THE WATER STRUCTURE IN THE ACTIVE CLEFT OF HUMAN CARBONIC ANHYDRASE B

A Monte Carlo simulation

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1. Introduction

The formation of H_2CO_3 from H_2O and CO_2 in the gas phase requires a substantial activation energy, ~52 kcal/mol, and is a thermodynamically unfavourable process [1,2]. In aqueous solution the activation energy is lower, ~18 kcal/mol [3], but still high enough to constitute in principle a rate-limiting step in biochemical processes. However, in living cells the hydration and dehydration reaction:

$$H_2O + CO_2 \rightleftarrows H_2CO_3 \ (HCO_3^- + H^+)$$

is catalyzed by the enzyme carbonic anhydrase (CA), which is an extremely efficient catalyst with one of the highest turnover numbers known [4]. The tertiary structure of the enzyme has been determined by X-ray diffraction methods [5] and several models for the reaction mechanism have been proposed [6–8]. The enzyme and the reactions related to it have been studied with a number of kinetic and spectroscopic methods and recently it has been the subject of several theoretical investigations [1,9–12].

The active site of CA appears as a relatively small cavity (~10 Å deep and less wide) of approximately conical shape, with the base of the cone towards the surface of the enzyme, and the vertex of the cone pointing to the interior of the enzyme. Nearly at the vertex there is a Zn^{2+} bound to 3 histidine residues. In the vicinity of the Zn^{2+}, there is a glutamic acid residue, which can be either in the neutral or in the ionic form, and which has been proposed to be important for the catalytic activity of CA [8]. The cavity is sufficiently wide to accomodate several water molecules. No unambiguous experimental information about the number and/or positions of the water molecules in the active cleft is presently available, making the determination of the water structure in the active cleft an important task. We have therefore performed Monte Carlo simulations of the water structure in the active cleft of CA using analytical pair potentials obtained from quantum mechanical calculations [13–15].

2. Iso-energy contour maps of the cavity

A simulation of the solvation of the whole enzyme, requiring several hundreds of water molecules, is not possible. The system was reduced to a tractable size by 'cutting out' the active cleft and the amino acids bordering it (see fig.1). In the Monte Carlo simulation and in the iso-energy contour maps 27 amino acid residues were included [16]. The coordinates used for the enzyme were those obtained from the X-ray study [5].

The first information that may be obtained is a more precise shape of the active cleft of CA. This can be accomplished by calculating iso-energy contour maps [17] for several cross-sections perpendicular to the cone axis of the active cavity (see fig.1). The iso-energy contour maps were obtained by calculating a two-dimensional grid in which, at each *x,y*-coordinate of the water oxygen atom, the orientation of the water molecule was optimized. The requirement for

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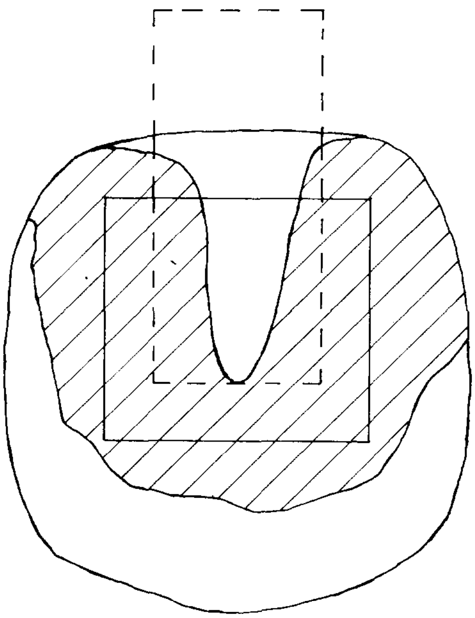


Fig.1. Schematic picture of a cut through the active cleft of carbonic anhydrase. All amino acids within the indicated cylinder (full lines) were included in the iso-energy contour maps and in the Monte Carlo simulation. The small cylinder (dashed lines) defined the boundaries used in the Monte Carlo simulation. The dimensions of the large cylinder are r = 7 Å and h = 16 Å and of the small cylinder r = 4.5 Å and h = 22 Å.

