EUROPEAN CHITIN SOCIETY

NEWSLETTER

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July, 2007
No. 23

EDITORIAL

We are now in the run-up to our next conference which as you know will be held in Antalya, Turkey from 8th-11th of September. After this we should be back on a 2-year cycle on the odd-numbered years, at least until there is some international agreement for timetabling chitin/chitosan conferences. At the risk of being repetitious I would point out that this means we are now back on schedule to hold a EUCHIS meeting in 2011, the bicentenary of the discovery of chitin by Braconnot. Any suggestions for where the 2011 meeting should be held?

I know that the venue should not be the main reason for deciding to go to a particular conference but contrary to popular belief scientists are also humans. I recall being told some years ago that the American Chemical Society was surprised to find that they had a much larger attendance when they held their conference in Hawaii than when they held it in Chicago – I would have thought that was, in modern parlance, a 'no-brainer'. That being so I would expect this year's meeting in Antalya to be standing room only, as the photographs that I have seen show the area to be really beautiful. Please read the announcement in this *Newsletter* from the conference organisers and make sure you register in time.

In this issue we have, in addition to the conference announcement, an article by Drs Eijsink and S Horn with a suggested new approach to strategies for improving enzymatic depolymerisation of polysaccharides in biotechnological processes, a report by Professor Kjell Vårum on the IVth SIAQ meeting held at Natal, Brazil in early May, and three PhD thesis abstracts from Braconnot Prize candidates.

Finally I am sure all the EUCHIS member join me in wishing our Honorary President, Professor Olav Smidsrod, a speedy and full recover from his recent illness.

George Roberts
Honorary Secretary

8th International Conference of The European Chitin Society
8-11 September 2007
Divan Hotel Antalya Talya

Dear EUCHIS Members,

The organizers of the upcoming 8th International Conference of the European Chitin Society, which will be held between 8-11 September in Antalya, Turkey, wish to remind you of their invitation to participate actively by making an oral and/or poster and/or presentation.

With more than 20 invited and keynote speakers from around the world, together with scheduled round-table discussion chaired by leading authorities the conference will be an opportunity to update yourself on the recent advances in chemical, enzymological and regulatory aspects of chitin and chitosan and their applications.

Attendees wishing to make an oral or poster presentation must first submit an abstract and all the details for doing so can be found on the conference website on www.euchis07.hacettepe.edu.tr
Please note that:

The deadline for submitting abstracts is extended to : 20th July

So remind your colleagues and students to prepare and submit their abstracts NOW.

Accommodation deadline – July 20th
There are not many days remaining to take advantage of the conference hotel rates being offered to EUCHIS delegates and accompanying persons. After July 6, the congress organizers cannot guarantee any hotel room availability and rates charged by the hotels are subject to change. To make your reservation, contact the organization secretariat (info@palma-travel.com).

Conference publications

1. **Proceedings Book**
   A multi-volume book (Advances in Chitin Science) will be published including full papers of works presented at the Conference. The paper length will be 6 printed pages for short oral presentations and posters, and 8 pages for invited lectures.
   The deadline for submitting full paper manuscript is August 6th.

2. **Abstract Book**
   A separate Abstracts Book will be published with abstracts of works presented at the Conference. It will be distributed among full rate participants at the beginning of the Conference.

See you all soon in Antalya!

Prof. Dr. Sevda Senel
EUCHIS'07 Chair
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From chitin to biofuels: fundamental insight into the enzymatic conversion of recalcitrant polysaccharide biomass

In a recent paper in PNAS entitled *"Costs and benefits of processivity in enzymatic degradation of recalcitrant polysaccharides"* [1], Horn *et al.* describe new principles of the enzymatic conversion of chitin that may have a general impact on the future development of enzyme technology for conversion of recalcitrant polysaccharides.

Enzymes acting on crystalline polysaccharides such as chitin or cellulose face the challenges of associating with the insoluble substrate, disrupting crystal packing, and guiding a single polymer chain into the catalytic centre. In addition to their catalytic domains, cellulases and chitinases often contain one or multiple carbohydrate-binding modules (CBMs), which are beneficial for enzyme efficiency because they adhere to and sometimes disrupt the substrate. Chitin-degrading microorganisms may also produce separate non-catalytic proteins whose function is to disrupt the crystallinity of the substrate, thus dramatically increasing the efficiency of hydrolysis by chitinases [2].

