Probing the Energetics of Dissociation of Carbonic Anhydrase-Ligand Complexes in the Gas Phase

Jinming Gao,* Qinyuan (Quincey) Wu,# Jeffrey Carbeck,* Q. Paula Lei,# Richard D. Smith,# and George M. Whitesides*

*Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138, and #Environmental Molecular Science Laboratory, Pacific Northwest National Laboratory, Richland, Washington 99352 USA

ABSTRACT This paper describes the use of electrospray ionization-Fourier transform ion cyclotron mass spectrometry (ESI-FTICR-MS) to study the relative stabilities of noncovalent complexes of carbonic anhydrase II (CAII, EC 4.2.1.1) and benzenesulfonamide inhibitors in the gas phase. Sustained off-resonance irradiation collision-induced dissociation (SORI-CID) was used to determine the energetics of dissociation of these CAII-sulfonamide complexes in the gas phase. When two molecules of a benzenesulfonamide (**1**) were bound simultaneously to one molecule of CAII, one of them was found to exhibit significantly weaker binding ($\Delta E_{50} = 0.4$ V, where E_{50} is defined as the amplitude of sustained off-resonance irradiation when 50% of the protein-ligand complexes are dissociated). In solution, the benzenesulfonamide group coordinates as an anion to a Zn(II) ion bound at the active site of the enzyme. The gas phase stability of the complex with the weakly bound inhibitor was the same as that of the inhibitor complexed with apoCAII (i.e., CAII with the Zn(II) ion removed from the binding site). These results indicate that specific interactions between the sulfonamide group on the inhibitor and the Zn(II) ion on CAII were preserved in the gas phase. Experiments also showed a higher gas phase stability for the complex of *para*-NO_2-benzenesulfonamide-CAII than that for *ortho*-NO_2-benzenesulfonamide-CAII complex. This result further suggests that steric interactions of the inhibitors with the binding pocket of CAII parallel those in solution. Overall, these results are consistent with the hypothesis that CAII retains, at least partially, the structure of its binding pocket in the gas phase on the time scale (seconds to minutes) of the ESI-FTICR measurements.

INTRODUCTION

Fundamental understanding of acid-base behavior has improved dramatically in the last 30 years, with major contributions to the subject, and especially to the understanding of solvation, derived from comparisons of proton transfer reactions in solution and in the vapor phase. Studies of protein-ligand complexes in the gas phase have a similar potential to clarify the role of solvation in noncovalent interactions. Our recent report comparing the stability of carbonic anhydrase-benzenesulfonamide complexes in the presence and absence of solvent highlighted the importance of hydrophobic interactions in aqueous media and of polar interactions in the gas phase (Wu et al., 1997). One of the key challenges in studying protein-ligand interactions in the gas phase is the knowledge of the structure of protein-ligand complexes at the molecular level. Unlike studies in the solution phase or in the solid state—where the structure of protein-ligand complexes can be determined by nuclear magnetic resonance or x-ray crystallography, respectively—there are currently no techniques that can determine the structure of these complexes in the gas phase. Many studies have suggested that, at least in some cases, proteins can be transferred to the gas phase by electrospray ionization (ESI), and that they may retain in the gas phase aspects of the higher order structures that characterize them in solution (Clemmer et al., 1995; Collings and Douglas, 1996; Covey and Douglas, 1993; Gross et al., 1996; Smith et al., 1992; Sullivan et al., 1996; Vonhelden et al., 1995; Winger et al., 1992; Wood et al., 1995; Wu et al., 1995). Recent ion mobility studies of ions derived from biomolecules produced by ESI have shown that different gas phase structures (generally less compact, lower mobility species) can be formed upon significant collisional activation (i.e., heating) in the mass spectrometer (Shelimov et al., 1997). Although large structural changes between solution and the gas phase can be distinguished, resolution is inadequate to detect more subtle modifications. For studies minimizing heating in the mass spectrometer and where desolvation is completed as ions are "freeze dried" by evaporation of solvent on a time scale of up to several seconds in the high vacuum of an ion cyclotron resonance trap, it remains uncertain what structural changes will occur on transfer from solution to the gas phase. Therefore the extent to which studies conducted in the mass spectrometer are directly relevant to structures and properties in solution is unclear. To address this issue, we describe in this paper a procedure to determine the energetics of dissociation of carbonic anhydrase II (CAII, EC 4.2.1.1)-benzenesulfonamide complexes in the gas phase by sustained off-resonance irradiation collision-induced dissociation (SORI-CID). Based on the energetic information, we

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Address reprint requests to Dr. George M. Whitesides, Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138. Tel.: 617-495-9431; Fax: 617-495-9857; E-mail: gwhitesides@gmwgroup.harvard.edu, or to Dr. Richard D. Smith, Environmental Molecular Science Laboratory, Pacific Northwest National Laboratory, Richland, WA 99352. Tel.: 509-376-0723; Fax: 509-376-2303; E-mail: rd_smith@pnl.gov.

