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Modelling Studies on the Binding of Substrate and Inhibitor to the Active Site of Human Sorbitol Dehydrogenase

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Abstract—This study reports a molecular modelling investigation of human sorbitol dehydrogenase complexed with the substrate sorbitol and the inhibitor WAY135 706 based on the structures of human β_3 alcohol dehydrogenase, human σ alcohol dehydrogenase and horse liver alcohol dehydrogenase. The tertiary structure of human β_3 alcohol dehydrogenase was used as a template for the construction of the model. The rms positional deviation between the main-chain atoms of the initial and final models of sorbitol dehydrogenase is 1.37 Å. Similar residue interactions exist between sorbitol dehydrogenase and both sorbitol and inhibitor. Binding of sorbitol in the substrate-binding site results in interactions with Lys-294, Tyr-50, His-69, Glu-150, and NAD^+ while WAY135 706 interacts with Ser-46, Lys-294 and Phe-59. The enzyme-inhibitor interactions revealed by this study will be useful in the design of more specific inhibitors. © 2000 Elsevier Science Ltd. All rights reserved.

Sorbitol dehydrogenase (SDH) catalyses the conversion of sorbitol to fructose with NAD^+ as the coenzyme.[1] Sorbitol dehydrogenase is the second enzyme involved in the polyol pathway of glucose metabolism. It is believed that this pathway is involved in the development of diabetic complications.[2] 4-[4-(*N,N* dimethylsulfamoyl) piperazino]-2-hydroxymethylpyrimidine (WAY 135 706/SDI) is a compound that inhibits SDH with an IC_{50} value of 1 μM.[3] Administration of SDI has been reported to attenuate the onset and severity of polyol-related complications by increasing blood and tissue levels of sorbitol.[4–6] Sorbitol dehydrogenase is a tetramer, has 356 residues (approximately 37 kDa per subunit) and contains one catalytic zinc atom per subunit.

The homologous alcohol dehydrogenase (ADH)[7] has a conserved zinc ligand motif (two cysteine residues and one histidine residue) and a hydrophobic substrate-binding pocket.[8] Major functional differences between ADH and SDH include the third zinc ligand in ADH (Cys) being replaced by a Glu in SDH.[9] While the crystal structure of sorbitol dehydrogenase is yet to be determined, a model of sheep liver sorbitol dehydrogenase was constructed using the high-resolution structure of alcohol dehydrogenase.[10] Amino acid sequence homology between sheep liver SDH and horse liver ADH revealed about 25% sequence identity.[10] Modelling studies have indicated extensive structural differences between the predicted SDH model and tertiary structure of ADH, particularly in the active site region.[10] In recent times crystal structures of many ADH have been solved.[11–13] From these structures and mutagenesis studies,[9] enough information is now available to construct a more accurate SDH model compared to the earlier model of the sheep liver SDH.[10] The model may be used to aid the design of more specific inhibitors of the enzyme.

Functionally related molecules that exhibit some degree of sequence homology have been shown to have similar tertiary structures.[14] The human sorbitol dehydrogenase amino acid sequence was compared with sequences of long chain alcohol dehydrogenases of known 3-D structures.[11,13,15] Sequence alignments of SDH and the ADH were carried out using Clustalw1.60 sequence alignment program,[16] introducing as few insertions/deletions as possible while conserving conformatonal aspects of the molecules (Fig. 1). The sequence alignment provided the SDH model with the zinc ligand motif, C44-H69-E150 suggested by site-directed mutagenesis.[9] The high-resolution crystal coordinates of human β_3 ADH, human σ ADH and horse liver ADH (PDB entries 1htb, 1agn and 1axe) were used to construct a model of human sorbitol dehydrogenase. The modelling program TURBO-FRODO version 5.5[17] was used to replace residues of the ADH with corresponding residues in the human SDH sequence, conserving the original secondary structure as

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much as possible. Human β_3 ADH was used as the template because it has the highest sequence homology (44%) with SDH. The rms positional deviation between the main-chain atoms of the initial and final SDH models is equal to 1.37 Å.

