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A structure based plasma protein pre-fractionation using conjoint immobilized metal/chelate affinity (IMA) system

Karan Karkra, Kishore K.R. Tetala, M.A. Vijayalakshmi*

Advanced Centre for Bioseparation Technology (CBST), VIT University, Vellore, Tamilnadu 632014, India

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ABSTRACT

The potential of immobilized metal/chelate affinity (IMA) in a continuous fashion, referred as conjoint approach, to pre-fractionate plasma proteins (in their native state) prior to LC–MS analysis was investigated in this study. Four transition metal-ions (Co (II), Zn (II), Ni (II) and Cu (II)) were individually chelated with IDA (iminodiacetic acid) coated CIM (Convective Interaction Media) disks and placed in a single housing in the following sequential order: IDA-Co (II) → IDA-Zn (II) → IDA-Ni (II) → IDA-Cu (II). The rationale behind this order is to retain proteins based on their specific requirement for surface exposed histidine topography. This structural pre-fractionation hypothesis was successfully proven using four human plasma proteins (fibrinogen, IgG, transferrin, and albumin) with varying histidine topographies. This conjoint IMA pre-fractionation strategy not only fractionated proteins (from plasma) based on their native surface histidine topography, but also identified 157 proteins from human plasma. The advantage of our conjoint IMA is its ability to fractionate proteins in their native state and reduce plasma complexity in a single step by employing single buffer system.

1. Introduction

In clinical proteomics, human blood plasma is the conventional candidate for biomarker studies as it traverses through all major body tissues thereby representing the entire body spectrum and ease of accessibility [1,2]. Plasma constitutes 60 – 90 mg/mL of total protein mass out of which 99% are represented by 22 high abundant proteins. Importantly, both albumin (HSA) & immunoglobulins (IgG's) constitute to nearly 75 – 80% of the mentioned 99% protein mass while other middle and low abundant proteins range from milli to zeptomolar levels [3]. This wide dynamic range of protein abundance that spans more than 10–12 orders of magnitude logically increases plasma complexity and enhances the challenge in finding potential biomarkers, often low abundant proteins [4]. In order to unmask the middle/low abundant proteins, sample pre-fractionation and immunodepletion strategies are an ideal choice. In literature, several pre-fractionation methodologies based on multidimension liquid affinity chromatography (AC) do exist [5–12]. Although they possess several widely known advantages, disadvantage like requirement of different binding and desorption conditions limit the possibility to design a continuous single system based on these AC fractionation methodologies. Currently, there is a need for simple continuous pre-fractionation technologies to unravel proteins and structural variations caused due to post translational modifications, pathologies etc.

Immobilized metal/chelate affinity (IMA), introduced by Porath et al. in 1975 [13], separates proteins and peptides based on electron donor-acceptor interactions between surface exposed amino acid, Histidine (His), with "intermediate transition metal ion(s)-chelating agent" complex present on a chromatography stationary phase [14]. This interaction occurs at near neutral pH and require high salt concentrations to overcome electrostatic interactions (Fig. 1A; mode of interaction) [15]. Protein elution is achieved using a competing eluting agent (e.g. imidazole) or by lowering buffer pH (protonation). Sulkowski et al. postulated the following ground rules for interaction of proteins/peptides with various transition metal-ions [15–19].

1. Both IDA-Co (II) and IDA-Zn (II) will retain proteins with surface exposed "His-clusters" only i.e. "-His-$(X)_n$-His- (n = 2, 3)" positioned in an α-helix section
2. IDA-Ni (II) will retain proteins that possess at least two surface exposed "His" residues and are even randomly distributed
3. IDA-Cu (II) will retain proteins even with single surface exposed "His" as well as multiple "His" and "His-clusters" also. Its affinity increases with increasing surface-accessible "His" number

* Corresponding author.
E-mail address: indviji@yahoo.com (M.A. Vijayalakshmi).

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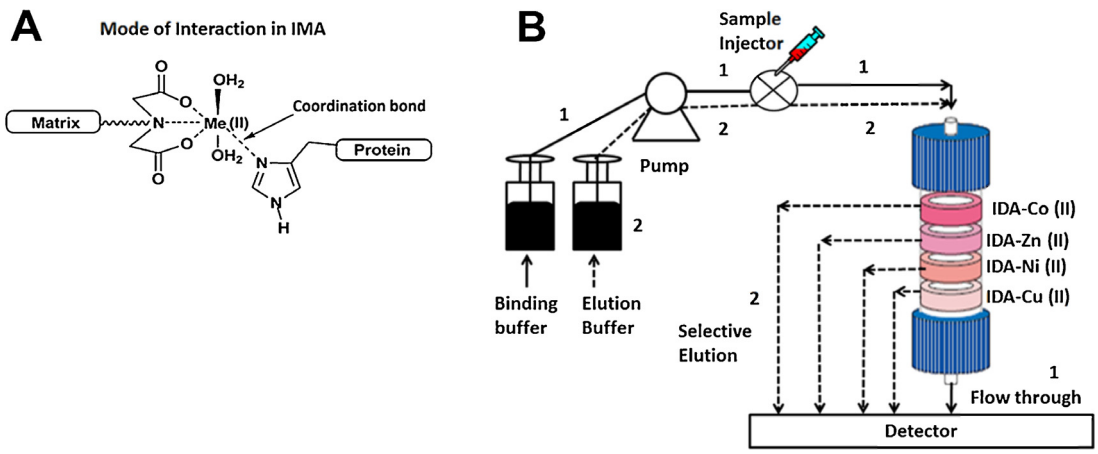

Fig. 1. Schematic representation of A) interaction mode of metal/chelate with surface exposed Histidine and B) design of Conjoint IMA system for protein fractionation, where "1" represent protein adsorption in a single step and "2" represent protein elution from each disk individually.

