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Wild-Type and E106Q Mutant Carbonic Anhydrase Complexed with Acetate

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Abstract

The molecular structures of the acetate complexes of wild-type human carbonic anhydrase II (HCAII) and of E106Q mutant human carbonic anhydrase II were solved with high completeness (89-91%) to 2.1 and 1.9 Å resolution, respectively. Both wild-type and mutant enzyme crystallize in space group $P2_1$ with cell dimensions a = 42.7, b = 41.7, c = 73.0 Å and β = 104.6°. The altered active-site hydrogen-bond network caused by the mutation results in a different binding of the inhibitor in the two complexes. In the mutant, but not in the wild-type complex, a carboxylate O atom is within hydrogen-bond distance of Thr199 Oγ1. In the wild-type enzyme ligand hydrogen bonding to this atom is normally only found for hydrogen-bond donors. The importance of this discrimination on catalysis by the enzyme is discussed briefly.

1. Introduction

Human carbonic anhydrase II (carbonate hydrolyase, E.C. 4.2.1.1) is a monomeric zinc enzyme that is abundant in erythrocytes (Keilin & Mann, 1940). It is catalytically the most efficient member of a group of six isoenzymes that have been demonstrated in humans (Khalifah & Silverman, 1991). The enzymatic reaction, hydration of carbon dioxide to bicarbonate or the reverse dehydration of bicarbonate, occurs at the zinc ion (Lindskog & Malmström, 1962). The zinc ion is tetrahedrally coordinated to three histidine residues and a water/hydroxide (Eriksson, Jones & Liljas, 1988; Håkansson, Carlsson, Svensson & Liljas, 1992) with a pK of ~7 (Steiner, Jonsson & Lindskog, 1975). Part of the active-site pocket is made up of hydrophobic residues belonging to the central β-sheet of the molecule. Substrate binding takes place between the zinc ion and the hydrophobic region where, in the free enzyme, the so called 'deep' water molecule is bound (Liang & Lipscomb, 1990; Merz, 1991; Håkansson & Wehnert, 1992; Xue, Vidgren *et al.*, 1993). Zinc-bound hydroxide becomes part of the bicarbonate product following nucleophilic attack on the carbon dioxide molecule (Coleman, 1967). A new water molecule replaces the bicarbonate and the hydroxide ion is probably regenerated through shuttling one of its protons *via* waters 318, 292 and His64 (Håkansson, Carlsson, Svensson & Liljas, 1992).

Analysis of some anion-inhibitor complexes of the enzyme revealed that only potential hydrogen-bond donors can replace the zinc-bound water/hydroxide and this is ascribed to a hydrogen-bond network through Glu106-Thr199-zinc water/hydroxide, where the threonine residue acts as a hydrogen-bond donor towards the glutamate and consequently as an acceptor towards the zinc-water site (Eriksson, Kylsten, Jones & Liljas, 1988). A catalytic function of this network has been proposed, where the bicarbonate substrate is destabilized by 'misorientation', since the hydroxyl group, rather than any of the carboxylate O atoms will be closest to the zinc ion resulting in a strong polarization of the hydroxyl-carbon bond (Håkansson, Carlsson, Svensson & Liljas, 1992). In addition, it orients one of the lone pairs of the hydroxide ion towards the presumed carbon dioxide binding site (Merz, 1990). The existence of a carboxylate binding site between the zinc ion and the hydrophobic region, common to bicarbonate, formate

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Table 1. *Soaking conditions and data collection*

Soaking experiments were performed in 80 m*M* citrate (wild type) or 25 m*M* MES (mutant) with 3*M* ammonium sulfate at pH 6 for 24 h.

	Wild-type carbonic anhydrase	E106Q mutant carbonic anhydrase
Acetate concentration (*M*)	0.3	0.5
Resolution (Å)	2.1	1.9
Completeness (%)	91	89
R_{merge} (%)	6.7	5.9

and related compounds like bisulfite and nitrate, has also been suggested as being involved in the 'misorientation' of bicarbonate (Fig. 1; Håkansson & Wehnert, 1992; Håkansson, 1992). In the present paper, acetate binding to wild-type carbonic anhydrase II and to E106Q mutant carbonic anhydrase II is described. In the mutant, the hydrogen-bond network is altered and the zinc-water site is accessible to hydrogen-bond donors as well as acceptors (Liang, Xue, Behravan, Jonsson & Lindskog, 1993; Xue, Liljas, Jonsson & Lindskog, 1993).

