Journal of Computer-Aided Molecular Design (2005) 19: 83–92
DOI 10.1007/s10822-005-1486-6

Homology modeling and molecular interaction field studies of α-glucosidases as a guide to structure-based design of novel proposed anti-HIV inhibitors

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Received 09 November 2004; accepted in revised form 27 January 2005

Key words: AIDS, giucosidase, homology modeling, molecular interacting field, saccharide

Summary

For AIDS therapy, there are currently a number of compounds available for multiple targets already approved by the FDA and in clinic, e.g. protease inhibitors, reverse transcriptase inhibitors (NRTI, NNRTI), fusion inhibitors, CCR4, CCR5 among others. Some pharmaceuticals act against the virus before the entrance of HIV into the host cells. One of these targets is the glucosidase protein. This novel fusion target has been recently explored because the synthesis of viral glycoproteins depends on the activity of enzymes, such as glucosidase and transferase, for the elaboration of the polysaccharides. In this work we have built an homology model of *Saccharomyces cerevisiae* glucosidase and superimposed all relevant glucosidase-like enzymes in complex with carbohydrates, and calculated as well molecular interaction fields in our *S. cerevisiae* active site model. Our results suggest that there are two saccharide binding sites which are the most important for the binding of inhibitors with this family of enzymes which supports the possibility of inhibitors containing only two sugar units. Based on these results, we have proposed a novel pseudo-dissacharide which is a potential pharmaceutical for AIDS treatment.

Introduction

The use of computer-aided molecular design has been very useful for investigating diseases, such as AIDS, cancer, allergies, Alzheimer and Chaga's disease [1–8]. AIDS, for example, has been for too long a major challenge to the international scientific community. From the beginning of the AIDS epidemic, some 20 million people have died and the estimate is that today, close to 40 million are living with the type 1 human immunodeficiency virus (HIV)/AIDS. About 14,000 people are being infected daily with AIDS worldwide [9]. For AIDS therapy, there are currently a number of compounds available for multiple targets approved by the FDA and in clinic, e.g. protease inhibitors, reverse transcriptase inhibitors (NRTI, NNRTI) and fusion inhibitors, CCR4, CCR5 among others [10–13]. In some cases, the action against the virus occurs before the entrance of HIV into the host cells. Among the recent targets used for drug development in AIDS treatment, glucosidases have been recently explored as novel fusion targets because the synthesis of viral glycoproteins depend on the activity of enzymes, such as glucosidase and transferase, for the elaboration of the polysaccharides [14, 15].

Glucosidase and saccharide-like compounds are potential therapeutic agents for the treatment

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of AIDS, as well as Gaucher's disease, cancer, diabetes, and can disrupt glycoprotein processing via direct-site irreversible glucosidase inhibition. Related compounds, such as hydroxycyclohexanes and their derivatives play an important role in this process because they mimic the disaccharide unit which is cleaved by glucosidases [15]. Hexahydroxycyclohexanes (inositols) and tetrahydroxycyclo-hexenes (conduritols) occur naturally. *Myo*-Inositol 1,4,5-triphosphate is crucially involved in cellular regulation, and inosamines, in which one or two hydroxyl groups are replaced by amino groups, are important constituents of some antibiotics [16]. As carba-sugars, hydroxycyclohexanes have a methylene group replacing the ring oxygen of normal sugars, and many in this class show interesting biological activity [17]. Thus, new synthetic carba-sugars, hydroxycyclohexanes and pseudo-dissacharides may introduce enantiomerically pure representatives of these types of compounds. They are of considerable interest in both theoretical and experimental biochemistry, involving molecular aided design and synthesis of novel pharmaceuticals for glucosidase inhibitors.

The enzymes glucosidase I and II are involved in key steps during the processing of N-linked oligosaccharides by cleaving three terminal glucose residues from the tetradeca-oligosaccharide moiety $Glc_3Man_9GlcNAc_2$ of an important intermediate N-linked oligosaccharide (Scheme 1). Inhibitors of such enzymes cause malformation of these oligosaccharides and have shown interesting anti-HIV activity, which is believed to arise from the prevention of a successful completion of gp120 in the viral reproduction.

The focus of the present work is to investigate glucosidase inhibitors including novel carba-sugars and molecular designed pseudo-disaccharides, as well as their inhibitors which indicate biological activity [17]. We have built an homology model of *Saccharomyces cerevisiae* glucosidase and superimposed all relevant glucosidase-like enzymes in complex with carbohydrates, and calculated as well molecular interaction fields in our *S. cerevisiae* active site model in order to propose novel glucosidase inhibitors. Our results suggest that there are two saccharide binding sites which are important to the binding of inhibitors of this family of enzymes. These two saccharide units were used to propose a novel inhibitor which is a potential pharmaceutical for AIDS treatment.