In the present work, the substrate sorbitol and inhibitor WAY135 706 were manually docked into the putative substrate-binding site of SDH. The substrate was modelled into the active site so that the second alcohol group interacts with the C4 of the nicotinamide ring and is present within liganding distance to the zinc atom. The alcohol group of the inhibitor molecule was positioned in the active site to occupy the same position of the second alcohol group of sorbitol. Hydrogen atoms, partial charges, atomic potentials and bond orders for the complexes were assigned using the automatic procedures within the InsightII 2.1 package (Biosym Technologies

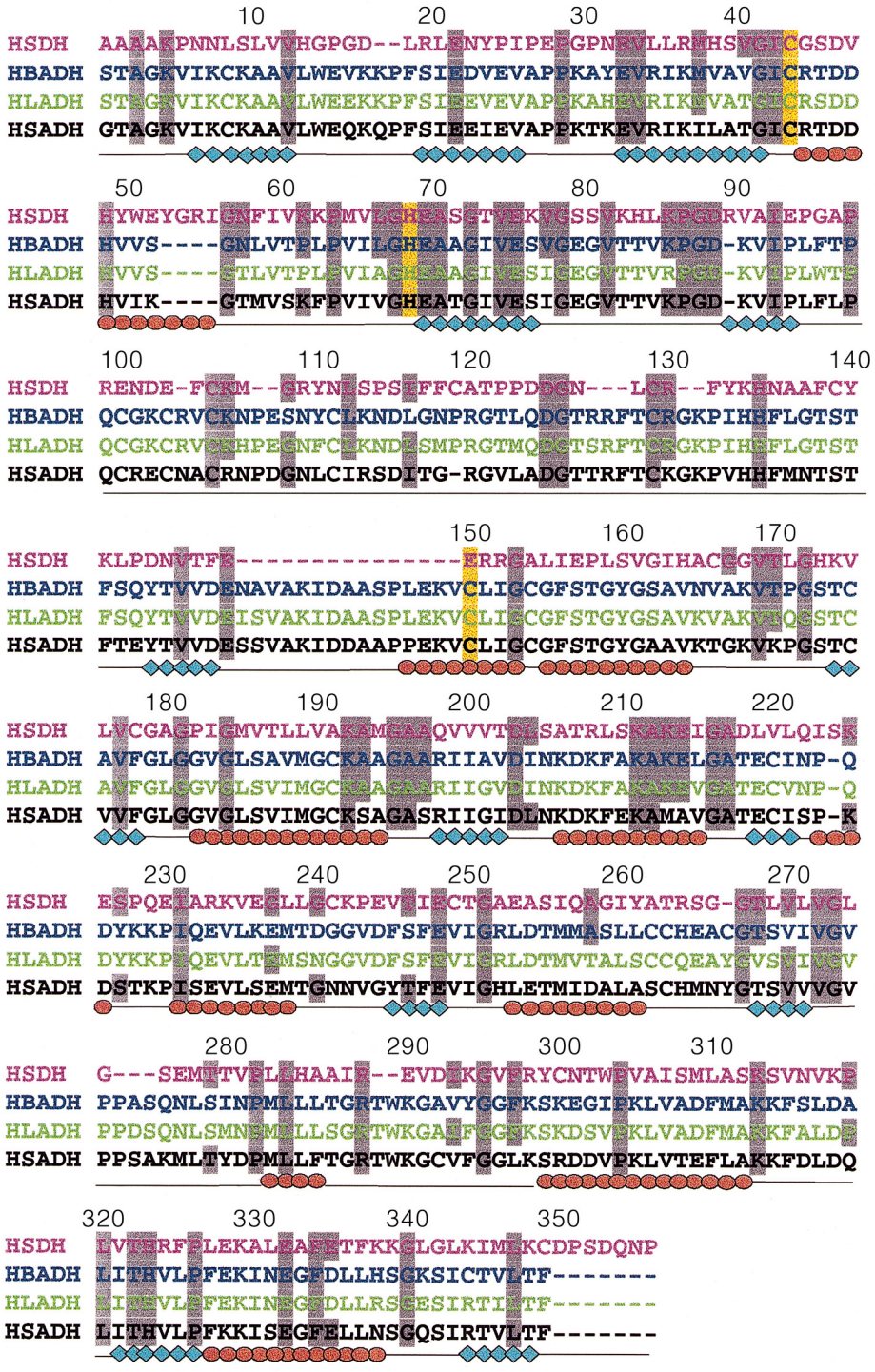

Figure 1. Amino acid sequence alignment of human sorbitol dehydrogenase (HSDH) with human β_3 alcohol dehydrogenase (HBADH), horse liver alcohol dehydrogenase (HLADH) and human σ alcohol dehydrogenase (HSADH). The shaded regions indicate residue identity. Zinc liganding residues are shown in yellow. Regions of α-helices and β-strands are designated by ● and ◆ respectively.

