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The structure of human carbonic anhydrase II in complex with bromide and azide

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The three-dimensional structure of human carbonic anhydrase II complexed with azide and with bromide was investigated crystallographically. Both of these non-protonated inhibitors replace the zinc and the 'deep' water, two catalytically important water molecules in the active site of the molecule. Both the azide and the bromide ions bind in a distorted tetrahedral manner 0.4 and 1.1 Å from the zinc water position, respectively, but are in close contact (2.0 and 2.6 Å, respectively) with the zinc ion.

Carbonic anhydrase; X-ray structure; Inhibitor; Azide; Bromide

1. INTRODUCTION

The zinc enzyme carbonic anhydrase (EC 4.2.1.1) catalyzes the reversible reaction

$$CO_2 + H_2O \longleftrightarrow HCO_3^- + H^+$$

in a wide variety of organisms and tissues [1].

The structure of the enzyme is composed of a ten stranded β-sheet surrounded on both sides by stretches of a mostly non-helical structure [2]. The active site is located in a 15-Å deep and 15-Å wide cavity between the β-sheet and the non-helical structure. Three histidine residues, 94, 96 and 119, all of which belong to the β-sheet, coordinate to the essential active site zinc ion. The fourth ligand is a water molecule at low pH (< 7) and a hydroxide ion at high pH (> 7) [3].

We have made extensive attempts to reach a structural understanding of inhibition, substrate binding and catalysis using isoenzyme II from human erythrocytes (HCAII). Crystallographic investigations of the complex with bisulfite have shown that one oxygen, presumably protonated, replaces the zinc water with tetrahedral coordination geometry [4,5]. The formate complex with HCAII on the other hand shows a penta-coordinated zinc ion [4], with both the inhibitor and the zinc water coordinated to the metal. The reason for this difference is that T199 Oγ1 can only function as a hydrogen bond acceptor in relation to the tetrahedral position on the zinc ion [6]. The binding of bicarbonate to the T200H mutant of HCAII [7] is consistent with the above observations. In all three cases, the non-protonated oxygens of the ligands were found in analogous positions. Spectroscopic investigations of some inhibitor complexes with cobalt(II) substituted carbonic anhydrase have been interpreted in terms of either tetra-or penta-coordination of the metal [8]. Other ions like bromide and azide, both unprotonated, seem to fall into another category with coordination between four and five. What this means in structural terms is the subject of the present crystallographic investigation.

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2. MATERIALS AND METHODS

2.1. *Data collection*

Crystals of HCAII were grown in complex with 1 mM Hg^{2+} in 2.4 M $(NH_4)_2SO_4$ 50 mM Tris-HCl, pH 8.5 [9]. The crystals were soaked in 50 mM Tris-HCl, pH 7.8, with 5 mM β-mercaptoethanol in order to remove the Hg^{2+} ions and subsequently in the inhibitor solutions shown in Table I.

The crystals belong to space group $P2_1$ with cell parameters a = 42.7 Å, b = 41.7 Å, c = 73.0 Å and β = 104.6°. Diffraction data were collected with a Siemens (Xentronics) multiwire area detector mounted on a Rigaku RU200HEB rotating anode X-ray generator (CuKa radiation, 0.3 mm focal spot, graphite monochromator, 0.5 mm collimator). The reflection data were integrated, reduced, merged and scaled using the program suite XENGEN [10]. The data collection statistics is summarized in Table I.

2.2. *Refinement*

The calculations were performed on a VAX station 3100. Restrained least square refinement was done with PROLSQ [11,12] using all diffraction data below 10 Å resolution. The 2 |Fo|–|Fc| and |Fo|–|Fc| electron density maps were calculated using the program suite CCP4 [13] and inspected on an Evans and Sutherland PS390 graphics workstation using the program FRODO [14,15]. The geometry of the azide ion was optimized by the real space refinement of FRODO.

The starting model for the refinement of the complexes was the refined structure of HCAII [4], where a few active site water molecules were removed. The inhibitor and solvent molecules were successively

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inserted in the active site of the molecule after some cycles of refinement. The refinement results are summarized in Table II.

