# EUROPEAN CHITIN SOCIETY NEWSLETTER

*No 17, June 15, 2004*

Edited by Martin G Peter, e-mail: peter@chem.uni-potsdam.de



# EDITORIAL

Dear Members of the European Chitin Society,

it is a long time ago that the last Newsletter had appeared, the delay being caused by various circumstances, but maily the permant facing of an overload of work by colleagues being active in their professional activities. At this time, EUCHIS does even not have a Secretary in charge, and thus I took the liberty to assemble some documents together in this paper which you might consider as Newsletter Number 17. We hope that this situation will change, soon.

I am writing to all members now in view of the soon coming-up 6th International Conference of the European Chitin Society, EUCHIS '04, will take place in POZNAŃ, POLAND, AUGUST 31 – SEPTEMBER 3, 2004. This will be an important event for EUCHIS, not only for freshing-up the contacts between members and scientific exchange, but also for matters of EUCHIS itself.

As set forth in the Statues of EUCHIS, a General Members Assembly shall take place during this meeting, and all members shall be informed about three months in advance about the agenda. Normally, this should be done by publication in the Newsletter.

Some of the topics listed in the Agenda for the General Assembly require further comments, as follows:

- New Board: Eight Board members must be elected new every second year, with a maximum time of service for four years. Nominations for the New Board are attached as file New Board 040615.DOC. You are invited to nominate additional persons. Note that only active members can nominate a member of the Board and that also this person must be an active member of EUCHIS.
- Prix Braconnot: nominations were received for Dr. Sabine Glöggler, Düsseldorf, and for Dr. Stanislaw Trzcinski, Torun. The Abstract of Dr. Glögglers thesis was published in Newsletter # 16, but is included here again.
- Financial Report: The financial report for 2002 was approved by the Board during a meeting in Montreal. It is included in this Newsletter. The report for 2003 shall be presented during the meeting in Poznan.

Various other topics are of interest to all members, first to mention the nomination of the Honorary President. Members will be also interested in the reports of the president and the treasurer, as well as gaining information about forthcoming activities, such as publications and conferences.

Last not least, we shall use the Newsletter to inform our members about articles, accepted for publication or having appeared in scientific journals. For sure, submission of such abstracts for incorporation into the Newsletter is certainly much lower than the number of papers actually published.

I hope that many of you will attend EUCHIS'04 and I am looking forward to meeting you in Poznan in about 2 ½ months.

With my best and personal wishes and greetings,

Martin G. Peter
President, EUCHIS

Potsdam, June 15, 2004

## EUROPEAN CHITIN SOCIETY

Minutes of the Board meeting of 28th August 2003

A meeting of the Board of the European Chitin Society was held at the Marriott Champlain Hotel, Montreal on the 28th August 2003 under the Chairmanship of the President, Professor Martin Peter.

The following members of the Board were present: Professor A Domard, Professor E Guibal, Dr G M Hall, Dr M Jaworska, Professor M Peter, Professor O Smidsrød, Professor M Spindler-Barth and Professor K M Vårum.

1. Secretary's Report

The Secretary reported that he had not published an edition of the newsletter as a result of his retirement from his University position. The loss of practical support from his department and other work activities had been the main causes. He thought that the situation would not improve in the foreseeable future and offered his resignation. The assistant Secretary, Prof Guibal, said he would take over the position for one year prior to the meeting in Montpellier which would occupy him thereafter. GMH would send EG all the relevant papers. MP reported that in future the Newsletter would appear in electronic form only unless a hard copy was specifically requested by a member. He also asked for contributions including activities and papers produced by Board members. Prof Vårum agreed to produce a summary of the current, Montreal, meeting.

2. Treasurer's Report

Prof Spindler-Barth gave a brief synopsis of the accounts at the end of 2002 which showed a surplus of about 7000 Euro which was a healthy position. Some costs would be incurred in setting up the Poznan and Montpellier meetings - although these costs were usually reimbursed at the end of meetings. The Prix Braconnot could be awarded in Poznan ( publicity for this was necessary by Board members) which would be an expense. The report was accepted by the Board .

