Thermochimica Acta 511 (2010) 189–193

Contents lists available at ScienceDirect

Thermochimica Acta

journal homepage: www.elsevier.com/locate/tca

Dissecting factors that contribute to ligand-binding energetics for family 18 chitinases

Anne Line Norberg, Vincent G.H. Eijsink, Morten Sørlie*

Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway

ARTICLE INFO

Article history:
Received 29 June 2010
Received in revised form 11 August 2010
Accepted 12 August 2010
Available online 19 August 2010

Keywords:
Isothermal titration calorimetry
Chitinase
Inhibition
Conformational changes

ABSTRACT

Inhibition of family 18 chitinases has several interesting applications. To this regard, it is important to understand the dependency of binding energetics with respect to the nature of the ligand as well as the chitinase. We have studied the binding of hexameric *N*-acetylglucosamine $(GlcNAc)_6$ to both glycon and aglycon subsites in chitinase B (ChiB) of *Serratia marcescens* and we compare the results with binding of allosamidin to ChiB (glycon subsites only, where products are released) and to chitinase A (ChiA) of *S. marcescens* (glycon subsites only, where polymeric substrates bind). The ΔG_r° values for the three binding processes were identical within experimental errors (−38 kJ/mol) while binding was driven by different factors, being solvation entropy ($-T\Delta S_{solv}^\circ$ = −52.3 ± 1.5 kJ/mol), conformational entropy ($-T\Delta S_{conf}^\circ$ −45.2 ± 2.0 kJ/mol) [27], and equal contributions of ΔH_r° and $-T\Delta S_{solv}^\circ$ (−23.4 ± 0.9 and −20.4 ± 3.1 kJ/mol) [29], respectively.

1. Introduction

Chitin, an insoluble linear polysaccharide consisting of repeated units of β-1,4-*N*-acetylglucosamine (GlcNAc), is common as a structural polymer in crustaceans, arthropods, fungi, and parasitic nematodes. Chitinases, the enzymes that degrade chitin, belong to the glycoside hydrolase enzyme families 18 and 19 [1]. As a consequence of the known and putative biological roles of chitinases, especially family 18 chitinases [2,3], inhibition of these enzymes is a target for the development of plant protecting compounds [4,5] and medicines for allergic and inflammatory disorders [6,7].

When designing inhibitors, it is important know and understand the energetics of the inhibitor–enzyme interaction. As an example, for FDA-approved HIV-1 protease inhibitors as well as statins, two classes of drugs for which complete thermodynamic information has been published, data suggest that best-in-class compounds that come into the market after several years are enthalpically better optimized than the original first-in class compounds, indicating that optimizing binding enthalpies is more favorable than optimizing binding entropies [8]. It is especially interesting to determine binding energetics of chitooligosaccharide (CHOS) based inhibitors since substrate based inhibitors hold a tremendous advantage in being very specific towards chitinases, and hence not likely to interfere with other enzymatic systems. The binding strength of such inhibitors can be tuned with the length of the oligomer (i.e. longer bind stronger) [9,10].

Binding of known family 18 chitinase CHOS-based inhibitors occurs in glycon subsites of the active site only or in both glycon and aglycon subsites (subsites are labeled according to the nomenclature for sugar-binding subsites in glycosyl hydrolases, where glycon subsites denoted −*n*, binding to the non-reducing end of the substrate and aglycon subsites are denoted +*n*, binding to the reducing end, with cleavage taking place between the −1 and +1 subsite [11]). As an example, Fig. 1 shows the overall structures and the subsites of chitinase A (ChiA) and chitinase B (ChiB) from *Serratia marcescens* that are the target enzymes used in this study. Known CHOS-based inhibitors bind to glycon subsites only: allosamidin [12] and CHOS thiazolines [9] that are intermediate analogues and CHOS lactones [13] that are transition state analogues. These CHOS-derivatives bind to the glycon subsites because the conformation of the sugar moiety at the "reducing end" of the oligomer resembles the structure of the transition state (^{4}E conformation) and thus has high affinity for the −1 subsite. In principle, it should be possible to develop CHOS-based inhibitors that bind to the whole of the active site, but this requires that the sugar moiety binding to the −1 subsite does not have an *N*-acetyl group [2]. Family 18 chitinases have an absolute preference for an *N*-acetylglucosamine sugar in the −1 subsite of the active site due to the need for the carbonyl group to act as a nucleophile in the first step of the hydrolysis [14].