Methodology

Sequence alignment and homology modeling procedures

Homology modeling of *S. cerevisiae* α-glucosidase was performed with the MODELLER program, which performs modeling by of spatial restraints [18]. In this method, the user provides an alignment of a sequence to be modeled with known related structures and MODELLER will automatically calculate an all-atom model. In general, the input to the program are restraints on the spatial structure of the amino acid sequence(s) and ligands to be modeled. The output is a 3D structure that best satisfies these restraints. The restraints can be related to distances, angles, dihedral angles as well as pairs of dihedral angles defined by atoms or pseudo atoms. MODELLER automatically obtains the restraints only from the known homologous structures and their alignment with the target sequence. The model of the 3D structure is obtained from optimization of a molecular probability density function (pdf). The molecular pdf is optimized with the variable target function procedure in Cartesian space that employs methods of conjugate gradients and molecular dynamics with simulated annealing.

Our model was obtained using the dimeric *Thermotoga maritima* 4-α-glucanotransferase/acarbose (modified) complex structure (PDB code 1LWJ) as a template [19], which was selected through a BLASTp search in the Protein Data Bank (PDB) for glucosidases. Sixty-six glucosidase-like proteins in complex with ligands were selected, whereas 26 are structurally similar. *T. maritima* 4-α-glucanotransferase, in complex with acarbose modified, is the only homologue, among these 26 complexes, that shares sequence identity higher than 30% with the α-glucosidase modeled herewith. The analyses was performed using pairwise alignments via the AMPS (Alignment of Multiple Protein Sequences) package [20]. Previous to modeling, a final alignment between the *T. maritima* 4-α-glucanotransferase and *S. cerevisiae* α-glucosidase sequences was obtained after

analysing the superposition of 26 complexes with respect to the α-carbons of residues of the respective active sites, using Insight II software [21], which allowed us to edit the previous alignment obtained from the Multalign module of AMPS [20]. This package performs a number of functions. These include pairwise sequence alignment and assessment of statistical significance, multiple alignment and additional functions that allow the inclusion of variable gap-penalties and specific weight schemes. The information of secondary structures in the template sequence was incorporated into this previous alignment using the MULTALIGN program of the AMPS package, with the restriction that all insertions and deletions were limited to regions outside the common core of α-helices and β-sheets. A gap penalty of 1000 was fixed for any deletion or insertion inside a secondary structure element. The alignment obtained was finally edited, investigating and considering aligned, the residues which are close in the space visualized in the structural superposition. This procedure results in a final alignment which is different from that based on the Dayhoff matrix used in AMPS [20].

Molecular interaction field calculations

Calculations were performed with the Classical Molecular Interaction Potential (cMIP) program [22]. The cMIP functional defines the potential around a macromolecule as the addition of steric and electrostatic contributions. The cMIP allows us to compute the electrostatic potential by considering: the Coulombic expression with (1) a constant dielectric model, (2) a linear distance-dependent dielectric model, (3) the Mehler-Solmajer sigmoidal distance-dependent dielectric model and (4) the potential obtained by solving numerically the linear Poisson equation. Finally, the van der Waals potential is computed using a Lennard-Jones expression (4). The total interaction energy was determined as the sum of the electrostatic with the van der Waals interactions and the energy maps were visualized on the Sybyl 6.9.2 software [23]. A focusing strategy was used to determine the effect of the protein and the solvent on the electrostatic potential at the binding site [4]. For tehis purpose, the model was initially enclosed in a box containing at least 40% empty space, and the Poisson equation is then solved numerically using a grid spacing of 1 Å. A box (centered at the Asp214 CA atom) containing all the residues of the binding site is built, whose size is subsequently scaled by a factor of 2, and each axis is enlarged ~3 Å. This procedure allows us to define a very conservative box containing the entire region of interest around the binding site. The Poisson equation is solved using a grid spacing of 0.5 Å, and the potential computed previously by using the initial box [4]. In order to obtain a quantitative evaluation of the potential interaction of ligands with the the *S. cerevisiae* glucosidase active site, the interaction energies between the model active site and three typical probes were computed. The probes used were a water molecule, a negative oxygen with charge $(q) = -0.3e^-$ and a positive oxygen (hydrophobic probe) with $q = 0.3e^-$, placed in a 0.5 Å spacing grid covering the overall binding site. Hydrogen atoms were added to the model using standard protonation states for the residues.

Results and discussion

Design of novel pseudo-disaccharides with potential glucosidase inhibitory activity requires knowledge of the tri-dimensional structure of the human and *S. cerevisiae* enzymes. The latter enzyme was used to investigate potential inhibitors due to their commercial availability. For these two enzymes there are no solved PDB structures, either native or complexed proteins. We have thus built an homology model of the *S. cerevisiae* glucosidase with one of the glucosidases homologues containing solved PDB structures. Among these structures we observed that *Thermotoga maritima* 4-α-glucanotransferase (PDB code 1LWJ) is the only one that shares an identity sequence larger than 30% with the *S. cerevisiae* glycosidase (34.3% with respect to the overall sequence). Residues of the two active sites show high identity sequence and are present in different domains of the overall protein sequence.

