FEBS Letters 350 (1994) 319–322

FEBS 14411

The structure of a complex between carbonic anhydrase II and a new inhibitor, trifluoromethane sulphonamide

K. Håkansson, A. Liljas*

Department of Molecular Biophysics, Chemical Center, University of Lund, Box 124, S-221 00 Lund, Sweden

Received 2 June 1994; revised version received 18 July 1994

Abstract

It has recently been shown that aliphatic sulphonamides are good inhibitors of carbonic anhydrase (CA) provided that the pK of the sulphonamide is low. We have determined the structure of the complex between CAII and $CF_3SO_2NH_2$ by X-ray crystallographic methods. The nitrogen of the sulphonamide is bound to the zinc ion of the enzyme in the usual manner. The other parts of the inhibitor show a different mode of binding from aromatic sulphonamides since the trifluoromethyl group is bound at the hydrophobic part of the active site instead of pointing out from the active site. It should be possible to design new inhibitors specific for the different isoenzymes, starting from the present structure.

Key words: Carbonic anhydrase: X-ray structure; Inhibitor; Trifluoromethane sulphonamide

1. Introduction

Carbonic anhydrase (carbonate hydro-lyase, EC 4.2.1.1) is a zinc enzyme that catalyzes the interconversion of CO_2 and HCO_3^- in a variety of organisms and tissues. Out of the seven known mammalian isoenzymes [1], CAI, CAII and CAIII [2–4] have been investigated crystallographically. As expected from the high degree of homology between these 30 kDa isoenzymes [1,5], they have analogous three dimensional structures, which resemble that of a hamburger, with a central β-sheet running through the molecule surrounded on both sides mostly by non-helical structures. The active site is in a cavity situated on the β-sheet. The active site zinc ion is tetrahedrally coordinated by three histidine residues belonging to this β-sheet [3] and a water/hydroxide molecule which in the high activity form CAII has a pK of around 7 [6]. In addition to high resolution refinement of free CAII many ligand complexes of this isoenzyme have been analyzed including several sulphonamides as well as the structure of mutants and metal substituted derivatives (reviewed in [7]). The inhibitor/substrate binding position is between the zinc and a part of the β-sheet consisting of hydrophobic side chains in a very stable and rigid stucture. The zinc water/hydroxide position can normally be occupied only by hydrogen bond donors due to the nearby lone pair of the T199Oγ1. The central step in all mechanistic models discussed today is the nucleophilic attack by the zinc bound hydroxide on the carbon dioxide carbon atom [7–12].

The interaction between carbonic anhydrase and the sulphonamides is of significant medical interest. Inhibition of carbonic anhydrase in the ciliary process by sulphonamide inhibitors is effective in the treatment of glaucoma [13,14]. Side effects caused by high doses of systemic drugs have urged the creation of a new generation of sulphonamide inhibitors which could be introduced topically in smaller amounts [13,15]. The common denominator of the sulphonamide drugs is the $-SO_2NH_2$ group. Aromatic sulphonamides normally bind strongly to the enzyme whereas aliphatic sulphonamides bind more weakly. However, recently Maren and Conroy discovered that trifluoromethane sulphonamide ($CF_3SO_2NH_2$) has a pK of 5.9 and a carbonic anhydrase II dissociation constant of 2 nM and that the dissociation constant of aliphatic sulfonamides is strongly related to the pK [14]. The affinity of this inhibitor for low activity forms of carbonic anhydrase is also unusually high. The structure of this inhibitor in complex with human carbonic anhydrase II is the topic of the present paper.

2. Materals and methods

2.1. Crystallization and soaking

Human carbonic anhydrase II was crystallized as described previously in 2.4 M ammonium sulphate, 50 mM Tris-HCl, pH 8.5, and 1 mM $HgCl_2$ [16]. Trifluoromethane sulphonamide was supplied by the Minnesota Mining and Manufacturing Company. The HCAII crystals were soaked in 3 M ammonium sulphate, 50 mM Tris, pH 7.8, for two days with 5 mM β-mercaptoethanol in order to remove the mercury and subsequently for two days with 1 mM $CF_3SO_2NH_2$.