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infer that CAII retains, at least partially, the structure of its binding pocket in the gas phase.

We chose CAII as a model protein because of the extensive knowledge of CAII and its inhibitors and our previous experience in studying these protein-ligand complexes in the gas phase (Cheng et al., 1995; Gao et al., 1996a; Wu et al., 1997). CAII is a spherical Zn(II)-containing metalloenzyme that catalyzes the hydration of CO_2 to bicarbonate (Dodgson et al., 1991). The Zn(II) ion is located at the bottom of a conical binding pocket, and it is chelated by three His residues from CAII in a tetrahedral configuration (Fig. 1). The fourth ligand is either a water molecule or, in this study, a sulfonamido anion ($RArSO_2NH^-$) on the ligand. A number of x-ray crystal structures of CAII complexed with sulfonamide ligands have been obtained under high resolution (<2.0 Å) (Alexander et al., 1993; Boriack et al., 1995; Boriack-Sjodin et al., 1995; Eriksson et al., 1988; Hakansson et al., 1992). These structures describe the interactions of the sulfonamide ligand with the binding pocket of CAII at the molecular level. X-ray crystal structures confirm the chelation of the sulfonamide group to the Zn(II) ion and demonstrate that the amino acid residues of CAII form a narrow binding pocket surrounding the phenyl ring of the benzenesulfonamide. Substituents introduced at the *para* position of the benzenesulfonamide (R_2 in Fig. 1) point toward the opening of the binding pocket and do not interact unfavorably with the narrow binding pocket on CAII; however, substituents introduced at the *ortho* position of benzenesulfonamide (R_1 in Fig. 1) decrease binding affinity to CAII in solution as a result of unfavorable steric interactions. Because repulsive van der Waals forces are not dependent on solvation, but are sensitive to the distance between the interacting atoms (Israelachvili, 1992), we hypothesized that structural isomers of benzenesulfonamide inhibitors would provide a good probe with which to examine the structure of the binding pocket of CAII in the vapor phase.

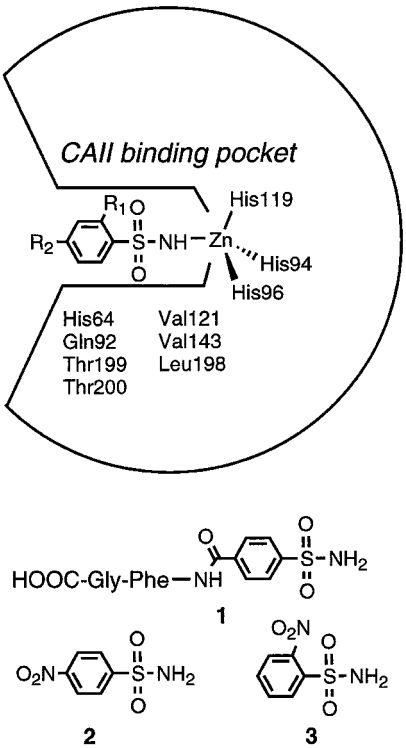

FIGURE 1 Schematic representation of the binding pocket of CAII. The Zn(II) ion is chelated by three His residues and a sulfonamide ligand in a tetrahedral configuration. The binding pocket surrounding the phenyl ring of the ligand is narrow and sensitive to the position of substitution on the benzenesulfonamides. The amino acid residues that form the binding pocket are listed with their numbered positions in the amino acid sequence. The chemical structures of three inhibitors (**1–3**) to CAII in this study are also shown.

MATERIALS AND METHODS

Materials

Human and bovine carbonic anhydrase II were purchased from Sigma (St. Louis, MO). The synthesis of ligand **1** was reported previously (Wu et al., 1997). Ligands **2** and **3** were provided by the Department of Pharmacology, Health Center, University of Florida. Uncoated fused silica capillaries with an internal diameter of 50 μm were purchased from Polymicro Technologies (Phoenix, AZ). NICK Spin columns containing G-50 Sephadex gel were purchased from Pharmacia Biotech (Piscataway, NJ).

