Molecular modelling of human gastric alcohol dehydrogenase (class IV) and substrate docking: differences towards the classical liver enzyme (class I)

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Abstract A three-dimensional model of the human class IV alcohol dehydrogenase has been calculated based upon the X-ray structure of the class I enzyme. As judged from the model, the substrate-binding site is wider than in class I, compatible with the differences in substrate specificities and the large difference in K_m value for ethanol. Substrate docking performed for the class I structure and the class IV model show all-*trans*-retinol and 11-*cis*-retinol to bind better to the class IV enzyme. The calculations also indicate that 16-hydroxyhexadecanoic acid binds in a different manner for the two enzyme classes. A simulation of coenzyme-binding indicates that the adenine ring of the coenzyme might be differently bound in class IV than in class I, decreasing the interactions with Asp-223 which is compatible with the higher k_{cat} values for class IV.

Key words: Alcohol dehydrogenase; Class specificity; Molecular modeling; Structural comparison; Substrate docking

1. Introduction

Human alcohol dehydrogenase is a zinc-dependent enzyme system with different classes and isozymes, containing 373–379-residue subunits [1]. These enzymes belong to the family of medium-chain dehydrogenases/reductases, MDR [2]. Presently, at least six different classes of mammalian alcohol dehydrogenase are known [1]. The inter-class amino acid residue differences give rise to different substrate specificities. Class I is the classical, ethanol-active liver enzyme, class II a variable form also in liver, class III a glutathione-dependent formaldehyde dehydrogenase of most tissues, and class IV a major form in the stomach and upper digestive tract, while the remaining classes are less well defined. Class IV possesses considerable activity with ethanol and has also drawn much attention because of its ability to use retinol as a substrate [3,4], thereby contributing to the formation of retinoic acid which is a regulatory factor in cellular growth and differentiation [5]. The class IV form has been enzymatically characterised [6] and its primary structure is known from human [7–11] and rat [12]. It is most closely related to the class I enzyme (69% residue identity), but exhibits several-fold higher K_m and k_{cat} values than the class I isozymes [10,11]. Also, the K_m and dissociation constants for NAD are much higher with class IV [10], consistent with the high k_{cat}. In order to acquire further knowledge about the structural properties that could explain the kinetic features of class IV, we have calculated a molecular model of the human enzyme based on the known three-dimensional structure of the human class I alcohol dehydrogenase [13]. We have also performed docking calculations between the enzyme and different substrates in order to evaluate binding characteristics.

2. Materials and methods

A three-dimensional model of human class IV alcohol dehydrogenase was obtained by adopting its amino acid sequence [7–11] into the known fold of the human class I β alcohol dehydrogenase subunit [13] using the program ICM (version 2.5, Molsoft LLC, Metuchen, NJ, USA; 1996). In the first step of model building, tethers were imposed between residues of the class I template structure and those of the class IV structure, and were then minimised. Subsequently, all methyl groups were rotated to minimise clashes, followed by iterative combined geometry and energy optimisation. After adjustments of polar hydrogen positions, the whole molecule was subject for free minimisation. Finally, the side chains were subjected to a biased Monte Carlo procedure [14] and loops around the substrate-binding and coenzyme-binding sites were minimised.

To study interactions between the enzyme and different substrates, a non-rigid docking procedure was utilised based upon a Monte Carlo procedure, allowing free movement of the substrate, the rotatable bonds of the substrate, and the χ angles of the substrate-binding residues at positions 48, 57, 93, 110, 115, 116, 140, 141, 294, 318, and with an additional distance restraint of 2.0–2.4 Å between the alcohol oxygen and the catalytic zinc ion. After the initial docking, the distance restraint was removed and the substrate and substrate-binding residues were subjected to energy minimisations. The binding energies were calculated with the program ICM using the REBEL (rapid-exact-boundary-element) method for electrostatic free energy, and the constant surface tension method with 20 cal/Å^2 for hydrophobic energy.

For the coenzyme docking calculations, the NAD was placed into the class IV model to occupy a position as in the class I structure. Subsequently, the coenzyme and coenzyme-binding residues were subjected to energy minimisations. For comparison, the same procedure was adopted to the class I structure.

3. Results and discussion

3.1. Model of the class IV enzyme

The human class IV alcohol dehydrogenase differs from the class I β form at 31% of the residues. The modelling shows that the class IV structure is compatible with the general fold of the class I enzyme, in accordance with the conservation of structurally important residues. The class IV structure has one residue less (Gly-117) compared to the class I structure. From the model it can be concluded that this difference gives a more open substrate-binding pocket in class IV than in class I (Fig. 1).