Another feature that overcomes the low accessibility of the substrate is the presence of long and deep, sometimes "tunnel-like", substrate-binding clefts, as revealed by the first crystal structures of cellulases (these cellulases are also called cellobiohydrolases). Such tunnel-like enzymes act processively, i.e. single carbohydrate chains are threaded through the active site cleft, while disaccharides are cleaved off at the catalytic centre (Figs. 1, 2). The general idea is that catalytic efficiency is improved by keeping the enzyme closely associated to the substrate in between subsequent hydrolytic reactions. Furthermore, by keeping the chain attached to the enzyme, it cannot re-associate with the insoluble material.

Since the successive sugar units in chitin (and cellulose) are rotated by 180 °, sliding of such polymers through the enzyme's active site will only result in productive binding for every second sugar, and the products of processive degradation are disaccharides (Fig. 2). This is particularly obvious in the case of family 18 chitinases, where catalysis is substrate-assisted and depends critically on a correctly positioned *N*-acetyl group on the sugar positioned in the -1 subsite of the enzyme (see Fig. 2). Due to a lack of catalytically crucial *N*-acetyl groups, chitosan can bind both productively and non-productively to chitinases. As explained in the legend of Fig. 2 and below, the combination of the soluble chitosan substrate with the substrate-assisted mechanism of a family 18 chitinase provides a unique model system for studying complex aspects of the enzymatic degradation of polysaccharides, such as processivity.

ChiB from *Serratia marcescens* is a processive family 18 chitinase with a deep substrate-binding cleft (Fig. 1). Because ChiB sharpens the ends of chitin microfibrils, it has been suggested that ChiB is an exochitinase ([3]; by analogy to the cellulase nomenclature, this would make ChiB a chitobiohydrolase). However, recent studies with chitosan have shown that ChiB tends to bind this substrate in an endo-fashion [4].

The substrate-binding clefts of ChiB and other chitinases (and cellobiohydrolases) are lined with aromatic residues that are thought to facilitate substrate binding and sliding of the polymer chain through the cleft during a processive mode of action (Fig. 1). There are a few examples showing that mutation of such residues impairs enzyme performance (e.g. [5-6]), but links to processivity are not clear. These previous studies were focused on aromatic residues located slightly outside the direct vicinity of the catalytic center.