To obtain more insight in the binding energetics of whole active site-binding inhibitors, we have studied the energetics for binding of $(GlcNAc)_6$ (Fig 2.) to ChiB-E144Q. From previous work, it is known that $(GlcNAc)_6$ binds to subsites −2 to +3 (with an "overhang" in

* Corresponding author. Tel.: +47 64965902; fax: +47 64965901.
E-mail address: morten.sorlie@umb.no (M. Sørlie).

0040-6031/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2010.08.013

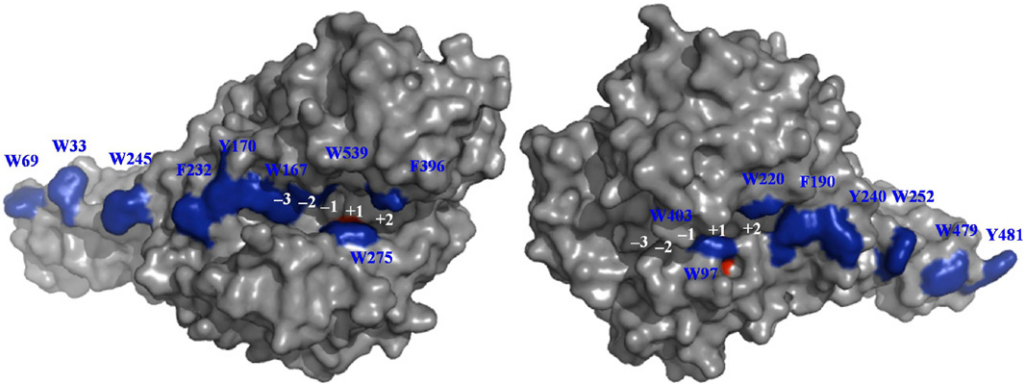

Fig. 1. Crystal structures of ChiA (left, [16] 1ctn), ChiB (right, [17] 1e15). Highlighted in blue are aromatic amino acids that are important for substrate binding; the catalytic acids, Glu^{315} and Glu^{144}, are colored red. These enzymes degrade chitin in a processive manner from the reducing end (ChiA; the polymeric substrate binds to glycon subsites) or the non-reducing end (ChiB; the polymeric substrate binds to aglycon subsites) [18]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

subsite "+4") and subsites −3 to +3 in ChiB [15]. We compare the results with data previously obtained for binding of the glycon-binding inhibitor allosamidin (Fig. 2) to both ChiB and ChiA.

2. Experimental

2.1. Proteins and chemicals

ChiB-E144Q from *S. marcescens* were over expressed in *Escherichia coli* and purified as described elsewhere [19,20]. $(GlcNAc)_6$ was purchased from Sigma–Aldrich (St. Louis, MO, U.S.A.).

2.2. Isothermal titration calorimetry experiments

ITC experiments were performed with a VP-ITC system from Microcal, Inc. (Northampton, MA) [21]. Solutions were thoroughly degassed prior to experiments to avoid air bubbles in the calorimeter. Standard ITC conditions were 250 μM of $(GlcNAc)_6$ in the syringe and 15 μM of ChiB-E144Q in the reaction cell in 20 mM potassium phosphate buffer of pH 6.0, 7.0, and 8.0, respectively. Aliquots of 8 μL were injected into the reaction cell at 180 s intervals at temperatures of 15, 17.5, 20, and 37 °C with a stirring speed of 260 rpm. The titrations were normally complete after 22–27 injections. At least three independent titrations were performed for each binding reaction. As a control experiment, 30 μM $(GlcNAc)_6$ was incubated with ChiB-E144Q (15 μM) for 75 min (equal the time for an ITC measurement) and analyzed using normal phase HPLC as described by Krokeide et al. to ensure that there are no enzymatic hydrolysis of $(GlcNAc)_6$ [22].