Of particular interest are the residues lining the substrate-binding site and the coenzyme-binding site in the class IV

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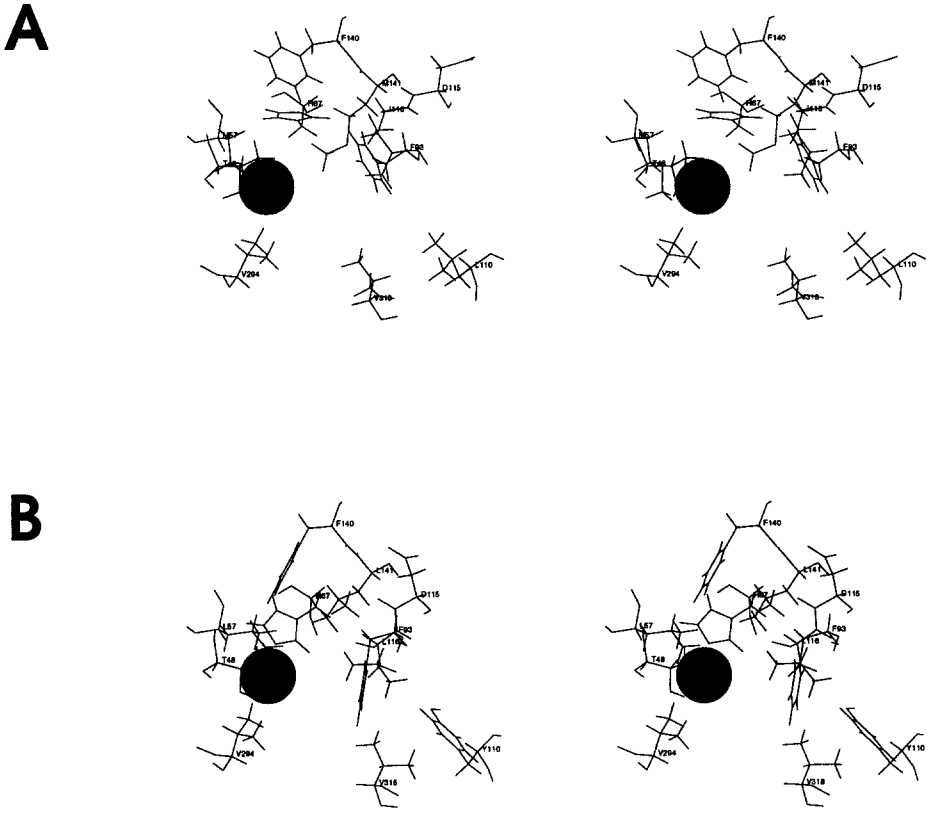

Fig. 1. Stereo views of the substrate-binding sites of the human class IV alcohol dehydrogenase model (A) and the human class I alcohol dehydrogenase (B). The catalytic zinc is shown as a sphere and substrate-binding amino acid residues as sticks. Residues are numbered according to the class I enzyme.

model. At the substrate-binding site, all but one exchange versus class I involve substitutions of a hydrophobic residue for another. The exception is a Leu/Tyr exchange (class IV/class I) at position 110 (Table 1). Among the coenzyme-binding residues, there are only two exchanges – Leu/Ile-224 and His/Arg-271. The contributions to the molecular surface of the substrate-binding residues were determined (Table 1). The sum of these contributions shows that the class IV model has a substrate-binding pocket with 406 Å^2 and class I one with 335 Å^2. This difference in size is compatible with the higher class IV K_m value for ethanol. A large substrate pocket size has also been proposed to explain the low affinity of class III for ethanol [15,16].

Table 1
Accessible surface areas of amino acid side chains lining the substrate-binding site in the human alcohol dehydrogenase class I β structure and class IV model

Position	Class I β		Class IV	
	Residue	Area (Å^2)	Residue	Area (Å^2)
48	Thr	22.4	Thr	28.9
57	*Leu*	*57.6*	*Met*	*47.9*
67	His	7.9	His	8.1
93	Phe	22.8	Phe	15.3
110	*Tyr*	*81.8*	*Leu*	*75.4*
116	*Leu*	*39.8*	*Ile*	*61.5*
140	Phe	10.2	Phe	34.1
141	*Leu*	*23.7*	*Met*	*33.3*
294	Val	41.7	Val	52.7
318	Val	26.6	Val	48.8
Sum		335		406

Positions with residue differences between the two classes are denoted in italics.