The model (Figure 1) was obtained as described in the previous section, indicates good stereochemistry as indicated by the general factor G (−0.25) and reasonable atomic contact quality (−0.92) as expected for a crystal structure with 2.5 Å resolution. Despite overall low sequence identity among the complex structures of the homologue glucosidase-like proteins, the active

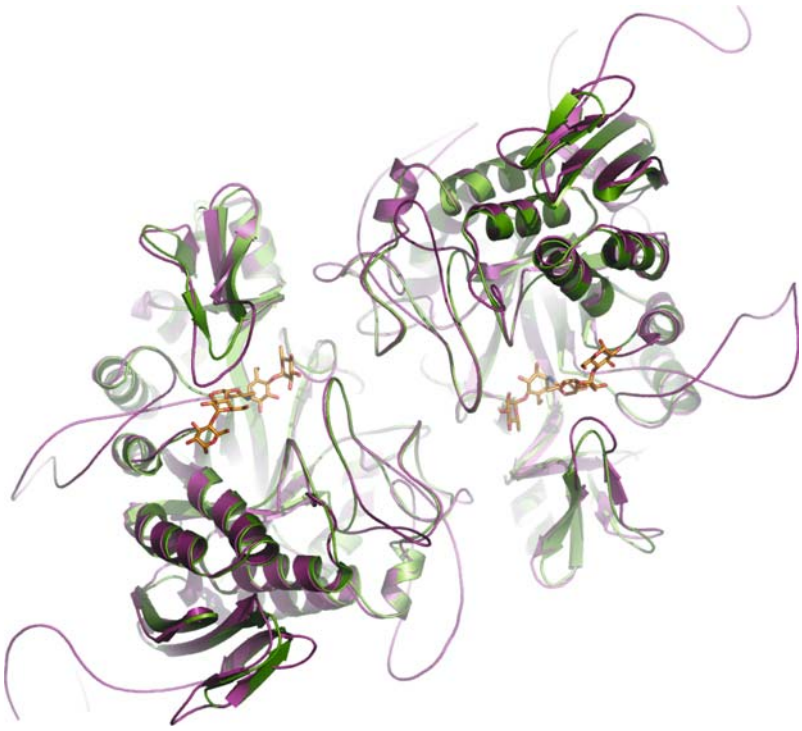

Figure 1. Ribbon diagram of the superposition of *Thermotoga maritima* 4-α-glucanotransferase/modified acarbose complex structure (PDB code 1LWJ), in green, with the *Saccharomyces cerevisiae* glucosidase model, in magenta. Superposition was done with respect to the atoms of identical residues found in both the active sites (RMS = 0.38). Modified acarbose of crystal complex is shown with carbon atoms in orange.

sites are structurally similar and reasonably well conserved.

In Figure 2 we observe that a modified acarbose, i.e., a pseudo-pentasaccharide interacts with some residues of *Thermotoga maritima* 4-α-glucanotransferase, such as Tyr54, His94, Phe150, Arg184, Asp186, His190, Glu216, Trp218, His277 and Asp278. Eight residues (Tyr54, His94, Phe150, Arg184, Asp186, Glu216, His277 and Asp278) interact with the ciclitol ring (valienamine moiety) of the modified acarbose. The ring at the opposite end of the nitrogen bridge (6-deoxyglucosyl moiety) interacts only with the His190 residue. The larger number of residues that interact with the second ring of the modified acarbose suggest that this region of interaction between ligand and active site of glucosidases should play the main role in saccharide-like ligand recognition. This region is structurally conserved at the catalytic domain. The acarbose and modified acarbose structures are given in Figure 3.

The valienamine moiety in acarbose is tightly bound by a stacking interaction with Tyr54 and multiple hydrogen bonds with other residues of the protein (Figure 2). The O2 and O3 hydroxyl groups form hydrogen bonds with both His277 and Asp278. The O2 hydroxyl group also makes hydrogen bonds with Glu216 OE2 and Arg184 NH2. The O6 hydroxyl group interacts with the side chains of His94 and Asp186. The nitrogen atom bridging the valienamine and 6-deoxyglucosyl moieties of the inhibitor is located at the scissile bond and is also hydrogen bonded with the side chain of Asp278. The O2 and O3 hydroxyl groups of the 6-deoxyglucosyl moiety forms hydrogen bonds with His190 NE2 and Glu216 OE1, respectively. If a glucosyl moiety was bound at this position whereas hydrogen bonds are formed, the hydroxyl group at O6 would interact with the side chain of Asp278. The valienamine moiety is flanked by the aromatic rings of Tyr54 and Phe150, making extensive hydrophobic π-stacking interactions (face to face) with the enzyme.

