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Modelling Studies of the Active Site of Human Sorbitol Dehydrogenase: An Approach to Structure-Based Inhibitor Design of the Enzyme

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Abstract—The program GRID was used to design novel potential inhibitors of human sorbitol dehydrogenase based on a model of the holoenzyme in complex with the inhibitor WAY135 706. Replacement of the methyl hydroxyl group of the inhibitor with methyl phosphate and methyl carboxylate functional groups increased the net binding energy of the complex by 2.0- and 1.7-fold, respectively. This study may be useful in the development of potent and more specific inhibitors of the enzyme.

Rapid increase in the number of three-dimensional structures of biological macromolecules generated by crystallographic and modelling methods has allowed a way for rational drug design.[1,2] Additionally, high resolution computer graphics and fast computational programs have allowed access to structural information depicting electrostatic potentials, active site hydrophobicity and possible enzyme–ligand interactions which aid the drug discovery process.[3] It has been demonstrated that it is possible to improve the pharmacological activity of a compound proven to inhibit an enzyme by systematic modification of its chemical structure resulting in enhancing of activity and lowering toxicity.[4]

Sorbitol dehydrogenase (SDH), the second enzyme of the polyol pathway, has been implicated in the development of the secondary complications of diabetes.[5,6] 4-[4-(*N,N*-dimethylsulfamoyl) piperazino]-2-hydroxymethylpyrimidine (WAY135 706) inhibits SDH with an IC_{50} value of 1 μM.[7] Administration of this compound has been reported to attenuate the onset and severity of polyol related complications by decreasing the ratio of free cytosolic NADH/NAD^+, regulating several metabolic pathways.[8,9] A recent crystal structure of rat SDH has confirmed the presence of a fourth zinc ligand in the active site of SDH, Glu-70.[10] This information along with recent modelling studies have identified several important residues within the active site of SDH that interact with WAY135 706 including Ser-46 OG (3.11 Å), Arg-298 NH2 (3.41 Å), and Phe-117 (4.9 Å).[11] In this study, based on a model of the holoenzyme–inhibitor complex we have designed several novel compounds that lead to enhanced binding energies of the complex.

The program GRID (version 18)[12] was used to search the active site of SDH holoenzyme model for the most suitable or favourable positions for a variety of probes. A total of 32 probes were tested which included the following groups: methyl, aromatic carbon, amino, amido and heterocyclic nitrogens, halogens, sulphur, carbonyl, ether and hydroxy oxygen, carboxy group, water, phosphate and other ions. Each probe was analysed independently using the InsightII 2.1 package (Biosym Technologies Inc., San Diego, CA, USA). Calculations were performed on a cube (35 Å per side) centred on the active site, with a grid spacing of 0.5 Å. The interaction energy between the probe and every atom within the protein structure was evaluated at each grid point. A dielectric constant of 80 was used to simulate a bulk aqueous phase, while areas as determined by GRID to be excluded from solvent were assigned a dielectric constant of 4 (i.e., the interior of the protein). The

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accompanying program GRIN was used to automatically assign atom types and charges for the protein, using the standard parameter file provided with GRID. The output was converted (using GINS supplied with GRID) into a form suitable for input to the Biosym utility contour, and contour maps were built up using steps of 1 kcal/mol. The contour map detailed a number of energy levels. Negative energy levels delineate regions at which ligand binding is particularly favoured and positive energy levels define the surface of the target. The contour map was then superimposed on the active site of the SDH model using InsightII. Superposition of the inhibitor WAY135 706 on the active site of SDH provided information on the predicted position of the probe with respect to the inhibitor. Summarised in Table 1 are the GRID analysis results for the probes with the most significant energy levels. These include six favoured probes suggested by the program GRID of which the amide probe is the least favoured and the phosphate probe is the most favoured.

Predicted regions of interactions between the active site of SDH and the phosphate, phenyl hydroxyl, carboxylate, amide, amine and hydroxyl probes are shown in Figure 1. The favoured regions of interactions are located in the vicinity of the CH_2OH group of the superimposed WAY135 706 molecule, in agreement with our initial modelling study on the binding of WAY135 706 to the active site.[11] From the GRID analysis results it was postulated that replacing the CH_2OH group of WAY135 706 with functional groups comprised of the five probes listed in Table 1 may improve the binding energies of the complex. Compounds **1**–**5** were then designed based on the structure of WAY135 706 to include the functional groups shown below so that each probe would be placed in its corresponding favored region of interaction.