2.3. Analysis of calorimetric data

ITC data were collected automatically using the Microcal Origin v.7.0 software accompanying the VP-ITC system [21]. Prior to further analysis, data were corrected for heat of dilution by subtracting the heat remaining after saturation of binding sites on the enzyme. Data were fitted using a non-linear least-squares algorithm using a single-site-binding model employed by the Origin software that accompanies the VP-ITC system. All data from the binding reactions fitted well with the single-site-binding model yielding the stoichiometry (n), equilibrium binding association constant (K_a), and the reaction enthalpy change (ΔH_r°) of the reaction. The value of n was found to be between 0.9 and 1.1 for all reactions. The reaction free energy change (ΔG_r°) and the reaction entropy change (ΔS_r°) were calculated from the relation described in Eq. (1).

$$\Delta G_r^\circ = -RT \ln K_a = \Delta H_r^\circ - T\Delta S_r^\circ \quad (1)$$

Errors are reported as standard deviations of at least three experiments at each temperature. A description of how the entropic term is parameterized has been described in detail previously [23,24].

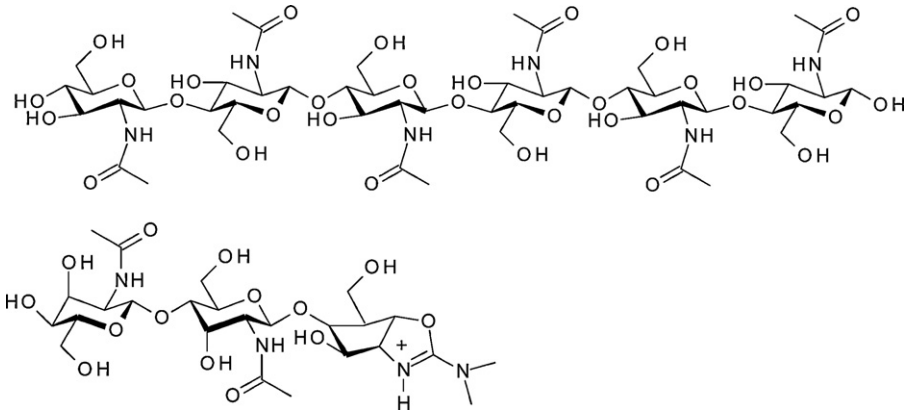

Fig. 2. Molecular structures of $(GlcNAc)_6$ (top) and allosamidin (bottom).

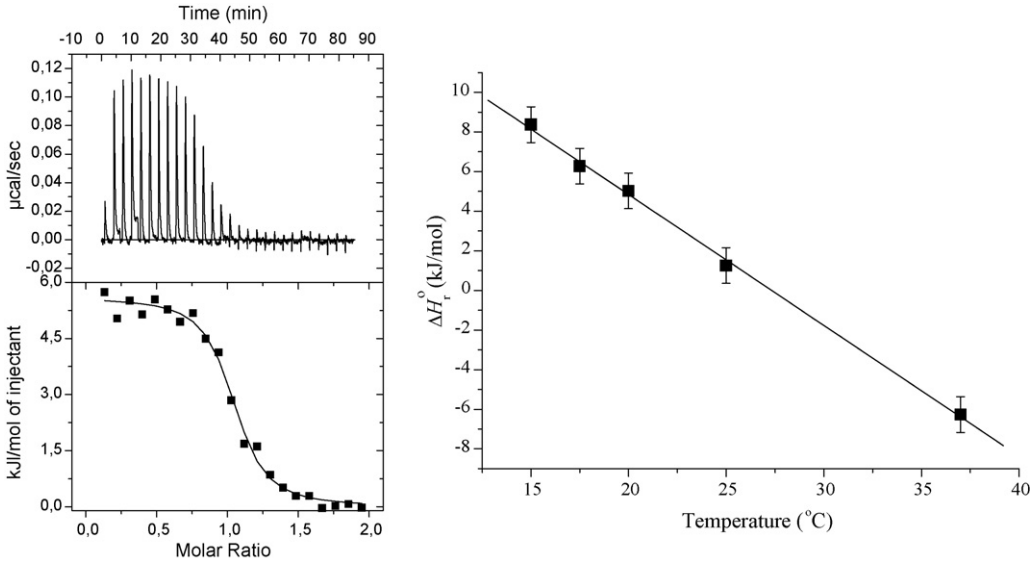

Fig. 3. Left panel, thermograms (top) and binding isotherms (bottom) for the titration of $(GlcNAc)_6$ (at $t = 20\,^{\circ}C$). Right panel, temperature dependence of $(GlcNAc)_6$ binding to ChiB-E144Q at pH 6.0. The plot of ΔH_r° vs. temperature yields the change of heat capacity (ΔC_p) as the slope. The value of ΔC_p is -661 ± 20 J/K mol.