3. Membership

This was still healthy and stabilised at about 125-30 people and the membership fee would remain unchanged. No fee for membership of the International Committee was necessary.

4. Conferences

Profs Domard and Guibal had made tentative bookings at the Palais de Congress in Montpellier for the first week in September (7th Euchis/10th ICCC). Costs and conference fees would be organised shortly.

The meeting of 2008 could be in Turkey (previously offered) or in Belfast (under the organisation of Dr Mike Healy). Any other formal offers should be made to the President and a choice made during the Poznan meeting.

KV noted that Euchis meetings were on a two year cycle and International meetings on a three year cycle so that they coincided every six years, as in Montpellier. Could the Euchis meetings also be on a three year cycle to avoid this. MP commented that coincidence tended to boost

attendance at European-based meetings by non-European attendees, especially from Japan. The matter could be discussed in Poznan at a General Assembly.

5. International Committee

MP reported that the Japanese community was interested in this committee although progress was slow. Such a committee could overcome the problem of conferences running close together and allow regional societies to organise the ICCC under guidance, e.g. in Asia in 2009 and the Iberoamerican Society in 2012. AD encouraged the interest of the Japanese community and their opinions were important if an international committee was needed at all. The committee should be formalised with good organisation.

6. Board Matters

MP suggested that Professor Olav Smidsrød should be the new Honorary President of Euchis and he could be proposed at the Poznan meeting. Prof Vårum could be the new President (MP at the end of his term) and we would need a new Secretary (and Assistant Secretary) as EG would be occupied with the Montpellier meeting. A new Vice President and four ordinary Board members would also be needed. Half the Board should be changed after two years and new members could come from Spain and Dr Senel from Turkey was a possibility.

7. Any Other Business

The next Summer School could be held in Montpellier but EG would need help with organisation and costs. MS-B thought the scheme was good for students and many of those who attended the Ulm school were in Montreal. Funding from the EU and Euchis was possible.

Oct. 13, 2003
Signed:
G.M. Hall, Secretary M.G. Peter, President

## 6th EUCHIS Conference, Poznan, Poland
## Agenda for the General Assembly

Location and time to be announced

## Agenda:

1 Report of the President
2 Honorary President: Nomination
3 New Board: Introduction of new member candidates and election
4 Report of the Secretary (Newsletter, Internet distribution)
5 Report of the Treasurer
6 Conference Proceedings
7 International Committee
8 Forthcoming Conferences
9 Prix Braconnot
10 Web-site and electronic communication
11 Varia

Any comments on the Agenda should be directed to M.G. Peter:
peter@chem.uni-potsdam.de

**EUCHIS: New Board, September 2004 – August 2006.**
**Nomination / suggestion of candidates.**

# Committee

| | | |
|---|---|---|
| Honorary President | Smidsrød (N) | suggested for Nomination |
| President | Varum (N) | Nomination accepted |
| Secretary | Roberts (UK) | Nomination accepted |
| Treasurer | Graeve, M. (D) | agreed |
| Vice President | Struszczyk (PL) ? | to be confirmed |
| Vice President | Domard (F) | |
| Assist. Secretary | N.N. | to be nominated |
| Assist. Treasurer | Spindler-Barth (D) | |

Members of the New Board: 10 nominated, 9 to be elected

| | | |
|---|---|---|
| Member | Feniche (I) | Nomination accepted |
| Member | Healy (UK) | Nomination accepted |
| Member | Heras (ES) | |
| Member | Gislason (IS) | |
| Member | Jaworska (PL) | |
| Member | Moerschbacher (D) | Nomination accepted |
| Member | Senel (TR) | Nomination accepted |
| Member | Eijsink (N) | Nomination accepted |
| Member | Duarte (P) | |
| Member | Gorovoj (UKR) | to be confirmed |

# PRIX BRACONNOT

**Nominations 2004**

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Sabine Glöggler
nominated by Prof. M. Spindler-Barth

PhD thesis, Department of General Zoology and Endocrinology, University of Ulm, Germany
Supervised and by Prof. M. Spindler-Barth

Characterisation of the Chitinase gene and investigation of the hormonal control of the Chitinase gene from the Dipteran *Chironomus tentans*.