Fourier transform ion cyclotron mass spectrometry

A 7-tesla Fourier transform ion cyclotron (FTICR) mass spectrometer equipped with an Odyssey data station (Finnigan FT/MS, Madison, WI) was used for these studies (Winger et al., 1993). Human and bovine carbonic anhydrase II were mixed with various benzenesulfonamide inhibitors in 10 mM aqueous NH_4OAc solution (pH 8.0). The solutions were infused into a modified Analytica (Branford, CT) ESI source that had a stainless steel inlet desolvation capillary (Winger et al., 1993). Typically, the infusion rate was 0.3 μl/min, as controlled by a syringe pump from Harvard Apparatus (South Natick, MA). The electrospray source voltage was ~2.5 kV. The capillary was resistively heated with ~20 W power, corresponding to conditions gentler than those typically used for desolvation in ESI-MS (i.e., ~30 W). Ions were transported to the FTICR cell through the magnetic field gradient, using two sets of RF-only quadrupole ion guides. The ICR cell in this study had dimensions 5 cm × 5 cm × 7.6 cm (Winger et al., 1993).

Determination of values of E_{50} by sustained off-resonance irradiation collision induced dissociation

The experimental procedures for off-resonance irradiation collision induced dissociation (SORI-CID) have been described previously (Gauthier et al., 1991; Marzluff et al., 1994; Senko et al., 1994; Wu et al., 1995). Briefly, CAII-inhibitor complex ions were selectively accumulated in the FTICR cell (Bruce et al., 1993) and then subjected to RF irradiation at 2000 Hz below their cyclotron frequency for 100 ms in the presence of nitrogen gas at a pressure of ~10^{-5} torr. Although the absolute pressure in the FTICR cell during activation was not known exactly, the pressure was precisely controlled, and the results were also confirmed by the measurement of similar relative stabilities under different SORI-CID conditions. In these studies, the amplitude of the irradiation was increased to cause collisional activation to induce the loss of the uncharged inhibitor(s) from the CAII complexes. In these cases, neither the inhibitors nor the protein underwent further fragmentation. No product ions corresponding to Zn(II)

ion loss from CAII have been observed from SORI-CID. The normalized relative intensities of the parent complex and the product ions were plotted against the irradiation amplitude, and the crossing point (E_{50}) of the two curves at 50% of the intensity provided a measure of the gas phase stability. The experimental uncertainty was measured to be ±2% for the relative intensity and ±0.1 V for the amplitude of irradiation. The spectra reported below are from 64 K data sets, and the time domain signal was apodized by a triangle function before Fourier transformation.

Determination of binding stoichiometry of CAII with ligand 1 in solution by capillary electrophoresis

Capillary electrophoresis (CE) experiments were conducted on a Beckman P/ACE 5500 system, using an uncoated capillary of fused silica (total length 47 cm, length from injecting end to detector 40 cm, 50-μm internal diameter) with an applied voltage of 15 kV at 25°C. The charge ladder of CAII was obtained by acetylating an aqueous solution of CAII (0.5 ml at 3 mg/ml in 4 mM borate buffer, pH 9.0) with acetic anhydride (5 μl at 100 mM in dioxane) for 1 min. The reaction product was purified on NICK spin columns spun at 2000 rpm for 4 min. The charge ladder of CAII was diluted and analyzed in 25 mM Tris–192 mM Gly buffer (pH 8.4). In the binding experiment, ligand **1** was prepared at 0.5 mM concentration in the Tris-Gly buffer, and the solution was used as the mobile phase for binding the charge ladder of CAII.

RESULTS AND DISCUSSION

Comparison of energetics of dissociation of CAII-ligand complex with that of apoCAII-ligand complex