Table 1. GRID analysis results showing the contour energy levels (kcal) within the active site of SDH for the best six probes

Probe	Max. energy (kcal)	Probe	Max. energy (kcal)
Phosphate	−14.7	Amine	−8.5
Carboxylate	−9.7	Phenyl hydroxyl	−8.0
Hydroxyl	−8.6	Amide	−6.5

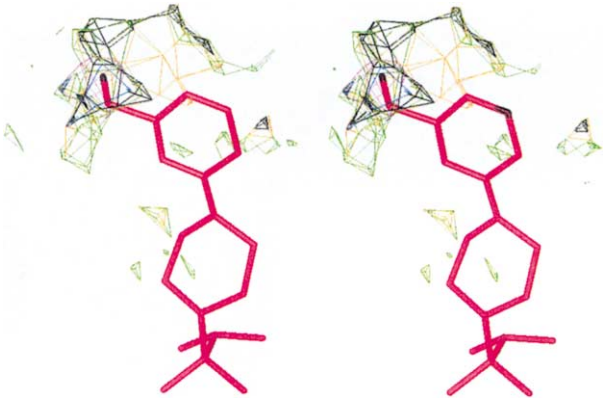
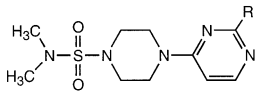


Figure 1. Stereoview showing contours of interaction energies between the active site of SDH and phosphate (pink), phenyl hydroxyl (orange), carboxylate (blue), amide (green), and hydroxyl (black) probes with the superimposed WAY135 706 molecule. The figure was prepared using InsightII (Biosym Technologies, San Diego, CA, USA).



1 (R = $CH_2OPO_3^{-2}$) **4** (R = $CONH_2$)

2 (R = CH_2COO^-) **5** (R = CH_2NH_2)

3 (R = 4-hydroxyl phenyl) **6** (R = CH_2OH)/WAY135 706

The designed compounds were manually docked into the active site of SDH, based on the orientation of WAY135 706.[11] Depending on the chemical structure of the functional group, a nitrogen or an oxygen atom was coordinated to the catalytic zinc atom. The functional group of compound **4** (see above) was positioned twice into the active site with either the oxygen (**4a**) or the nitrogen (**4b**) of the amide group coordinated to the zinc. Energy minimisation and molecular dynamics calculations were carried out using the Discover package (Biosym Technologies, San Diego, CA, USA) on an O2 (R10000) workstation (Silicon Graphics, Mountain View, CA, USA) following established procedures.[11] Briefly, energy minimisation 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). In order to prevent residues on the protein surface from deviating significantly from their positions, to accurately simulate electrostatic energies near the protein surface and to imitate an aqueous environment surrounding the active site, the active site of sorbitol dehydrogenase was solvated by a 15 Å layer of water molecules using the SOAK option of InsightII. Molecular dynamics were then performed using the 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. Finally, the resulting structures were extracted and energy minimised. The calculated binding energies between SDH and compounds **1**–**6** are listed in Table 2.

Molecular dynamics calculations revealed that compound **1** was the most favoured followed by compound **2**. Replacement of the methyl hydroxyl group of WAY135 706 (**6**) with methyl phosphate and methyl

Table 2. Protein interaction energies (kcal/mol) calculated between SDH residues and compounds **1**–**6**

Compd	Protein interaction energy (kcal/mol)	Compd	Protein interaction energy (kcal/mol)
1	−77.81	**4b**	−26.64
2	−65.20	**5**	−5.86
3	−53.86	**6**[a]	−38.03
4a	−50.05		

[a]The value reported by Darmanin and El-Kabbani[11] is amended after taking into account any energy loss due to displacement of water molecules in the active site by compound **6**.

carboxylate functional groups enhanced the net binding of the complex by 2.0- and 1.7-fold, respectively, after taking into account any energy loss due to the displacement of water molecules in the active site of the enzyme by the compounds (Table 2). Compounds **3** and **4a** enhanced the net binding energy by 1.4- and 1.3-fold, respectively. Compounds **4b** and **5** had no beneficial effect on the net binding energy of the complex. This study also revealed the possible interactions between the active site residues and the designed compounds. Residues present within van der Waals contacts of compound **1** and **2** are shown in Figure 2. Similar to the SDH/WAY135 706 model,[11] compounds **1** and **2** are hydrogen bonded to the side chains of Ser-46 OG (3.46 and 2.92 Å), Arg-298 NH2 (3.46 and 3.75 Å) and their pyrimidine rings π-stack against the side-chain of Phe-117 (4.2 and 4.8 Å). The enhanced binding of compound **1** (−77.81 kcal/mol) and **2** (−65.20 kcal/mol) compared to WAY135 706 (−38.03 kcal/mol) reflects the potential of forming new hydrogen bonding interactions