3. Results and discussion

3.1. Binding of $(GlcNAc)_6$ to ChiB-E144Q

The binding of $(GlcNAc)_6$ to ChiB-E144Q in 20 mM potassium phosphate at different temperatures (15-37 °C) and at a pH of 6.0 was studied by ITC. Fig. 2 shows a typical ITC thermogram and theoretical fit to the experimental data $t = 20\,^{\circ}C$. At this temperature, $(GlcNAc)_6$ binds with a $\Delta G_r^{\circ} = -38.0 \pm 1.3$ kJ/mol (Table 1). The binding is clearly entropically driven ($-T\Delta S_r^{\circ} = -43.0 \pm 1.8$ kJ/mol) with an enthalpic penalty ($\Delta H_r^{\circ} = 5.0 \pm 1.2$ kJ/mol). Determination of the temperature dependence of ΔH_r° yields the change in the reaction heat capacity ($\Delta C_{p,r}^{\circ}$). The change in reaction heat capacity for $(GlcNAc)_6$ binding to ChiB-E144Q was found to be -661 ± 20 J/K mol (Fig. 3). $(GlcNAc)_6$ binding to ChiB-E144Q was also analyzed at pH 7.0 and 8.0. The results show that the change in pH had only small effects on binding ($\Delta G_r^{\circ} = -36.0 \pm 1.5$ and 38.5 ± 1.8 kJ/mol, respectively) and little variation in the enthalpic and entropic terms ($\Delta H_r^{\circ} = 6.3 \pm 1.2$ and 7.1 ± 1.8 kJ/mol, respectively, and $-T\Delta S_r^{\circ} = -42.3 \pm 1.9$ and -45.6 ± 2.5 kJ/mol, respectively). Furthermore, there was little variation in the change in heat capacity with respect to pH as well with $\Delta C_{p,r}$ being -711 ± 29 J/K mol at pH 8.0 (not determined at pH 7.0).

ΔS_r° can be parameterized into three terms as shown in Equation 2 [25].

$$\Delta S_r^{\circ} = \Delta S_{solv}^{\circ} + \Delta S_{mix}^{\circ} + \Delta S_{conf}^{\circ} \quad (2)$$

As explained in the legend to Table 1, ΔS_{solv}° may be derived from $\Delta C_{p,r}^{\circ}$ [26–28] and ΔS_{mix} represent a fixed known "cratic" term [25], meaning that ΔS_{conf}° can be derived from ΔS_r°. The results, summarized in Table 1, show that at pH 6.0 $-T\Delta S_{solv}^{\circ}$ equals -52.3 ± 1.5 kJ/mol, the loss of translational entropy ($-T\Delta S_{mix}^{\circ}$) equals 9.7 kJ/K mol and the entropic effect of conformational changes, $-T\Delta S_{conf}^{\circ}$, equals -0.4 ± 2.3 J/K mol.

3.2. Differences in binding energetics

The free energies of binding for $(GlcNAc)_6$ and allosamidin to ChiB are identical within experimental errors. Both binding reactions come with an enthalpic penalty ($\Delta H_r^{\circ} = 5.0$ and 18.5 kJ/mol, respectively), and are thus entropically driven ($-T\Delta S_r^{\circ} = -43.0 \pm 1.8$ and -56.5 ± 1.7 kJ/mol, respectively). One would expect the longer ligand $(GlcNAc)_6$ that binds to six subsites to displace more solvent molecules than the shorter allosamidin that only binds to three subsites. This is reflected in $-T\Delta S_{solv}^{\circ}$ values of -52.3 ± 1.5

Table 1
Thermodynamic parameters for binding of $(GlcNAc)_6$ and allosamidin binding to ChiB[a] and for allosamidin binding to ChiA[a] at $t = 20\,^{\circ}C$, as determined by isothermal titration calorimetry.