3. RESULTS

3.1. *The complex with azide*

The binding of the azide ligand in the active site of the enzyme is shown in Fig. 1. The N3 atom replaces the zinc water and the N1 atom replaces the 'deep' water in the native structure. The differences in location between N3 and the zinc water, and between N1 and the deep water are both 0.4 Å. The distances between N3 and the zinc ion is 2.0 Å, and between N3 and T199 Oγ1 3.2 Å. The N1 atom is 3.4 Å from T199 N and 3.7 Å from T199 Oγ1. The only water which is within hydrogen bond distance to the azide is water 318. It is 2.6 Å away from N3 and 3.4 Å away from N2. Water 389 is not observed. The native position of this water is 3.1 Å away from the azide N2 atom, which has no lone pair available for hydrogen bonding.

3.2. *The complex with bromide at pH 6.0*

The structure of this complex is shown in Fig. 2. The bromide ion replaces both the zinc water and the deep water in the active site of the enzyme, 2.5 Å away from the zinc ion. The distances to T199 Oγ1 is 3.6 Å, and to T199 N 3.8 Å. The difference between N2 in the azide complex and the bromide ion is 0.2 Å. The distances to the site of replaced water molecules are 1.1 Å (zinc water) and 1.6 Å (deep water).

Table I

Soaking conditions and data collection

	Azide complex (pH 7.8)	Bromide complex (pH 7.8)	Bromide complex (pH 6.0)
Inhibitor conc. (mM)	20	110	540
2Θ angle (°)	19	20	17
Deg/frame (°)	0.25	0.25	0.25
Resolution (Å)	1.84	1.80	1.91
Completeness (%)	86.8	82.5	69.4
R_{merge} (%)	6.8	6.0	6.3

All experiments were performed in 3 M $(NH_4)_2SO_4$ with 50 mM Tris (pH 7.8) or 80 mM citrate (pH 6.0). Soaking time was 24 h.

3.3. *The complex with bromide at pH 7.8*

The structure of this complex is displayed in Fig. 3. The occupancy of bromide was refined to 40% and the

Table II

Refinement results

	Target sigma	Native (pH 7.8) [2]	Azide complex (pH 7.8)	Bromide complex (pH 7.8)	Bromide complex (pH 6.0)
R^*_{cryst}		0.150	0.161	0.150	0.183
Mean B (Å^2)		15.0	13.8	13.8	11.7
Error in coordinates (Å) according to Luzatti plot		0.14	0.17	0.16	0.22
Protein atoms			2,060	2,060	2,060
Solvent atoms		220	220	218	219
RMS values					
Distances					
Bond distance (Å)	0.020	0.021	0.021	0.020	0.020
Angle distance (Å)	0.030	0.038	0.036	0.038	0.036
Planar 1–4 distance (Å)	0.050	0.056	0.048	0.049	0.054
Miscellaneous					
Plane groups (Å)	0.020	0.020	0.018	0.018	0.017
Chiral centers (Å^3)	0.150	0.237	0.229	0.212	0.207
Non-bonded distances					
Single torsion (Å)	0.200	0.163	0.159	0.159	0.162
Multiple torsion (Å)	0.200	0.167	0.163	0.163	0.180
Possible X–Y H-bond (Å)	0.200	0.146	0.150	0.158	0.172
Torsion angles					
Planar (°)	3.0	4.5	3.3	3.2	2.8
Staggered (°)	15.0	16.3	16.6	16.7	18.5
Orthonormal (°)	20.0	31.0	30.5	30.7	30.5
Thermal restraints					
Main chain bond (Å^2)	1.000	1.074	0.969	0.963	0.883
Main chain angle (Å^2)	1.500	1.703	1.532	1.536	1.427
Side chain bond (Å^2)	1.500	2.328	1.986	1.999	1.486
Side chain angle (Å^2)	2.000	3.634	3.055	3.092	2.187