## Summary

The chitinase gene from the epithelial cellline from the dipteran *Chironomus tentans* was isolated and sequenced. Analysis of the genomic structure revealed the presence of four exons and three introns. A unique property of the *Chironomus* chitinase is the presence of an intron within the catalytic domain and the presence of a 637bp intron with a gene like structure of so far unknown function.
Comparison with chitinase genes from other dipterans and lepidoterans revealed pronounced species specific differences, which might be important for the application of chitinase inhibitors for insect pest control.
As suggested previously by immunohistochemical techniques, Southern blots indicated also that only one chitinase is present in *Chironomus tentans*. This is in contrast to the dipteran *Drosophila*, where seven chitinase genes were described. Besides the well know hormonal regulation by the molting hormone 20-0H-ecdysone on the transcriptional level chitinase specific mRNA stability and mRNA-degradtion are also regulated by the molting hormone, presumably mediated by AU-rich domains present in the 3'-end. The half-life time of chitinase specific mRNA of about 20 hours is reduced to about 50% after hormone treatment, which is in contrast to the long term stability of the chitinase enzyme.

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Dr. Stanisław Trzciński

nominated by Prof. H. Struszczyk

**The kinetics of degradation and characterisation of selected physicochemical properties of degraded chitosans**

The doctor's thesis was submitted to the Faculty of Chemistry, Nicolaus Copernicus University of Toruń, Poland, as the partial fulfilment of the requirements for the academic title of the *Doctor of Philosophy* (Ph.D.).

Date of graduation: June 12, 2002.

*Summary of the doctor's thesis*

The doctor's thesis is devoted to kinetic investigations of physical (by means of ultrasound action), chemical (by means of hydrogen peroxide action) and combined (by means of ultrasound assisted hydrogen peroxide action) degradation of chitosans differing in initial degree

