Short communication

Anti Zn antibodies: Cross reactivity and competitive binding with heavy metals[☆]

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Abstract

Monoclonal antibodies of IgM class, specific to IDA-Zn were used for evaluating their Zn^{2+} binding efficiency in the presence of trace metal ions such as Cr^{3+} Cr^{6+}, Cu^{2+} and Cd^{2+}. In the present work, antibody raised against the hapten IDA-Zn(II) was pre-incubated with different metal ions and the binding capacity to the specific hapten was tested using ELISA and immobilized metal ion affinity chromatography (IMAC) techniques. IMAC was carried out with the free antibody and antibody pre-incubated with selected heavy metal ions using Sepharose IDA-Zn^{2+} column and the same samples were tested using a hapten specific ELISA with non-protein hapten carrier. Different effects were observed after pre-incubation with metal ions. Cr^{3+} exhibited synergistic binding where as antagonism was detected with Cd^{2+}. The synergistic effect observed with Cr^{3+} suggests involvement of binding sites other than that of zinc and conformational changes that result from Cr^{3+} binding. It is probable that, this binding event also increases the accessibility of the zinc binding sites on IgM. On the same lines, the antagonism observed with Cd^{2+} could be attributed to structural changes resulting in reduced accessibility to zinc binding sites. In case of Cr^{6+}, no appreciable change in binding to IDA-Zn was observed while Cu^{2+} showed competitive binding.

1. Introduction

Metal catalyzed enzymatic reactions are widely known biochemical reactions, especially the synthetic organic reactions [1]. In organic synthesis, metal catalyzed reactions compete with enzymes in efficiency and ability to control the stereo chemical outcome [2,3]. Thus incorporation of metal-binding sites into proteins is a step towards construction of metalloenzymes. Most metal-binding sites have been incorporated into proteins to impart stability [4], regulate activity [5], aid in purification [6] or mediate their assembly [7].

Systematic studies on the composition of metal coordination site provide valuable information concerning the metals and amino acid residues involved in biological functions. Metals such as Zn, Cu, etc., were identified for their role in substrate activation and catalysis. In addition, the binding of metal ions is shown to improve the structural stability of proteins. Histidine and cysteine residues are known to be involved in the binding of catalytically important metal ions [8].

Using the complementarities of (a) IDA-Zn(II) binding sites HisXXXHis [9] and (b) the Ag-Ab recognition, anti IDA-Zn(II) IgM antibodies were prepared and studied using ELISA and immobilized metal ion affinity chromatography (IMAC) methods. In addition the influence of second metal binding on this Ab-Ag [IDA-Zn(II)] recognition was studied. Using the elution pattern on IMAC column, the possibility of any structural change induced by metal ion binding was also explored. To achieve this, using ELISA and IMAC, differential binding of IgM to IDA-Zn

on prior incubation of the antibody with Cr^{3+}, Cr^{6+}, Cu^{2+} and Cd^{2+} was studied.

2. Experimental

2.1. Chemicals and reagents

Bovine serum albumin (BSA) was purchased from Aldrich Chemical Co., St. Louis, MO, USA. Iminodiacetate (IDA), horseradish peroxidate (HRP), *O*-phenylene diamine dihydrochoride, buffer chemicals and EDTA were purchased from Sigma Chemical Co., Steinheim, Germany. Avidin was purchased from Biosys, Canada. All the experiments were conducted using Millipore water.

2.2. Sample preparation

Biotin-PEG-IDA (BPI) was synthesized as reported by Ehteshami et al. [10]. Anti IDA-Zn(II) monoclonal antibodies were prepared by Dr. Nedonchelle [11a] and the ascite fluid of the stable clone 8D27 containing IgM was used for this study without further purification. Ascite fluid has effectively been used previously for establishing the absence of non-specific binding to the hapten and by the present non-protein carrier ELISA method. The clones with specific paratopes to IDA-Zn(II) were selected [11b]. One hundred microlitre of $2\,\mu g/mL$ of ascite fluid was pre-incubated with two different concentrations of the metal ion (10 and $50\,\mu M$). Control consisted of ascite fluid without metal pre-incubation.