3.2. *Docking of different substrates*

The model was utilised to evaluate the differences toward the class I structure in substrate binding. For each substrate, the binding energies were calculated as the difference between the complex on the one hand and the enzyme and substrate alone on the other (Table 2). The decrease in water-accessible surfaces was calculated and the distances between the alcohol/aldehyde group and the catalytic zinc were measured (Table 2).

The largest differences between binding energies for the class I structure and the class IV model are seen with all-*trans*-retinol having a binding energy of −9.0 kcal/mol for class IV, but −6.0 kcal/mol for class I. The distance of 2.4 Å between the -OH group of that substrate and catalytic zinc is also the smallest for the substrates investigated, reflecting a tight binding, consistent with another study [11]. This correlates with the fact that all-*trans*-retinol is a better substrate for class IV than for the class I isozymes [4]. Also, for 11-*cis*-retinol, the binding energies are lower for class IV (−5.0 kcal/mol) than for class I (−2.1 kcal/mol). Due to the instability of this retinoid, it has not been generally tested as a substrate for alcohol dehydrogenase. Our model predicts that it may be a better substrate for class IV than for class I.

For 16-hydroxyhexadecanoate, the binding energies are of the same range, but the substrate is then differently bound. In the wider substrate-binding pocket of the class IV model, 16-hydroxyhexadecanoate binds deeper down in the substrate pocket (Fig. 2), while in the class I pocket it is bound in an