of polymerisation ($x$) and degree of *N*-acetylation (in term of the molar fraction of 2-acetamido-2-deoxy-**D**-glucopiranose units, $F_A$).
The aim of the thesis was 1) to establish the effect of complex molecular structure of chitosan ($x$, $F_A$) and supermolecular arrangement (aggregates) on the general rate parameter ($k$) of degradation in relation to experimental conditions, 2) to define the molecular structure of degradation products, 3) to indicate the probable mechanisms of the chitosan chain scission, including the side reactions, and additionally 4) to check the effect of ultrasonic action on heterogeneous de-*N*-acetylation of α-chitin.
In order to cope with these tasks, the following experimental techniques were used: 1) viscometry, 2) size exclusion chromatography, 3) high field $^{1}H$ NMR, $^{13}C$ CP/MAS NMR spectrometry, 4) FTIR spectroscopy, 5) UV spectroscopy.
Kinetic studies were carried out using the degradation time dependence of intrinsic viscosity of chitosan in 1.0 % (w/v) or 0.5 % (w/v) solutions in 0.1 or 1.0 M acetic acid (sonication experiments) and in 1.0 % (w/v) solutions in 0.1 M acetic acid with 2 % (w/w) hydrogen peroxide (oxidative and combined degradation experiments). The obtained data were handled using Schmid mid-point scission model. Additionally, for data of hydrogen peroxide induced degradation, the random scission model was applied.
The results sonochemical degradation showed that supermolecular structures of chitosan molecules (aggregates), which are $F_A$ and concentration dependent affect the value of $k$: the increase of $F_A$ value caused the increase of sonochemical degradation rate (effect of $x$ was separated). The concentration dependence of aggregates on the sonochemical degradation rate is shown by lower differences in numerical values of $k$ for 0.5 % (w/v) chitosan solutions [2.2 and 1.0 ($k \times 10^{12}$) mol×L$^{-1}$×h$^{-1}$], in comparison with those obtained for 1.0 % (w/v) chitosan solutions [2.0 and 0.46 ($k \times 10^{12}$) mol×L$^{-1}$×h$^{-1}$], for $F_A$ equal [0.28] and [0.10], respectively.
The $F_A$ dependence of peroxide induced degradation of chitosan has the opposite direction: $k$ is inversely proportional to $F_A$ value, and for chitosan with $F_A$ [0.01] it is 4.5 h$^{-1}$ ($k \times 10^{3}$), what is 8 times of the $k$ value for chitosan with $F_A$ [0.28]. This indicates the strong influence of the acceptor effect of protonated amine group at the C(2) position in the glucopyranose ring. This means that the scission of chitosan molecules is not random, and the limiting stage of the reaction is the dissociation of anomeric proton, what was confirmed by NMR studies.
The influence of experimental conditions (temperature, concentration) on sonochemical degradation of chitosan solutions, established in the thesis, is consistent with general considerations on the mechanism of ultrasonic degradation. The detailed studies of the temperature dependence of the conformation of chitosans in solution (e.g. the evaluation of the temperature coefficient of intrinsic viscosity) allowed to conclude that the increase of $k$ could be connected not only with the increased intensity of cavitation at lower temperature, but also with the expansion of chitosan coils.
The combined degradation of chitosan, introduced for the first time by the author of the thesis, is characterised by the two times higher value of $k$ at the initial stage of the process in comparison to hydrogen peroxide degradation. This is caused by the acceleration of hydroxide radicals formation by sonolytic destruction of hydrogen peroxide and simultaneous sonication itself , hence the limiting degree of polymerisation is reached earlier. Generally, it can be stated that the combined mode of degradation is the most interesting among studied in the thesis, and may be effectively used for chitooligomers production.
Size exclusion chromatography analysis which followed the alternation of the molecular weight distribution during the course of degradation showed that the sonochemical process of chitosan degradation is non-random, with preferential breaking near a midpoint of the chitosan chain. The hydrogen peroxide induced process of chitosan degradation also indicates features of the non-random process (intermediate increase of polydispersity index). In this case, however, the reason is the efficiency of chain scission, which depends on the accessibility of anomeric protons to the radicals attack. It was also established that during sonochemical or hydrogen peroxide induced

degradation, the type of molecular weight distribution is being changed from logarithmic normal to exponential. In the case of combined degradation the final type of molecular weight distribution is Flory's type.

Spectroscopic investigations showed that side reactions are possible during degradation of chitosan, e.g.: the oxidation at the C(1) position, specially in the case of action of hydroxide radicals in the presence of atmospheric oxygen, and the oxidation at the C(1) and C(4) positions, when sonolysis occurs due to mechanical scission of the β-(1→4)-glycosidic bonds. The presence of new carbonyls were confirmed by $^{13}C$ NMR analysis and FTIR spectroscopy, and low intensity of corresponding signals and bands suggests that the described reactions take place at chain ends. Spectroscopic analysis additionally showed that there are no remarkable changes in $F_A$ values during degradation, though the small decrease of pH (for degradation performed at neutral pH in hydrogen peroxide solution) suggests, that de-*N*-acetylation and/or oxidation of amine groups are possible.

The application of the action of ultrasound, which is a known homogenising agent, was found to be the effective method for the decrease of the polydispersity of *N*-acetylation degree of chitosan obtained by the heterogeneous de-*N*-acetylation of α-chitin.