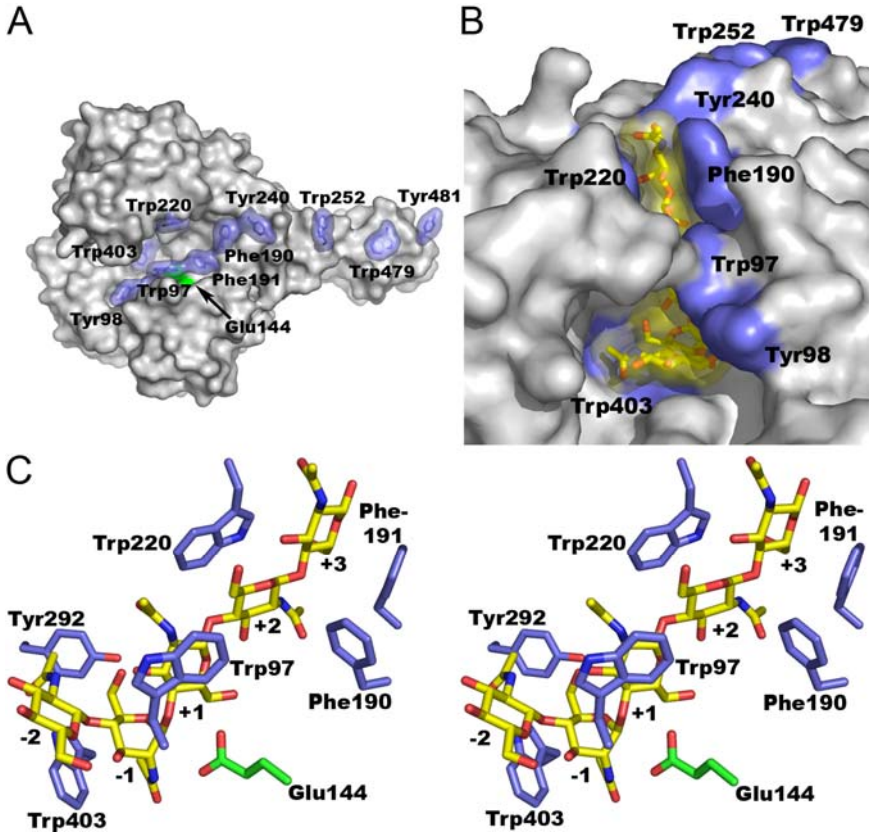

Figure 1. Enzyme-substrate interactions in ChiB. (*A*) Surface representation showing aromatic side chains lining the substrate-binding cleft and the binding-surface of the chitin-binding domain (extending to the right; residues 479 and 481). The catalytic Glu144 is coloured green. (*B*) Surface representation of the E144Q mutant in complex with chitopentaose [$(GlcNAc)_5$] bound to subsites –2 to +3 [7], showing that the substrate-binding cleft has a closed "roof" when substrate is bound. $(GlcNAc)_5$ is shown with a yellow van der Waals surface. The surfaces of aromatic residues in the protein are blue. (*C*) Stereo picture showing $(GlcNAc)_5$ and aromatic residues near the catalytic centre. Individual sugars in the pentamer are labelled by the number of the enzyme subsite (from –2 to +3) to which they bind. Picture and legend taken from reference 1.

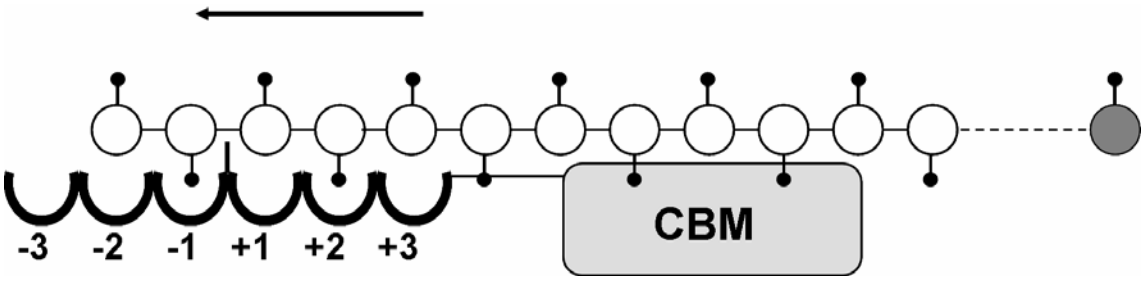

Figure 2. Schematic picture of ChiB in complex with a single chitin chain. The enzyme has six subsites, numbered –3 to +3; CBM stands for the carbohydrate-binding module.The reducing end sugar is coloured grey. A correctly positioned *N*-acetylgroup (symbolized by small black balls on sticks) in the –1 subsite is essential for catalysis (which is "substrate-assisted") to occur. Initial binding of the substrate will produce an odd- or even-numbered "overhang" leading to an odd- or even-numbered product (a trimer or dimer in case of exo-activity). The scheme shows the situation during subsequent processive action when only dimers are produced. The arrow indicates the direction of the sliding of the substrate through the active site cleft. In the case of chitosan, complexes formed during processive action may be non-productive because the sugar bound in the –1 subsite may lack the *N*-acetyl group. This leads to the production of longer even-numbered oligomers that is diagnostic for processivity. Picture and legend taken from reference 1.

Horn *et al.* [1] mutated aromatic residues in the substrate-binding cleft of ChiB that were located close to the catalytic center. Analysis of mutational effects using standard substrates (chitin, chito-oligosaccharides) revealed two particularly interesting mutants (W97A, W220A, in subsites +1 and +2), which were analyzed in more detail using the chitosan model substrate. The data for the most remarkable mutant, W97A, are depicted in Figure 3.