2. Materials and methods

Crystals of mutant carbonic anhydrase were grown in 2.3 *M* ammonium sulfate, 50 m*M* Tris-H_2SO_4 and 3 m*M* dithiothreitol (Tilander, Strandberg & Fridborg, 1965). Crystals of human carbonic anhydrase II were grown in the same buffer with 2.4 *M* ammonium sulfate and 1 m*M* $HgCl_2$. The mercury was removed with 3 *M* ammonium sulfate, 50 m*M* Tris pH 7.8 and 5 m*M* β-mercaptoethanol. Soaking conditions and some details of the data collection are shown in Table 1. Data collection, reduction and integration of the wild-type enzyme complex was performed with a Siemens area detector and 2θ = 17° essentially as described by Håkansson, Carlsson, Svensson & Liljas (1992). Mutant-complex data were integrated with *XDS* (Kabsch, 1988*a*,*b*). Both data sets were refined with *PROLSQ* (Finzel, 1987; Hendrickson, 1985) and the refinement statistics are shown in Table 2 (Håkansson, Carlsson, Svensson & Liljas, 1992, and references therein). Coordinates and *F* values have been deposited with the Protein Data Bank (Bernstein *et al.*, 1977). The accession numbers are 1CAY (wild-type complex) and 1CAZ (mutant complex).*

Table 2. *Refinement statistics of wild-type and E106Q mutant carbonic anhydrate* II *complexed with acetate*

$R_{cryst} = \Sigma(|F_o| - |F_c|)/\Sigma|F_o|$.

	Target σ	Wild-type enzyme with acetate	Mutant enzyme with acetate
R_{cryst}		0.141	0.153
Mean *B* (Å²)		15.1	12.6
Error in coordinates (Å) according to Luzzati plot		0.16	0.17
R.m.s. values			
Distances			
Bond distance (Å)	0.020	0.020	0.020
Angle distance (Å)	0.030	0.040	0.038
Planar 1–4 distance (Å)	0.050	0.050	0.049
Miscellaneous			
Plane groups (Å)	0.020	0.019	0.018
Chiral centres (Å³)	0.150	0.226	0.202
Non-bonded distances			
Single torsion (Å)	0.200	0.157	0.159
Multiple torsion (Å)	0.200	0.163	0.163
Possible *X*···*Y* hydrogen bond (Å)	0.200	0.164	0.154
Torsion angles			
Planar (°)	3.0	3.5	3.3
Staggered (°)	15.0	16.3	17.4
Orthonormal (°)	20.0	30.6	30.6
Thermal restraints			
Main-chain bond (Å²)	1.000	0.986	0.982
Main-chain angle (Å²)	1.500	1.620	1.585
Side-chain bond (Å²)	1.500	2.144	2.077
Side-chain angle (Å²)	2.000	3.347	3.201

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 1CAY, 1CAZ, R1CAYSF, R1CAZSF). Free copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England (Supplementary Publication No. SUP 37101). A list of deposited data is given at the end of this issue.