Saccharomyces cerevisiae glucosidase contains 66 homologue proteins with solved structures as determined by a PDB search with the aid of the BLASTp program. Of these structures, only 26 are complexes which can be structurally superimposed

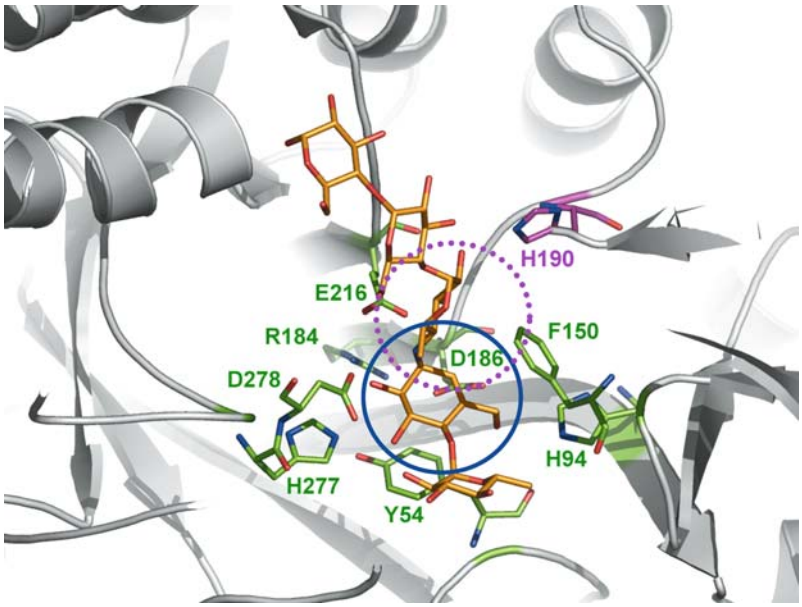

Figure 2. Ribbon diagram of the *Thermotoga maritima* 4-α-glucanotransferase/modified acarbose complex structure, in gray. Selected residues of the active site that interact with the modified acarbose (carbon atoms in orange) are shown. Residues that interact with valienamine moiety (surrounded by a blue circle, bold line) of the ligand are represented with carbon atoms in green. His190 (carbon atoms in magenta) is the only residue that interacts with the 6-deoxyglucosyl ring (surrounded by a magenta circle, dot line) of modified acarbose.

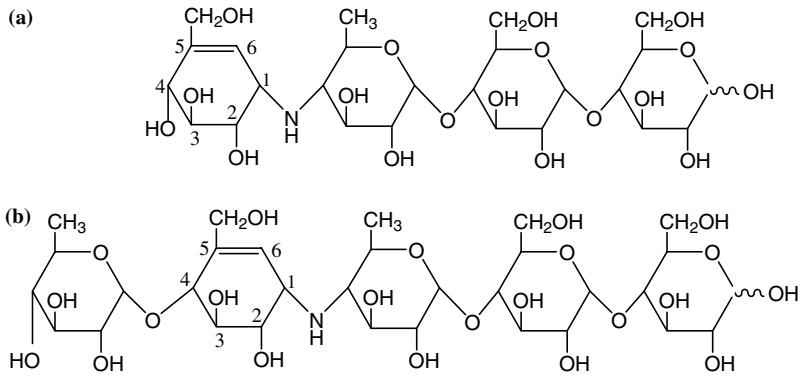

Figure 3. Acarbose (a) and modified acarbose (b). The numbered rings corresponds to the ciclitol.

with the *S. cerevisiae* model. The superposition of the structures of these 26 complexes with the model described above, with respect to the α-carbons of residues of the respective active sites, allows us to position each of the ligands, such as ciclodextrine, deoxy-nojirimicine, acarbose, glucose and others in the interior of the active site of this glucosidase, for comparison. The superposition of 26 complexes (Figure 4) indicates that in the region of the catalytic cavity less accessible to the solvent only two rings of the ligands have similar spatial orientation (structural *consensus*). In contrast, the other saccharide units of the ligands do not indicate good structural alignment. Interactions that occur between one of the rings and the residues of the catalytic site differ only by one ring, the one with the best structural *consensus* (indicated by a blue circle in Figures 2, 4 and 6) participate effectively in most of the interactions observed in the enzymes.

The most important aminoacids involved in the interactions of our model with these ligands would

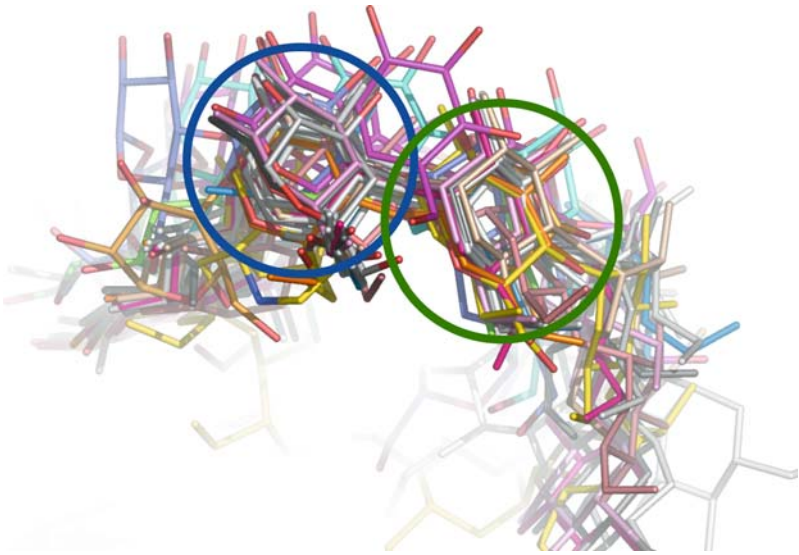

Figure 4. Superposition of 26 complex structures of homologue glucosidase-like proteins. Two preferencial pirane binding sites are shown with circles, whereas the ring surrounded by the blue circle was analyzed to be the most important to bind saccharides.