4. The metal-ions will not retain any protein if the "His" is not surface exposed or no "His" is present within the protein structure (e.g. duck lysozyme)

Based on these fundamental rules of IMA, we designed, in this study, a continuous pre-fractionation device, which we refer to as conjoint IMA system, by placing the four metal-ion chelated IDA matrix i.e. CIM (Convective Interaction Media) disk in a single housing as shown in Fig. 1B: IDA-Co(II) → IDA-Zn (II) → IDA-Ni (II) → IDA-Cu (II). The rationale to choose CIM disks (over chromatography based resins) is due to their proven immense advantages [20–22] and the possibility to place the four metal/chelate disks in a single housing that limit sample dilution during protein pre-fractionation process. The developed conjoint IMA successfully fractionated plasma proteins based on surface exposed "His" surface topography in a single step using a single buffer system.

2. Material and methods

2.1. Chemicals and reagents

Ammonium bicarbonate, copper (II) sulphate pentahydrate, cobalt (II) chloride hexahydrate and imidazole were obtained from Merck (USA). Glycine, sodium hydroxide (NaOH), ethylene diamine tetra acetic acid disodium salt (EDTA.disodium salt), sodium chloride (NaCl), and sodium acetate were procured from Sisco Research Laboratories (Mumbai). Zinc (II) sulphate heptahydrate, nickel (II) sulphate hexahydrate, guanidium hydrochloride (GnCl), morpholinopropane sulfonic acid (MOPS), morpholinoethane sulfonic acid (MES), dithiothretol (DTT), iodoacetamide, trypsin from porcine, sodium phosphate monobasic and brilliant blue G (Bradford estimation), human serum albumin (HSA), human IgG (Cohn fraction II & III), human fibrinogen, and human transferrin were purchased from Sigma-Aldrich (India). HPLC grade formic acid (FA) and LC–MS grade acetonitrile containing 0.1% FA were purchased from Fluka analytical (India). CIM-IDA disks (12 mm × 3 mm, 0.34 mL) were generously gifted by BIA separations d.o.o. (Ljubljana, Slovenia). Macrosep® advance centrifugal device 3 kDa filters were purchased from Pall life Sciences (India). Milli Q water used in all the experiments was obtained from MilliQ water purification system of Millipore (Bedford, USA). All other chemicals and reagents used were of analytical grade. All experiments performed in study were done in triplicates.

2.2. Instrumentation

Charging of IDA-CIM disks with intermediate transition metal-ions Co (II), Zn (II), Ni (II) and Cu (II) was done using AKTA FPLC system (Amersham Bioscience, Uppsala, Sweden). Fractionation experiments were performed using AKTA FPLC system consisting of a P-920 pump connected to an injector with 500 μL loop and a UPC-900 monitor to measure UV absorption (wavelength set at 280 nm), pH and conductivity. Off-line protein determination was performed using Beckman Coulter DU® 730 spectrophotometer (Fullerton, CA, USA). For SDS-PAGE analysis, Bio-Rad Mini-PROTEAN system (Hercules, CA, USA) was used.

2.3. Human plasma

Blood (10 mL) was collected from a single healthy individual into a tube containing EDTA as an anticoagulant (1.5 mg/mL) followed by centrifugation at 1000*g* for 10 min at 4 °C. The obtained supernatant i.e. plasma was collected and aliquots of 500 μL were stored at −80 °C until further use. Protein concentration of the above plasma (~60 mg/mL) was determined using Bradford assay (UV–vis spectrophotometer at a wavelength of 595 nm). Human Serum Albumin (HSA) was used as a reference protein.

2.4. Preparation of metal/chelate CIM disks

IDA-CIM disks (column volume: 0.34 mL) were charged with individual intermediate transition metal-ions (Co (II) from Cobalt chloride hexahydrate; Zn (II) from Zinc sulphate hexahydrate; Ni (II) from Nickel sulphate hexahydrate and Cu (II) from copper sulphate pentahydrate) as follows: The CIM-IDA disk were first equilibrated with 40 mL milliQ water for 40 min with a flow rate of 1 mL/min. Subsequently, the disks were washed with 50 mL aqueous solution containing 100 mM intermediate transition metal-ions at a flow rate of 1 mL/min and with a final milliQ water wash to remove unbound metal-ions from the disks.

2.5. Conjoint IMA CIM device

2.5.1. Model proteins: individual vs mixture

Four human plasma proteins (fibrinogen, IgG, transferrin and albumin) were used individually and in a mixture at a concentration of 100 μg/100 μL in binding buffer (20 mM MES + 20 mM MOPS + 20 mM sodium acetate + 0.5 M NaCl; pH 7). The four metal/chelate CIM disks were placed in a single housing (Fig. 1B) in the following sequential