ΔG_r° [b]	ΔH_r° [b]	$-T\Delta S_r^{\circ}$ [b]	$-T\Delta S_{solv}^{\circ}$ [b,c]	$-T\Delta S_{conf}^{\circ}$ [b,d]	ΔS_r° [e]	$\Delta C_{p,r}^{\circ}$ [e,f]
ChiB-$(GlcNAc)_6$						
-38.0 ± 1.3	5.0 ± 1.2	-43.0 ± 1.8	-52.3 ± 1.5	-0.4 ± 2.3	147 ± 4	-661 ± 20
ChiB-allosamidin[a]						
-38.0 ± 1.0	18.5 ± 0.9	-56.5 ± 1.7	-21.0 ± 1.1	-45.2 ± 2.0	193 ± 4	-263 ± 16
ChiA-allosamidin[b]						
-39.3 ± 0.9	-23.4 ± 0.9	-15.9 ± 1.7	-20.4 ± 3.1	-5.2 ± 3.5	54 ± 4	-255 ± 52

[a] The values at $t = 20\,^{\circ}C$ are calculated from the ones at $t = 30\,^{\circ}C$ by using the temperature dependence on ΔH_r° and ΔS_r°, from Cederkvist et al. [22] and Baban et al. [28], respectively, to be able to compare to those of the $(GlcNAc)_6$–ChiB-E144Q binding that has low ΔH_r° at $t = 30\,^{\circ}C$.
[b] kJ/mol.
[c] $\Delta S_{solv}^{\circ} = \Delta C_p \ln(T_{293\ K}/T_{385\ K})$ [25–27].
[d] Derived using $\Delta S_r^{\circ} = \Delta S_{solv}^{\circ} + \Delta S_{mix}^{\circ} + \Delta S_{conf}^{\circ}$ where $\Delta S_{mix}^{\circ} = R\ln(1/55.5) = -33$ J/K mol ("cratic" term) [24].
[e] J/K mol.
[f] These data are derived from the temperature dependence of ΔH_r°.

and -21.0 ± 1.1 kJ/mol, respectively, i.e. a much more favorable $-T\Delta S^{\circ}_{solv}$ for the hexamer. Interestingly, there is a huge difference in the conformational entropy change. While binding of $(GlcNAc)_6$ to ChiB results in $-T\Delta S^{\circ}_{conf}$ of -0.4 ± 2.3 kJ/mol, allosamidin binding to the same protein is accompanied with an $-T\Delta S^{\circ}_{conf}$ of -45.2 ± 2.0 kJ/mol. One obvious explanation for this great difference is that long ligands are more flexible than short, and that the conformational entropy loss upon binding for this reason will be greater. Similar trends have been observed for the binding of xylosaccharides to xylanases [10]. Another explanation may be that the allosamizoline group in allosamidin is preformed while −1 sugar in $(GlcNAc)_6$ must undergo a chair – boat conformational transformation and, hence, lose conformational entropy. While this conformational entropy change to our knowledge has not been calculated for GlcNAc in CHOS, it has been shown to be less than 11% (<12 J/K mol) of the free energy change for the same conformational transformation for glucose in dextran [30].

Another factor may be the topology and the structure of the ligand-binding sites of the enzymes. ChiB contains several loops with relatively high *B*-factors that primarily interact with ligands binding in aglycon subsites, and not in the glycon subsites where allosamidin binds [31]. Rigidification of these loops upon binding of $(GlcNAc)_6$ may contribute to the relatively unfavorable ΔS°_{conf} for binding of this hexamer. Support for this comes from allosamidin binding to ChiA. For this interaction, $-T\Delta S^{\circ}_{solv}$ (-20.4 ± 3.1 kJ/mol) is the same as for binding to ChiB (-21.0 ± 1.1 kJ/mol) but $-T\Delta S^{\circ}_{conf}$ is 40 kJ/mol less favorable than for ChiB (-5.2 ± 3.5 kJ/mol vs. -45.2 ± 2.0 kJ/mol) [23,29]. Even though allosamidin binds to glycon subsites in both chitinases, the difference is that allosamidin binds to ChiA in "substrate-binding sites" (i.e., where the chitin chain would be binding during processive hydrolysis) compared to "product-binding sites" in ChiB (i.e., where chitobiose is released from the enzyme during processive hydrolysis). Judged from the structure, flexible loops interacting with the polymeric substrate are less prominent in ChiA than in ChiB, but ChiA does indeed contain some inserted loops absent in ChiB that are affected by substrate binding in the glycon subsites [32]. Thus, one could a priori expect relatively unfavorable – $T\Delta S^{\circ}_{conf}$ terms for hexamer binding to ChiB and allosamidin binding to ChiA, as is indeed observed.