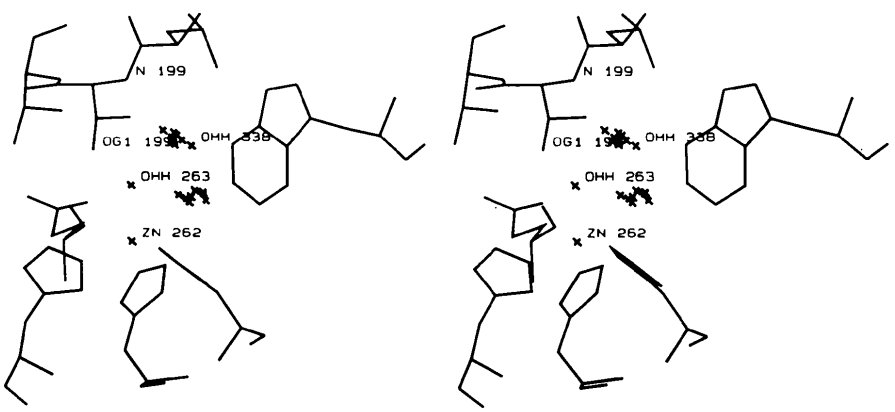

Fig. 1. The positions of partially negatively charged atoms of some of the carbonic anhydrase-ligand complexes. Included are, in addition to the native water molecules 263 and 338 (zinc and deep water), atoms from formate, bisulfite, nitrate, cyanate, Diamox, aminobenzolamide as complexed to native human carbonic anhydrase II, and the carboxylate O atoms of bicarbonate as complexed to cobalt(II)-substituted carbonic anhydrase and to T200H mutant carbonic anhydrase (Håkansson, 1992, and references therein).

Table 3. *Geometries in the active site of wild-type carbonic anhydrase complexed with acetate*

Distances (Zn—*X*) and angles (*X*—Zn—*Y*) around the zinc ion.

	Distances (Å)	Angles (°) CH_3CO_2 O2	His94 Nε2	His96 Nε2	His119 Nδ1
Wat 263	2.05	67.6	111.0	92.5	129.9
CH_3CO_2 O2	2.42		97.9	156.7	86.8
His94 Nε2	2.24			100.7	114.8
His96 Nε2	2.21				97.8
His119 Nδ1	2.13				

3. Results

3.1. *Wild-type carbonic anhydrase* II *complexed with acetate* (*Fig. 2a*)

The structure of this complex is similar to the carbonic anhydrase-nitrate complex (Mangani & Håkansson, 1992). The inhibitor replaces waters 338 and 389. One of the carboxylate O atoms is hydrogen bonded to Thr199 N (3.0 Å) and the other has a distance to the zinc of 2.4 Å, and to the native zinc-water position of 1.7 Å. The zinc water itself is displaced 0.8 Å from its native position. The carboxylate O atoms differ in positions from the corresponding atoms in the formate complex by 1.0 and 0.5 Å, respectively. This is probably due to steric influence from some of the hydrophobic residues in the active site. The methyl group of the inhibitor makes van der Waals interactions with Val143 (3.3 Å), Leu198 (3.9 Å) and Trp209 (3.6 Å). None of the carboxylate O atoms come closer than 3.8 Å to Thr199 Oγ1. The zinc coordination geometry is shown in Table 3.

3.2. *E106Q mutant carbonic anhydrase complexed with acetate* (*Fig. 2b*)

In this complex one of the carboxylate O atoms is coordinated to the zinc at a distance of 2.1 Å from the metal and 0.6 Å from the native zinc-water position. The zinc water and waters 338 and 389 are all replaced by the inhibitor in this complex. Water 318 is displaced by 1.3 Å from its native position to a position 2.6 Å from the zinc and 2.0 Å from the native zinc water. The coordination number thus seems to be 4, but the geometry deviates significantly from a tetrahedral arrangement and approaches a trigonal bipyramid if water 318 is included. An important feature is that the zinc-coordinated carboxylate O atom is hydrogen bonded to Thr199 Oγ1 (2.8 Å) in this mutant. The other carboxylate O atom, although differing from its