X-ray crystal structures of CAII-sulfonamide complexes in the solid state show that the sulfonamido anion chelates to the Zn(II) ion in the center of the enzyme (Alexander et al., 1993; Boriack et al., 1995; Boriack-Sjodin et al., 1995; Eriksson et al., 1988; Hakansson et al., 1992). To examine whether this chelation is maintained in the gas phase, we compared the energetics of dissociation of CAII-sulfonamide complex with that of apoCAII-sulfonamide complex (i.e., the complex involving CAII with the Zn(II) ion removed from the active site). We hypothesized that if the sulfonamide-Zn(II) chelation is maintained in the gas phase, more energy would be required to dissociate the CAII-sulfonamide complex than the apoCAII-sulfonamide complex. The logic supporting this hypothesis is clear, but its correctness is difficult to prove. Binding of a sulfonamido anion to CAII in solution has two components: a polar interaction between the $ArSO_2NH^-$ anion and the Zn(II) cation, and a hydrophobic interaction between the phenyl ring and the nonpolar pocket into which it fits. The hydrophobic interaction has both a van der Waals component and an entropic component (resulting from the release of ordered water molecules on binding the ligand). In the vapor phase, the polar component between the $ArSO_2NH^-$ and Zn(II) ion would be preserved and would probably increase in importance, because the mean dielectric constant screened by the ions would be lower in the vapor phase. The van der Waals component would also persist and be substantially more important in the vapor phase than in solution (because removal of the aryl group from the binding pocket would not be accompanied by its replacement by water); there is no analog in the vapor phase of the entropic contribution to binding coming from release of water. Our hypothesis that apoCAII would bind less tightly than CAII in the vapor phase is therefore based on the assumption that the polar contribution from the $ArSO_2NH^-$ to Zn(II) interaction dominates in the vapor phase as it does in solution (Dodgson et al., 1991). We believe this hypothesis to be plausible, but we have not tried to support it directly and independently through either experiment or computation.

We mixed approximately equimolar concentrations (2.5 μM each) of **1** and bovine CAII (BCAII) at neutral pH for introduction to the mass spectrometer by electrospray ionization. Peaks were obtained that corresponded to the complexes of one molecule of each of the ligand and BCAII, i.e., $(BCAII \cdot \mathbf{1})^{9+}$ and $(BCAII \cdot \mathbf{1})^{10+}$. This observation correctly reflected the 1:1 binding stoichiometry in solution under similar conditions (Dodgson et al., 1991). In contrast, no complexes were detected when an equimolar solution of apoBCAII and ligand **1** was examined under identical conditions. It was possible, however, to form small amounts of the complexes corresponding to apoBCAII · **1** when the ratio of **1**/apoBCAII was increased to 3 (i.e., 2.5 μM of apoBCAII and 7.5 μM of the ligand). The charge states of these complexes were predominantly 9+ and 10+, similar to the results obtained by electrospraying pure BCAII under the same conditions. The narrow distribution of the charge states in the gas phase and their low values compared to those for the denatured protein suggest that BCAII-sulfonamide complex retains its compact structure under these experimental conditions (Carbeck et al., 1999; Cheng et al., 1995; Light-Wahl et al., 1994).

We used selective ion accumulation to trap only the complex ions for the SORI-CID experiments (for example, the top spectrum in Fig. 2 *A*, where the irradiation energy is zero). As the complex ions were irradiated, they dissociated to $BCAII^{10+}$ and a neutral ligand molecule. Fig. 2 *A* shows a SORI-CID spectrum of $(BCAII \cdot \mathbf{1})^{10+}$ ions after irradiation at different amplitudes. As the energy of irradiation increased, we observed a decrease in the peak intensity of the $(BCAII \cdot \mathbf{1})^{10+}$ complex and an increase in that of the dissociated $BCAII^{10+}$ ions. Fig. 2 *B* plots the normalized relative intensities of the complexes and their dissociation products versus the extent of SORI-CID activation for the $(BCAII \cdot \mathbf{1})^{10+}$ complexes. A measure of the relative stability of the protein-ligand complexes in the gas phase is obtained from the amplitude of SORI irradiation, where 50% of the parent ions are dissociated, i.e., at the crossing point of the parent and product ion curves (E_{50}, Fig. 2 *B*). Here we define E_{50} as the amplitude of sustained off-resonance irradiation where 50% of the protein-ligand complexes are dissociated. The value of E_{50} is a measure of the dissociation energy of protein-ligand complexes in the gas phase. We determined the values of ΔE_{50} to be 0.4 ± 0.1 Volts between the $(BCAII \cdot \mathbf{1})^{10+}$ and $(apoBCAII \cdot \mathbf{1})^{10+}$ complexes. This difference in E_{50} is significant because the