Effective enzymes need to make sure that their products are not bound too strongly. The occurrence of aromatic residues in the active site clefts of ChiA and ChiB indicate that the enzymes are optimized to bind the polymeric substrate (in glycon subsites in ChiA and in aglycon subsites in ChiB), while the product is bound more loosely [18,33]. Indeed, our previous studies, summarized in Table 1, show that binding of allosamidin to substrate-binding subsites in ChiA is accompanied by a favorable enthalpic change in contrast to what is observed binding to product binding sites in ChiB (-23.4 ± 0.9 kJ/mol vs. 18.5 ± 0.9 kJ/mol, respectively). Surprisingly, the characterization of binding of $(GlcNAc)_6$ to ChiB in the present study showed only a minor enthalpic improvement as compared to binding of allosamidin, and the overall enthalpic term still disfavors binding. Structural studies show that $(GlcNAc)_6$ has several favorable interactions in the aglycon subsites [31]. Somewhat unexpectedly, it is only the favorable effect of ΔS_{solv} that drives binding of $(GlcNAc)_6$.

Lastly, the binding of $(GlcNAc)_6$ to ChiB-144Q was not dependent of pH contrasting to what is observed for the binding of allosamidin to both ChiA and ChiB where the binding enthalpy changes decreases with increasing pH [23,29]. The pH dependencies for the latter interactions have been attributed to the deprotonation of the Asp^{313}–Glu^{315} and Asp^{142}–Glu^{144} diads for ChiA and ChiB, respectively, and the formation of favorable electrostatic–electrostatic interactions with the positively charged allosamizoline group of allosamidin [23,29]. For the $(GlcNAc)_6$–ChiB interaction, this cannot only be due to the fact that there are no titratable groups on the ligands, but must also imply that the titratable groups remaining in the catalytic center of ChiB after mutating the catalytic Glu^{144} to a non-titratable glutamine are not significantly titrated in the pH 6.0–8.0 range. This is in accordance with the results of previous experimental and bioinformatics studies showing that only remaining titratable group with a pK_a in or near the physiological pH interval is Asp^{142} and that this residue has a distorted pK_a value near, and possibly well above 8.0 [20].

4. Concluding remarks

The results described in this work show that binding energetics for ligands to family 18 chitinases greatly depend on both the nature of ligand and details of the active site architecture of the enzyme. To some extent, the observed energetics can be explained by chemical considerations and structural information on ligand binding, but, on the other hand, the energetics of binding of $(GlcNAc)_6$ showed some surprising features. This whole active site-binding compound showed good affinity, comparable with that of allosamidin, but did not show the expected favorable enthalpic term in binding energetics. The two active human chitinases, chitotriosidase (HCHT) and acidic mammalian chitinase (AMCase) that are possible therapeutic targets [7,34] contain many aromatic residues in both their glycon and aglycon subsites, but the roles of these residue e.g. in determining processivity and its direction agree not yet known. Future rational inhibitor design will require more in-depth knowledge of the enzymes in addition to the type of measurements described in this study.

Acknowledgements

We are grateful for the funding from the Norwegian Research Council for a research grant (Project 177542/V30) and the ITC instrument (Project 155518/V00).