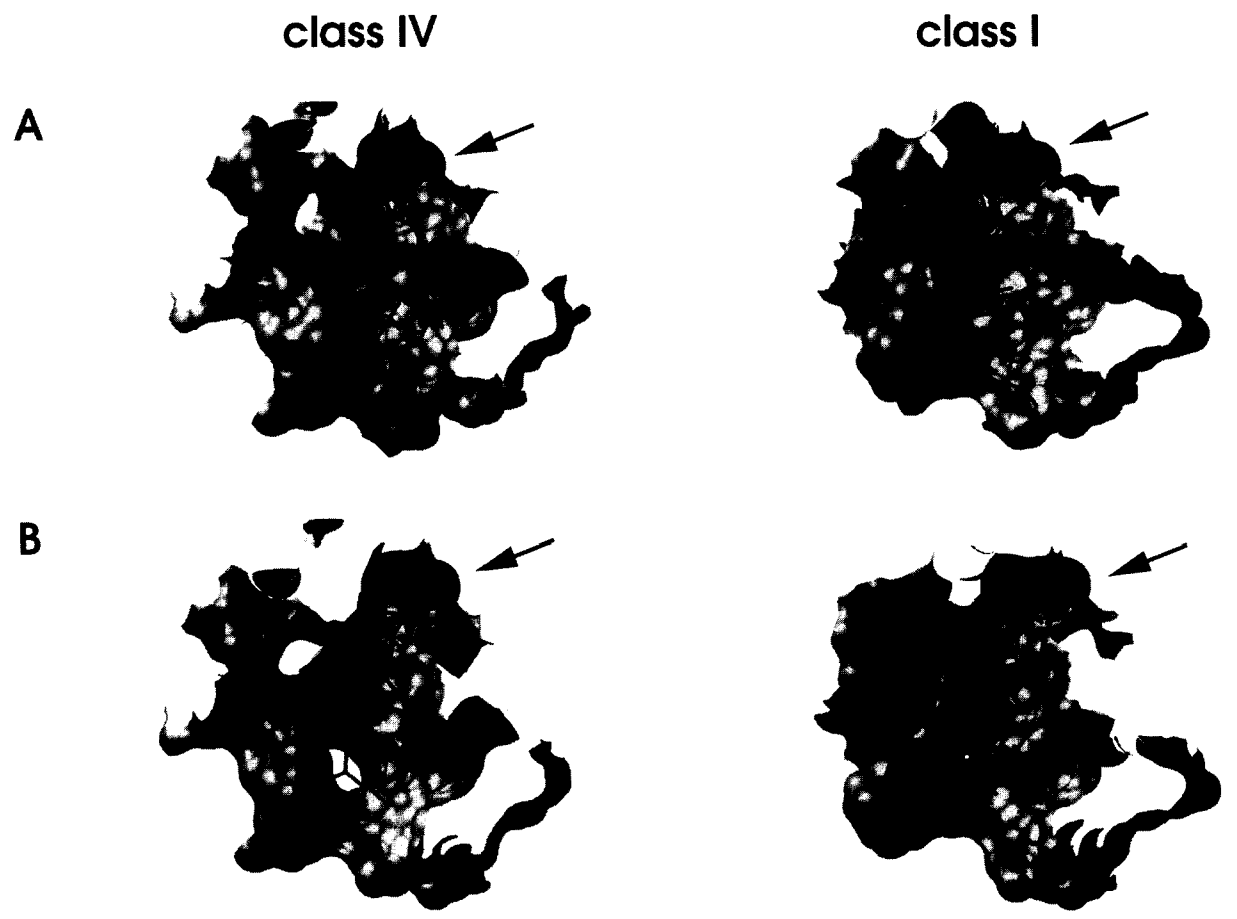

Fig. 2. Comparison of the substrate-binding sites between class IV (left) and class I (right) with 16-hydroxyhexadecanoate (A) and all-*trans*-retinol (B) bound. The accessible surface is visualized for the amino acid residues 48, 56–59, 67, 93, 110, 114–119, 140, 141, 174, 294–298, 318 and 319. The substrate molecules are shown as stick models. The arrows point at the catalytic zinc.

elongated form. The different binding does not affect the K_m values [6,17].

In contrast, with ethanol, hexanal, *trans*-2-hexenal, octanol, octanal, 4-hydroxynonenal, nitrobenzaldehyde, and 12-hy-

Table 2
Binding energies, differences in water-accessible surface, and distances between the substrate alcohol/aldehyde group and the catalytic zinc for different substrates docked to the class I structure and the class IV model

Enzyme	Substrate	Binding energy (kcal/mol)	Difference in water-accessible surface (Å^2)	Distance (Å)
Class I	all-*trans*-retinol	−6.0	−713.7	2.9
Class IV	all-*trans*-retinol	−9.0	−781.1	2.4
Class I	11-*cis*-retinol	−2.1	−725.5	2.8
Class IV	11-*cis*-retinol	−5.0	−729.9	2.6
Class I	12-hydroxydodecanoate	−1.9	−622.3	3.0
Class IV	12-hydroxydodecanoate	−3.2	−674.9	3.1
Class I	16-hydroxyhexadecanoate	−3.9	−777.1	2.8
Class IV	16-hydroxyhexadecanoate	−4.3	−814.6	2.8
Class I	ethanol	−2.0	−227.5	2.8
Class IV	ethanol	−2.0	−251.5	2.7
Class I	hexanal	−2.3	−398.7	2.6
Class IV	hexanal	−2.0	−411.1	2.8
Class I	*trans*-2-hexenal	−2.2	−373.0	2.7
Class IV	*trans*-2-hexenal	−3.1	−407.6	2.6
Class I	octanal	−3.1	−427.5	3.2
Class IV	octanal	−2.3	−463.1	2.8
Class I	octanol	−4.4	−470.4	2.6
Class IV	octanol	−3.1	−513.2	2.8
Class I	4-hydroxynonenal	−3.0	−445.7	2.6
Class IV	4-hydroxynonenal	−3.2	−548.4	2.6
Class I	nitrobenzaldehyde	−2.9	−407.2	3.3
Class IV	nitrobenzaldehyde	−2.8	−403.7	2.6

droxydodecanoate, the binding energies are similar for the class I and class IV structures. In both cases, the size of the substrate pocket is large enough to harbour these substrates.

3.3. Coenzyme binding

Coenzyme docking calculations were performed for the class I structure and the class IV model after initial positioning of the coenzyme and subsequent energy minimisation of the coenzyme and binding residues. In the class I structure, the coenzyme does not change its position, confirming the correct fit. However, in the class IV model, the adenine ring of the coenzyme becomes differently positioned. This is caused by a small difference in the model at Arg-47, apparently induced by side-chain replacements more distantly positioned in the molecule. As a consequence of the difference in exact coenzyme positioning, the interactions with Asp-223 become weaker in the class IV model which is compatible with its decreased coenzyme binding and the higher k_{cat} values of class IV [10,11]. Similar effects may also be derived from changes at positions 54, 224 and 271, or indirectly, through other residues [10] affecting the general backbone folding. The present model is compatible with our previous observations that the weak binding cannot be related to a single amino acid exchange but to several substitutions, some of them far from the active site [10].

3.4. Conclusions

The present modelling and docking show that the class IV alcohol dehydrogenase has a fold generally compatible with that of the class I enzyme. The substrate-binding pocket is wider, causing a better binding of all-*trans*-retinol and 11-*cis*-retinol, and the coenzyme is differently bound, apparently because of amino acid substitutions at several positions. These variations offer an explanation for the distinct catalytic properties of classes IV and I.

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