2.3. Methods

2.3.1. ELISA procedure

ELISA was carried out as reported by Nedonchelle et al. [11b]. Briefly, Avidine DX (Biosys) was diluted with coating buffer (50 mM carbonate buffer, pH 9.5) to $10\,\mu g/mL$ and $100\,\mu L$ of this was used for coating the well. Incubation was carried out for 1 h at 37 °C. It was then washed with phosphate buffer saline (PBS) of pH 7.0. Bovine serum albumin ($200\,\mu L$ of 1% BSA) was used for blocking at 4 °C overnight and washed with PBS–Tween-20 mixture. The hapten carrier conjugate (Zn-IDA-PEG-Biotin) was obtained by mixing $100\,\mu M$ BPI in 10 mL of 100 mM zinc sulfate solution and incubating for 2 h at 37 °C. One hundred microlitre of this mixture was directly added to each well and incubated for 90 min at 37 °C. It was then washed with PBS-T. Ascite fluid containing IgM (without pre-incubation and pre-incubated with different metal ions) was added to each well and incubated at 37 °C for 2 h and washed with PBS-T. This was followed by addition of $100\,\mu L$ of HRP-secondary antibody and incubated at 37 °C for 1 h and washed with PBS-T. To each well, $100\,\mu L$ of 3 mM *O*-phenylene diamine dihydrochloride was added and incubated at room temperature for 30 min in dark. The reaction was stopped by adding $100\,\mu L$ of 2N HCl. The ELISA readings were taken at 490 nm. Each reaction was carried out in triplicate and mean of these readings was used for conclusions and obtaining the graph (Fig. 1).
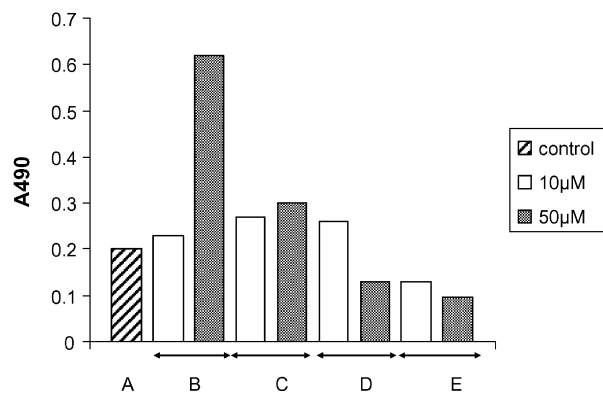

Fig. 1. Effect of a second metal ion on the binding efficiency of Zn^{2+} to the anti IDA-Zn(II) IgM antibodies by ELISA. Ascite fluid ($100\,\mu L$ of $2\,\mu g/mL$) was pre-incubated with two different concentration of the metal of interest ($10\,\mu M$, $50\,\mu M$). Control consisted of ascite fluid without metal pre-incubation. ELISA was carried out as described in Section 2. (A) Control; (B) Cr^{3+}; (C) Cr^{6+}; (D) Cu^{2+}; (E) Cd^{2+}.

2.3.2. Chromatographic procedure on Sepharose-IDA-Zn^{2+}

Sepharose was coupled with IDA [12]. Two microlitre of chelating Sepharose fast flow was packed into column (10 cm length × 1 cm internal diameter). The column was loaded with 50 mM $ZnSO_4$ at flow rate of 0.5 mL/min. Unbound Zn^{2+} was washed by using five column volumes of water, followed by five column volumes of 100 mM acetate buffer pH 4.0 containing 0.5 M NaCl. The gel was equilibrated with 10 column volumes of phosphate buffer in 0.5 M NaCl (pH 7.0). One hundred microlitre of $2\,\mu g/mL$ of ascite fluid pre-incubated with $10\,\mu M$ Cr^{3+} and Cd^{2+}, respectively, was directly introduced into the Sepharose-IDA-Zn column in two separate experiments. Elution was done employing discontinuous pH gradient with 50 mM phosphate buffer in 0.5 M NaCl at pH 7.0, 6.5 and 6.0, 100 mM acetate buffer in 0.5 M NaCl with pH 5.0 and 4.0. Fractions (1 mL) were collected and the absorbance of each of these fractions was monitored at 280 nm. The protein content was estimated by Bradford method using BSA as standard. The column was stripped with 50 mM EDTA. The experiment was repeated twice and the average was used to derive the graph (Fig. 2). Mab activity was shown by 2nd *Y*-axis (Fig. 2).

