A Large Difference in the Thermodynamics of Binding of Isofagomine and 1-Deoxynojirimycin to β-Glucosidase

Anne Bülow, Igor W. Plesner, and Mikael Bols*

Department of Chemistry, Aarhus University
DK-8000 Aarhus, Denmark

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Azasugar inhibitors of glycosidases and related enzymes are subject to intense current interest.[1−3] Some time ago it was found that a subtle change in the classical azasugar inhibitor 1-deoxynojirimycin (**1**, Figure 1), by moving the nitrogen to the anomeric position to form isofagomine (**2**), gave a much more potent β-glucosidase inhibitor.[4] This appears to be general, and some of the strongest glycosidase inhibitors have been found among the isofagomines.[5−8] However, it is unclear why these molecules are so strong β-glycosidase inhibitors, though some explanations have been offered.[1,6,7] If **2** is a transition state analogue it implies that the transition state resembles **A** rather than the distorted intermediate **B** suggested in the Phillips mechanism.[6] Another puzzle is that **2** is a tight binder even though it lacks a 2-hydroxyl group. That hydroxyl group is very important for binding in **1** and other inhibitors[9] as witnessed by **3** (Figure 1) being a very poor inhibitor. Solving these puzzles may give important insight into how these enzymes work.

We therefore believe it pertinent to report our recent discovery of a remarkable difference in the mode of binding of **1** and **2** to almond β-glucosidase, an enzyme that **2** inhibits very strongly. These modes may be characterized by the very different thermodynamic functions associated with the binding process. For **1**, **2**, and some related inhibitors we have measured standard enthalpy and entropy of binding to the enzyme. This was done by measuring K_i at a series of temperatures, and plotting $-\ln K_i$ vs $1/T$ in a van't Hoff plot, from which ΔH^θ and ΔS^θ were calculated (Table 1). The inhibitors used were **1**, (±)-**2**, **4**, **5**, and (±)-**6**, which were synthesized by previously published methods[10−13] or obtained commercially.

The thermodynamic data show (Table 1) that the binding of **1** to the enzyme is driven by a negative enthalpy, which means that the binding of **1** is favorable in terms of bond energies. The entropy is relatively unimportant for the binding. This is what one would predict from what is known about the thermodynamics of carbohydrate−protein interaction; Lemieux et al. have shown[14−16] that a negative enthalpy is associated with many carbohydrate−lectin bindings, while the entropy plays a relatively minor role.

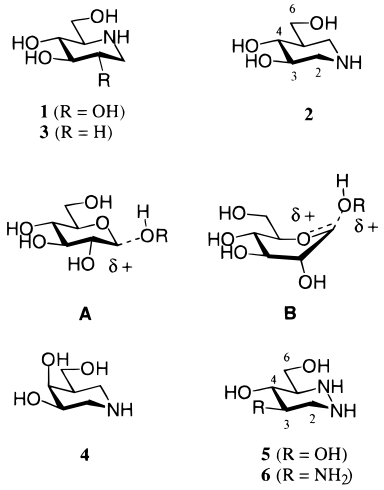

Figure 1. The chemical strucure of the competitive inhibitors **1**−**2** and **4**−**6** and transition states **A** and **B**.

Table 1. Thermodynamic Parameters for the Binding of the Five Competitive Inhibitors to Almond β-Glucosidase at pH 6.8[a]

	K_i (μM, 25 °C)	ΔH (kJ/mol)	ΔS (J/molK)
1	26.3	-25.7	1.3
5, R = OH	0.18	-1.5	123.1
6, R= NH_2*	48	4.3	97.2
2, R = OH*	0.27	58.6	323.8
4, R = epi-OH	0.097	58.7	331.0

[a] An asterisk indicates the inhibitor is racemic.

The binding of isofagomine **2**, on the other hand, is very different: The enthalpy is positive, and there is a very large positive entropy. This means that the binding of **2** is unfavorable in terms of bond energies, but this is more than compensated for by the large entropy increase. Iso-*galacto*-fagomine **4**, which also is a strong almond β-glucosidase inhibitor,[5,7] has a virtually identical thermodynamic profile.[17] 1-Azafagomine **5**, another potent β-glucosidase inhibitor,[12] which resembles both **1** and **2**, has a binding enthalpy close to zero, and a large positive entropy. The entropy gain is considerably smaller than that of **2** though. The 3-amino analogue of **5**, **6**, has a similar energy profile though the binding ethalpy is 6 kJ/mol larger.

From these data some interesting observations can be made. It is clear that the presence of an anomeric nitrogen atom does not contribute to binding through bond energy by decreasing ΔH.