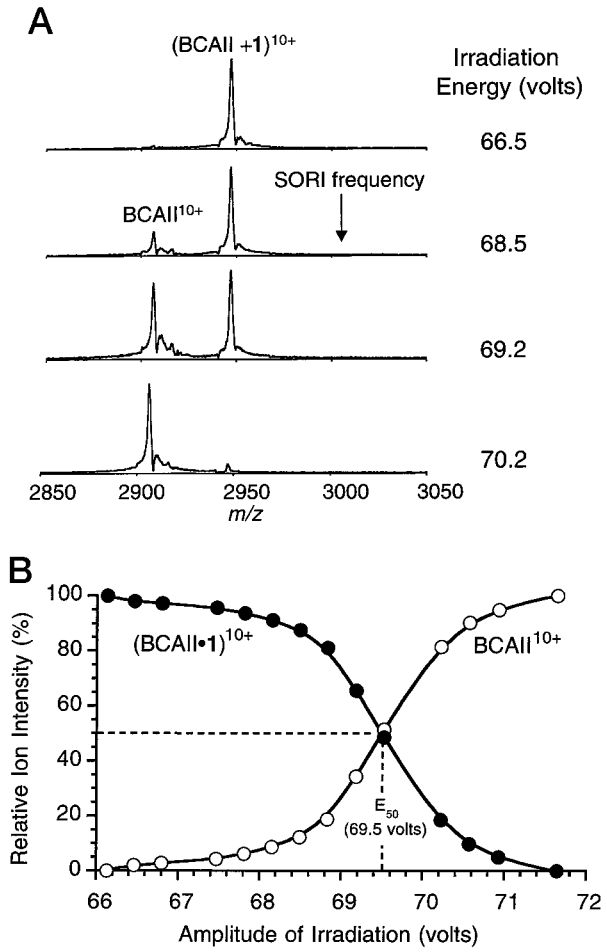

FIGURE 2 (*A*) SORI-CID spectrum of the (BCAII · **1**)$^{10+}$ complex. As the amplitude of irradiation increases from the top to the bottom spectrum, the (BCAII · **1**)$^{10+}$ complex dissociates to BCAII^{10+} and a neutral inhibitor molecule. The arrows pointing to the *m/z* axis correspond to the frequency of SORI irradiation. (*B*) Plot of the normalized ion abundances for the (BCAII · **1**)$^{10+}$ complexes (*filled symbols*) and their dissociation products (*open symbols*) versus the amplitudes of SORI irradiation. The amplitudes of irradiation at the crossing point of the two curves at 50% of the intensity (E_{50}) provides a measure of the relative gas phase stability. The experimental uncertainty was ±2% for the relative ion intensity and ±0.1 V for the amplitude of irradiation.

complexes vary from completely stable to completely dissociated over a range of ~2 V (Fig. 2 *B*). We suggest that the higher value of E_{50} for the BCAII-sulfonamide complex compared to the apoBCAII-sulfonamide complex is consistent with the hypothesis that the sulfonamide group retains its binding to Zn(II) ion in the gas phase.

Association of two molecules of ligand 1 with BCAII

Formation of the apoBCAII-sulfonamide complex requires a relatively high concentration of sulfonamide ligand in solution. Under similar conditions, BCAII was detected to form a complex with two molecules of ligand **1**, BCAII · 2**1**, in the gas phase. The charge states of the complex were predominantly 9+ and 10+, similar to those of pure BCAII. Because nonspecific formation of an adduct resulting from the ESI process can sometimes be observed (Smith et al., 1992), we hypothesize that the equivalent of inhibitor that is more weakly bound does not bind to the Zn(II) ion in the binding pocket. In SORI-CID experiments, (BCAII · 2**1**)$^{10+}$ complex was converted into (BCAII · **1**)$^{10+}$ ions (by loss of a neutral inhibitor molecule). We determined the value of E_{50} for the loss of one equivalent of **1** from the (CAII · 2**1**)$^{10+}$ complex to be 69.1 ± 0.1 Volts. The value of E_{50} of this complex was found to be the same (within experimental uncertainty) as that for the loss of **1** from (apoBCAII · **1**)$^{10+}$ ion under otherwise identical conditions.

We have no information regarding the sites on BCAII and apoBCAII interacting with the weakly bound inhibitor molecule. All of the x-ray crystal structures of CAII-sulfonamide complexes show the binding of only one sulfonamide ligand to the Zn(II) ion in the binding pocket (Boriack et al., 1995; Eriksson et al., 1988; Hakansson et al., 1992). Solution ^{19}F NMR studies, however, showed that some fluorosubstituted benzenesulfonamides can bind to CAII in a 2:1 binding stoichiometry; in addition, the authors suggested that both sulfonamide ligands were bound to Zn(II) in the active site (Dugad et al., 1989; Dugad and Gerig, 1988). To determine whether the BCAII · 2**1** complex observed in the gas phase has already been formed in solution, we used affinity capillary electrophoresis (ACE) (Chu et al., 1995) to determine the binding stoichiometry for association of ligand **1** to BCAII in solution.