References

[1] B. Henrissat, G.J. Davies, Structural and sequence-based classification of glycoside hydrolases, Curr. Opin. Struct. Biol. 7 (1997) 637–644.
[2] F.H. Cederkvist, M.P. Parmer, K.M. Vårum, V.G.H. Eijsink, M. Sørlie, Inhibition of a family 18 chitinase by chitooligosaccharides, Carbohydr. Polym. 74 (2008) 41–49.
[3] O.A. Andersen, A. Nathubhai, M.J. Dixon, I.M. Eggleston, D.M. Van Aalten, Structure-based dissection of the natural product cyclopentapeptide chitinase inhibitor argifin, Chem. Biol. 15 (2008) 295–301.
[4] H. Izumida, M. Nishijima, T. Takadera, A.M. Nomoto, H. Sano, The effect of chitinase inhibitors, cyclo(Arg-Pro) against cell separation of Saccharomyces cerevisiae and the morphological change of Candida albicans, J. Antibiot. (Tokyo) 49 (1996) 829–831.
[5] J. Saguez, F. Dubois, C. Vincent, J.C. Laberche, B.S. Sangwan-Norreel, P. Giordanengo, Differential aphicidal effects of chitinase inhibitors on the polyphagous homopteran Myzus persicae (Sulzer), Pest. Manage. Sci. 62 (2006) 1150–1154.
[6] L.E. Donnelly, P.J. Barnes, Acidic mammalian chitinase—a potential target for asthma therapy, Trends Pharmacol. Sci. 25 (2004) 509–511.
[7] Z. Zhu, T. Zheng, R.J. Homer, Y.K. Kim, N.Y. Chen, L. Cohn, Q. Hamid, J.A. Elias, Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation, Science 304 (2004) 1678–1682.
[8] E. Freire, Do enthalpy and entropy distinguish first in class from best in class? Drug Discov. Today 13 (2008) 869–874.
[9] J.M. Macdonald, C.A. Tarling, E.J. Taylor, R.J. Dennis, D.S. Myers, S. Knapp, G.J. Davies, S.G. Withers, Chitinase inhibition by chitobiose and chitotriose thiazolines, Angew. Chem. Int. Ed. Engl. 49 (2010) 2599–2602.
[10] G. Zolotnitsky, U. Cogan, N. Adir, V. Solomon, G. Shoham, Y. Shoham, Mapping glycoside hydrolase substrate subsites by isothermal titration calorimetry, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 11275–11280.
[11] G.J. Davies, K.S. Wilson, B. Henrissat, Nomenclature for sugar-binding subsites in glycosyl hydrolases, Biochem. J. 321 (1997) 557–559.
[12] S. Sakuda, A. Isogai, S. Matsumoto, A. Suzuki, K. Koseki, The structure of allosamidin, a novel insect chitinase inhibitor, produced by Streptomyces Sp, Tetrahedron Lett. 27 (1986) 2475–2478.

