Talanta 102 (2012) 164–170

Contents lists available at SciVerse ScienceDirect

Talanta

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

Advances of CE-ICP-MS in speciation analysis related to metalloproteomics of anticancer drugs ☆

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ARTICLE INFO

Available online 20 July 2012

Keywords:
Capillary electrophoresis
ICP-MS
Metallodrugs
Proteins
Mode of action

ABSTRACT

The mode of action of metal-based anticancer drugs, including their accumulation in blood, transport, delivery to cancer cell, and cell processing (together with release of an active form and possibly targeting) is largely dependent on protein binding. Among analytical methods capable of providing a better understanding of metallodrug–protein interactions, capillary electrophoresis (CE) with inductively coupled plasma mass spectrometry (ICP-MS) detection is arguably a premier technique. Since its advent to the area of metallodrug proteomics in 2004 [1], the benefits of CE-ICP-MS became evident, stimulating further research and methodological developments. This hyphenated technique's merits comprise an ability to separate rapidly and efficiently the parent drug and protein-bound drug form(s), with no alteration of original speciation in the sample, to identify the metal-containing species due to specific ICP-MS response, to measure the binding parameters (e.g. rate and equilibrium constants), and finally to quantify the metal–protein adducts in real-world samples. This review is aimed on offering the reader a summary of applications of CE-ICP-MS to various metallodrug–protein systems, with a focus on experimental strategies in use for the assessment of binding reactivity and affinity, monitoring in vitro cellular transformations and serum binding profiles, and ex vivo metallodrug–proteomic analysis.

1. Introduction

In the recent years, capillary electrophoresis (CE) hyphenated with inductively coupled plasma mass spectrometry (ICP-MS) has become one of the preferred techniques for speciation analysis. This is the effect of a number of factors, including improvements in the methodology of interfacing CE and ICP-MS instruments, significant advances in the development of new approaches for the analysis of various element species in different types of samples, and the existence of a vast body of literature that supports understanding and implementation of this combined technique [2–5]. An area of great potential of CE-ICP-MS is identification and determination of different chemical species of an element in biological systems, where the method has attracted significant attention in the last decade [6–9]. A highly intensive, element-specific, and interference-free response provided by ICP-MS makes CE separations tailor-made to elucidating the essentiality, toxicity, and in general, biological functions of trace elements. Furthermore, when such an element is an active drug component, as, for instance, platinum in the case of platinum-based anticancer drugs, CE-ICP-MS appears to be one of few analytical tools that enable a better knowledge about the destiny of the drug in the body and its mechanism of action at the molecular level [10,11]. In this field of biospeciation analysis, CE offers an attractive alternative to HPLC-based speciation schemes. With the renowned capability of CE in the analysis of metal species [12,13], this platform does not only provide superior separation efficiency within shorter time, simplicity in separation hardware, and lower consumption of sample and reagents but more importantly, it minimizes an impact on the equilibrium between different metal species existing in a given model system or a real biological sample. An inferior sensitivity of CE compared to HPLC seems to be no great analytical challenge, as the administered levels of metallodrugs are fairly high to deal with in the direct analysis of clinical samples, even using quadrupole ICP-MS.

Abbreviations: BGE, Background electrolyte; CE, Capillary electrophoresis; EOF, Electro osmotic flow; ICP-MS, Inductively coupled plasma mass spectrometry

☆ Dedicated to Professor Yuri A. Zolotov on the occasion of his 80th birthday.

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http://dx.doi.org/10.1016/j.talanta.2012.07.031

Reactions with various biological molecules in the human body indisputably play a key role in metabolism and therapeutic action of metal-based drugs. Many such interactions, particularly those with proteins as binding partners, have been evaluated in recent CE-ICP-MS research, as discussed below. Interest in these studies stems from the well-known fact that the rate and degree of the transformation upon binding toward serum proteins regulate the cellular uptake and accumulation of the drug in tumor tissue and determine differences in efficacy, activity and toxicity, as well as the overall distribution and the excretion of individual drugs [14,15]. There exist different ways how we can investigate this important component of preclinical development of anticancer metallodrugs by CE-ICP-MS. The binding behavior can be assessed (and compared) kinetically, in terms of rate constants. Next or in parallel, it is essential to analyze the binding equilibrium, with the aim of determining the association (binding) constant and/or the number of protein sites participating in drug binding, i.e. its stoichiometry. Such information would afford a more faithful interpretation of protein-mediated metabolism of anticancer agents after intravenous administration or after the uptake of an oral drug in the bloodstream. Stability of the protein–drug adducts in the presence of various bioligands and /or reductants at simulated extracellular or intracellular conditions or, oppositely, their inclination to respective speciation changes is another important line of CE-ICP-MS research. Using such in vitro screening, one may gain an insight into biotransformations accompanying the storage and delivery of metallodrugs, their intracellular activation, and interaction with drugable cell targets. Evolution of the protein–drug conjugates under real serum or plasma circumstances can be monitored directly, taking advantage of relative freedom of CE from proteinaceous sample matrices (compared to HPLC). There is no need to emphasize that experiments with blood constitutions give a more inclusive picture of the in vivo state. In situations when a specific protein-bound form dominates drug's speciation in blood (or its fractions), the drug of interest can be subject to quantification through measuring metal loading of the protein adduct, e.g. via drug/protein ratios. This actually moves CE-ICP-MS forward to the realm of quantitative biospeciation analysis.