Determination of the stoichiometry of binding of BCAII and 1 in solution: BCAII · 1

We used ACE in combination with protein charge ladders (Colton et al., 1997; Gao et al., 1996b) to determine the stoichiometry of binding in solution. The charge ladder of BCAII was formed by acetylation of the ϵ-amino groups on Lys residues of BCAII. Upon each modification, the net charge of the derivative of BCAII increased by one unit of negative charge, as one unit of positive charge on the lysine ϵ-ammonium group was neutralized. Capillary electrophoresis separates the charge ladder of BCAII into "rungs" of peaks, which provides an internal scale in integral units of charge (Fig. 3). When ligand **1** is bound to the charge ladder of BCAII, the peaks in the ladder will shift as a result of the change in effective charges of protein-ligand complexes. The magnitude of the shift depends on the binding affinity, binding stoichiometry, and net charge on the ligand. When one molecule of ligand **1** is fully bound to one molecule of BCAII, the charge on the protein-ligand complex will decrease by one unit because of the introduction of a carboxylate group on ligand **1**. If two molecules of ligand **1** are bound simultaneously to one molecule of BCAII, a charge decrease of two units will be expected. We carried out binding experiments in which the concentration of li-

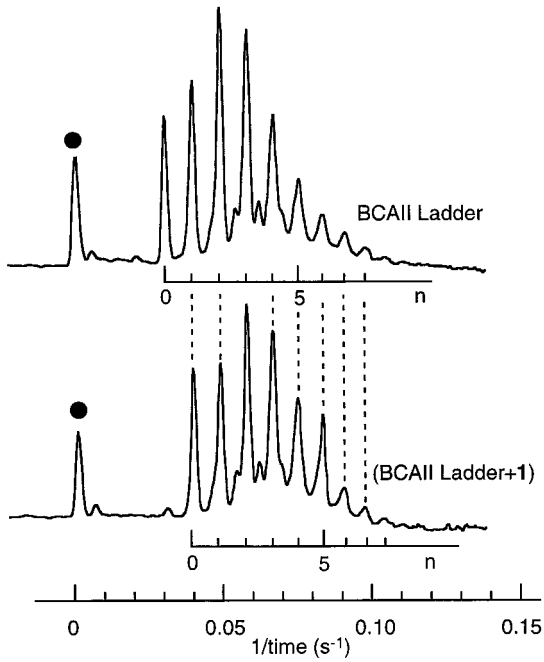

FIGURE 3 Determination of binding stoichiometry of BCAII-sulfonamide complexes in solution. Binding of ligand **1** to the charge ladder of BCAII shifted the mobility of BCAII and its derivatives. By comparing the mobility shift as a result of binding with the internal charge scale from charge ladders of BCAII, we determined a 1:1 binding stoichiometry for BCAII · **1** complexes. Both electropherograms were presented on a 1/time axis, which directly reflects the electrophoretic mobility for each derivatives of BCAII. *para*-Methoxybenzylalcohol was used as a neutral marker (indicated by the *filled circle*). *n* refers to the number of positively charged ϵ-ammonium groups transformed into its neutral *N*-acetyl derivatives of BCAII.

gand **1** is at 0.5 mM, much higher than its concentration for the MS experiment and the dissociation constant of the ligand from BCAII (K_d = 15 nM) (Wu et al., 1997). The mobility shift upon binding is equal to one unit of charge, as shown by the internal scale of charge ladders (Fig. 3), demonstrating that the binding stoichiometry of ligand **1** to BCAII is 1:1 in solution. Therefore, the second equivalent of inhibitor **1** is more likely to be condensed on the surface of CAII (at a non-Zn(II) site) during the ESI desolvation process than it is to be associated with the active site. Whether the similarity in the values of E_{50} for the complexes of (BCAII · 2**1**)$^{10+}$ and (apoBCAII · **1**)$^{10+}$ suggests similar binding sites for these molecules of **1** cannot be determined from the available data.

Binding of isomeric inhibitors

Results from comparison of CAII-sulfonamide and apoCAII-sulfonamide complexes suggest that the sulfonamide ligand retains its binding to the Zn(II) ion in CAII after transfer to the gas phase. However, the extent to which the conformation of the binding pocket surrounding the Zn(II) ion of CAII is preserved in the gas phase remains to be determined. We have examined the use of isomeric inhibitors to probe the structure of the binding pocket of CAII in the gas phase. Our study is based on the hypothesis that the energetics of dissociation (or values of E_{50}) of CAII-sulfonamide complexes reflects their molecular interactions and surface contact in the gas phase. By choosing ligands that minimize differences in electrostatic interactions, we hope to use the energetics of dissociation to reveal steric interactions and to suggest the structure of the binding pocket of CAII in the gas phase. Similar steric models have been applied to probe the active site structures of chymotrypsin and subtilisin in solution (Bosshard and Berger, 1974; Karasaki and Ohno, 1978; Keller et al., 1991).