(1) Heightman, T. D.; Vasella, A. T. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 750−770.
(2) Zechel, D. L.; Withers, S. G. *Acc. Chem. Res.* **2000**, *33*, 11−18.
(3) Stütz, A. E. *Iminosugars as glycosidase inhibitors: nojirimycin and beond;* Wiley-VCH: Weinheim, 1999.
(4) Jespersen, T. M.; Dong, W.; Skrydstrup, T.; Sierks, M. R.; Lundt, I.; Bols, M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1778−9.
(5) Ichikawa, Y.; Igarashi, Y. *Tetrahedron Lett.* **1995**, *36*, 4585−6.
(6) Bols, M. *Acc. Chem. Res.* **1998**, *31*, 1−8.
(7) Ichikawa, Y.; Igarashi, Y.; Ichikawa M.; Suhura Y. *J. Am. Chem. Soc.* **1998**, *120*, 5854.
(8) Williams, S. J.; Hoos, R.; Withers, S. G. *J. Am. Chem. Soc.* **2000**, *122*, 2223−5.
(9) Panday, N.; Meyyappan, M.; Vasella, A. *Helv. Chim. Acta* **2000**, *83*, 513−38.
(10) Liang, X.; Lohse, A.; Bols, M. Unpublished.
(11) Hansen, S. U.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2000**, 911−15.
(12) Ernholt, B. V.; Thomsen, I. B.; Lohse, A.; Jensen, K. B.; Hazell, R. G.; Plesner, I.; Liang, X.; Jacobsen, A.; Bols, M. *Chem. Eur. J.* **2000**, *6*, 278−287.
(13) Lohse, A.; Jensen, H. H.; Bach, P.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2000**, 659−665.
(14) Spohr, U.; Hindsgaul, O.; Lemieux, R. U. *Can. J. Chem.* **1985**, *63*, 2644−52.
(15) Hindsgaul, O.; Khare, D. P.; Bach, M.; Lemieux, R. U. *Can. J. Chem.* **1985**, *63*, 2653−8.
(16) Lemieux, R. U.; Du, M.-H.; Spohr, U.; Acharya, S.; Surolia, A. *Can. J. Chem.* **1994**, *72*, 158−63.

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Thus when comparing **5** and **1** it is likely that the 24 kJ/mol lower binding enthalpy in **1** is caused by the presence of a strong hydrogen bond to the 2-hydroxyl group in **1**, which is lacking in **5**. The value is very close to the 30−40 kJ/mol by which the 2-hydroxyl group is believed to stabilize the transition state.[9] 1-Azafagomine (**5**) has a 60 kJ/mol lower binding enthalpy than **2**, suggesting this to be the bond energy obtained from having a ring nitrogen in place of ring oxygen. This energy could be obtained from forming a strong hydrogen bond or a salt bridge between the catalytic carboxylic acid group of the enzyme and ring nitrogen. Both **5** (pK_a 5.3) and **1** (pK_a 6.7) should be largely unprotonated when entering the active site, and formation of a salt bridge would be expected to result in an enthalpy gain. The inhibition of β-glucosidase by **5** has been shown to be independent of pH in the range 5−8,[18] while the inhibition by **1** decreases strongly with decreasing pH.[19] This shows that for both compounds the inhibiting species is the free amine, but given the large enthalpy decrease on binding it is conceivable that it become protonated inside the active site.

Isofagomine (**2**, pK_a 8.6), on the other hand, presumably forms a salt bridge with the nucleophilic carboxylate of the enzyme. However, since **2** would be expected to be largely protonated when entering the active site at this pH, and the carboxylate also is ionized in the empty active site, it is quite reasonable that little enthalpy is gained by forming this salt bridge, especially because this interaction must replace similar interactions between the enzyme and water and inhibitor and water.

This suggests that the protonated **2** is the inhibiting species. To support this idea we tested the inhibition of (±)-**2** at pH 5.0 and found the K_i to be 0.75 μM, compared with the K_i at pH 6.8 of 0.20 μM. This showed that the protonated **2** indeed could be the inhibiting species, as the change in inhibition is relatively small and reflects the very small change in the concentration of that species when going from pH 6.8 to 5.0.

The presence of an anomeric nitrogen atom in compounds **2** and **4**−**6** appears to result in a large entropy gain on binding. Thus, when the inhibitor, protonated at the anomeric nitrogen, forms a salt bridge with the nucleophilic carboxylate, more disorder occurs. This can to some extent be explained by the expulsion of water molecules that were involved in solvating the nucleophilic carboxylate in the empty enzyme, which are no longer needed when this group is paired with the inhibitor. This solvation might be a designed feature in the enzyme that makes that carboxylate more reactive. However, the entropy gain is so large that it does not appear to be the only explanation; perhaps the carboxylate is paired with other groups in the enzyme in an ordered fashion that is disrupted when the carboxylate reacts, or is complexed, with the inhibitor.

The observations reported here imply that the 2-hydroxyl group of **1** and the nitrogen of **2** are not interacting with the same group and a 2-hydroxy analogue of **2** would be a more potent inhibitor than **2**. They also support the notion that the enzyme stabilizes a permanent positive charge at the anomeric position, and that **A** indeed could be a transition state in the β-glucosidase catalyzed glycoside cleavage.

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Supporting Information Available: A van't Hoff plot of the binding of the 5 inhibitors to β-glucosidase (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Though **4** has a K_i that is three times lower than (±)-**2** the true difference in potency is only 1.5 because the latter compound is racemic. It may seem extraordinary that the *galacto* isomer **4** is a slightly more potent inhibitor than the *gluco* isomer on inhibition of a glucosidase; however, the same phenomena is seen for glycoimidazole inhibitors and deoxynojirimycins on this enzyme.[3]

(18) Bols, M.; Hazell, R. G.; Thomsen, I. B. *Chem. Eur. J.* **1997**, *3*, 940−47.

(19) Dale, M. P.; Ensley, H. E.; Kern, K.; Sastry, K. A. R.; Byers, L. D. *Biochemistry* **1985**, *24*, 3530−9.