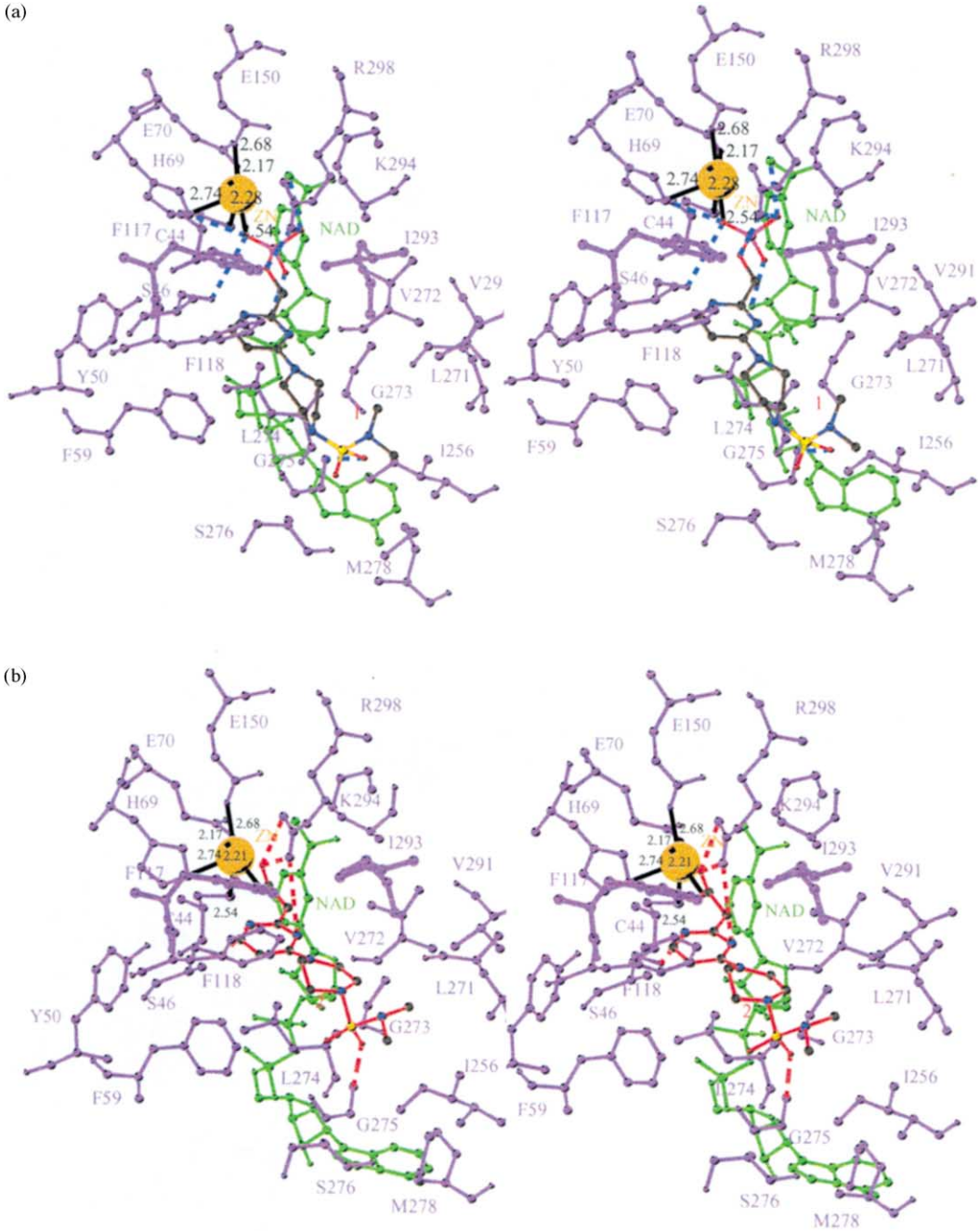


Figure 2. Stereoview of compounds **1** (R = methyl phosphate) and **2** (R = methyl carboxylate) modelled into the active site of SDH. Residues within 4 Å of the compounds and hydrogen bonds (dashed lines) are shown. (a) Hydrogen bonds exist between compound **1** and Ser 46 OG (3.46 Å), Arg-298 NH2 (3.46 Å and 3.15 Å), Gly-275 N (3.35 Å), NAD^+ amide nitrogen (2.6 Å) and nicotinamide ribose oxygen (3.14 Å) and π-stacking occurs with Phe-117 (4.2 Å). The liganding distance between the phosphate oxygen of compound **1** and the catalytic zinc is equal to 2.28 Å. (b) Hydrogen bonds are shown between compound **2** and Ser-46 OG (2.92 Å), Arg-298 NH2 (3.75Å and 2.81 Å) and NH1 (3.02 Å), Gly-275 N (2.94 Å) and π-stacking occurs with Phe-117 (4.8 Å). The liganding distance between the carboxylate oxygen of compound **2** and the catalytic zinc of SDH is equal to 2.21 Å. The figures were prepared after molecular dynamics calculations using MOLSCRIPT.[13]