# EUCHIS Financial Report 2002

**(per December 31, 2002) Account at Deutsche Bank, Bonn**

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|---|---|---|---|---|---|
| **POSITIVA** | | | | | |
| **Balance per 31.12.2001** | **EUR** | **2.253.81** | | | |
| **members fees** | | | | | |
| **- collective members** | | | | | |
| **- active members** | | | **EUR** | **2.184.08** | |
| **- associate members** | | | **EUR** | **416.24** | |
| **- student members** | | | **EUR** | **176.00** | |
| Reembursment Summerschool 2001 | | | **EUR** | **1.960.58** | |
| | | | **EUR** | **2.600.00** | |
| | **EUR** | **2.253.81** | **EUR** | **7.876.90** | |
| **total** | | | | | **EUR 10.130,71** |
| **NEGATIVA** | | | | | |
| Bank charges | | | EUR | -174,31 | |
| **Prix Braconnot 2002** | | | **EUR** | **-1.000,00** | |
| **Printing costs Newsletter** | | | **EUR** | **-1.545,91** | |
| **Office expenses** | | | **EUR** | **-260,00** | |
| **Internet charges** | | | **EUR** | **-71,88** | |
| **total** | | | **EUR** | **-3.052,10** | **EUR -3.052,10** |
| **Balance per December 31 2002** | | | | | **EUR 7.078,61** |

Bremen, 31.12.2002

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Dr. Martin Graeve

# FORTHCOMING MEETINGS

**2004, 6th Euchis, Poznan, Poland, AUGUST 31 – SEPTEMBER 3, 2004:**

**http://www.ior.poznan.pl/Euchis/**

**2nd Iberoamerican Symposium on Chitin, Cordoba, Spain, 27 - 29 September 2004**

**no website known, 2nd cicrular can be downloaded from the EUCHIS Website "Announcements"**

**2006, 7th Euchis/10th ICCC, Montpellier, France**

**No web address yet established**

## EUCHIS Members publications, 2002 - 2004: Abstracts of papers

please submit your abstracts to M.G. Peter: peter@chem.uni-potsdam.de

**Chitosan supports the initial attachment and spreading of osteoblasts preferentially over fibroblasts.**

A. Fakhry, G. B. Schneider, R. Zaharias and S. Senel,
Biomaterials 25 (2004) 2075–2079

The aim of this study was to determine chitosan's effect on osteoblast and fibroblast cell attachment. Mouse MC3T3-E1 osteoblasts and 3T3 .broblasts were grown in the presence of serum on two commercially available chitosans, Chitosan-H (CH) and Protasan CL212 (PR). Cell attachment and immunofluuorescent analysis at various time points were done to analyze initial phenotypic profiles. At 1 h, significantly ($P<0.05$) fewer fibrobroblasts attached to CH or PR than serum-coated substrates. Osteoblast attachment to the same biopolymers at 1 h was significantly greater than those seen with fibroblasts. At 24 h, levels of cell attachment for fibroblasts to both CH and PR significantly increased and were similar to levels seen in osteoblast cultures at both 1 and 24 h. Morphologically, immunofluorescent analysis showed that osteoblasts plated on the biopolymers were attached and beginning to spread at 1 h, whereas fibroblasts appeared more rounded. At 24 h, fibroblasts plated on CH or PR revealed a heterogeneous population of round and semi-spread cells. In comparison, osteoblasts displayed phenotypes that were well spread with a developed cytoskeleton. These results suggest that CH and PR support the initial attachment and spreading of osteoblasts preferentially over fibroblasts, and that manipulation of the biopolymer can alter the level of attachment and spreading.

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**Effect of chitosan on a periodontal pathogen Porphyromonas gingivalis**

G. Ikinci, S. Senel, H. Akincibay, S. Kas¸ S. Ercis¸ C.G. Wilson, A.A. Hincal
International Journal of Pharmaceutics 235 (2002) 121–127

Local delivery systems of antimicrobial agents for treatment of the periodontal diseases received considerable attention during the past decade due to the disadvantages of the systemic administration. An ideal formulation should exhibit ease of delivery, a good retention at the application site, and a controlled release of the drug. The application of bioadhesive gels provides a long stay in the oral cavity, adequate drug penetration, high efficacy and acceptability.

