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Assessment of metals bound to marine plankton proteins and to dissolved proteins in seawater

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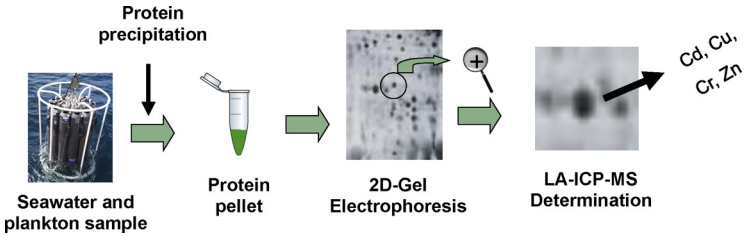
HIGHLIGHTS

- Study of the metals bound to marine proteins in seawater and marine phytoplankton.
- Use of the 2D PAGE and LA–ICP-MS in the study of marine proteins.
- The presence of metals in dissolved marine proteins was demonstrated by the first time.

GRAPHICAL ABSTRACT



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ABSTRACT

Studies based on laser ablation–inductively coupled plasma-mass spectrometry (LA–ICP-MS) have been performed to assess metal bound to dissolved proteins and proteins from marine plankton after two-dimensional polyacrylamide gel electrophoresis (2D PAGE). Dissolved proteins were preconcentrated from surface seawater (60 L) by tangential ultrafiltration with 10 kDa molecular weight cut-off (MWCO) membranes and further centrifugal ultrafiltration (10 kDa) before proteins isolation by methanol/chloroform/water precipitation. Proteins isolation from plankton was assessed after different trichloroacetic acid (TCA)/acetone and methanol washing stages, and further proteins extraction with a phenol solution. LA–ICP-MS analysis of the electrophoretic profiles obtained for dissolved proteins shows the presence of Cd, Cr, Cu, and Zn in five spots analyzed. These proteins exhibit quite similar molecular weights (within the 10–14 kDa range) and pIs (from 5.8 to 7.3). Cd, Cr, Cu, and Zn have also been found to be associated to proteins isolated from plankton samples. In this case, Cd has been found to be bound to proteins of quite different molecular weight (9, 13 and 22 kDa) and pIs (4.5, 5.2, 5.5, and 10). However, trace elements such as Cr, Cu and Zn appear to be mainly bound to plankton proteins of low molecular weight and variable pI.

1. Introduction

Plankton plays an important role in the ocean's carbon cycle. Phytoplankton in surface seawater, mainly photosynthetic cyanobacteria and single-celled algae [1], is responsible for about half the photosynthetic fixation of carbon (primary production) on Earth, and it is a primary producer of dissolved organic matter (DOM) in oceans [1,2]. DOM assessment in marine ecosystems is important because of the complexing properties of DOM for several dissolved substances which conditions the fate of trace metals and hydrophobic organic contaminants by marine biota [3].

In addition to various macro- and micro-nutrients, non-essential or toxic metals are present at low concentrations in seawater. Trace metals can be captured by phytoplankton, and introduced into the marine food chain by producing extracellular organic matter with metal complexing properties [4]. Some

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metals, such as Cu, Zn, Fe, Cr and Co, are essential micronutrients for phytoplankton and act as co-factors in different enzymatic reactions. As an example, Fe is involved in phytoplankton growth; whereas, Cu plays an important role in photosynthesis. Other metals such as Pb and Cd are non-essential or toxic. These elements may displace bioactive trace metals in enzymes and thus they can alter the proper enzymes activity. Obviously, these changes produce significant effects at the different trophic levels in the aquatic food chain [5,6]. Therefore, metal–proteins complexes and metalloproteins are an important fraction of DOM as well as important constituents in marine plankton. Metal-binding proteins with high-affinity interactions are considered as metalloproteins, while metal-binding proteins with low-affinity interactions (easily broken) are referred to metal–protein complexes. Both, metalloproteins and metal–protein complexes, can be involved in complex biochemical reactions and can participate in several biological functions [7,8]. For this reason, metal-binding proteins assessment in phytoplankton, as well as the determination and characterization of DOM and dissolved proteins in water are issues of interest.

One or two-dimensional polyacrylamide gel electrophoresis (1D or 2D-PAGE) with subsequent laser ablation inductively coupled plasma mass spectrometry (LA–ICP-MS) has been increasingly used for the analysis of metalloproteins and metal–protein complexes [9]. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is commonly used for the separation of protein mixtures on the basis of their molecular size. This technique applied in the one-dimensional mode has also been used for isolating dissolved proteins, but it fails when resolving complex protein mixtures [10–12]. In this case, 2D-PAGE is a powerful and sensitive technique for separating individual proteins from complex samples. In the first dimension, proteins are separated according to their isoelectric point (pI) by isoelectric focusing (IEF); whereas, separation according to the molecular weight (Mw) is achieved in the second dimension. 2D-PAGE has been used for characterizing Mws of both particulate and dissolved proteins in surface seawater, and proteins from marine plankton [13–15].