[13] G. Vaaje-Kolstad, A. Vasella, M.G. Peter, C. Netter, D.R. Houston, B. Westereng, B. Synstad, V.G. Eijsink, D.M. Van Aalten, Interactions of a family 18 chitinase with the designedinhibitor HM508, and its degradation product,chitobiono-delta-lactone, J. Biol. Chem. (2004).
[14] A.C. Terwisscha van Scheltinga, S. Armand, K.H. Kalk, A. Isogai, B. Henrissat, B.W. Dijkstra, Stereochemistry of chitin hydrolysis by a plant chitinase/lysozyme and X-ray structure of a complex with allosamidin: evidence for substrate assisted catalysis, Biochemistry 34 (1995) 15619–15623.
[15] S.J. Horn, M. Sørlie, G. Vaaje-Kolstad, A.L. Norberg, B. Synstad, K.M. Vårum, V.G.H. Eijsink, Comparative studies of chitinases A, B and C from *Serratia marcescens*, Biocatal. Biotransform. 24 (2006) 39–53.
[16] A. Perrakis, I. Tews, Z. Dauter, A.B. Oppenheim, I. Chet, K.S. Wilson, C.E. Vorgias, Crystal structure of a bacterial chitinase at 2.3 A resolution, Structure 2 (1994) 1169–1180.
[17] D.M.F. van Aalten, B. Synstad, M.B. Brurberg, E. Hough, B.W. Riise, V.G.H. Eijsink, R.K. Wierenga, Structure of a two-domain chitotriosidase from *Serratia marcescens* at 1.9-angstrom resolution, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 5842–5847.
[18] H. Zakariassen, B.B. Aam, S.J. Horn, K.M. Varum, M. Sorlie, V.G. Eijsink, Aromatic residues in the catalytic center of chitinase A from *Serratia marcescens* affect processivity, enzyme activity, and biomass converting efficiency, J. Biol. Chem. 284 (2009) 10610–10617.
[19] M.B. Brurberg, I.F. Nes, V.G.H. Eijsink, Comparative studies of chitinases A and B from *Serratia marcescens*, Microbiology 142 (1996) 1581–1589.
[20] B. Synstad, S. Gåseidnes, D.M.F. van Aalten, G. Vriend, J.E. Nielsen, V.G.H. Eijsink, Mutational and computational analysis of the role of conserved residues in the active site of a family 18 chitinase, Eur. J. Biochem. 271 (2004) 253–262.
[21] T. Wiseman, S. Williston, J.F. Brandts, L.N. Lin, Rapid measurement of binding constants and heats of binding using a new titration calorimeter, Anal. Biochem. 179 (1989) 131–137.
[22] I.-M. Krokeide, B. Synstad, S. Gåseidnes, S.J. Horn, V.G.H. Eijsink, M. Sørlie, Natural substrate assay for chitinases using high-performance liquid chromatography: a comparison with existing assays, Anal. Biochem. 363 (2007) 128–134.
[23] F.H. Cederkvist, S.F. Saua, V. Karlsen, S. Sakuda, V.G.H. Eijsink, M. Sørlie, Thermodynamic analysis of allosamidin binding to a family 18 chitinase, Biochemistry 46 (2007) 12347–12354.
[24] H. Zakariassen, M. Sørlie, Heat capacity changes in heme protein–ligand interactions, Thermochim. Acta 464 (2007) 24–28.
[25] B.M. Baker, K.P. Murphy, Dissecting the energetics of a protein–protein interaction: the binding of ovomucoid third domain to elastase, J. Mol. Biol. 268 (1997) 557–569.
[26] R.L. Baldwin, Temperature dependence of the hydrophobic interaction in protein folding, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 8069–8072.
[27] K.P. Murphy, P.L. Privalov, S.J. Gill, Common features of protein unfolding and dissolution of hydrophobic compounds, Science 247 (1990) 559–561.
[28] K.P. Murphy, Hydration and convergence temperatures—on the use and interpretation of correlation plots, Biophys. Chem. 51 (1994) 311–326.
[29] J. Baban, S. Fjeld, S. Sakuda, V.G.H. Eijsink, M. Sørlie, The roles of three *Serratia marcescens* chitinases in chitin conversion are reflected in different thermodynamic signatures of allosamidin binding, J. Phys. Chem. B 114 (2010) 6144–6149.
[30] R.G. Haverkamp, A.T. Marshall, M.A.K. Williams, Entropic and enthalpic contributions to the chair—boat conformational transformation in Dextran under single molecule stretching, J. Phys. Chem. B 111 (2007) 13653–13657.
[31] D.M.F. van Aalten, D. Komander, B. Synstad, S. Gåseidnes, M.G. Peter, V.G.H. Eijsink, Structural insights into the catalytic mechanism of a family 18 exo-chitinase, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 8979–8984.
[32] Y. Papanikolau, G. Prag, G. Tavlas, C.E. Vorgias, A.B. Oppenheim, K. Petratos, High resolution structural analyses of mutant chitinase A complexes with substrates provide new insight into the mechanism of catalysis, Biochemistry 40 (2001) 11338–11343.
[33] H. Zakariassen, V.G.H. Eijsink, M. Sørlie, Signatures of activation parameters reveal substrate-dependent rate determining steps in polysaccharide turnover by a family 18 chitinase, Carbohydr. Polym. 81 (2010) 14–20.
[34] F. Fusetti, H. von Moeller, D. Houston, H.J. Rozeboom, B.W. Dijkstra, R.G. Boot, J.M.F.G. Aerts, D.M.F. van Aalten, Structure of human chitotriosidase. Implications for specific inhibitor design and function of mammalian chitinase-like lectins, J. Biol. Chem. 277 (2002) 25537–25544.