We chose *para*- and *ortho*-NO_2-benzenesulfonamide inhibitors (**2** and **3** in Fig. 1, respectively) as model ligands for this study. Nitro groups introduced at the *para* and *ortho* positions have similar electron withdrawing effects on the acidity of the sulfonamide group (Hansch et al., 1985; Kakeya et al., 1969a,b). Fig. 4 *A* shows mass spectra of BCAII and HCAII, and of their complexes with *para*- and *ortho*-NO_2-benzenesulfonamide inhibitors. Mass spectra were obtained after ESI from 10 mM NH_4OAc solution (pH 7) containing 20 μM (each) of BCAII and HCAII with 50 μM of one inhibitor. Because the isomeric inhibitors have the same mass, we determined the stability of the complexes of the enzymes with each ligand individually. The mass spectra show that the relative gas phase abundance of the enzyme-inhibitor complexes for the *para*-substituted inhibitor (**2**) are more than four times greater than that for the *ortho*-substituted inhibitors (**3**). This result parallels the relative binding affinities of the two ligands in solution (the dissociation constants, K_d, are 5.5×10^{-7} M and 3.5×10^{-6} M for **2** and **3**, respectively (Hansch et al., 1985; Kakeya et al., 1969a,b); that is, a factor of 6), as we demonstrated previously (Cheng et al., 1995). Selective accumulation of the complex ions followed by SORI-CID experiments determined the values of E_{50} for the (HCAII · **2**)$^{10+}$ and (HCAII · **3**)$^{10+}$ complexes to be 63.7 ± 0.1 and 62.9 ± 0.1 V, respectively (Fig. 4 *B*). The relative stability of HCAII with *para*-substituted ligand is significantly higher than that with the *ortho*-substituted counterpart (ΔE_{50} = 0.8 V).

X-ray crystallography of the complex of CAII with sulfonamide ligands has shown that the binding pocket is very narrow and sensitive to the location of substituents on the benzenesulfonamides. Quantitative structure and activity relationship (Hansch, 1993) showed that, in solution, a bulky group such as -NO_2 or -$COOCH_3$, in the *ortho* position of benzenesulfonamide, has unfavorable steric interactions with the binding pocket on CAII and thus reduces the binding affinity relative to the *para*-substituted inhibitor (Hansch et al., 1985; Kakeya et al., 1969a,b). In the gas phase, complexes of *ortho*-NO_2-benzenesulfonamide with CAII was also less stable than its *para*-substituted counterpart. These data demonstrate that steric interactions observed in solution were maintained in the gas phase and strongly suggest that the binding pocket of CAII retains at least some structural similarity to that in solution.