$$*R_{cryst} = \frac{\Sigma||Fo|-|Fc||}{\Sigma|Fo|}$$

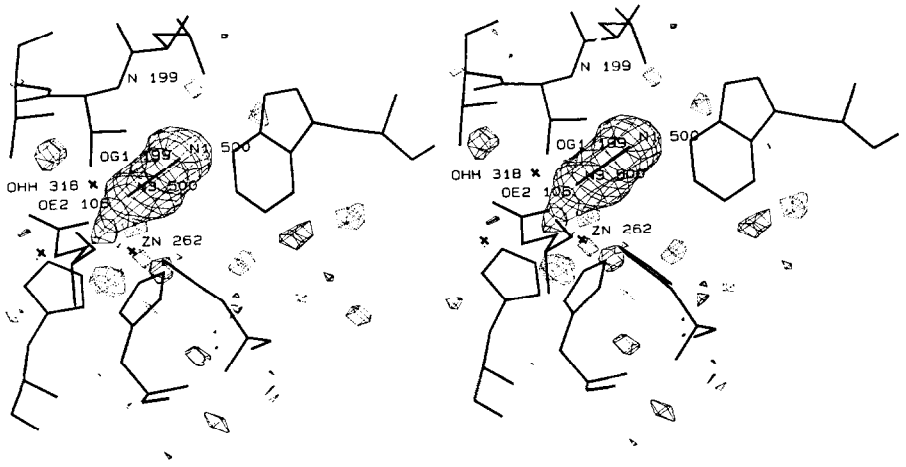

Fig. 1. A stereo view of the active site in the HCAII complex with azide. The azide replaces the zinc water and the deep water, with one nitrogen (N3) in close contact with the zinc and another (N1) hydrogen bonded to Thr-199N. Positive (continuous lines) and negative (broken lines) |Fo|–|Fc| maps are drawn at 3 σ.

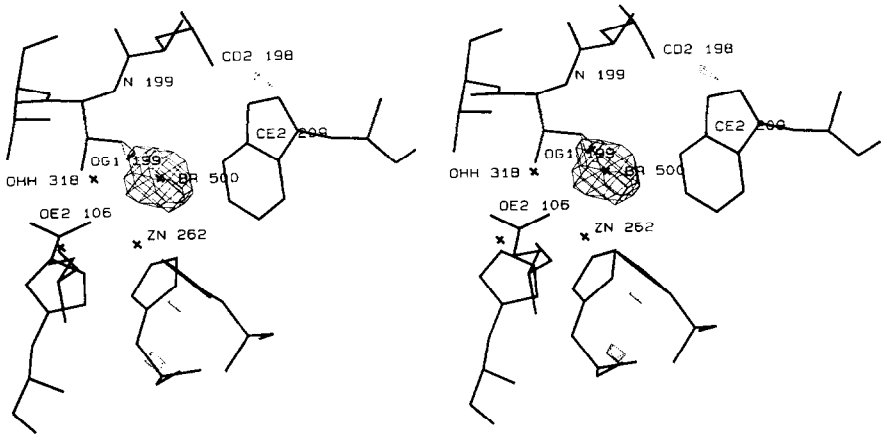

Fig. 2. The active site in HCAII complex with bromide at pH 6.0. The bromide binds to the zinc ion at distance of 2.5 Å. Both the zinc water and the deep water are absent. Positive (continuous lines) and negative (broken lines) |Fo|–|Fc| maps are drawn at 3.5 σ.

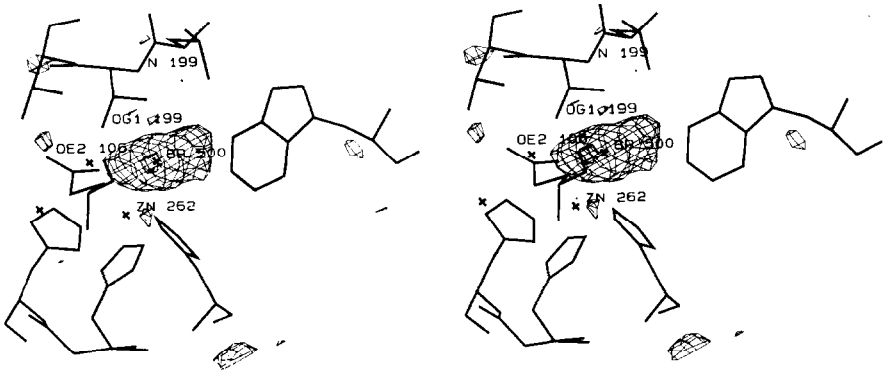

Fig. 3. The active site in HCAII complex with bromide at pH 7.8. The structure is virtually identical to that of pH 6.0 (Fig. 2). Positive (continuous lines) and negative (broken lines) |Fo|–|Fc| maps are drawn at 3 σ.