In dentistry and oral medicine, various applications of chitosan, which is a bioadhesive polymer have been proposed due to its favorable properties such as biocompatibility and biodegradability. The aim of this study was to determine the antimicrobial activity of chitosan formulations either in gel or film form against a periodontal pathogen, Porphyromonas gingivalis. The viscosity, bioadhesive properties and antimicrobial activity of chitosans at different molecular weight and deacetylation degree were evaluated in the absence or presence of chlorhexidine gluconate

(Chx), incorporated into the formulations at 0.1 and 0.2% concentrations. The flow property of the gels were found to be suitable for topical application on the oral mucosa and to syringe into the periodontal pocket. Bioadhesion of the gels and films examined ex-vivo using fresh porcine buccal mucosa showed that both the film and gel formulations exert bioadhesive properties and was not affected by incorporation of Chx. Chitosan is shown to have an antimicrobial activity against P. gingivalis and this was higher with high molecular weight chitosan. The combination

of chitosan with Chx showed a higher activity when compared to that of Chx alone, which would provide Chx application at lower concentrations thus avoiding its unwanted side effects.

Chitosan films and gels seem to be promising delivery systems for local therapy of periodontal diseases with its bioadhesive property and antimicrobial activity.

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**The release of model macromolecules may be controlled by the hydrophobicity of palmitoyl glycol chitosan hydrogels**
Lee Martin , Clive G. Wilson , Fariba Koosha , Laurence Tetley , Alexander I. Gray , Sevda Senel , Ijeoma F. Uchegbu
Journal of Controlled Release 80 (2002) 87–100

A non-covalently cross-linked palmitoyl glycol chitosan (GCP) hydrogel has been evaluated as an erodible controlled release system for the delivery of hydrophilic macromolecules. Samples of GCP with hydrophobicity decreasing in the order 1 GCP12.GCP11.GCP21 were synthesised and characterised by H NMR. Hydrogels were prepared by freeze-drying an aqueous dispersion of the polymer in the presence or absence of either a model macromolecule fluorescein isothiocyanatedextran (FITC-dextran, MW 4400), and/or amphiphilic derivatives Gelucire 50/13 or vitamin E d-a-tocopherol polyethylene glycol succinate. Gels were analysed for aqueous hydration, FITC-dextran release, and bioadhesion, and imaged by scanning electron microscopy. The gels were highly porous and could be hydrated to up to 953 their original weight without an appreciable volume change and most gels eventually eroded. Hydration and erosion were governed by the hydrophobicity of the gel and the presence of the amphiphilic additives. GCP gels could be loaded with up to 27.5% (w/w) of FITC-dextran by freeze-drying a dispersion of GCP in a solution of FITC-dextran. The controlled release of FITC-dextran was governed by the hydrophobicity of the gel following the trend GCP21.GCP11.GCP12. GCP gels were bioadhesive but less so than hydroxypropylmethylcellulose, Carbopol 974NF (7:3) tablets.

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**Superabsorbent materials from shellfish waste - a review.**
Dutkiewicz, J. K.
Journal of Biomaterial Materials Research. 2002, 63 (3), 373-381.

Highly absorbing materials based on polyelectrolyte polymers can absorb up to 50 grams of body fluid per gram of dry mass. Currently available synthetic superabsorbents are not biodegradable and do not offer any value-added functions to personal and medical-care products. Various academic and industrial research groups made significant efforts toward development of new absorbents from natural polymers, which could decompose in landfills. Basic substrates in these studies have been mainly cellulose and starch. Commercial synthetic polyacrylate superabsorbents as well as carboxymethylated cellulose and starch are polyanionic. On the other hand, polycationic polymers seem to have potential functional advantages over the polyanionic natural-based counterparts in terms of antimicrobial and other biological, value-added properties. Chitosan becomes polycationic in acid media and can be cross-linked in various controlled ways to produce superabsorbent polymers. This review provides basic information about new, highly absorbent materials based on chitosan salts, their properties and preparation.