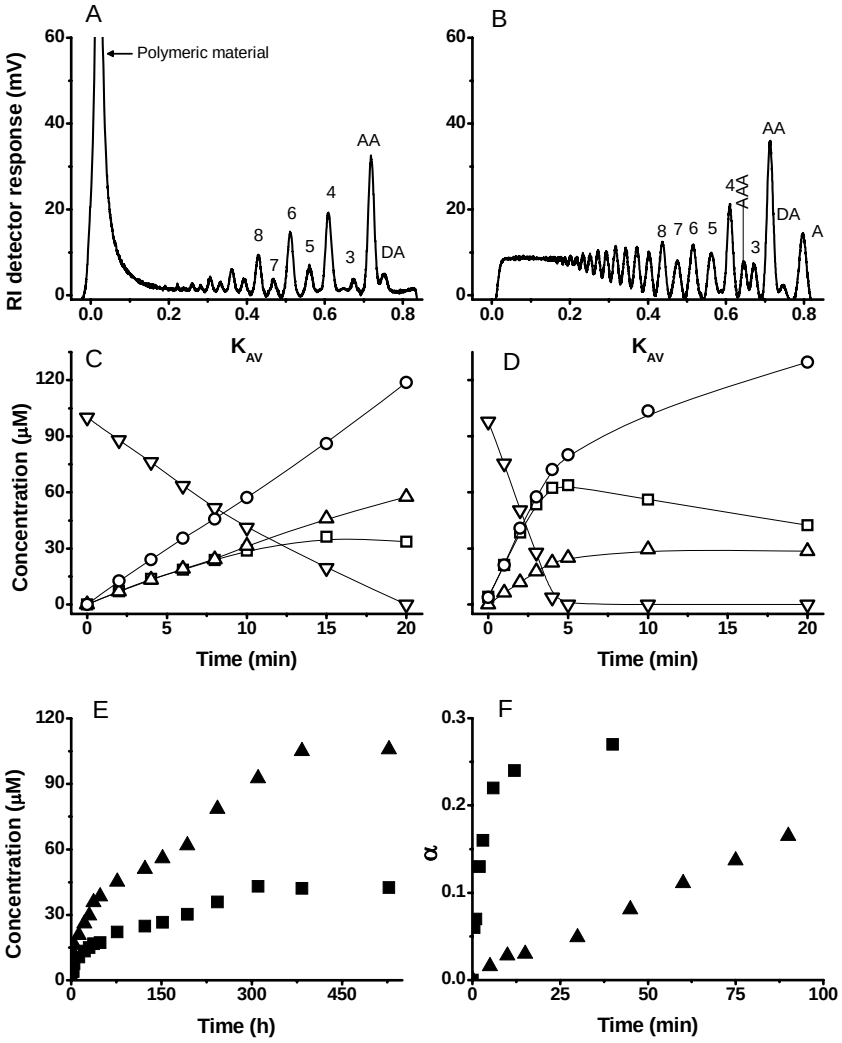

Figure 3. Characteristics of wild-type ChiB and the W97A mutant. (*A, B*) Size exclusion chromatography of products formed during degradation of chitosan (65 % acetylated water-soluble chitin with random distribution of acetylated units) with ChiB (*A*) and W97A (*B*), after cleavage of 14 % of the glycosidic bonds (i.e. $\alpha = 0.14$). The products are labelled by chain length or, for the shortest products, by sequence (A = acetylated unit; D = deacetylated unit). (*C, D*) Degradation of chitohexaose with ChiB (*C*) and W97A (*D*). Inverted triangles, $(GlcNAc)_6$; Squares, $(GlcNAc)_4$; Triangles, $(GlcNAc)_3$; Circles, $(GlcNAc)_2$. (*E*) Degradation of chitin with ChiB (triangles) and W97A (squares). (*F*) Time-curve for chitosan degradation with ChiB (triangles) and W97A (squares). α, fraction of cleaved glycosidic bonds (complete conversion of the substrate to exclusively dimers would yield $\alpha = 0.50$). Picture and legend taken from reference 1.

The results show that the single W97A mutation almost completely abolishes processivity (Fig. 3A,B; see also Figs 3C and 3D which show that the GlcNAc hexamer is converted processively to three dimers by the wild-type enzyme, whereas it is converted to a tetramer

and a dimer by the W97A mutant). Furthermore, as expected, the non-processive W97A mutant is less efficient than wild-type in degradation of chitin (Fig. 3E). Interestingly, the W97A displayed a 4-fold increase in activity towards the soluble GlcNAc hexamer (Fig. 3C,D). The increased activity towards soluble substrates was even more prominent for chitosan. For this soluble polymeric substrate the activity of the W97A mutant was a dramatic 29-fold higher than the activity of the wild-type enzyme.

These results show that a processive mode of action comes at a large cost in terms of enzyme speed. While such a mode of action is beneficial when dealing with crystalline chitin, it makes the enzymatic degradation process unnecessarily slow in cases where the substrate consists of single polymer chains, e.g. in chitosan or perhaps in heavily disrupted crystals. Thus, in some cases it might be better to focus strategies for enzymatic depolymerization of polysaccharide biomass on improving substrate accessibility for non-processive enzymes rather than on improving the properties of processive enzymes. This insight is of considerable importance for the ongoing massive efforts in the development of enzyme technology for efficient enzymatic hydrolysis of lignocellulosic biomass. Development of such technology is a prerequisite for the commercial introduction of second generation bioethanol.