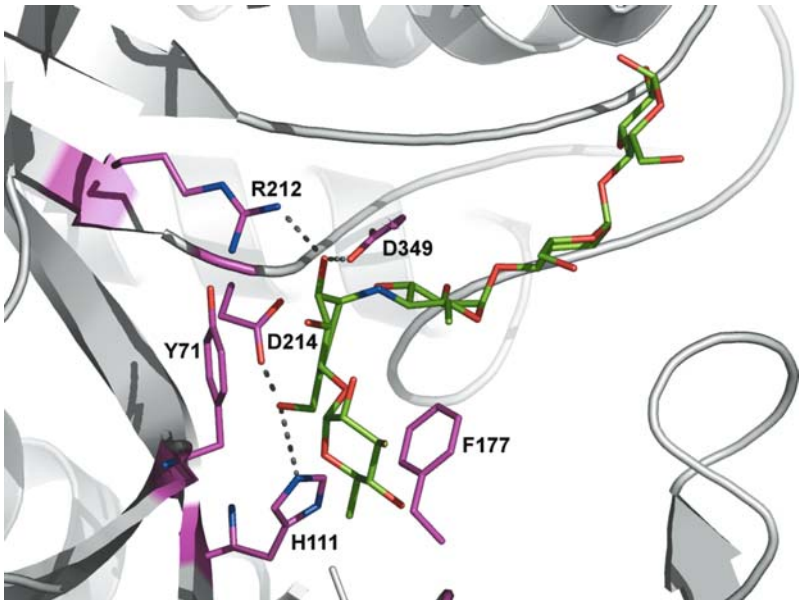

Figure 5. Ribbons diagram of the superposition of the model of *Saccharomyces cerevisiae* glucosidase (in gray) superimposed with the *Thermotoga maritima* 4-α-glucanotransferase/modified acarbose complex structure (ligand with carbon atoms in green). Selected residues of the *S. cerevisiae* glucosidase are shown (in magenta), which would interact with modified acarbose. Interactions between the valienamine ring and residues of the active site are represented by dash lines.

be Tyr71, Asp349, Phe177, Arg212 and Asp214 (Figure 5). These residues show identity sequence with the template sequence. The residues Arg212 and Asp349 can interact via hydrogen bonding with the secondary hydroxyl of the valienamine moiety. On the other hand, His111 and Asp214 have the same type of interaction, involving the primary hydroxyl of the same sugar unit, and can act as electron acceptors or donors. In the interior of the catalytic site, Asp214 and Arg212 are bonded by a salt bridge, whose conformational restriction favors the interaction of these conserved

residues, respectively with the primary and secondary pirane system. On the other hand, Tyr71 is adequately positioned to make stacking with the same sugar ring via interaction with the oxygen heteroatom (from the carbohydrate) or with the double bond of the carba-sugar derivatives, such as acarbose. Positioning this pirane ligand ring in an horizontal plane, it is possible to visualize the Tyr71 residue above the plane and the Phe177 residue bellow, flanked by the monossacharide.

The superposition involving one glucose ligand (PDB code 1JG9), a covalently bonded oligossacharide (PDB code 1CXL), acarbose (PDB code 1LWJ) and deoxy-nojirimicin (PDB code 1I75), allows the visualization of a certain degree of structural alignment, whereas the piperidine system of deoxy-nojirimicin is slightly displaced. In the model of *S. cerevisiae* glucosidase, the second region of largest structural consensus previously described (green circle in Figure 6) is occupied by voluminous residues such as Met69. We also considered a third region with large structural consensus between the 26 complexes (magenta circle in Figure 6) as the likely additional ligand site to be explored in the design of novel pseudodissacharides.

In the complex with PDB code 1I75, the nitrogen of the deoxy-nojirimicin is located in the hydrophobic region, composed by residues such as Phe183, Leu194 and Tyr195 (corresponding to Trp154, Leu176 and Phe177 of the *S. cerevisiae* glucosidase model, respectively). The presence of an alkylic chain bound to nitrogen, as observed in the most potent inhibitor N-butyl-1-deoxy-nojirimicin, should fill and adjust to this space. This last compound presents the highest activity with respect to glucosidases when compared with deoxy-nojirimicin.