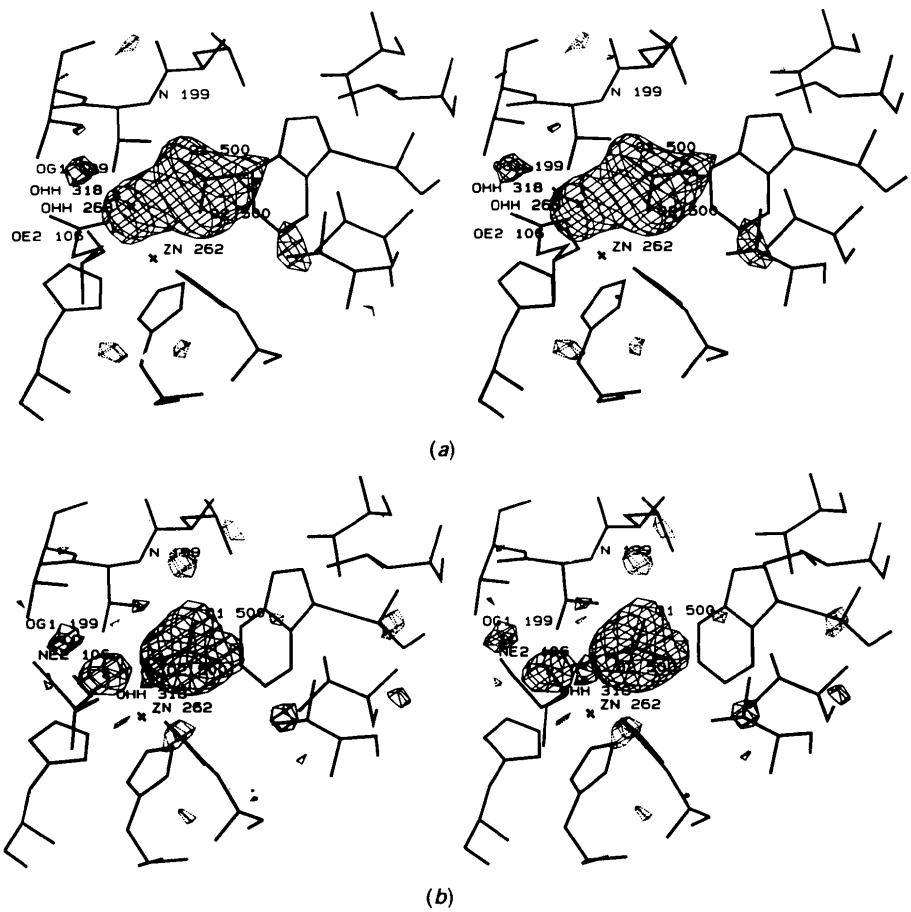

Fig. 2. (*a*) The wild-type carbonic anhydrase-acetate complex. (*b*) The E106Q mutant carbonic anhydrase-acetate complex. Difference electron-density maps were calculated after refinement of native coordinates without waters 263, 338 and in (*b*) also without water 318. Positive (continuous lines) and negative (broken lines) $|F_o| - |F_c|$ contours were drawn at $+3\sigma$.

Table 4. *Geometries in the active site of E106Q mutant carbonic anhydrase complexed with acetate*

Distances (Zn—*X*) and angles (*X*—Zn—*Y*) around the zinc ion.

	Distances (Å)	Angles (°) CH_3CO_2 O2	His94 Nε2	His96 Nε2	His119 Nδ1
Wat 318	2.63	66.8	83.5	86.0	158.9
CH_3CO_2 O2	2.13		127.0	120.2	92.9
His94 Nε2	2.10			99.1	114.9
His96 Nε2	2.22				100.3
His119 Nδ1	2.17				

homologue in the wild-type complex by 1.6 Å, is still hydrogen bonded to Thr199 N at a distance of 3.0 Å. The coordination geometry of the zinc ion is shown in Table 4.

4. Discussion

The structures described in this paper are in good agreement with our current view of the inhibition and catalytic properties of carbonic anhydrase II. One of the keystones in the mode of operation of the enzyme is the exclusion of atoms that are not hydrogen-bond donors from coordination in the zinc-water position by virtue of the Glu106···Thr199 hydrogen-bond network. This rule has only a few exceptions, where relatively soft (polarizable) ions like bromide bind with distorted geometry around the metal (Jönsson, Håkansson & Liljas, 1993). Hence the bicarbonate carboxylate group is excluded from the zinc-water position and instead binds to a rather well defined carboxylate-binding site between the zinc-water position and the cluster of hydrophobic active-site residues (Fig. 1). This idea is supported by the behaviour of acetate, where the hydroxyl group of the substrate is replaced by a methyl group. In the wild-type enzyme complex, the acetate carboxylate group binds to this carboxylate-binding site, similar to most inhibitors with two partly negatively charged atoms. The hydrogen-bond acceptor Thr199 Oγ1 prevents the carboxylate O atoms from entering the zinc-water position, which would be the most stable coordination for a tetrahedral organic zinc ion complex but which would also be catalytically unproductive. In the enzyme, this site is reserved for the bicarbonate hydroxyl group. The methyl group of acetate cannot of course mimic the bicarbonate hydroxyl in this sense but is instead oriented towards the hydrophobic side of the active site where it makes van der Waals interactions with several residues. In the E106Q mutant complex, on the other hand, the hydrogen-bond network mentioned above is reversed and Thr199 Oγ1 can act either as a hydrogen-bond donor or acceptor *vis-à-vis* the zinc-water position. Consequently, acetate (and by inference bicarbonate) can bind with a carboxylate O atom close to the zinc-water position. For bicarbonate, destabilization of the hydroxyl-carbon bond, and hence dehydration, fails to occur at a high rate. The mutant binds bicarbonate much stronger than the wild-type enzyme does (Liang, Xue, Behravan, Jonsson & Lindskog, 1993) and it is reasonable to assume that this is also true for acetate, due to the electrostatic interaction between the negatively charged oxygen and the zinc ion. Another reason for the loss of activity might be that the hydroxide (in the hydration reaction) might have its proton, rather than a lone pair, oriented in the substrate direction. The whole process of regenerating an active nucleophilic hydroxide is thus slowed down. In addition, as a result of dipole alteration of the Thr199···zinc-water hydrogen bond, the hydroxide will be less nucleophilic than in wild-type carbonic anhydrase.