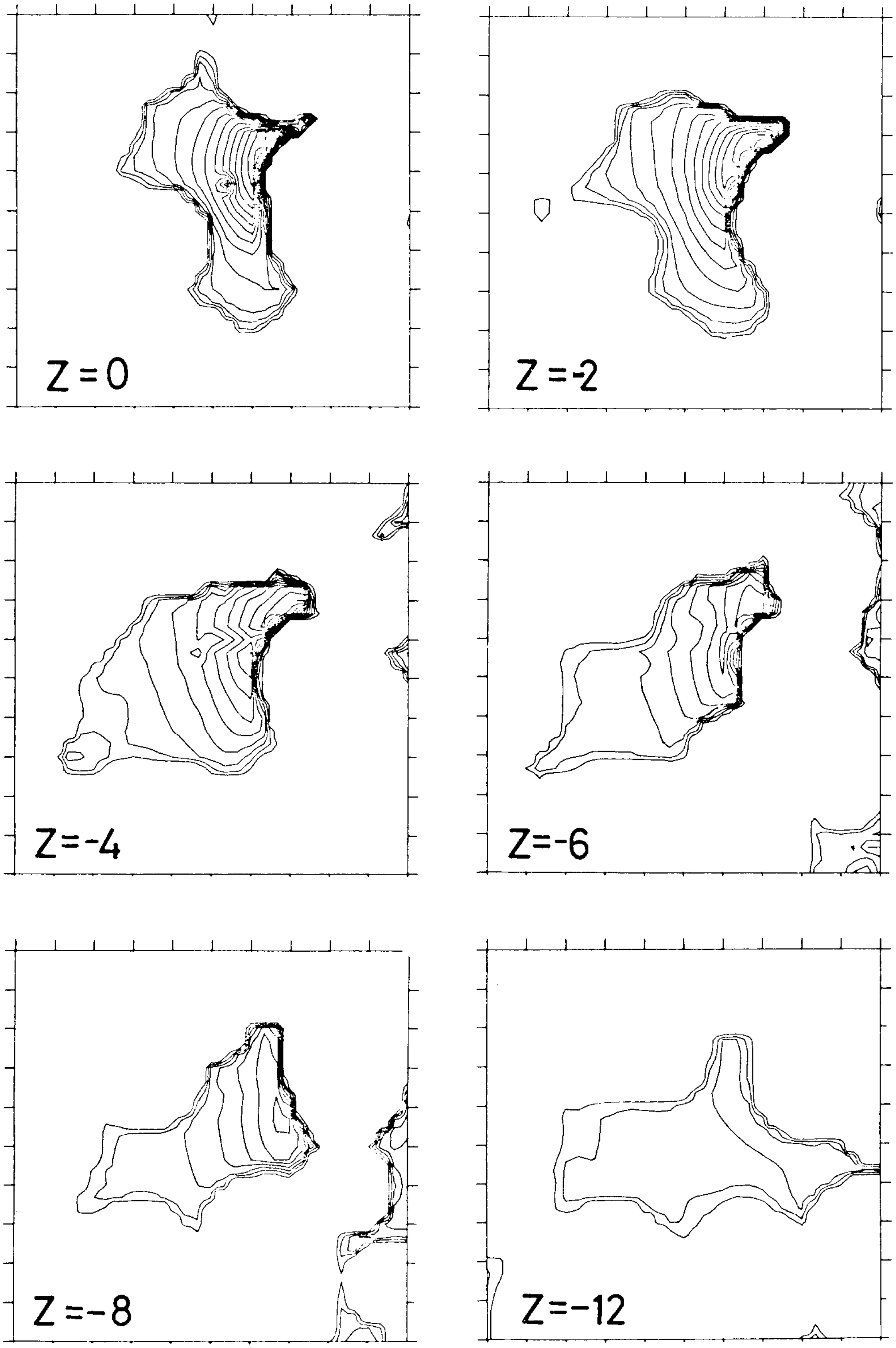
calculating iso-energy contours is a knowledge of the intermolecular potentials. The potentials needed are those between water and Zn^{2+} and between water and the amino acids [14,15]. The Zn^{2+} has been placed at the origin of the coordinate system, thus the 'cone' of the active cavity begins at a z-coordinate of ~0 and extends down to $z \approx -10$ Å (the approximate axis of the cone has been taken as z-axis). Further down the active cavity opens up in an irregular way reaching the surface of the enzyme. The iso-energy contour maps have been calculated for 6 parallel planes perpendicular to the z-axis and following the variation of the contours it is possible to form a quantitative image of the shape of the cavity, as experienced by 1 water molecule. It is noted that this shape does not have to coincide with the one obtained, for example, by attributing van der Waal's radii to the atoms of the residues: the hydrophilic and/or hydrophobic character of each protein atom relative the water molecule is included in the analytical atom–atom pair potentials and therefore the representation provided by the iso-energy contours is a quantitative one.