2.2. Data collection and processing

X-ray diffraction data were collected with a Siemens area detector system using a Rigaku RU200BEH rotating anode running at 40 kV and 100 mA with a 0.3 mm focus as the X-ray source. A graphite monochromator was used to obtain CuKα radiation. The detector to crystal distance was 8.5 cm and the collimator width 0.5 mm. The goniostat was a Siemens three-axis system and 2Θ was set to 17°. The crystal was rotated 185° around ω with a step size of 0.25° and 150 s exposure time per frame. The procedure was repeated after changing φ by 90°. A single crystal was used for the entire data set. The program

*Corresponding author. Fax: (46) (46) 104543.

SSDI 0014-5793(94)00798-5

package Xengen [17] was used for data reduction. Data collection statistics are shown in Table 1.

2.3. Refinement and graphics

Restrained least square refinement [18] was performed at 1.93 Å resolution using the program PROFFT [19]. The starting model was the native HCAII structure [9] where some of the active site water molecules were removed. $2|Fo|-|Fc|$ and $|Fo|-|Fc|$ electron density maps were calculated with the CCP4 program suite [20]. The refinement was performed iteratively with graphics work where inhibitor and solvent molecules were inserted successively to fit the calculated maps prior to the next series of refinement cycles. The results were visualized on an Evans and Sutherland ESV graphics station using the program O [21]. Plots were prepared by the program ESVodo [22]. Temperature factors were refined isotropically. Coordinate root mean-square deviations were estimated from Luzzati plots [23]. Refinement statistics are shown in Table 2. Coordinates and F values have been deposited with the Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton, NY 11973, USA, from which copies are available [24]. The accession number is 1BCD.

3. Results and discussion

The active site structure of the HCAII trifluoromethane sulphonamide complex is shown in Fig. 1. A comparision between the present trifluoromethane sulphonamide binding and that of the aromatic sulphonamide acetazolamide [25] is shown in Fig. 2. The positions for the sulphonamide nitrogen atoms are similar, but compared to acetazolamide, the trifluoromethane sulphonamide inhibitor is rotated 180° around the sulphur-nitrogen bond. As a result, both oxygens of the trifluoromethane sulphonamide inhibitor are oriented more outwards from the active site cleft differing from the closest oxygens in acetazolamide with 2.0 and 2.5 Å. Likewise, the orientation of the aliphatic trifluoromethyl group differs markedly from that of the aromatic ring in acetazolamide, the former pointing inward and the latter outward from the active site cleft. The smaller aliphatic group can make close contacts in the hydrophobic part of the active site which is impossible for bulky aromatic groups. Its fluoro atoms are within 4 Å of several of the hydrophobic active site residues like Val_{121}, Val_{143}, Leu_{198}, Thr_{199}, Trp_{209} and a water molecule (OHH 461) as well as the zinc ion and its ligands.

Table 2
Refinement results

	Target sigma	Result
R_{cryst}		0.154
Mean B (Å^2)		13.3
Error in coordinates (Å) according to Luzzati plot		0.17
RMS values		
Distances		
Bond distance (Å)	0.020	0.020
Angle distance (Å)	0.030	0.039
Planar 1–4 distance (Å)	0.050	0.049
Miscellaneous		
Plane groups (Å)	0.020	0.019
Chiral centers (Å^3)	0.150	0.215
Non-bonded distances		
Single torsion (Å)	0.200	0.157
Multiple torsion (Å)	0.200	0.165
Possible X–Y H bond (Å)	0.200	0.160
Torsion angles		
Planar (°)	3.0	3.4
Staggered (°)	15.0	16.6
Orthonormal (°)	20.0	30.8
Thermal restraints		
Main-chain bond (Å^2)	1.000	0.978
Main-chain angle (Å^2)	1.500	1.537
Side-chain bond (Å^2)	1.500	1.979
Side-chain angle (Å^2)	2.000	2.970

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

Water molecules 263, 318, 338 and 389 are replaced by the inhibitor. Water molecule 292 is also absent in the inhibitor–enzyme complex. This water molecule is hydrogen bonded to His^{64} in native carbonic anhydrase [9]. Instead a new water (OHH499) forms hydrogen bonds with His^{64}, T200Oγ1 and water 359. This is due to the fact that the side chain of His^{64} is rotated 180° around the Cβ–Cγ bond from its native conformation [9].