3. Results and discussion

Many factors are known to affect the protein–metal ion interaction [13–15]. In order to study the specific binding of the hapten IDA-Zn to the anti IDA-Zn monoclonal antibodies and any competitive/synergic binding of the metals, different metal ions such as chromium (hard acid), copper (borderline acid) and cadmium (soft acid) were chosen.

3.1. ELISA experiments

Effect of antibodies incubated with different metal ions on its binding to the biotin-PEG-IDA-Zn (BPI-Zn) is given in Fig. 1. Results indicate that, when antibody was incubated with Cr^{3+} at $10\,\mu M$ concentration, binding with IDA-Zn was almost same as
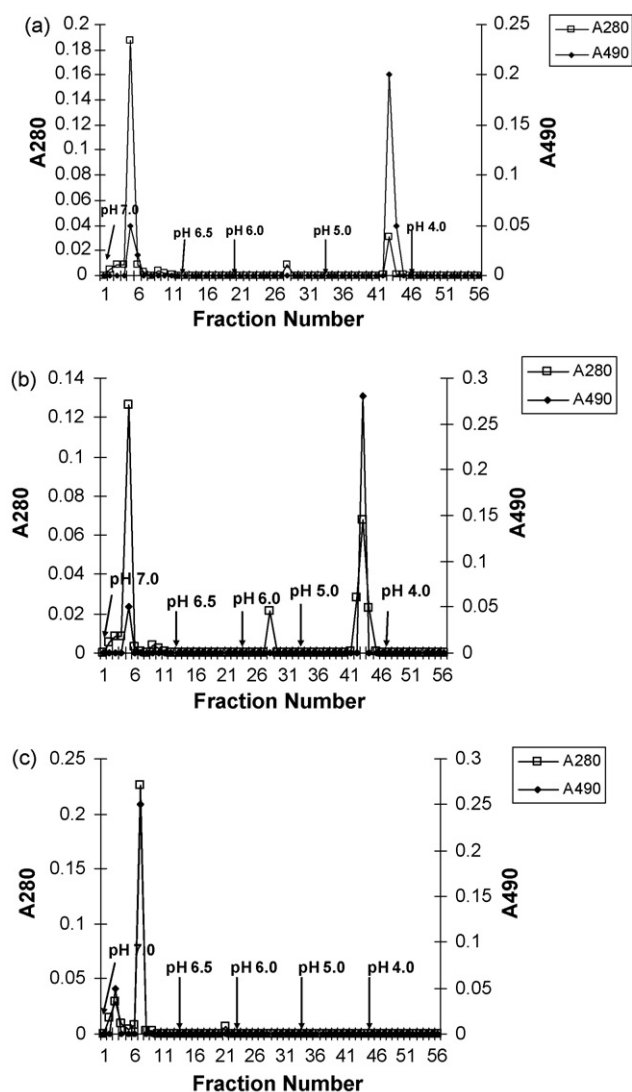
Fig. 2. IMAC profiles of anti IDA-Zn(II) IgM antibodies onto Sepharose-IDA-Zn^{2+} column. Ascite fluid (100 μL of 2 μg/mL) pre-incubated with 10 μM of the metal ion of interest. This mixture was loaded onto 2 mL IMAC column and chromatography was carried out as described in Section 2. Control consisted of ascite fluid without metal pre-incubation. Mab activity by ELISA was shown in 2nd Y-axis. (a) Control; (b) Cr^{3+}; (c) Cd^{2+}.

initial binding. IgM incubated with Cr^{3+} at a concentration of 50 μM, binding with IDA-Zn is increased (Fig. 1). It is known that hard acceptors effectively bind hard donors and soft acceptors prefer soft donors to form stable compounds [15,16]. Cr^{3+} ions being hard acid, prefers hard bases such as COO^-, i.e. amino acids containing carboxyl groups such as aspartic acid, glutamic acid, etc. Binding sites of Cr^{3+} on antibody are different from zinc binding sites, which mostly constitute histidine moieties. Consequently, Cr^{3+} may bind to IgM at different binding sites. This binding might induce structural changes, increasing accessibility to zinc binding sites. IgM incubated with Cr^{6+} at 10 and 50 μM concentrations showed no appreciable change in IDA-Zn binding (Fig. 1), indicating that Cr^{6+} did not bring any significant structural changes in IgM. This differential effect could be attributed to the change in oxidation state of the metal

ion which might in turn affect its ligand choice and emphasizes the importance of steric factors in addition to coordination geometry and coordination number in metal ion binding.