Inc., San Diego, CA). Arginine, lysine, aspartate and glutamate amino acids were charged while the histidines were uncharged, with hydrogen atoms fixed at the Nε. Energy minimisation was performed to relieve steric strain associated with the coordinates using the Discover 2.7 package (Biosym Technologies Inc., San Diego, CA) on an O2 (R10000) workstation (Silicon Graphics, Mountain View, CA). The constant valence force field incorporating the simple harmonic function for bond stretching and excluding all non-diagonal terms was used (cut-off distance of 31 Å). Calculations were done using the algorithms steepest descents and conjugate gradients (down to a maximum atomic root-mean-square derivative of 10.0 and 0.01 kcal/Å respectively). Molecular dynamics were then performed using leapfrog algorithms in Discover. Dynamics were equilibrated for 2 ps with time steps of 1 fs and then continued for 4 ps with time steps of 2 fs at 350 K. The resulting structure was extracted and energy minimised. This protocol was found to be an effective method for exploring conformational space with a protein–ligand complex close to its lowest energy structure. The calculated binding energies are listed in Table 1.

Molecular modelling of the substrate and inhibitor into the active site of sorbitol dehydrogenase revealed the possible interactions between SDH and both sorbitol and SDI. Figure 2a illustrates residues present within 4

Table 1. Intermolecular binding enthalpies (kcal/mol) between sorbitol dehydrogenase (SDH) residues and (a) sorbitol and (b) inhibitor (SDI)

SDH residues	Sorbitol ΔH (kcal/mol)	SDH residues	SDI ΔH (kcal/mol)
Lys-294	−7.64	Phe-59	−8.27
Phe-59	−4.91	Ser-46	−6.90
Ser-46	−4.75	Lys-294	−6.81
Ile-293	−3.55	Phe-117	−5.39
Glu-150	−3.41	Phe-118	−3.87
Tyr-50	−3.13	Tyr-50	−2.78
Phe-117	−2.25	Leu-274	−1.94
Asp-47	−2.18	Ile-293	−1.89
Phe-118	−1.81	Arg-151	−1.57
Tyr-110	−0.99	His-69	−0.91
Leu-274	−0.69	Cys-44	−0.74
Cys-44	−0.66		
Total energy (kcal/mol)	−14.72	Total energy (kcal/mol)	−45.04