As expected, the nitrogen atom of the inhibitor is bound directly to the zinc ion with tetrahedral coordina-

Table 1
Data collection summary

Shell lower limit (Å)	Number of scaled observations	Number of unique reflections	Bragg possible	Completeness to lower limit (%)	Average intensities	Average I/sig(I)	R_{sym} (%)
3.50	12,734	3,233	3,252	99.4	288	43.0	6.2
2.78	10,099	3,161	3,186	99.3	142	14.7	10.6
2.43	8,323	3,091	3,166	98.8	77	6.7	16.0
2.21	7,107	2,977	3,141	97.8	58	4.7	19.1
2.05	5,966	2,854	3,139	96.4	45	3.2	22.9
1.93	2,237	1,467	3,152	88.2	39	2.2	27.0
All data	46,466	16,783	19,036	88.2	118	13.9	8.3

$$R_{sym} = 100\,\frac{\Sigma\Sigma\,|<I>-I_i|}{\Sigma I_i}$$ where $<I>$ is the average of I_i over all symmetry equivalents.

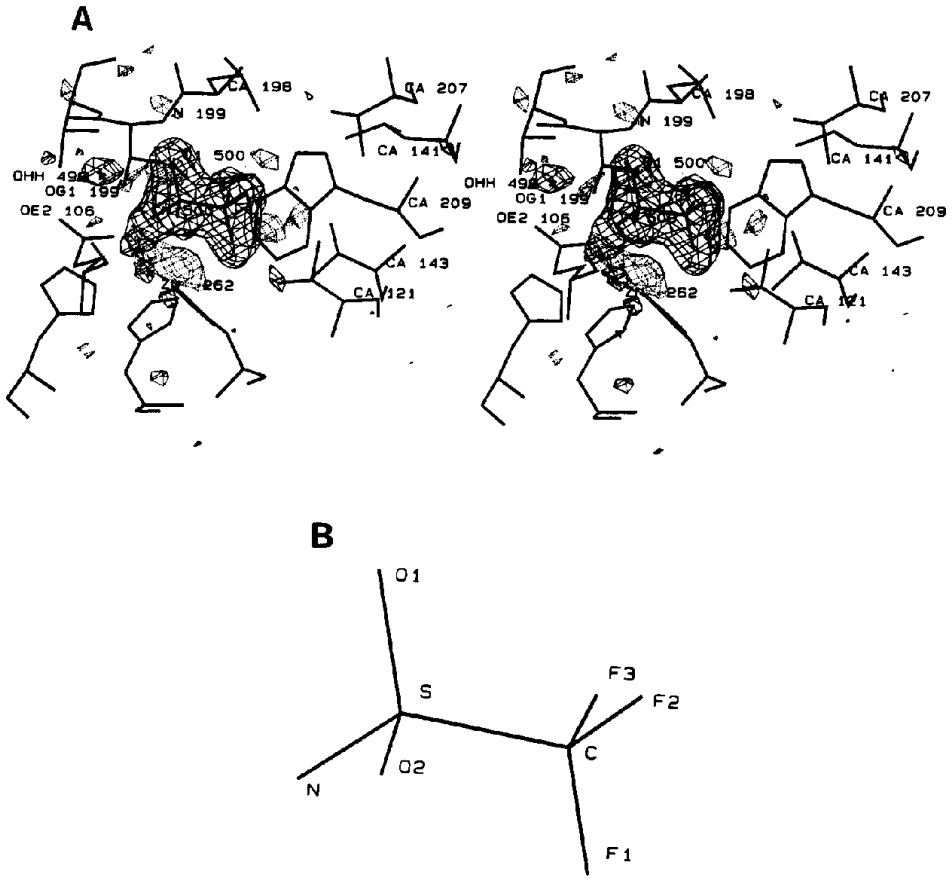

Fig. 1. (A) The active site of carbonic anhydrase complexed with the inhibitor $CF_3SO_2NH^-$. Difference electron maps were calculated after refinement of native coordinates without waters 263, 292, 318, 338 and 389. Positive (continous lines) and negative (broken lines) $|Fo|-|Fc|$ contours were drawn at $\pm 3\ \sigma$. (B) The inhibitor from (A) with all atoms labelled.

tion (Table 3) 2.7 Å from T199Oγ1. Ligands with this property – a negative charge and a proton available on the same atom – include OH^- and SH^- in addition to members of the sulphonamide family. These ligands all bind strongly [6,13,26] to the zinc water position [9,15,25,27,28] because they interact electrostatically with the zinc ion by virtue of their negative charge and in addition they are hydrogen bond donors to the lone pair of T199Oγ1.