3. Monte Carlo simulations

The iso-energy contour maps give information about the interactions in the cavity felt by 1 water molecule. In order to obtain knowledge about the actual solvent structure (e.g., coordination numbers, probability densities) it is necessary to perform some kind of solvent simulation. In this work it was done by applying the Monte Carlo technique 20 to a sample of 20 water molecules at 300 K confined to the active cavity by a cylindrical box ranging from $z = 0$ to $z = -22.0$ Å and with a radius of 4.5 Å. The amino acids of the enzyme were considered as a solute and were kept at fixed positions. In some additional calculations the dimensions of the cylinder were varied with only minor changes in the final probability distributions. The cylinder is longer than the cavity (see fig.1). This was done in order to provide a water reservoir to fill the cavity. The radius of the box was chosen in order to avoid a solvation of the outside of the cavity, since this is an artificial surface created by cutting out only a minor part of the enzyme. A total of 430 000 configurations were generated, 350 000 for the equilibration and 80 000 for the calculation of statistical averages. The average water–water interaction energy in the cleft was –10.5 kJ/mol which, compared to the same value for pure water, –35.6 kJ/mol [18], indicates that the water structure in the active cleft is somewhat different from that in pure water.

In fig.3 is shown the radial distribution function

Fig.2. Iso-energy contour maps for 6 parallel cross-sections cutting through the cavity, parallel to the x,y-plane and at the indicated z-coordinates (a.u.). The contour-to-contour separation is 5 kcal/mol with the outermost contour corresponding to 0 kcal/mol. The separation between consecutive marks on the perimeter is 2 a.u. The Zn^{2+} defines the origin with respect to which the geometrical centre of each drawing has the coordinate $x = -3$ and $y = -1$ a.u. (1 a.u. = 0.529 Å).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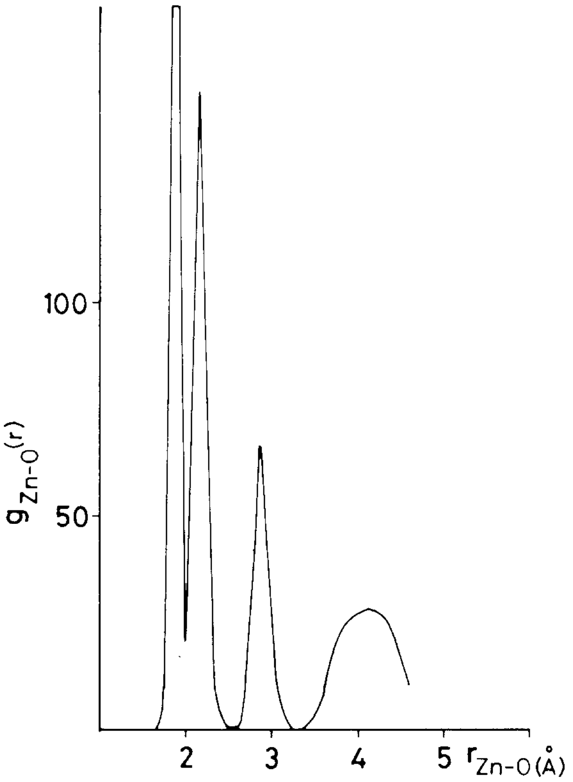

Fig.3. Radial distribution function, $g_{Zn-O}(r)$, for water oxygen atoms around Zn^{2+}, in the enzyme cavity. The first 3 peaks correspond to 3 water molecules and the following peak contains 4 water molecules.

$g_{Zn-O}(r)$ up to 5 Å away from the Zn^{2+}. Upon integrating the first three peaks, corresponding to an approximate first solvation sphere, it is found that they contain 1 water molecule each, while the fourth peak corresponds to 4 water molecules. Another representation of the water distribution in the active cleft is provided in fig.4, where the probability distributions of oxygen and hydrogen atoms are shown. The probability distributions are presented for volumes obtained by segmenting the cylinder with planes parallel to the base and at an interval of 1 Å. From the distribution functions shown in fig.4, it is possible to obtain an approximate orientation of the water molecules in the cleft. The x- and y-coordinates are given in the figure while the z-coordinate has an uncertainty of the sampled volume thickness (1 Å).

4. Discussion

The results suggest that the Zn^{2+} in CA is at least 5-coordinated, or even 6-coordinated, in contrast to what has been suggested for Co-containing bovine carbonic anhydrase [19]. As the glutamic acid (Glu 105) has been considered in its ionic form, the results here approximately correspond to a situation with high pH. From the probability density plots (fig.4) it is also apparent that even several Å away from the Zn^{2+}, there is a high degree of order in the water structure in the active cleft.