In case of IgM incubated with 10 μM concentration of Cu^{2+}, binding with IDA-Zn remains the same as that of the control but in case of IgM incubated with 50 μM concentration of Cu^{2+}, binding of IgM with IDA-Zn was reduced (Fig. 1). This could arise from competition between Cu^{2+} and Zn^{2+} ions for histidine moieties at higher concentration. Cu^{2+} is known to bind to nitrogeneous bases particularly the histidine side chains.

In case of IgM incubated with Cd^{2+}, with increase in concentration of Cd^{2+}, there was a corresponding decrease in zinc binding (Fig. 1). Cd^{2+} is a soft Lewis acid (large polarisability) and shows high affinity to soft Lewis bases such as the sulfhydryl side chains of cysteine. Due to this property, Cd^{2+} may bind to SH group of IgM which in turn could induce structural changes resulting in the antagonism observed.

3.2. Immobilized metal ion affinity chromatography experiments

Results described above using ELISA, lead to the hypothesis that Cr^{3+} and Cd^{2+} have different binding sites on IgM and exhibited synergic and antagonistic effect, respectively. Here, antibody incubated with Cr^{3+} was loaded onto the Sepharose-IDA-Zn^{2+} column and relatively higher amounts were eluted at pH 5.0.

In the second set, IgM incubated with Cd^{2+} was loaded onto the IDA-Zn column and no binding was observed (Fig. 2c). This correlates the results obtained using ELISA. Control IMAC of IgM on IDA-Zn without any metal ion pre-incubation is comparatively depicted in Fig. 2a. Mab activity by ELISA was presented in the 2nd Y-axis to indicate the peaks in the chromatogram corresponding to IgM (Fig. 2a–c).

In conclusion, the synergic effect observed with Cr^{3+} suggests the involvement of different binding sites associated with structural perturbations. Cd^{2+} exhibited antagonism clearly indicating occlusion of the zinc binding sites in the antibody. In the case of copper, competitive binding was observed.

Acknowledgements

References

[1] J. Tainer, V. Roberts, E. Getzoft, Curr. Opin. Biotechnol. 2 (1991) 582.
[2] R. Noyori, Science 248 (1990) 1194.
[3] G. Crispino, P.Ho.K. Sharpless, Science 259 (1993) 64.
[4] S. Toma, S. Campagnol, I. Margaret, R. Gianna, G. Grandi, M. Bolognesi, V. De Fillippis, A. Fontana, Biochemistry 30 (1991) 97.
[5] J. Higaki, B. Haymore, S. Chen, R. Fletterick, C. Craik, Biochemistry 29 (1990) 8582.

[6] M. Smith, J. Cook, T. Furman, P. Gesellehen, D. Smith, H. Hsiung, in: M. Ladisch, R. Willison, C. Painton, S. Builder (Eds.), Protein Purification, American Chemical Society, Washington, DC, 1990, p. 168.

[7] M. Ghadiri, C. Soares, C. Choi, J. Am. Chem. Soc. 114 (1992) 4000.

[8] B.L. Vallee, D.S. Auld, Biochemistry 29 (1990) 5647.

[9] E. Sulkowski, Bioessays 10 (1989) 170.

[10] G. Ehteshami, J. Porath, R. Guzman, J. Mol. Recognit. 9 (1996) 733.

[11] (a) E. Nedonchelle, Ph.D. Thesis, LIM Tech., Universite de Technologie, Compiegne, France, 2000;

(b) E. Nedonchelle, C. Leduc, X. Garcia, R. Guzman, M.A. Vijayalakshmi, Ann. N. Y. Acad. Sci. 864 (1998) 106.

[12] V. Boden, C. Colin, J. Barbet, J.M. le Doussal, M.A. Vijayalakshmi, Bioconjugate Chem. 6 (1995) 373.

[13] D. Ghosh, V.L. Pecoraro, Curr. Opin. Chem. Biol. 9 (2005) 97.

[14] H.Y. Shrivastava, A. Dhathathreyan, B.U. Nair, Chem. Phys. Lett. 369 (2003) 534.

[15] R.G. Pearson, J. Chem. Educ. 45 (1968) 581.

[16] R.G. Pearson, J. Chem. Educ. 45 (1968) 643.