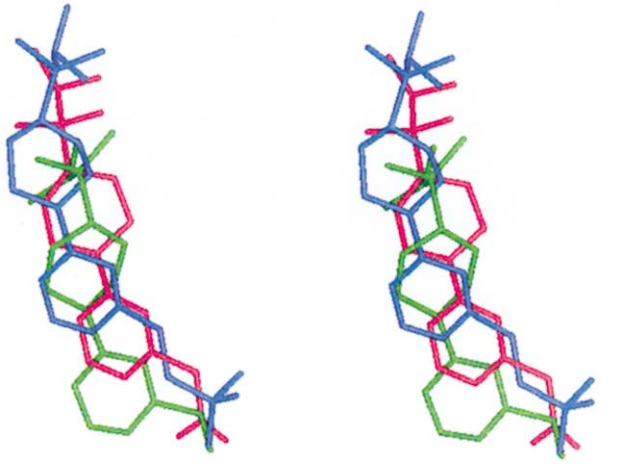


Figure 3. Stereoview of inhibitor WAY135 706 (compound **6**, green) superimposed with compound **1** (blue) and compound **2** (red) within the active site of sorbitol dehydrogenase. The figure was prepared using MOLSCRIPT[13] after molecular dynamics calculations.

with the holoenzyme (Fig. 2 and 3). Compound **1** forms new hydrogen bonds with Gly-275 N (3.35 Å), NAD^+ amide nitrogen (2.60 Å), nicotinamide ribose oxygen (3.14 Å) and Arg-298 NH2 (3.15 Å). The liganding distance between the phosphate oxygen of compound **1** and the catalytic zinc of SDH is equal to 2.28 Å. Compound **2** forms additional hydrogen bonds with Gly-275 N (2.94 Å) and Arg-298 NH1 (3.02 Å) and NH2 (2.81 Å). The liganding distance between the carboxylate oxygen and the catalytic zinc is equal to 2.21 Å.

In conclusion, the replacement of the methyl hydroxyl group of the SDH inhibitor WAY135 706 (**6**) with new functional groups (**1**–**4**) suggested by the program GRID enhances the binding energies of the enzyme–inhibitor complex. The complex with the maximum binding energy has a methyl phosphate as the functional group (**1**). Our results indicate that compounds **1**–**4** may aid the development of potent and more specific inhibitors of human sorbitol dehydrogenase.

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References and Notes

1. Wlodawer, A.; Vondrasek, J. *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 249.
2. von Itzstein, M.; Dyason, J. C.; Oliver, S. W.; White, H. F.; Wu, W.-Y. W.; Kok, G. B.; Pegg, M. S. *J. Med. Chem.* **1996**, *39*, 388.
3. Ealick, S. E.; Sudhakar Babu, Y.; Bugg, C. E.; Erion, M. D.; Guida, W. C.; Montgomery, J. A.; Secrist, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *188*, 11540.
4. Appelt, K.; Bacquet, C.; Bartlett, C.; Booth, S.; Freer, M.; Fuhry, M.; Gehring, M.; Herrmann, S.; Howland, E.; Janson, C.; Jones, T.; Kan, C.-C.; Kathardekar, V.; Lewis, K.; Marzoni, G.; Matthews, D.; Mohr, C.; Moomaw, E.; Morse, C.; Oatley, S.; Ogden, R.; Reddy, M.; Reich, S.; Schoettlin, W.; Smith, W.; Varney, M.; Villafranca, J.; Ward, R.; Webber, S.; Webber, S. E.; Welsh, K.; White, J. *J. Med. Chem.* **1991**, *34*, 1925.
5. 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.
6. Jeffery, J.; Jörnvall, H. *Advances in Enzymology and Related Areas of Molecular Biology*; Wiley: New York, 1988; 61, 47.
7. 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.
8. Tilton, R. G.; Chang, K.; Nyengaard, J. R.; Van Den Enden, M.; Ido, Y.; Williamson, J. R. *Diabetes* **1995**, *44*, 234.
9. Lee, A.; Chung, S. *FASEB* **1999**, *13*, 23.
10. Johansson, K.; El-Ahmad, M.; Kaiser, C.; Jörnvall, H.; Eklund, H.; Höög, J.; Ramaswamy, S. *Chemico-Biol. Interactions.* **2001**, *130–132*, 351.
11. Darmanin, C.; El-Kabbani, O. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1101.
12. Goodford, P. *J. Med. Chem.* **1985**, *28*, 849.
13. Kraulis, P. J. *J. Appl. Cryst.* **1991**, *24*, 946.