In the following sections of the present review the aforementioned research domains will be critically scrutinized, after a brief consideration of advanced separation/detection methodology used in metallodrug–protein studies. Challenges that remain in applying CE-ICP-MS in investigations of protein-binding behavior of metallodrugs and future steps to be taken toward method's betterment for further narrowing the gaps in our understanding of the roles of proteins in the mechanism of action of metal-based anticancer drugs will also be brought into focus.

2. CE-ICP-MS methodology in metallodrug proteomics

2.1. Sample preparation

It makes no sense to accentuate that the interaction of metal-based drugs with serum transport proteins should be assessed under conditions as close as possible to extracellular physiological environment. Otherwise, all binding data acquired turn to be simply irrelevant. Therefore, the binding experiments are typically carried out in incubation solution comprising 10 mM phosphate buffer [1,16–18,21–24] or 4 mM disodium hydrogenphosphate–25 mM sodium bicarbonate buffer [19] at pH 7.4 and 100 mM NaCl (at 37 °C). Given the total salt concentration (about 0.154 M) and a pH of 7.43 in human plasma, these buffers appear to be a good choice to simulate a real-world situation. However, the carbonate-containing buffer was found to be unstable to perform prolonged binding [17]. It should also be mentioned that maintaining such ionic strength within the background electrolyte (BGE) presents a challenging problem when using ICP-MS detection (see below).

The initial concentration of proteins is commonly kept at 5×10^{-5} M, which approximates satisfactorily the physiological level for transferrin (3.5×10^{-5} M) but about ten times lower than the actual concentration of albumin (ca. 6×10^{-4} M). Notably, serum proteins other than albumin and apo-transferrin (hereafter referred to as transferrin) have not been examined (until a very recent attempt on holo-transferrin [20] and a single report exploring a partially Fe(III)-loaded transferrin [19]). A reasonable explanation is behind their relative abundance and pertinent transport functions. The drug concentration in the incubated mixture is varied from a 1:1 to 20:1 M ratio to the protein, being a reasonable estimate of the factual drug-to-protein proportion at the stage of intravenous administration (or entering the bloodstream). In most cases, this concentration range also guarantees the complete (equilibrium) binding conditions.

In case of real serum or plasma samples, their treatment should be performed in a gentle way so that the integrity of the protein–drug adducts is not deteriorated. This is accomplished by a moderate dilution (10-fold at maximum) with the physiological buffer [21,22] or water [23].

2.2. Separation

Ideally, the BGE composition is to be the same as incubation solution (or blood electrolyte composition). This is believed to preserve the protein-bound analytes of scrutiny against possible changes in speciation in a CE system and in addition, minimizes electrodispersion effects. However, high-salt electrolytes disturb the coupling to ICP-MS by producing unstable spraying conditions and frequently clogging the nebulizer by deposits. Next, the electric current may reach a value unacceptable to maintain stable performance of the interface. Moreover, use of simulated physiological buffer solution may lead to poor resolution, low intensity of adduct response, and lack of signal repeatability [16]. Therefore, neutral BGEs void of sodium chloride have been used in most of metallodrug–protein studies by CE-ICP-MS, with the preference given to phosphate buffer electrolytes (10–15 mM) [1,16–18], using bare fused-silica capillaries. These BGEs ensure good resolution of the target species (including adducts of different proteins) [16] and rather high electroosmotic flow (EOF) to transport them rapidly past the detector, as can be seen in Fig. 1. Another BGE based on a 10 mM ammonium carbonate solution showed less favorable separation ability [19]. Occasionally, a cationic surfactant is added to the BGE accompanied by polarity reversal of the power supply [16]. This favors the separation of different protein adducts (by virtue of ion-pairing interaction and possibly micellar partitioning effects) and improves the recovery of metal species from the capillary. For hydrophobic, oral metal-based drug, displaying high affinity to fused-silica capillary walls and hence poor recovery, a zwitterionic separation electrolyte of Good's buffer type was found more suitable than commonly used phosphate buffer system [23]. The rationale of employing such BGEs is that they can be applied at high concentration (without increasing the current) so that analyte adsorption is competitively suppressed.

When analyzing serum and plasma samples, protein adsorption onto the surface of uncoated capillary wall can also be overwhelming. The use of capillaries semi-permanently coated with a cationic polymer (hexadimethrine bromide) was proposed to eliminate this problem [21,22]. The coating led to a positively charged surface, a reversal of the EOF, and consequently, to a change of voltage polarity and requiring acidic BGE conditions.