In the complex 4-α-glucanotransferase of *T. maritima*/modified acarbose (pentassacharide), the nitrogen of glucosidic bridge makes strong interaction via hydrogen bond with the conserved residue of Asp278 (corresponding to Asp349 of the *S. cerevisiae* glucosidase model), at a distance of 2.73 Å (Figure 2). The introduction of an additional sugar unit in acarbose (fifth unit bonded to ciclitol) allows an accommodation of the ligand in the cavity of this complex, whereas in the *S. cerevisiae* glucosidase this space is occupied by Met69 (Figure 6). This observation supports the need of maintaining the ciclitol group free in one of the extremities of the molecule for design purposes of

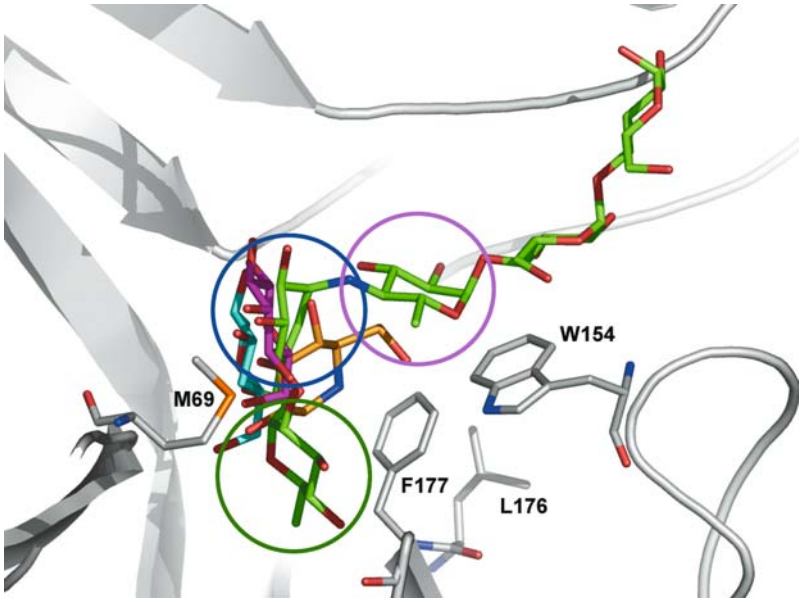

Figure 6. Superposition of the *S. cerevisiae* glucosidase model (carbon atoms in gray) with the structures of 4 complexes of homologue proteins selected from PDB. The two most important saccharide binding sites are shown surrounded by the blue and green circles, previously described for Figure 4. The second most important site (surrounded by the green circle) is occupied, in the *S. cerevisiae* glucosidase model, by Met69, but a third site (6-deoxyglucosyl ring, surrounded by a magenta circle) could also play a important role in the binding of sugar units.

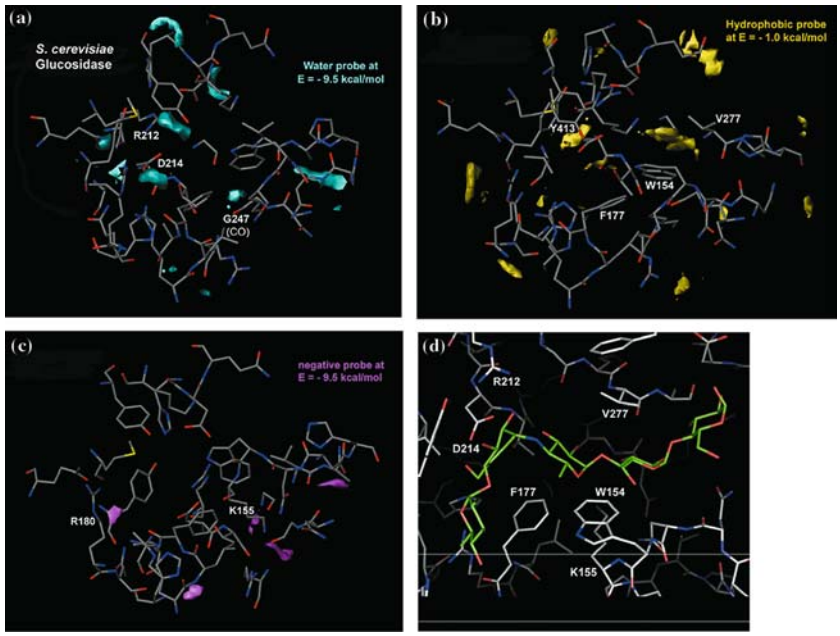

Figure 7. Energy contours corresponding to the interaction of the *S. cerevisiae* and three probe groups: water (a), hydrophobic (b) and negative (c). In A, the energy is contoured at −9.5 kcal/mol and it is mainly due to the Arg212 and Asp214 residues. In 5B, the energy is contoured at −1.0 kcal/mol, and it is mainly due to the Trp154, Phe177, V277 and Tyr413 residues. In 5C, the energy is contoured at −9.5 kcal/mol, and it is mainly due to the Arg180 and Lys155 residues. In (d), the *S. cerevisiae* glucosidase model (carbon atoms in gray) superimposed to modified acarbose (carbon atoms in green) of the *Thermotoga maritima* 4-α-glucanotransferase structure. Selected residues of 7a–c are shown.

novel active pseudo-dissacharides with respect to *S. cerevisiae* glucosidase.