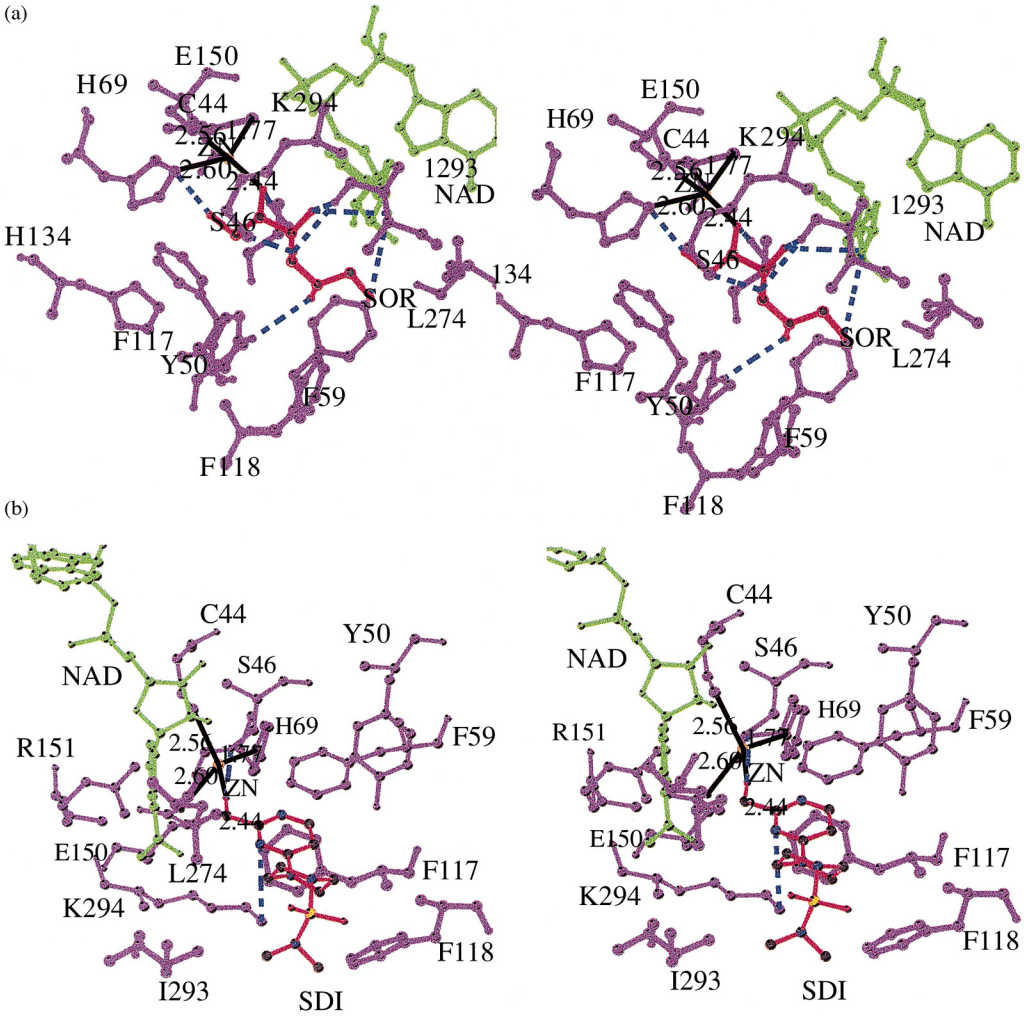

Figure 2. Stereoview of the active site of sorbitol dehydrogenase model. (a) Residues within 4 Å of sorbitol in the substrate-binding site of human sorbitol dehydrogenase. (b) The active site residues of SDH within 4 Å of the inhibitor. These figures were prepared using MOLSCRIPT.[18]

Å of sorbitol in the active site of the energy minimised SDH/sorbitol structure. Molecular dynamics calculations revealed that the C1, C2, C3, C4, C5 and C6 hydroxyl groups of sorbitol are within hydrogen bonding distance with His-69 Nϵ (2.47 Å), Ser-46 OG (2.57 Å), Ile-293 O (2.95 Å to O4 and 2.74 Å to O3), Lys-294 NZ (2.87 Å), Tyr-50 OH (3.34 Å) and NAD+ nicotinamide carbonyl oxygen (3.337 Å to O3 and 3.77 Å to O6). The C2 hydroxyl oxygen of sorbitol is coordinated to the zinc atom (2.44 Å). Sorbitol also makes contacts with the 5 apolar residues: Phe-59, Phe-117, Phe-118, His-135 and Leu-274. Additionally the zinc ligand residue Cys-44 is within van der Waals contacts with sorbitol. Molecular dynamics calculations also revealed the possible interactions of the inhibitor WAY135 706 with sorbitol dehydrogenase. The inhibitor is hydrogen bonded with Ser-46 OG (2.58 Å) and Lys-294 NZ (3.03 Å) and van der Waals contacts are made with the 7 apolar residues: Tyr-50, Phe-59, His-69, Phe-117, Phe-118, Leu-274 and Ile-293. Additionally contacts are present between SDI and Glu-150, Arg-151, and NAD^+. The pyrimidine ring of the inhibitor π- stacks against the side chain of Phe-59 (Fig. 2b). This stacking is likely to contribute to the tight binding of SDI. An important residue of ADH, Ser-46 is hydrogen bonded to the hydroxyl group of SDI coordinated to catalytic zinc. It was reported that this hydrogen bond could function in a proton relay system to facilitate removal of proton from the alcohol.[19] This may prove to be the same for SDH.

