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Thermodynamics of binding copper ion by myelin basic protein

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Abstract

The interaction of myelin basic protein (MBP) from bovine central nervous system with divalent copper ion was studied by equilibrium dialysis and isothermal titration calorimetry techniques at 27 °C in Tris buffer solution at pH = 7.2. MBP has two binding sites for copper ion. The intrinsic association equilibrium constants are 0.083 and 1.740 μM^{-1} in the first and second binding sites, respectively. Hence, occupation of the first site has produced an appreciable enhancement 21 of the binding affinity of the second site. A new representation of titration calorimetric data, as well as, the Scatchard plot, shows positive cooperativity in two binding sites for copper ions. The molar enthalpies of binding are −13.5 and −14.8 kJ mol^{-1} in the first and second binding sites, respectively. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Myelin basic protein; Copper; Metal binding; Isothermal titration calorimetry

1. Introduction

Myelin basic protein (MBP) is one of the most important proteins of the myelin sheath [1,2]. Various aspects of MBP (MW = 18 500), including its immunological properties have been summarized in several reviews [3–5]. The structure of MBP, interaction of this protein with other molecules, particularly lipids, the influence of other molecules on the structure of the protein and the nature of its association with the myelin membrane have been reviewed by Smith [6].

In aqueous solution, the thermodynamically stable state of MBP is a flexible coil in which the protein contains about 20% β-sheet secondary structure [7,8]. MBP lacks both the disulfide bonds and the prosthetic groups which stabilize the folded form of many globular proteins, and it contains an unusually large number of positively charged amino acid residues spread throughout the polypeptide chain which will generate strong intramolecular electrostatic repulsions. Hence, MBP has a little secondary structure [6].

Contrary to the effect of Ca^{2+}, 0.1–1.0 mM Zn^{2+}, Co^{2+}, or Cu^{2+} inhibits dissociation of MBP from the membrane [9]. The mechanism by which these ions exert this effect has not been established, but these results showed the complex ion–protein and ion–lipid interactions which may occur within myelin. Binding of Cd, Co, Cu, Hg, Mn, Pb, Zn, Ca and Mg ions by isolated MBP of bovine central nervous system (CNS) have been recently assessed by centrifugal equilibrium dialysis [10]. These metal ions were bound in the order of Hg > Cu > Zn > Mg > Cd > Co, exempting Mn, Pb and Ca.

The current investigation focuses on the interaction between MBP and copper ions by equilibrium dialysis and isothermal titration calorimetry methods. A new presentation for calorimetric data analysis according

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to the Scatchard plot [11–13] was also introduced and applied in the present work.

2. Experimental

2.1. Materials

MBP from bovine CNS and Tris–HCl were obtained from Sigma Chemical Co. Copper nitrate was purchased from Merck Co. All other materials and reagents were of analytical grades, and solutions were made in double-distilled water. Tris–HCl solution with 30 mM concentration, pH = 7.2, was used as a buffer.

2.2. Methods

2.2.1. Equilibrium dialysis

Experiments were carried out at 300 K using an MBP solution with a concentration of 0.25 mg ml^{-1}, of which 2 ml aliquots were placed in dialysis bags and equilibrated with 2 ml of the copper solution, covering the required concentrations range for over 96 h. Corrections for inequalities arising from Donnan effects were negligible at the ionic strength used. The free copper concentrations in equilibrium with complexes of MBP–copper were assayed by the atomic absorption (Perkin-Elmer, model 603) method. The molecular weight of MBP was taken to be 18 500.

2.2.2. Isothermal titration calorimetry

The isothermal titration micro-calorimetric experiments were performed with the four-channel commercial micro-calorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. Each channel is a twin heat-conduction calorimeter where the heat-flow sensor is a semi-conducting thermopile (multi-junction thermocouple plates) positioned between the vessel holders and the surrounding heat sink. The insertion vessel was made from stainless steel. Copper solution (100 μM) was injected by use of a Hamilton syringe into the calorimetric stirred titration vessel, which contained 1.8 ml MBP, 0.25 mg ml^{-1}, in Tris buffer (30 mM), pH = 7.2. Thin (0.15 mm i.d.) stainless steel hypodermic needles, permanently fixed to the syringe, reached directly into the calorimetric vessel. Injection of copper solution into the perfusion vessel was repeated 21 times, and each injection included 35 μl reagent, except the last injection that was 350 μl. The calorimetric signal was measured by a digital voltmeter that was part of a computerized recording system. The heat of each injection was calculated by the "Thermometric Digitam 3" software program. The heat of dilution of the copper solution was measured as described above except MBP was excluded. The enthalpy of dilution was subtracted from the enthalpy of MBP–copper interaction. The enthalpy of dilution of MBP is negligible. The micro-calorimeter was frequently calibrated electrically during the course of the study.