We have performed molecular interaction field (MIF) calculations and the results obtained agree with the observation of two consensus carbohydrate binding sites, since the residues of *S. cerevisiae* glucosidase that have the most important interactions with the three probe groups are close to the two sugar units described previously as the structural *consensus* among the 26 complexes. In Figure 7a, the interaction of this enzyme with the water probe is energy contoured at −9.5 kcal/mol, and it is mainly due to the Arg212 and Asp214 residues. In Figure 7b, the interaction of the enzyme with the hydrophobic probe is energy contoured at −1.0 kcal/mol, and it is mainly due to the Trp154, Phe177, V277 and Tyr413 residues. In Figure 7c, the interaction of the enzyme with the negative probe is energy contoured at −9.5 kcal/mol, and it is mainly due to the Arg180 and Lys155 residues. For comparison, the *S. cerevisiae* glucosidase model superimposed with the modified acarbose of *T. maritima* 4-α-glucanotransferase is shown in Figure 7d.

One proposal would be the variation of the acarbose, maintaining the double bond of the ciclitol group (for stacking interaction with Tyr71 and Phe177), eliminating the hydroxy-methylene group at position 5 and introducing a new hydroxyl (secondary) in position 6. This inversion of hydroxyl positions could allow an interaction with the new residue Thr215, present in *S. cerevisiae* glucosidase, in addition to increasing the proximity of the hydroxyl group in C-6 with the residue Asp214. In our structure a hydroxyl group was also removed from ciclitol at position 2. These modifications can

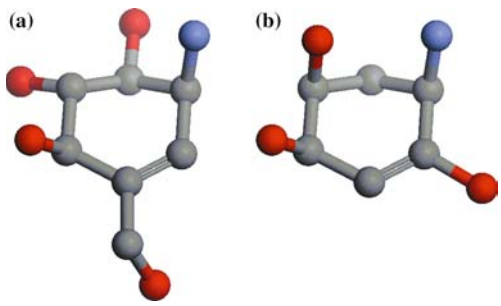

Figure 8. (a) The ciclitol group of acarbose. (b) The novel ciclitol group proposed in this work.

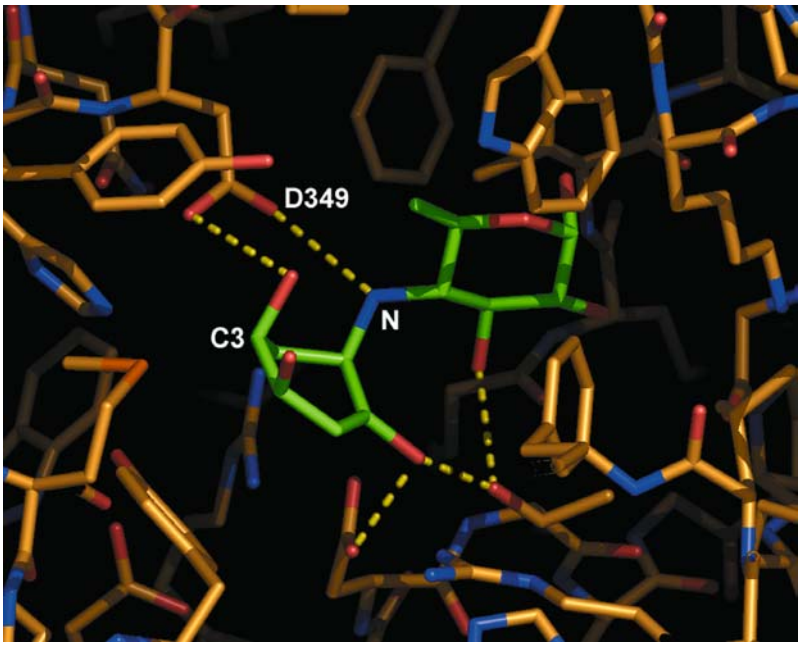

Figure 9. Main interactions (in dash lines) between the pseudo-dissacharide proposed (carbon atoms in green) and residues of catalytic site of *S. cerevisiae* glucosidase model. Asp349 of the model would interact with the hydroxyl in C-3 bind the nitrogen bridge binding the glucose unit of the novel inhibitor. The structure of the proposed inhibitor was placed in the active site by superposition with the modified acarbose of the complex with *T. Marítima* 4-α-glucanotransferase.

be visualized in Figure 8 for the two ciclitol rings above described. It is worthy to comment that the ciclitol groups of the acarbose (valienamine moiety) as well as our structural moiety are not local energy minima, but graphical representations to aid the comprehension of the structural similarity between them. However, our structure is near a minimum local of energy (−515.658 a.u.), such as calculated using density functional theory at B3LYP/6-31G* level in the gas-phase [Silva, C.H.T.P., Carvalho, I. and Taft, C.A., unpublished results].

In this novel proposed glucosidase inhibitor, the interaction of the hydroxyl located in C-2 of the acarbose ciclitol with the Asp349 residue would be substituted by the interactions with a hydroxyl in C-3 of the new inhibitor, with an inverted configuration to that found in the acarbose modified complexed to 4-α-glucanotransferase (PDB code 1LWJ), in order to allow a stronger interaction with this aspartic acid. The semi-equatorial configuration of the nitrogen bridge binding the glucose unit of acarbose should be maintained in the new derivatives with the objective of attaining an interaction with Asp349. Figure 9 shows how this potential inhibitor could interact with great structural complementarity and a network of interactions inside the catalytic site of the *S. cerevisiae* glucosidase model. This novel glucosidase inhibitor constitutes a homologous series of pseudo-dissaccharides which are being synthesized in our laboratory and tested against *S. cerevisiae* glucosidase as potential anti-HIV pharmaceuticals.