In conclusion the inhibitor was found to have fewer hydrogen bonds with the holoenzyme (Ser-46 and Lys-294) when compared to sorbitol (His-69, Ser-46, Ile-293, Lys-294, Tyr-50 and NAD^+). However the stacking of the pyrimidine ring of the inhibitor against the side chain of Phe-59 plays an important role in the binding. Comparisons between the first model[11] and this model indicate similar interactions between SDH and substrate. The important residues in both cases were found to be Lys-294, Phe-59 and Ser-46. This is supported by the calculated binding energy values shown in Table 1. However the zinc ligands are different due to the differences in sequence alignments and hence structural models. The hydrogen bonds between SDH and substrate are also different. The primary alcohol group of sorbitol is hydrogen bonded to both Glu-150 and Lys-294 in the earlier model,[11] in this model however, Lys-294 is hydrogen bonded to the C4 hydroxyl group of the substrate and Glu-150 is present within van der Waals contacts. While most of the enzyme–ligand interactions remain the same in the two models, the new model agrees more favourably with mutagenesis results that have recently become available in the literature.[9]

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References

1. Jeffery, J.; Jörnvall, H. *Advances Enzymology Related Areas of Molecular Biology* **1988**, *61*, 47.
2. Williams, J. R.; Chang, K.; Frangos, M.; Hasan, K. S.; Ido, Y.; Kawamura, T.; Nyengaard, J. R.; Van Den Enden, M.; Kilo, C.; Tilton, R. G. *Diabetes* **1993**, *42*, 801.
3. Kador, P. F.; Inoue, J.; Secchi, E. F.; Lizak, M. J.; Rodriguez, L.; Mori, K.; Greentree, W.; Bleesing, K.; Lackner, P. A.; Sato, S. *Exp. Eye. Res.* **1998**, *67*, 203.
4. Gerssen, K.; Utz, R.; Grötsch, H.; Lang, H.-J.; Nimmesgern, H. *Arzneim-Forsch/Drug Res.* **1994**, *44*, 103243.
5. Tilton, R. G.; Chang, K.; Nyengaard, J. R.; Van Den Enden, M.; Ido, Y.; Williamson, J. R. *Diabetes* **1995**, *44*, 234.
6. Cameron, N. E.; Cotter, M. A.; Basso, M.; Hohman, T. C. *Diabetologia* **1997**, *40*, 271.
7. Jeffery, J.; Chesters, S. J.; Mills, C.; Sadler, P. J.; Jörnvall, H. *EMBO J.* **1984**, *3*, 357.
8. Jörnvall, H.; Persson, M.; Jeffery, J. *Proc. Natl. Acad. Sci. USA* **1981**, *75*, 4226.
9. Karlsson, C.; Jörnvall, H.; Höög, J. In *Enzymology and Molecular Biology Carbonyl Metabolism 5;* Weiner, H., Ed.; Plenum: New York. 1995, p 397.
10. Eklund, H.; Horjales, E.; Jörnvall, H.; Brändén, C.; Jeffery, J. *Biochem.* **1985**, *24*, 8005.
11. Eklund, H.; Nordström, B.; Zeppezauer, E.; Söderlund, G.; Ohlsson, I.; Boiwe, T.; Söderberg, B.-O.; Brändén, C.-I.; Åkeson, Å. *J. Mol. Biol.* **1976**, *102*, 27.
12. Davis, G.; Bosron, W.; Stone, C.; Owusu-Deky, K.; Hurley, T. *J. Biol. Chem.* **1996**, *271*, 17057.
13. Xie, P.; Parsons, S. H.; Speckhard, D. C.; Bosron, W. F.; Hurley, T. D. *J. Biol. Chem.* **1997**, *272*, 18558.
14. Creighton, T.E., In *Proteins, Structures and Molecular Properties*; W.H. Freeman; New York, 1993; Vol 1, p 507.
15. Davis, J. G.; Borson, W. F.; Store, C. L.; Owusu-Dekyi, K.; Hurley, T. D. *J. Biol. Chem.* **1996**, *271*, 17057.
16. Thompson, J. D.; Higgins, D. G.; Gibson, T. J. *Nucleic Acids Res.* **1994**, *22*, 4673.
17. Roussel, A.; Fontecilla-Camps, J.C.; Cambillau, C. *Acta Crystallog.* **1990**, Sect. A, 46, C66.
18. Kraulis, P. J. *J. Appl. Cryst.* **1991**, *24*, 946.
19. Eklund, H.; Plapp, B. V.; Samama, J. P.; Brändén, C. *J. Biol. Chem.* **1982**, *257*, 14349.