MS analyses, thereby highlighting a field of analytical chemistry that experienced tremendous progress in the past few years.

1.1. ICP-MS: a versatile technique for multiplexed bioanalytical analysis

ICP-MS is a technique for quantitative multi-element analysis. Traditionally, ICP-MS has been used to analyze the element composition of virtually any type of sample after acid digestion or via direct solid sampling by laser ablation (LA). ICP-MS technology is based on hard ionization by a 7000–10,000 K hot plasma, which vaporizes, atomizes and ionizes the introduced sample. After mass filtering of the ions produced, pre-selected mass-to-charge ratios (m/z) covering the periodic table of elements are detected. As a consequence of the hard ionization, this technology is independent of molecular structure and exhibits less matrix interference in comparison to MS techniques based on soft ionization [1]. Moreover, ICP-MS offers sub-ng L^{-1} (ppt) limits of detection (LODs), up to nine orders of linear dynamic range, versatile coupled applications as well as high accuracy and precision. For these reasons, ICP-MS has received increased attention as a powerful bioanalytical technique for the analysis of proteins and nucleic acids [2]. ICP-MS analysis of biomolecules is achieved by direct or indirect labelling with metals, or via hetero-atoms (ICP-MS detectable elements inherent to the biomolecule such as phosphorus, sulfur or metals in metallo-proteins) [3]. In this context, a unique feature of ICP-MS is the possibility for hyphenation to virtually any technique available for separation of biomolecules, including the online separation techniques flow cytometry (FC), capillary electrophoresis (CE), size-exclusion chromatography (SEC), ion-exchange chromatography (IEC) and reversed-phase high-performance liquid chromatography (RP-HPLC) [4]. Moreover, biomolecules separated by polyacrylamide-gel electrophoresis (PAGE) can be analyzed directly by LA-ICP-MS or after transfer of the separated sample to a membrane e.g. using the Western blotting or Northern blotting techniques [5,6].

1.2. Lanthanides as labels for ICP-MS-based detection of proteins and nucleic acids

The lanthanide series consists of the 15 elements lanthanum (La), cerium (Ce) praseodymium (Pr), neodymium (Nd), promethium (Pm, which occurs artificially and is radioactive with a half-life of ~20 years), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yt) and lutetium (Lu), with a total of 38 isotopes. Some 30 years ago, time-resolved fluorescence (TRF) bioassays for sensitive protein and nucleic-acid analysis were developed. TRF assays were based on the enhanced and long-lived fluorescence of lanthanide ions Eu^{3+}, Sm^{3+}, Tb^{3+} and Dy^{3+} when bound to chelating compounds [7]. This allowed discrimination between short-lived background fluorescence and lanthanide fluorescence using time-delayed detection. In addition to their TRF properties, the distinct and non-overlapping emission lines of Eu, Sm, Tb and Dy made them ideally suited to be labels in multiplexed assays. However, due to the inherent dependence on fluorescence, these assays were in principle limited to four analytes. In contrast, lanthanide detection by ICP-MS increases the theoretical multiplex potential to 38, if using enriched isotopes as labels. In this context, ICP-MS based analysis of lanthanides is highly sensitive due to several parameters:

(1) lanthanides are low-abundance elements in nature and consequently the background levels in biological sample matrices are low;
(2) polyatomic interferences are rarely significant; and,
(3) the ionization efficiency of lanthanides is high due to low first-ionization potentials.

Furthermore, the lanthanide elements have similar chemical properties and are thus highly suited for developing multiplexed assays based on ICP-MS [8]. In 2001, Zhang et al. [9] were the first to publish a method for detecting proteins via lanthanide-labelled antibodies based on ICP-MS. The antigen was immuno-reacted with a biotin-labelled antibody, which subsequently was conjugated with Eu-labelled streptavidin. In the following years, a number of studies tested the potential of lanthanide-labelled antibodies for multiplexed protein analysis using ICP-MS detection [10]. Encouraged by the success of these experiments, various bioconjugation techniques and lanthanide-chelating tags have since been developed and validated to improve the sensitivity and the multiplexing capacity of these assays.

2. Lanthanide-chelating tags and bioconjugation approaches

Lanthanide-chelating tags are composed of hetero-bifunctional crosslinkers that contain a reactive group and a lanthanide chelator. Specific lanthanide labelling of biomolecules can only be realized using a lanthanide-chelating tag containing a reactive group that permits conjugation to a functional group on the biomolecule (bioconjugation). Bioconjugation results in a covalent bond between the biomolecule and the chelating tag. In addition, the chelating tags most commonly used result in lanthanide-chelate complexes with very high thermodynamic stability and consequently lanthanide labels are very stable [11].