Table III

Geometries in the active site of carbonic anhydrase II complexed with azide: distances (X–Zn) and angles (X–Zn–Y) of the zinc ion ligands

	Distance (Å)	Angles (°)		
		His 94 Nε2	His 96 Nε2	His 119 Nδ1
Azi 500 N3	2.02	102.8	116.4	120.2
His 94 Nε2	2.20		105.9	112.5
His 96 Nε2	2.18			98.6
His 119 Nδ1	2.12			

electron density maps were consequently difficult to interpret. The bromide ion replaces both the zinc water and the deep water, as was found at pH 6. Its distance to the zinc ion is 2.6 Å. The distances to T199 Oγ1 and to T199 N are 3.5 Å and 3.8 Å, respectively. Thus there are no significant differences between the two structures, however, at higher pH, hydroxide ions compete for coordination to the zinc ion. The enzyme was therefore not saturated with respect to bromide in the pH 7.8 experiment, but it is reasonable to assume that, once bound, the structure of the bromide complex is independent of pH [2]. The electron density of water 389 is rather weak, and at a distance of 3.4 Å from the bromide ion. The site is probably occupied only when there is no bromide bound.

3.4. *General*

The conformation of the peptide chain was, in all three cases, unaffected by inhibitor binding. The root mean square deviations for Cα atoms from the native positions were 0.07, 0.17 and 0.08 Å, respectively.

4. DISCUSSION

Both azide and bromide replace the zinc water as well as the deep water, and the coordination number of the zinc ion is four in both cases. The coordination is significantly distorted from normal tetrahedral geometry (Tables III and IV). The inhibitors cannot bind close to T199 Oγ1 due to their lack of protons and to the lone pair the former keeps oriented towards the tetrahedral zinc water position. Bromide thus behaves essentially as iodide (Kannan, K.K., personal communication) with respect to its coordination to the metal. The fact that these two ligands can replace the zinc water without donating a proton to T199 Oγ1 might be due to their softness (polarizability), compared to oxygen. Soft anions have a higher affinity for relatively soft cations like zinc and, conversely, hard anions have a higher affinity for hard cations like alkali and alkaline earth metal ions [16]. The higher polarizability of the bromide and azide ions might alleviate the confrontation between its electron orbitals and the lone pair of T199 Oγ1. That the hydrogen bond network in these complexes really is intact is supported by the increased distances between inhibitor and Thr199 Oγ1, as compared to previously analyzed tetrahedral complexes. Furthermore, no previously investigated anion complex (listed in [4]) has suggested reversal of or drastic changes in the Glu^{106}–Thr^{199} hydrogen bond network in native HCAII.

Thus we conclude that hard anions with oxygen ligands binding to the tetrahedral position require that they are proton donors, but that soft anions might bind tetrahedrally, with distortions in coordination geometry if they lack protons.

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Table IV

Geometries in the active site of carbonic anhydrase II complexed with bromide at pH 6.0 (pH 7.8 in brackets): distances (X–Zn) and angles (X–Zn–Y) of the zinc ion ligands

	Distance (Å)	Angles (°)		
		His 94 Nε2	His 96 Nε2	His 119 Nδ1
Br 500	2.48 (2.56)	100.1 (104.9)	135.0 (138.3)	100.2 (96.5)
His 94 Nε2	2.27 (2.21)		108.5 (103.1)	114.4 (117.9)
His 96 Nε2	2.25 (2.15)			98.8 (97.0)
His 119 Nδ1	2.26 (2.11)			

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