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Thermodynamics of binding of a sulfonamide inhibitor to metal-mutated carbonic anhydrase as studied by affinity capillary electrophoresis

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ABSTRACT

By affinity capillary electrophoresis (ACE), the thermodynamic binding constants of a sulfonamide (SA) inhibitor to bovine carbonic anhydrase II (CA) and metal mutated variants (M-CAs) were evaluated. 1-(4-Aminosulfonylphenylazo)-2-naphthol-6,8-disulfonate was used as the SA in the electrophoretic buffer for ACE. The Scatchard analysis of the dependence of the electrophoretic mobility of native CA on the SA concentration provided the binding constant to be $K_b = (2.29 \pm 0.05) \times 10^6\ M^{-1}$ (at pH 8.4, 25 °C). On the other hand, apoCA showed far smaller value [$K_b = (3.76 \pm 0.14) \times 10^2\ M^{-1}$], suggesting that the coordination of SA to the Zn^{II} center controlled the binding thermodynamics. The ACE of M-CAs showed the same behaviors as native CA but with different K_b values. For example, Co–CA adopting the same tetrahedral coordination geometry as native CA exhibited the largest K_b value [$(2.55 \pm 0.05) \times 10^6\ M^{-1}$] among the M-CAs. In contrast, Mn– and Ni–CA, which adopted the octahedral coordination geometry, had K_b values that were about two orders of magnitude lower. Because the hydrophobic cavity of CA around the active center pre-organized the orientation of SA, thereby fixing the ligating NH^- moiety to the apex of the tetrahedron supported by three basal His_3 of CA, metals such as Zn and Co at the center of M-CA gave the most stable CA–SA complex. However, pre-organization was not favored for octahedral geometry. Thus, pre-organization of SA was the key to facilitating the tetrahedral coordination geometry of the Zn^{II} active center of CA.

1. Introduction

Metalloenzymes harness a specific metal ion to be accommodated in a peculiar coordination environment, facilitating their catalytic functions including hydrolysis, redox reactions, and isomerization [1–3]. No other metal species can effectively serve as the catalytic center. For example, carbonic anhydrase (CA) catalyzes the hydration of carbon dioxide and dehydration of bicarbonate through the Zn^{II} center in a tetrahedral coordination geometry supported by three histidine residues (His_3) of CA (Fig. 1a) [4]. Substitution of the Zn^{II} center with other divalent metal ions, such as Cd^{II}, Cu^{II}, and Hg^{II}, leads to a substantial reduction or loss of catalytic function [5]. Thus, the metalloenzyme should harbor a specific metal species that is most appropriate for the coordination environment not only to facilitate the catalytic activity of the enzyme but also such that high kinetic and thermodynamic stability is retained. Substitution of the ligating groups around the active center of CA with other amino acid residues by mutation has shown that metal-binding selectivity arises from the coordination geometry and number to overwhelm the selectivity; this is called the Irving–Williams series, in which the thermodynamic stability of metal ions to small ligands intrinsically follows the order $Mn^{II} < Fe^{II} < Co^{II} < Ni^{II} < Cu^{II} > Zn^{II}$ [6]. Thus, the tetrahedral coordination environment is indispensable for CA to retain the kinetically labile Zn^{II} as the catalytic center.

Inhibitors that strongly inhibit enzymatic activity are commonly believed to be good analogs for the transition state of the catalytic reaction. If this were true, comparison of the binding ability of the inhibitor to a series of metallo variants of a metalloenzyme should also clarify the most suitable metal species to be accommodated by the coordination environment of the enzyme. In the case of CA, the highest-affinity class of inhibitors is the arylsulfonamides as shown by the small dissociation constants of the complex (in the picomolar to micromolar range) [4]. Also crystallographic analyses of the arylsulfonamide–CA complexes have clearly shown the resemblance to the transition state of CO_2 hydrolysis and HCO_3^- dehydration (Fig. 1) [4,7,8]. Herein, we report the evaluation of the binding constant of SA to metallo variants of bovine CA II (M-CAs). To the best of our knowledge, quantitative evaluation of the binding constants for M-CAs is unprecedented. Our data revealed that metals that adopt tetrahedral coordination geometry, such as Zn^{II} and Co^{II}, formed thermodynamically more stable M-CA–SA complexes than Mn^{II}, Ni^{II}, Cu^{II}, and Cd^{II}. Moreover, the pre-organization of SA through the hydrophobic cavity played an important role in setting up the NH^- group of SA to occupy the apex of the tetrahedral coordination geometry of Zn^{II}. Affinity capillary electrophoresis (ACE) [9–11] was used in this study to demonstrate the applicability

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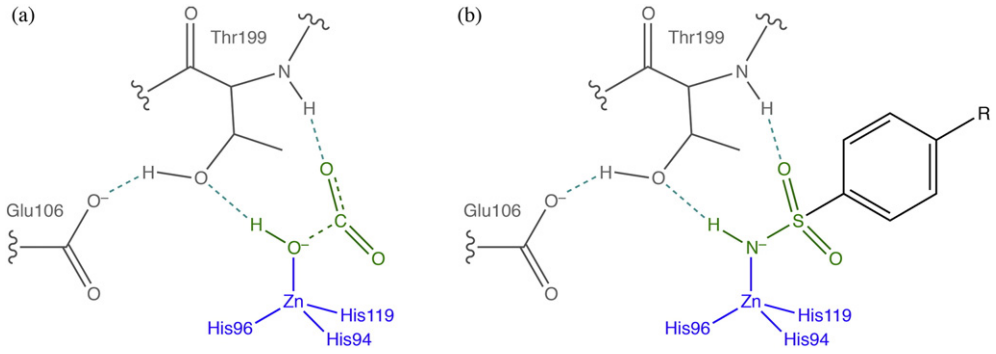

Fig. 1. Schematic drawings of the catalytic center of CA showing (a) transition state of the hydration/dehydration reaction and (b) binding of an arylsulfonamide.

of the method, including the ability to use CA samples without accurate determination of the concentration and the tolerance of the method to the presence of impurities such as isozymes as well as apo and native CAs in the M-CA sample due to incomplete substitution of Zn^{II}.