The O1 atom of the inhibitor is hydrogen bonded to T199N (2.9 Å) and makes van der Waals' contacts with several of the atoms of residues 198 and 199. The two oxygens of the aromatic sulphonamides bind at approximately the same site as cyanate [29], which is isoelectronic with carbon dioxide whereas the oxygens of trifluoromethane sulphonamide bind differently. As pointed out by Kumar et al. [30] the sulphonamide group can be considered as a transition state analogue mimicing a hydroxide reacting with carbon dioxide. The new binding mode raises the question of which sulphonamide complex is the best representation of the transition state. Since most inhibitors bind close to the positions of the oxygens of aromatic sulphonamides we are inclined to

Table 3
Geometries in the active site of carbonic anhydrase complexed with $CF_3SO_2NH^-$

	Distance	His^{94} Nε2	His^{96} Nε2	His^{119} Nδ1
$CF_3SO_2NH^-$N	2.10	115.2	102.9	113.5
His^{94} Nε2	2.17		102.3	116.3
His^{96} Nε2	2.25			104.2
His^{119} Nδ1	2.07			

Distances (Zn-X) and angles (X-Zn-Y) around the zinc ion in Ångström and degrees.

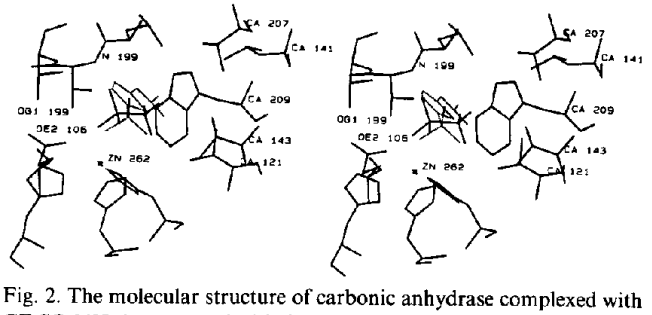

Fig. 2. The molecular structure of carbonic anhydrase complexed with $CF_3SO_2NH^-$ is compared with the complex with acetazolamide (broken lines). The polypeptide chain of the acetazolamide complex [25] is not shown since the conformations are virtually the same in the two cases.

believe that this is the best representation of the transition state [7].

Aromatic sulphonamide inhibitors bind more strongly to the high activity forms of carbonic anhydrase than to the low activity forms [13]. This is true also for the aliphatic trifluoromethane sulphonamide [14] although it is bound in a way different from the aromatic sulphonamides.

In conclusion, the factors giving the trifluoromethanesulphonamide high affinity for carbonic anhydrase is the low pK enabling a negatively charged nitrogen to be bound to the zinc ion, a small hydrophobic moiety placed in the hydrophobic part of the enzyme active site and finally the oxygens that are hydrogen bonded (O1) or exposed to the solvent (O2). Since inhibiton of ciliary CAIV is the most critical factor in the in vivo action (in rabbits at least) of $CF_3SO_2NH_2$ [14] a structural study of sulphonamide inhibitors to this isoenzyme in combination with the current results may be a useful guide for developing new topical sulphonamides for pharmaceutical usage.

Acknowledgement: We are grateful to Prof. T.H. Maren for suggesting this study, providing us with the inhibitor and for valuable discussions. We thank the Natural Science Research Council (NFR), Swedish Council for Planning and Coordination of Research (FRN), NUTEK, the SE-bank, and the Knut and Alice Wallenberg foundation for funding.