3. Results and discussion

Fig. 1 shows the Scatchard plot, $v/[Cu^{2+}]_f$ versus v, where $[Cu^{2+}]_f$ is the free concentration of copper ion and v defined to be moles of bound copper ions per mole of total MBP. The shapes of the Scatchard plots are clearly characteristic of different types of cooperativity. A concave downward curve, as shown in Fig. 1, describes a system with positive cooperativity. For obtaining approximated values of binding parameters, it might be possible to fit the binding data to Hill equation [14]

$$v = \frac{g(K[Cu^{2+}]_f)^n}{1 + (K[Cu^{2+}]_f)^n} \quad (1)$$

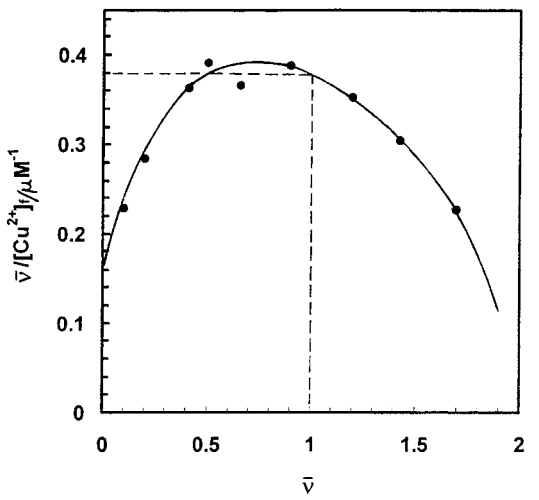

Fig. 1. The Scatchard plot of binding copper ion by MBP at 27 °C. The best-fit curve of the experimental binding data was transformed to a Scatchard plot using Eq. (1) with $g = 2$, $K = 0.38\ \mu M^{-1}$ and $n = 1.6$.

where g, K and n are the number of binding sites, binding constant, and Hill coefficient, respectively. The binding data for the binding of copper ions to MBP have been fitted to the Hill equation using a computer program for non-linear least-square fitting [15]. The results are $g = 2$, $K = 0.38\ \mu M^{-1}$ and $n = 1.6$. The best-fit curve of the experimental binding data was then transformed to a Scatchard plot as shown in Fig. 1. A simple method for calculating intrinsic association equilibrium constants for system with two cooperative sites (K_1 and K_2) has been introduced from the Scatchard plot [16]. It has been shown that, in the limit as v approaches 0, $v/[Cu^{2+}]_f = 2K_1$ and when $v = 1$, or at half-saturation, $v/[Cu^{2+}]_f = (K_1K_2)^{1/2}$. Thus, K_1 can be obtained from the ordinate intercept of a Scatchard plot and K_2 is derived from the value of $v/[Cu^{2+}]_f$ at half-saturation. The results obtained from Fig. 1 are $K_1 = 0.083\ \mu M^{-1}$ and $K_2 = 1.740\ \mu M^{-1}$. So, occupation of the first site has produced an appreciable enhancement 21 of the binding affinity of the second site. The Hill coefficient can also be obtained from the inverse of unoccupied fraction of sites at the maximum point of the Scatchard plot [12]

$$n = \frac{1}{1 - (v_{max}/g)} = \frac{1}{1 - (0.75/2)} = 1.6 \quad (2)$$

where v_{max} is the v value at the maximum point of Scatchard plot.

The raw data obtained from isothermal titration calorimetry of MBP interaction with copper ion was shown in Fig. 2. Fig. 2(a) is showing the heat of each injection and Fig. 2(b) is showing the heat of related to each total concentration of copper ion, $[Cu^{2+}]_t$. These raw calorimetric data can be used to show the heat of binding copper ions per mole of MBP (ΔH) versus total concentration of copper ions (Fig. 3(a)) or versus moles of bound copper ions per mole of total MBP (v) (Fig. 3(b)). Fig. 3(b) was obtained from the combination of data in Figs. 1 and 3(a) using Eq. (3)

$$[Cu^{2+}]_t = [Cu^{2+}]_f + [Cu^{2+}]_b = [Cu^{2+}]_f + v[MBP]_t \quad (3)$$

where $[Cu^{2+}]_b$ is the bound concentration of copper ion and [MBP] is the total concentration of MBP. Fig. 3(b) shows that the molar enthalpies of binding

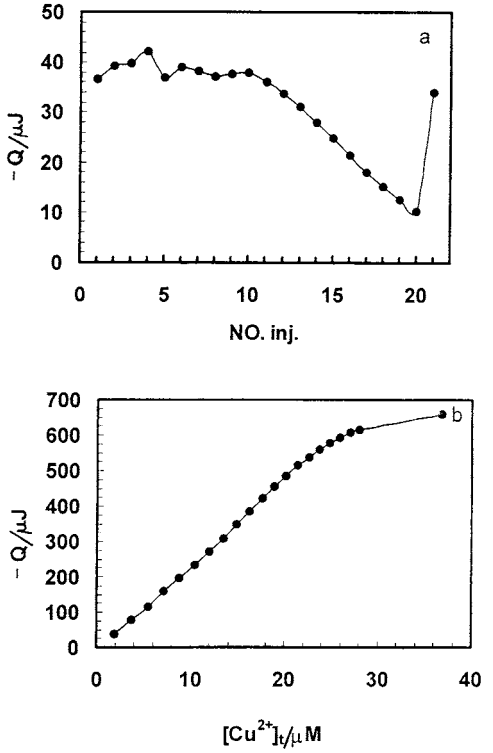

Fig. 2. (a) The heat of copper binding on MBP for 21 automatic cumulative injections, each of 35 μl, of copper, 70 μM, into the sample cell containing 1.8 ml MBP solution at a concentration of 0.25 mg ml^{-1}. The last injection was 350 μl. (b) The heat of binding vs. total concentration of copper ion, calculated from (a).

are -13.5 and -14.8 kJ mol^{-1} in the first and second binding sites, respectively.