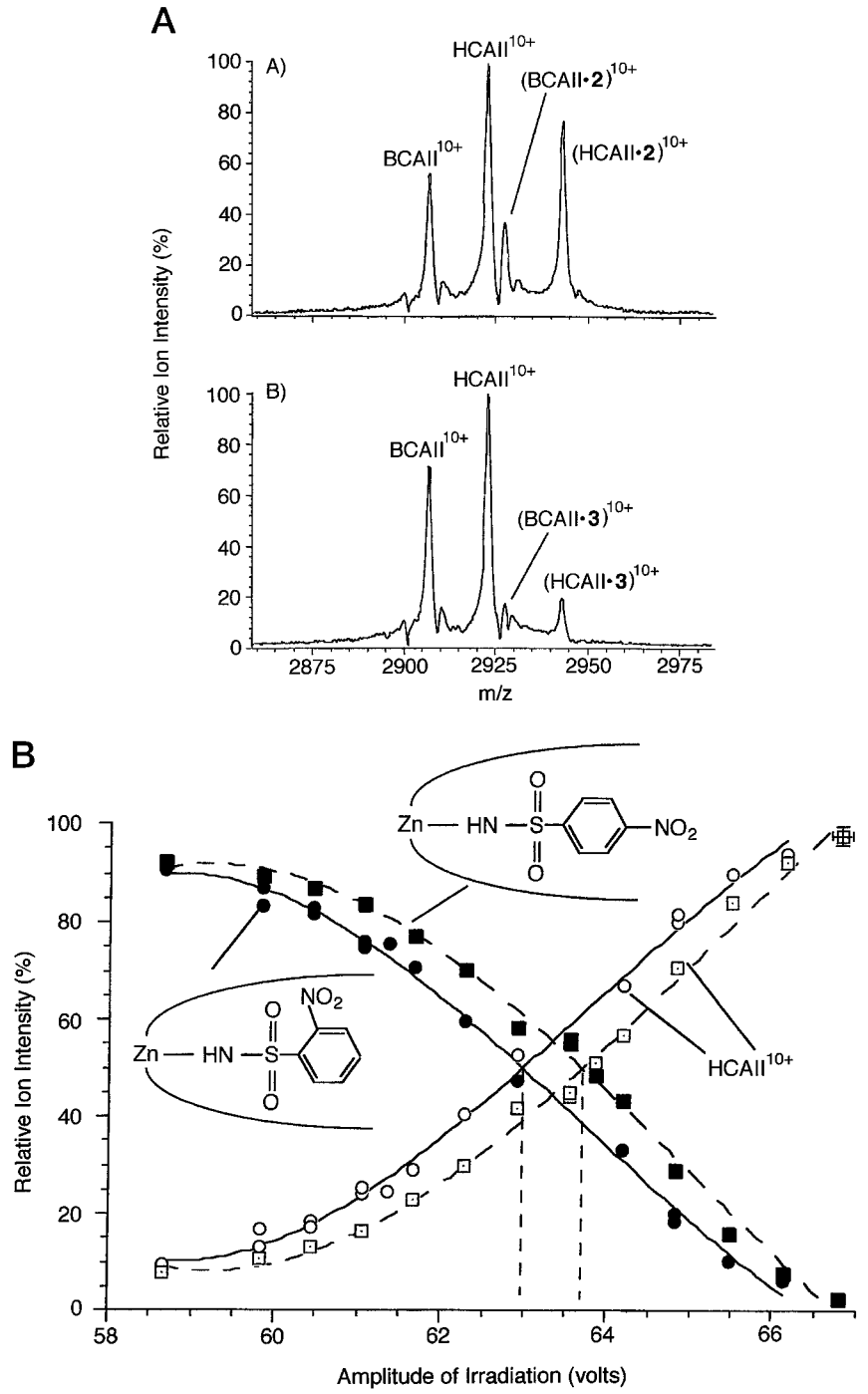

FIGURE 4 (*A* and *B*) Electrospray ionization mass spectra showing ions of BCAII and HCAII and their noncovalent complexes with inhibitors 2 and 3, respectively. The solution concentrations of these two inhibitors and other experimental conditions were identical. (*C*) Plot of the normalized ion intensities of $(\text{HCAII} \cdot \mathbf{2})^{10+}$ and $(\text{HCAII} \cdot \mathbf{3})^{10+}$ (*filled symbols*) and the dissociated HCAII^{10+} ions (*open symbols*) as a function of the extent of SORI irradiation. The value of E_{50} for $(\text{HCAII} \cdot \mathbf{2})^{10+}$ is 63.7 ± 0.1 V; that for $(\text{HCAII} \cdot \mathbf{3})^{10+}$ is 62.9 ± 0.1 V. The experimental uncertainty was ±2% for the relative ion intensity and ±0.1 V for the amplitude of irradiation.

CONCLUSION

This paper explores the structure of the binding pocket of CAII-benzenesulfonamide complexes in the gas phase based on the energetics of dissociation of these complexes. Comparison of values of E_{50}—the energy required to dissociate 50% of the CAII-ligand complexes in the gas phase—for CAII · **1** and apoCAII · **1** complexes demonstrates that the CAII · **1** complex is more stable than the apoCAII · **1** complex (ΔE_{50} = 0.4 V) and suggests that specific interactions between the sulfonamide group on the ligand and Zn(II) ion on CAII are maintained in the gas phase. This inference is supported by the observation that the value of E_{50} for apoCAII · **1** complex is the same as that for the weakly bound (and presumably not Zn(II) associated) equivalent of **1** dissociating from CAII · 2**1**. Experiments with *para*-NO_2- and *ortho*-NO_2-substituted benzenesulfonamides further demonstrate that steric interactions between the substituents on the ligands and the binding pocket of CAII parallel those in solution. These results are all consistent with a model of the binding pocket of CAII in which at least some structural aspects are preserved in the

gas phase on the time scale (seconds to minutes) of studies in ESI-FTICR. Use of differently substituted benzenesulfonamide ligands as steric probes will allow detailed mapping of the structure of the binding pocket of CAII in the gas phase.

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