Conclusion

In this work we have built an homology model of *S. cerevisiae* glucosidase and superimposed all relevant glucosidase-like enzymes in complex with carbohydrates, and calculated as well molecular interaction fields (MIF) for our model. The MIF calculations agree with the results obtained from the superposition of all glucosidase-like proteins in complex with ligands, i.e., there are two saccharide binding sites which are the most important to bind inhibitors of this family of enzymes. This novel discovery of the existence of two binding sites supports the possibility of inhibitors containing only two sugar units. Based on these results, we have proposed a novel

pseudo-dissacharide which is a potential pharmaceutical for AIDS treatment.

Acknowledgements

We acknowledge financial support from CNPq, CAPES, FAPESP and FAPERJ. We thank Renato Murillo for his participation. We gratefully acknowledge Prof. Dr. Josep L. Gelpí and Modesto Orozco, from the Universitat of Barcelona, for kindly providing the cMIP program.

References

1. Martins, J.B.L., Taft, C.A., Perez, M.A., Stamato, F.M.L.G. and Longo, E., Int. J. Quant. Chem., 69 (1998) 117.
2. Pavão, A.C., Taft, C.A., Guimarães, T.C.F., Leão, M.B.C., Mohallem, J.R., Lester, W.A.Jr., J. Phys. Chem. A, 105 (2001) 5 – Invited International Paper: Special edition dedicated to Per-Olov LÖwdin.
3. Martins, J.B.L., Perez, M.A., Longo, E., Taft, C.A., Arissawa, M., Stamato, F.G.L. and Tostes, J.G.R., Int. J. Quant. Chem., 90 (2002) 575.
4. Fradera, X., de la Cruz, X., Silva, C.H.T.P., Gelpi, J.L., Luque, F.S. and Orozco, M., Bioinformatics, 18 (2002) 939.
5. Arissawa, M., Taft, C.A., Felcman, J., Silva, C.H.T. and Taft, C.A., Int. J. Quant. Chem., 93 (2003) 422.
6. Silva, C.H.T.P., Almeida, P. and Taft, C.A., J. Mol. Model., 10 (2004) 38.
7. Silva, C.H.T.P., Sanches, S.M. and Taft, C.A., J. Mol. Graph. Model., 23 (2004) 89.
8. Carvalho, I. and Melo, E.B., Carbohyd. Res., 339 (2004) 361.
9. Walker, P.R., Worobey, M., Rambaut, A., Holmes, E.C. and Pybus, O.G., Nature, 422 (2003) 6933.
10. Noor, M.A., Parker, R.A., O'Mara, E., Grasela, D.M., Currie, A., Hodder, S.L., Fiedorek, F.T. and Haas, D.W., AIDS, 18 (2004) 2137.
11. Dando, T.M. and Scott, L.J., Drugs, 65 (2005) 285.
12. Veiga, A.S., Santos, N.C., Loura, L.M., Fedorov, A. and Castanho, M.A., J. Am. Chem. Soc., 126 (2004) 14758.
13. Princen, K., Hatse, S., Vermeire, K., Aquaro, S., De Clercq, E., Gerlach, L.O., Rosenkilde, M., Schwartz, T.W., Skerlj, R., Bridger, G. and Schols, D., J. Virol., 78 (2004) 12996.
14. Mehta, A., Zitzmann, N., Rudd, P.M., Block, T.M. and Dwek, R.A., FEBS Lett., 430 (1998) 17.
15. Asano, N., Nishida, M., Kato, A., Kizu, H., Matsui, K., Shimada, Y., Itoh, T, Baba, M., Watson, A.A., Nash, R.J., Lilley, P.M.de, Watkin, D.J. and Fleet, G.W.J., J. Med. Chem., 41 (1998) 2565.
16. Billington, D.C., Perron-Sierra, F., Picard, I., Beaubras, S., Duhault, J., Espinal, J. and Challal, S., Bioorg. Med. Chem. Lett., 19 (1994) 2307.
17. Dalko, P.I. and Sinay, P., Angew. Chem. Int. Edit., 38 (1999) 773.
18. Sali, A. and Blundell, T.L., J. Mol. Biol., 212 (1990) 403.
19. Roujeinikova, A., Raasch, C., Sedelnikova, S., Liebl, W. and Rice, D.W., J. Mol. Biol., 321 (2002) 149.
20. Barton, G. and Sternberg, M.J.E., J. Mol. Biol., 198 (1987) 327.
21. Insight II. Accelrys. CA, San Diego, 2004.
22. Gelpi, J.L., Kalko, S.G., Barril, X., Cirera, J., Cruz, X. de la, Luque, F.J. and Orozco, M., Proteins, 45 (1991) 110.
23. Blanco, M., J. Comp. Chem., 12 (1991) 237.