A new representation of titration calorimetric data, very similar to the Scatchard plot (replacing v by Q, because of $Q \propto v$) has been shown in Fig. 4. We have recently reported such representation of titration calorimetric data; however, all plots were linear [17,18] indicating non-cooperative systems in ligand binding. Now, a concave downward curve, as shown in Fig. 4, describes a system with positive cooperativity in copper ions binding to MBP. Also, the Hill coefficient can be obtained using Eq. (4)

$$n = \frac{1}{1 - (Q_{max}/Q')} = \frac{1}{1 - (260/690)} = 1.6 \quad (4)$$

This equation is very similar to Eq. (2), which Q_{max} and Q' are heat values at the maximum point and

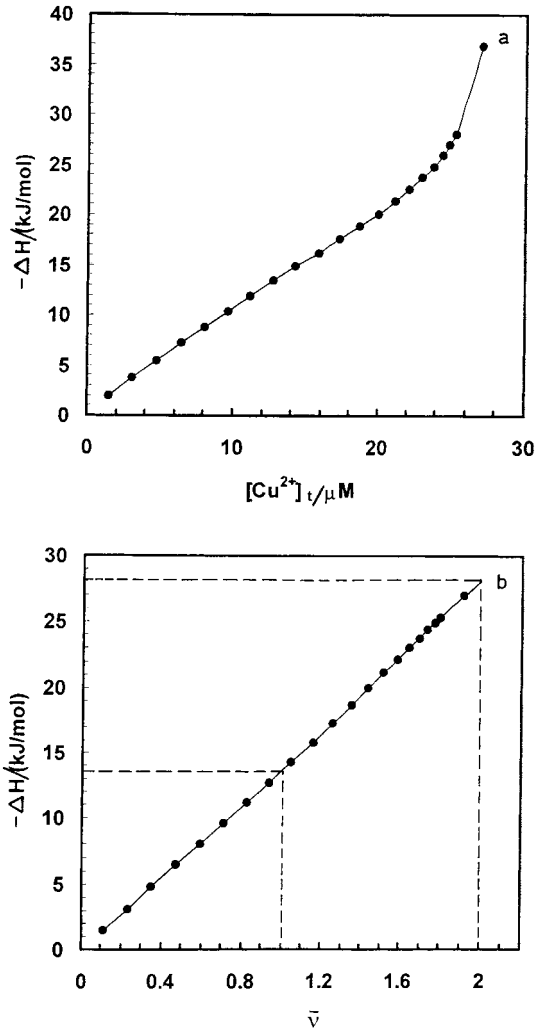

Fig. 3. (a) The heat of binding copper ions per mole of MBP (ΔH) vs. total concentration of copper ions, calculated from Fig. 2(b). (b) The heat of binding copper ions per mole of MBP (ΔH) vs. moles of bound copper ions per mole of total MBP (ν), calculated from (a) and Eq. (3).

horizontal-intercept of pseudo-Scatchard plot (in μJ), respectively.

It is concluded that MBP has two positive cooperative binding exothermic sites for copper ion. Occupation of the first site is produced an appreciable enhancement 21 of the binding affinity of the second site. A new representation of isothermal titration calorimetric data as a pseudo-Scatchard plot, which is used in this system, can be developed for other

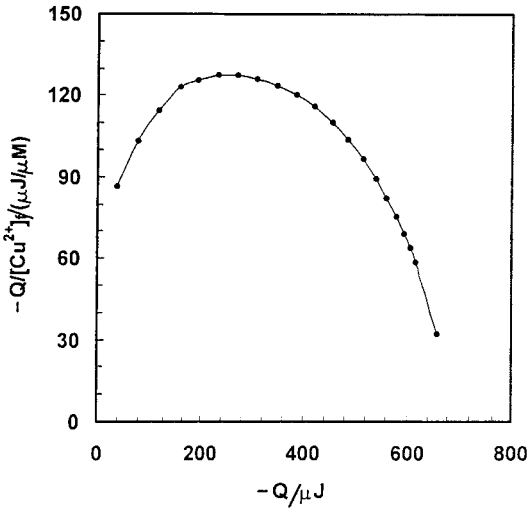

Fig. 4. A new representation of titration calorimetric data, very similar to the Scatchard plot, shows positive cooperativity in two binding sites for copper ions.

systems to find the type of cooperativity in the binding and calculating of the Hill coefficient.

Acknowledgements

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