The choice of adequate sample preparation methods is important for obtaining reliable results by electrophoresis analysis. Different protein extraction procedures from plankton have been reported [16–19]. Jiménez et al. [16] have found that a treatment with a buffer solution consisting of 25 mM Tris–HCl and a commercial cocktail protease inhibitor, followed by protein precipitation with acetone was the most efficient procedure for protein extraction from plankton. Recently, García-Otero et al. [17] have proposed a protein extraction method for plankton which combines different washing stages with trichloroacetic acid (TCA)/acetone, and with methanol, before proteins extraction with phenol. Regarding dissolved proteins, main problems arise from the low concentrations in seawater and from the presence of high levels of dissolved salts. Therefore, pre-concentration methods for isolating the dissolved proteins from the saline matrix are needed. The literature shows that tangential ultrafiltration (UF) procedures are more adequate than solid phase extraction (SPE) methods for isolating dissolved compounds of high molecular weight such as dissolved proteins [10,11,13–15,20,21]. The selection of UF membranes of 10 kDa molecular weight cut-off guarantees the successful isolation of dissolved proteins in a minimum volume of retentate, which offers high pre-concentrations factors [22]. Further salts removal is also needed when performing chromatographic and electrophoresis analysis [22]. When dealing with dissolved protein, the formation of the protein pellets guarantees that most of dissolved salts remains in the liquid phase of the retentate. However, some protein precipitation procedures such as those based on ice-cold acetone (protein pellet formation at −20 °C for 2 h) [23] have proved to be unsuccessful for further protein separation by OFFGEL electrophoresis [24]. In these cases, trichloroacetic acid as a precipitating reagent [10,20], and combined procedures based on water and methanol soluble interferences (salts) removal before protein precipitation by chloroform [20] have offered reliable electrophoresis separations [24].

The assessment of metals bound to proteins is commonly performed by direct analysis of gels by LA–ICP-MS. In addition to the development by Jiménez et al. [16] for assessing metals bound to proteins from plankton, LA–ICP-MS methods after 1D and 2D electrophoresis have been performed for detecting P in standard phosphorylated proteins [25], for assessing Cu-, Zn- and Fe-containing human brain proteins [26], Zn-containing proteins in slug (*Genus Arion*) tissues [27], Se-containing proteins from sunflower leaves [28], Cd- and Zn-binding proteins in *Spinacia oleracea* [29], and determining Cu, Fe, Zn, Mn and Pb in metalloproteins from rat kidney [30]. Other developments involve the assessment of metal–humic acid complexes [31].

There are few developments regarding metal bound to proteins from plankton. Jiménez et al. [16] have recently applied LA–ICP-MS to assess metal bound to plankton proteins after 1D electrophoresis. However, this application has been performed with a plankton-based certified reference material (BCR-414), and the assessment of metal bound to proteins from fresh marine plankton remains un-reported. In addition, although various published papers on dissolved proteins characterization in seawater by 1D and 2D electrophoresis can be found in the literature, LA–ICP-MS developments focused on determining/identifying metals bound to dissolved proteins have not yet been addressed. The aim of the current work has been the application of LA–ICP-MS for determining metal-containing proteins from fresh marine phytoplankton and dissolved proteins in seawater. Detection/determination of trace metals associated to the isolated proteins has been performed after 2D-PAGE. Possibilities and problems found when applying the proposed methodology for assessing metals binding dissolved proteins and proteins from fresh phytoplankton are fully discussed.

2. Experimental

2.1. Apparatus

LA–ICP-MS measurements were performed with an UP-213 Nd-YAG LA system operating at 213 nm (New Wave Research, Huntingdon, UK) coupled to an ICP-MS (Elan 6000, Perkin Elmer Sciex, Toronto, Canada). Hitachi double-beam spectrophotometer model U-2010 (Hitachi, Berkshire, UK) equipped with 10 mm quartz cells was used for all UV–vis measurements. Isoelectrofocusing was performed with a Protean IEF System from Bio-Rad (Hercules, CA, USA), and second dimension was run in a Protean XL (BioRad). The gels were vacuum dried before LA–ICP-MS measurements with a model 583 gel drier (Bio-Rad). The tangential flow ultrafiltration (UF) system consisted of a Masterflex I/P pump (Millipore, Bedford, MA, USA), a Prep/Scale-TFF Cartridge (Millipore) with a polyethersulfone membrane (nominal Mw cut-off 10 kDa), and a Pre/Scale-TFF Holder (Millipore) equipped with a pressure gauge. Centrifugal ultrafiltration was performed with an Alresa Digtor centrifuge (Madrid, Spain). Other laboratory devices were an ultracentrifuge Laborzentrifugen model 2K15 (Sigma, Osterode, Germany), a centrifuge Centromix (Selecta, Barcelona, Spain), a Reax top shaker from Heidoph (Schwabach, Germany), and an Basic 20 pH-meter with a glass–calomel electrode (Crison Instruments S.A., Barcelona, Spain).

2.2. Reagents and material

Ultrapure water, resistance 18 MΩcm, was obtained from a Milli-Q water-purification system (Millipore). Centrifugal