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Carbonic Anhydrase-Inhibitor Binding: From Solution to the Gas Phase

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Many biochemical processes are mediated by the formation and dissociation of noncovalent complexes between proteins and ligands, and the role of solvent in these processes remains a fundamentally and practically important problem in biophysics. Electrospray ionization-mass spectrometry (ESI-MS) is emerging as a powerful tool for the study of noncovalent interactions in the gas phase.[1] Still unclear are the extent of structural changes as a result of solvent removal during electrospray ionization and the interactions that govern stability of the desolvated species in the gas phase. Many recent studies have suggested that at least some aspects of the higher order structure of proteins can be retained after transfer into the gas phase.[2,3]

In this report, we compare the kinetic stabilities of noncovalent complexes between bovine carbonic anhydrase II (BCAII, EC 4.2.1.1) and *para*-substituted benzenesulfonamide inhibitors[4] in the gas phase and in solution. These BCAII−inhibitor systems are attractive model systems due to the stability of carbonic anhydrase (CA) and its well characterized structure and ligand complexes, providing a basis for inferences regarding the protein structure in the gas phase and its ligand interactions. CA is a roughly spherical Zn(II) metalloenzyme having a conical binding pocket which catalyzes the hydration of CO_2 to bicarbonate. A large body of data correlate structures of sulfonamide ligands with their binding constants to CA.[5] A set of eight inhibitors was selected for this study, covering a wide range of binding affinities and varying in the length of their tails and aromatic content. The results demonstrate that relative stabilities of BCAII-inhibitor complexes differ substantially between the gas and liquid phases and also show the dominant role of polar surface interactions in the gas phase.

The relative complex stabilities in the gas phase were measured using a Fourier transform ion cyclotron resonance mass spectrometer and sustained off-resonance irradiation-collision induced dissociation (SORI-CID).[6,7] The off-rates (k_{off}; the dissociation rate for a complex in solution) and binding constants (K_b) of these complexes in solution were measured using fluorescence spectroscopy (see Table 1).[3c,8] The results summarized in Table 1 show that the kinetic stabilities of the protein−ligand complexes in the gas phase and the kinetic stabilities or thermodynamic stabilities of the complexes in solution are not directly related. The off-rates are primarily correlated with the hydrophobicity[9] of the first amino acid residue at the *para*-position of the benzenesulfonamide inhibitors but *not* with the total hydrophobicity of their amino acid tails (Table 1). These and previous results indicated that hydrophobic interactions between protein and ligand are important in binding in solution.[10]

The gas phase stabilities of these complexes were found to have no direct correlation with the hydrophobicity of the inhibitors but rather increased with the length of the inhibitor tail. The gas phase data (Table 1) show that as the number of amino acid residues increases for an inhibitor, a higher amplitude of irradiation (E_{50})[6] is required for its removal from the BCAII−inhibitor complex. A plot of total polar molecular surface area[11] of the inhibitor vs. E_{50} shows a clear correlation (Figure 1) for both the inhibitors with (**3**−**6**) and without (**1**, **2**, **7**, and **8**) aromatic amino acid residues. The polar surface area refers to that portion of the molecular surface which is due to N or O atoms in the structure (which are typically partially charged and/or contribute to hydrogen bonding). The molecular surface is

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(6) A 10 mM NH_4OAc solution containing 35 μM of BCAII and 60 μM of a benzenesulfonamide inhibitor was electrosprayed into a 7 Tesla Fourier transform ion cyclotron mass spectrometer (FTICR-MS),[7a] and the protein−inhibitor complex ions (10+ charge state) was selectively accumulated in the FTICR cell.[7b] The complexes were subjected to sustained off-resonance irradiation (SORI)[2g,7c−e] at 2000 Hz below their cyclotron frequency for 100 ms with nitrogen gas of $\sim 10^{-5}$ Torr pressure and detected after a delay of ~5 s. (We neglect the small m/z dependence of the collisional activation process since the protein-ligand complexes have only very small m/z differences; a full treatment of the activation process is in preparation.) All reported experiments were performed under identical instrumental conditions. Although the absolute pressure in the FTICR cell during activation was not exactly known, 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, and the complexes dissociated to form only BCAII (10+) and the uncharged inhibitor (i.e., neither further fragmentation nor Zn^{2+} loss from BCAII occurred). The normalized relative intensities of the complex and BCAII were then plotted against the irradiation amplitude, and the crossing point of the two curves (E_{50}; 50% intensity for the complex and BCAII) provided a measure of the gas phase stabilities. The experimental uncertainty was measured to be ± 2% for the relative intensity and ±0.1 V for the amplitude of irradiation, on the basis of multiple determinations.