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**Chitin and chitosan - Highlights from the Chitin Symposium 2002 in Acapulco, Mexico.**
Goycoolea, F. M., Shirai, K. & Peter, M. G.
*Macromolecular Bioscience.* **2003**, *3***,** 510.

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**Structure of the D142N mutant of the family 18 chitinase ChiB from Serratia marcescens and its complex with allosamidin.**
Vaaje-Kolstad, G., Houston, D. R., Rao, F. V., Peter, M. G., Synstad, B., van Aalten, D. M. F. & Eijsink, V. G. H.
*Biochimica Et Biophysica Acta-Proteins and Proteomics.* **2004**, *1696***,** 103-111.

Catalysis by ChiB, a family 18 chitinase from Serratia marcescens, involves a conformational change of Asp142 which is part of a characteristic D140XD142XE144 sequence motif In the free enzyme Asp142 points towards Asp140, whereas it rotates towards the catalytic acid, Glu144, upon ligand binding. Mutation of Asp142 to Asn reduced k(cat) and affinity for allosamidin, a competitive inhibitor. The X-ray structure of the D142N mutant showed that Asn142 points towards Glu144 in the absence of a ligand. The active site also showed other structural adjustments (Tyr10, Ser93) that had previously been observed in the wild-type enzyme upon substrate binding. The X- ray structure of a complex of D142N with allosamidin, a pseudotrisaccharide competitive inhibitor, was essentially identical to that of the wild-type enzyme in complex with the same compound. Thus, the reduced allosamidin affinity in the mutant is not caused by structural changes but solely by the loss of electrostatic interactions with Asp142. The importance of electrostatics was further confirmed by the pH dependence of catalysis and allosamidin inhibition. The pH-dependent apparent affinities for allosamidin were not correlated with k(cat), indicating that it is probably better to view the inhibitor as a mimic of the oxazolinium ion reaction intermediate than as a transition state analogue. 

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**Interactions of a family 18 chitinase with the designed inhibitor HM508 and its degradation product, chitobiono-delta- lactone.**
Vaaje-Kolstad, G., Vasella, A., Peter, M. G., Netter, C., Houston, D. R., Westereng, B., Synstad, B., Eijsink, V. G. H. & van Aalten, D. M. F.
*Journal of Biological Chemistry.* **2004**, *279***,** 3612-3619.

We describe enzymological and structural analyses of the interaction between the family 18 chitinase ChiB from Serratia marcescens and the designed inhibitor N,N'-diacetylchitobionoxime-N-phenylcarbamate (HM508). HM508 acts as a competitive inhibitor of this enzyme with a K-i in the 50 muM range. Active site mutants of ChiB show K-i values ranging from 1 to 200 muM, providing insight into some of the interactions that determine inhibitor affinity. Interestingly, the wild type enzyme slowly degrades HM508, but the inhibitor is essentially stable in the presence of the moderately active D142N mutant of ChiB. The crystal structure of the D142N-HM508 complex revealed that the two sugar moieties bind to the -2 and -1 subsites, whereas the phenyl group interacts with aromatic side chains that line the +1 and +2 subsites. Enzymatic degradation of HM508, as well as a Trp-->Ala mutation in the +2 subsite of ChiB, led to reduced affinity for the inhibitor, showing that interactions between the phenyl group and the enzyme contribute to binding. Interestingly, a complex of enzymatically degraded HM508 with the wild type enzyme showed a chitobiono-delta- lactone bound in the -2 and -1 subsites, despite the fact that the equilibrium between the lactone and the hydroxy acid forms in solution lies far toward the latter. This shows that the active site preferentially binds the E-4 conformation of the -1 sugar, which resembles the proposed transition state of the reaction.