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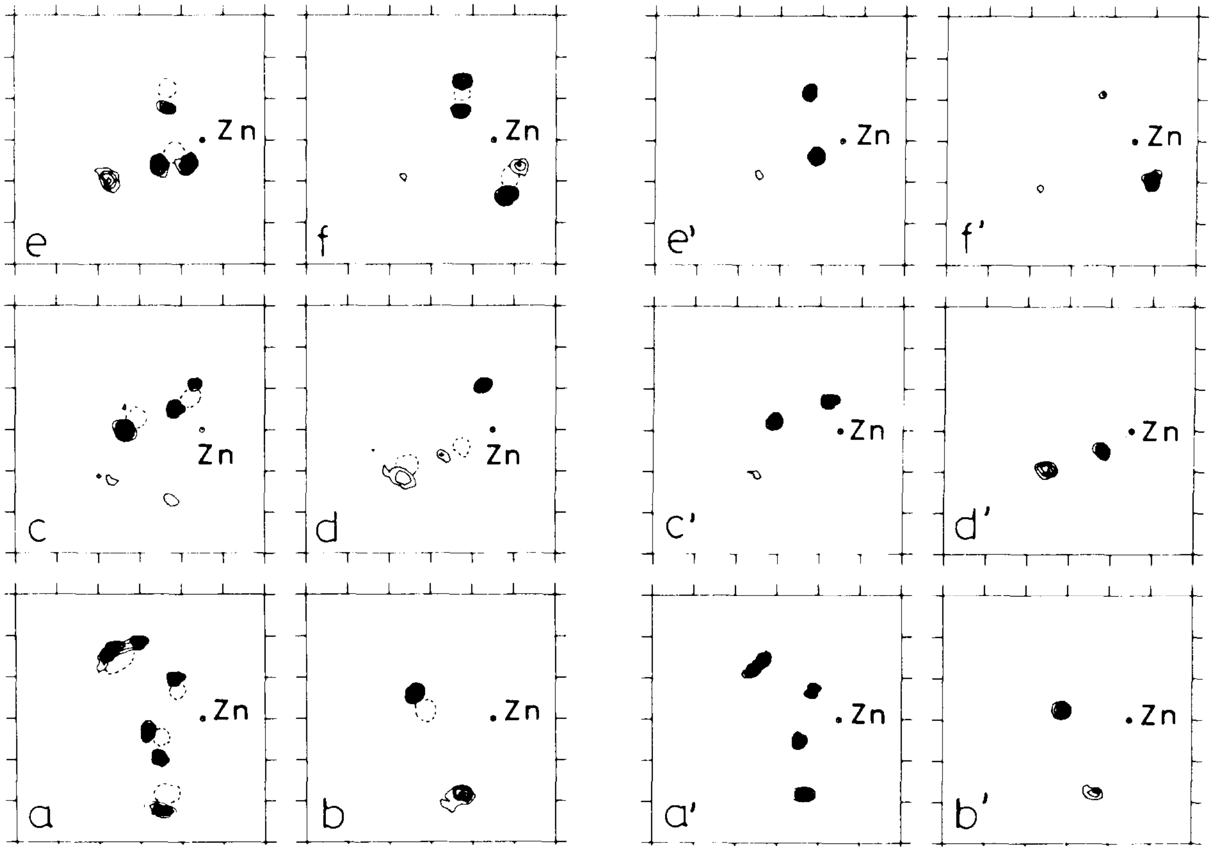

Fig.4. Probability density maps for water hydrogens (insert a–f) and oxygens (insert a' to f'). The maps correspond to cross sections through the cavity and parallel to the x,y-plane at the following z-values: a, a', $\bar{z}$ = −0.5 Å; b, b', $\bar{z}$ = −1.5 Å; c, c', $\bar{z}$ = −2.5 Å; d, d', $\bar{z}$ = −3.5 Å; e, e', $\bar{z}$ = −4.5 Å; f, f', $\bar{z}$ = −5.5 Å. The probability maps show the probability of finding a H or O atom at the given x,y-coordinate and with the z-coordinate in the range $\bar{z}$ ± 0.5 Å. In order to facilitate the reading of the maps, the oxygen maps have been added to the appropriate hydrogen maps (dashed lines). The contour separation is 0.25 atom/Å^3 and the interval between consecutive marks on the perimeter is 2 Å.

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