2. Experimental

2.1. Equipment

For measurement of absorption spectra, a Shimadzu UV-1800 UV–vis spectrophotometer was used. Capillary electrophoresis was carried out using an Agilent CE 7100 instrument equipped with a fused silica capillary (inner diameter = 50 μm; outer diameter = 375 μm) supplied by GL-Sciences Inc. A TOA-DKK pH meter (HM-25R) with a combined glass electrode (GST-5425C) was used to measure the pH. ^{1}H NMR spectra were obtained using a Bruker DPX-400 spectrometer. A Yamato Coolnics Circulator CTE42W was used to maintain the temperature.

2.2. Materials

The CA used throughout the study was from bovine serum (>98% purity, cat. no. C3934) purchased from Sigma-Aldrich (St. Louis, MO, USA), the main content of which was identified as BCA II by capillary electrophoresis of the CA isozyme II from bovine erythrocytes (C2522) purchased from Sigma-Aldrich (see the Supplementary data). Metal-substituted CAs were prepared by simply mixing a metal salt solution and apoCA obtained by dialysis of CA with dipicolinic acid [12] (see the Supplementary data for details). For dialysis of CA, a Visking seamless cellulose tubing (UC-24-32-100) was used. The sulfonamide inhibitor 1-(4-aminosulfonylphenylazo)-2-naphthol-6,8-disulfonate (SA1, Fig. 2) was prepared *via* a diazo-coupling reaction (see the Supplementary data). All other chemicals were of reagent grade. Deionized (d.i.) water prepared with an Elix Advantage 5 Water Purification System (Merck Millipore) was used throughout the study.

2.3. ACE

Prior to the ACE run, the capillary (total length $L = 80.6$ cm, effective length $l = 71.9$ cm) was thoroughly washed with a mixture of 0.01 M SDS, 0.01 M NaOH, d.i. water, and the electrophoretic buffer (25 mM Tris-phosphate buffer [pH 8.4] containing 0–7.62 mM SA1). The sample solution of CA, apoCA, or M-CA in 25 mM Tris–HCl (pH 8.4) was prepared by mixing the solutions of the components at room temperature for 1–12 h (see Section 1.8 of the Supplementary data for the exact conditions and compositions). It was injected into the anodic end of the capillary by applying 50 mbar for 3 s, and capillary electrophoresis-mediated separation was then implemented by applying 30 kV under absorption detection at 200 nm at the cathodic end. The observed electrophoretic mobility (μ_{obs}) was calculated by Eq. (1),

$$\mu_{obs} = (l \times L)/(t_m \times V) \quad (1)$$

where t_m is the migration time of a peak. The electro-osmotic mobility (μ_{eo}) was estimated using Eq. (1) for the electro-osmotic flow (EOF) peak. The observed electrophoretic mobility of CA ($\mu_{CA,obs}$) at a certain SA1 concentration in the electrophoretic buffer was calculated by subtracting μ_{eo} from μ_{obs} (Eq. (2)).

$$\mu_{CA,obs} = \mu_{obs} - \mu_{eo} \quad (2)$$

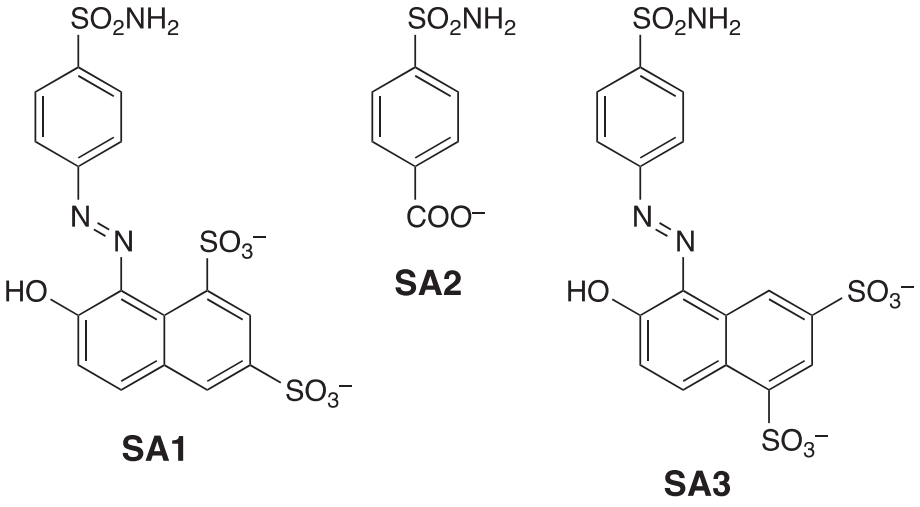

Fig. 2. Structures of sulfonamides.