References

[1] Tashian, R.E., Hewett-Emmet, D. and Venta, P.J. (1991) In: Carbonic Anhydrase: From Biochemistry and Genetics to Physiology and Clinical Medicine (Botrè, F., Gros., G. and Storey, B.T. Eds.), pp. 151–161, VCH Verlagsgesellschaft mbH.
[2] Kannan, K.K., Notstrand, B., Fridborg, K., Lövgren, S., Ohlsson, A. and Petef, M. (1975) Proc. Natl. Acad. Sci. USA 72, 51–55.
[3] Liljas, A. Kannan, K.K., Bergstén, P.-C., Waara, I., Fridborg, K., Strandberg, B., Carlbom, U., Järup, L., Lövgren, S. and Petef, M. (1972) Nature New Biol. 235, 131–137.
[4] Eriksson, A.E. and Liljas, A. (1993) Proteins 16, 29–42.
[5] Hewett-Emmet, D. and Tashian, R.E. (1991) In: The Carbonic Anhydrases: Cellular Physiology and Molecular Genetics (Dodgson, S.J., Tashian, R.E., Gros, G. and Carter, N.D. Eds.), pp. 15–32, Plenum Press, NY.
[6] Steiner, H., Jonsson, B.H. and Lindskog, S. (1975) Eur. J. Biochem. 59, 253–259.
[7] Liljas, A., Håkansson, K., Jonsson, B.H. and Xue, Y. (1994) Eur. J. Biochem. 219, 1–10.
[8] Coleman, J.E. (1967) J. Biol. Chem. 242, 5212–5219.
[9] Håkansson, K., Carlsson, M., Svensson, L.A. and Liljas, A. (1992) J. Mol. Biol. 227, 1192–1204.
[10] Eriksson, A.E., Kylsten, P.M., Jones, T.A. and Liljas, A. (1988) Proteins 4, 283–293.
[11] Liang, J.-Y. and Lipscomb, W.N. (1987) Biochemistry 26, 5293–5301.
[12] Lindskog, S. and Liljas, A. (1993) Curr. Op. Struct. Biol. 3, 915–920.
[13] Maren, T.H. (1987) Drug. Dev. Res. 10, 255–276.
[14] Maren, T.H. and Conroy, T.H. (1993) J. Biol. Chem. 268, 26233–26239.
[15] Baldwin, J.J., Ponticello, G.S., Anderson, P.S., Christy, M.E., Murcko, M.A., Randall, W.C., Schwam, H., Sugrue, M.F., Springer, J.P., Gautheron, P., Grove, J., Mallorga, P., Viader, M.-P., McKeever, B.M. and Navia, M.A. (1989) J. Med. Chem. 32, 2510–2513.
[16] Tilander, B., Strandberg, B. and Fridborg, K. (1965) J. Mol. Biol. 12, 740–760.
[17] Howard, A.J., Gilliland, G.L., Finzel, B.C., Poulos, T.L., Ohlendorf, D.H. and Salemme, F.R. (1987) J. Appl. Crystalogr. 20, 383–387.
[18] Hendrickson, W.A. (1985) Methods Enzymol. 115, 252–270.
[19] Finzel, B.C. (1987) J. Appl. Crystallogr. 20, 53–55.
[20] CCP4, 1979, The SERC (UK) Collaborative Computing Project No. 4, a suite of programs for protein crystallography, distributed from Daresbury Laboratory, Warrington, WA4 4AD, UK.
[21] Jones, T.A., Zou, J.-Y., Cowan, S.W. and Kjeldgaard, M. (1991) Acta Crystallogr. A47, 110–119.
[22] Pflugrath, J.W., Saper, M.A. and Quicho, F.A. (1984) In: Methods in Applied Crystallography and Computing (Hall, S. and Ashida, T. Eds.) pp. 404–407, Clarendon Press, Oxford.
[23] Luzzati, V. (1952) Acta Crystallogr. 5, 802–810.
[24] Bernstein, F.C., Koetzle, T.F., Williams, G.J.B., Meyer, E.G., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M. (1977) J. Mol. Biol. 112, 535–542.
[25] Vidgren, J., Liljas, A. and Walker, N.P.C. (1990) Int. J. Biol. Macromol. 12, 342–344.
[26] Thorslund, A. and Lindskog, S. (1967) Eur. J. Biochem. 3, 117–123.
[27] Mangani, S. and Håkansson, K. (1992) Eur. J. Biochem. 210, 867–871.
[28] Lindahl, M., Svensson, A. and Liljas A. (1993) Proteins, 177–182.
[29] Vidgren, J., Svensson, A. and Liljas, A. (1993) Int. J. Biol. Macromol. 15, 97–100.
[30] Kumar, K., King, R.W. and Carey, P.R. (1976) Biochemistry 15, 2195–2202.