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References

Bernstein, F. C., Koetzle, T. F., Williams, G. J. B., Meyer, E. G., Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T. & Tasumi, M. (1977). *J. Mol. Biol.* **112**, 535-542.

Coleman, J. E. (1967). *J. Biol. Chem.* **242**, 5212-5219.

Eriksson, A. E., Jones, T. A. & Liljas, A. (1988). *Proteins*, **4**, 274-282.

Eriksson, A. E., Kylsten, P. M., Jones, T. A. & Liljas, A. (1988). *Proteins*, **4**, 283-293.

Finzel, B. C. (1987). *J. Appl. Cryst.* **20**, 53-55.

Håkansson, K. (1992). Doctoral thesis, Univ. of Lund, Sweden.

Håkansson, K., Carlsson, M., Svensson, L. A. & Liljas, A. (1992). *J. Mol. Biol.* **227**, 1192-1204.

Håkansson, K. & Wehnert, A. (1992). *J. Mol. Biol.* **228**, 1212-1218.

Hendrickson, W. A. (1985). *Methods Enzymol.* **115**, 252-270.

Jönsson, B. M., Håkansson, K. & Liljas, A. (1993). *FEBS Lett.* **322**, 186-190.

Kabsch, W. (1988*a*). *J. Appl. Cryst.* **21**, 67-71.

Kabsch, W. (1988*b*). *J. Appl. Cryst.* **21**, 916-924.

Keilin, D. & Mann, T. (1940). *Biochem. J.* **34**, 1163-1176.

Khalifah, R. G. & Silverman, D. N. (1991). *The Carbonic Anhydrases*, edited by S. J. Dodgson, R. E. Tashian, G. Gros & N. D. Carter, pp. 49-70. New York: Plenum Press.

Liang, J. Y. & Lipscomb, W. N. (1990). *Proc. Natl Acad. Sci. USA*, **87**, 3675-3679.

Liang, Z., Xue, Y., Behravan, G., Jonsson, B.-H. & Lindskog, S. (1993). *Eur. J. Biochem.* **211**, 821-827.

Lindskog, S. & Malmström, B. G. (1962). *J. Biol. Chem.* **237**, 1129-1137.

Mangani, S. & Håkansson, K. (1992). *Eur. J. Biochem.* **210**, 867-871.

Merz, K. M. (1990). *J. Mol. Biol.* **214**, 799-802.

Merz, K. M. (1991). *J. Am. Chem. Soc.* **113**, 406-411.

Steiner, H., Jonsson, B. H. & Lindskog, S. (1975). *Eur. J. Biochem.* **59**, 253-259.

Tilander, B., Strandberg, B. & Fridborg, K. (1965). *J. Mol. Biol.* **12**, 740-760.

Xue, Y., Liljas, A., Jonsson, B. H. & Lindskog, S. (1993). *Proteins*, **17**, 93-106.

Xue, Y., Vidgren, J., Svensson, A., Liljas, A., Jonsson, B. H. & Lindskog, S. (1993). *Proteins*, **15**, 80-87.