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Table 1. The Stabilities of BCAII-Inhibitor Complexes in Solution and in the Gas Phase. The Inhibitors were Ordered according to their Kinetic Stabilities (off-rates) in Solution

ID	R[a]	k_{off}[b] (10^{-3} s^{-1})	K_b[b] (10^6 M^{-1})	first residue (hydrophobicity)[c]	hydrophobicity[c,d]	polar surface area (A^2)[e]	E_{50} (V)[f]
1	-(Leu)$_2$-Ala(β)-OH	9.3	140	Leu (−3.98)	−8.83	69.0	69.8
2	-Leu-OH	10.0	110	Leu (−3.98)	−3.98	52.0	66.3
3	-Phe-Asn-OH	29.0	50	Phe (−2.04)	5.53	71.0	71.6
4	-Phe-Gly-OH	30.0	67	Phe (−2.04)	−2.04	61.0	69.2
5	-Phe-Asp-OH	34.0	24	Phe (−2.04)	7.62	73.0	72.1
6	-(Gly)$_3$-CPh-OH	54.0	10	Gly (0.0)	1.08	83.0	73.7
7	-Asn-OH	54.0	2.7	Asn (7.58)	7.58	71.0	68.8
8	-(Gly)$_3$-Nle-OH	68.0	4.4	Gly (0.0)	−3.98	85.0	72.6

[a] R is the amino acid tail (with L configuration) of an inhibitor having structure p-H_2NO_2S-C_6H_4-CO-R. CPh = −HNCH($CH_2C_6H_4$-p-Cl)CO− and Nle = −HNCH($(CH_2)_3CH_3$)CO−. [b] The values of k_{off} and K_b were measured in 20 mM phosphate buffer (pH = 7.5) at 37 °C. The experimental uncertainty is ±10% from multiple experiments. [c] The relative hydrophobicity (referenced to Gly as 0 kcal mol^{-1}) of the amino acid was taken from ref 9. The values of Tyr and Leu were used for CPh and Nle, respectively. A more negative value indicates a higher hydrophobicity. [d] Total side-chain hydrophobicity of the inhibitor tail R. [e] The polar molecular surface area of the inhibitor was calculated using the QUANTA 3.3 program.[11] [f] The uncertainty of the measurement of E_{50} is ±0.1 V,[6] as determined from multiple experiments. Similar results were also obtained using different irradiation conditions.

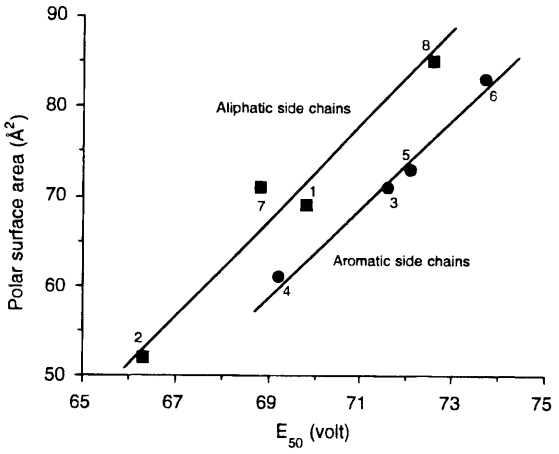

Figure 1. Plot of polar surface areas of the *para*-substituted benzenesulfonamide inhibitors vs. the gas phase stabilities of the inhibitor-BCAII complexes, as measured by the amplitude of SORI-irradiation (E_{50}; Table 1).[6] For the same polar surface area, inhibitors with aromatic amino acid residues (circles) show stronger binding with BCAII in the gas phase than those with aliphatic side chains.

the surface traced out by the edge of a probe sphere (1.4 Å radius) that is rolled over the surface of the molecular structure. The polar molecular surface area was calculated using the Connolly surface algorithm.[11] Polar surface area was found to increase monotonically with the number of amino acid residues of the inhibitors. These correlations suggest that the major attractive forces for noncovalent protein−ligand binding in the gas phase are due to interactions between the polar surfaces through electrostatic or hydrogen bonding interactions and that the inhibitor tail has collapsed to the protein surface in the gas phase, contributing significantly to complex stability. Figure 1 also shows that for inhibitors having the same polar surface area, an aromatic amino acid side chain results in a stronger binding interaction with the protein in the gas phase than does an aliphatic side chain. These results suggest that van der Waals interactions involving the more polarizable π electrons[12] of the aromatic amino acid residues contribute to stabilizing the protein−ligand complexes in the gas phase to a greater extent than dispersion interactions involving the aliphatic hydrocarbons.

In conclusion, the dissociation (off-rates) of BCAII−inhibitor complexes in solution are mainly affected by hydrophobic interactions between the inhibitor and the enzyme, while their corresponding gas phase stabilities appear to be primarily determined by polar surface interactions. These results emphasize the role of solvation in protein−ligand interactions and are of general significance for understanding the differences in inter- and intramolecular interactions between solution and the gas phase. The demonstrated absence of a direct correlation between the gas phase and solution complex stabilities also suggests that inferences regarding solution binding based on the stability of the corresponding gas phase complexes must be made with great caution. In addition, ESI-MS measurements should be conducted under conditions that minimize dissociation of complexes if they are to correctly reflect the relative abundances of the protein−ligand complexes in solution, as we have suggested previously.[3c]

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