Written by Svein Horn and Vincent Eijsink. The Figures and several text fragments were taken from reference number 1.

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The IVth Iberoamerican Chitin Symposium
May 6 to 9, 2007, Natal, Brazil

The Iberoamerican Chitin Society (SIAQ) is an active chitin society recruiting its members both from Europe and Central/South America. Previous SIAQ-meetings have been arranged in La Habana (2000), Acapulco (2002) and Cordoba (2004), and the IVth Iberoamerican Chitin Symposium (IV SIAQ) was arranged this year in Natal, Brazil. The beautiful city of Natal (population: ca. 700 000) is the capital of Rio Grande do Norte State, the most easterly location in South-America with convenient direct access to Europe and Central/South America.
Presentations at SIAQ are allowed in three languages (english, spanish and portuguese), which represents a challenge to the participants. I would encourage SIAQ to consider using only english as the official language in their future conferences.
There are large resources of chitin in South-America, and some of them are currently not explored. No invited lectures were focusing on the raw materials from Central/South America at the IV SIAQ, although such resources are unique and abundant, such as the Caribbean spiny lobster (*Panulirus argus*). I would actually encourage SIAQ to focus on their resources in a future conference, it should be an interesting theme both to their own members and to international attendants.

From the opening ceremony of the IV SIAQ. From left: Sergio Paulo Campana Filho (Secretary of SIAQ), Waldo Argüelles Monal (president of SIAQ), José Ivonildo do Rego (Rector of Universidade Federal do Rio Grande do Norte), Fernando Ribeiro (Representative of the brazilian government company for petroleum PETROBRAS) and Otom Anselmo de Oliveira (Dean of Departamento de Química of the Universidade Federal do Rio Grande do Norte).

Thirteen invited lectures were presented, spanning a wide range of characterization / application of chitin/chitosan, from metal ion interactions to biomedical applications. Dr. Galo Cardenas (Chile) presented a lecture entitled 'Biomedical Applications of Chitosan Acetate Composite Films' with results from the treatment of various injuries (such as surgery scarf, burns and ulcers) on both animal models and humans. The presented results from the treatment of human injuries using chitosan films were convincing and unique in the sense that it could probably not have been performed elsewhere. This is an interesting application of chitosan, although it is not an easy task to objectively study the process of (human) wound healing.

The other presentations were divided into 5 sessions:

- Sources, Production and Technological Processes
- Enzymology, Biochemistry and Biological Aspects
- Characterization. Chemical and Physicochemical Properties
- Chemical Modifications and Advanced Materials
- Applications in Agriculture, Food, Biotechnology, Medicine, Environmental and Other Fields

The symposium was arranged at a hotel ca. 6 km from the center of Natal, where all facilities (lecture halls, meals, and accommodation) were available, which was very convenient to the participants. The Banquet Dinner was served in the same hotel in a truly latin-american style with nice food and lots of music that attracted all participants to the dancing floor!

The activity of the Iberoamerican Chitin Society is impressive, and it seems that SIAQ manages to encourage their members to participate and present their most recent results at regular meetings. I do hope they will continue this work to encourage their members to further work in the field.

Kjell M. Vårum

Elaboration and study of the physical and biological properties of a multi-membrane bioreactor.

Sebastien Ladet
Laboratory of Polymer Materials and Biomaterials, University of Lyon 1, France

Defended January 16th, 2007.
Supervisor: Professor Alain Domard Co-supervisor: Professor Laurent David

The so called "third generation" of biomaterials have been developed which are designed to be both resorbable and bioactive. The goal in biomaterial design should be to define a biological problem and to develop its physico-chemical structure inspired from those of the living tissues. Since most of the living tissues have a multilayer structure, such as, cornea, blood vessels, intervertebral discs and skin, thus several multilayered biomaterials were developed.

This work aims at developing a new and simple method allowing the formation of multi-membrane "onion-like" architectures (**Fig. 1a**) from a bioactive polysaccharide initially in the form of a physical hydrogel. These materials, based on chitosan physical hydrogels, were designed to be used as bioreactors for tissue engineering applications. These systems taking advantage of the bioactivity of the chitosan[1] could offer a rapid regeneration of complex multi-layered or multi-membrane tissues by a one shoot multi-cellular culture (**Fig. 1b**) in a bio-inspired architecture.

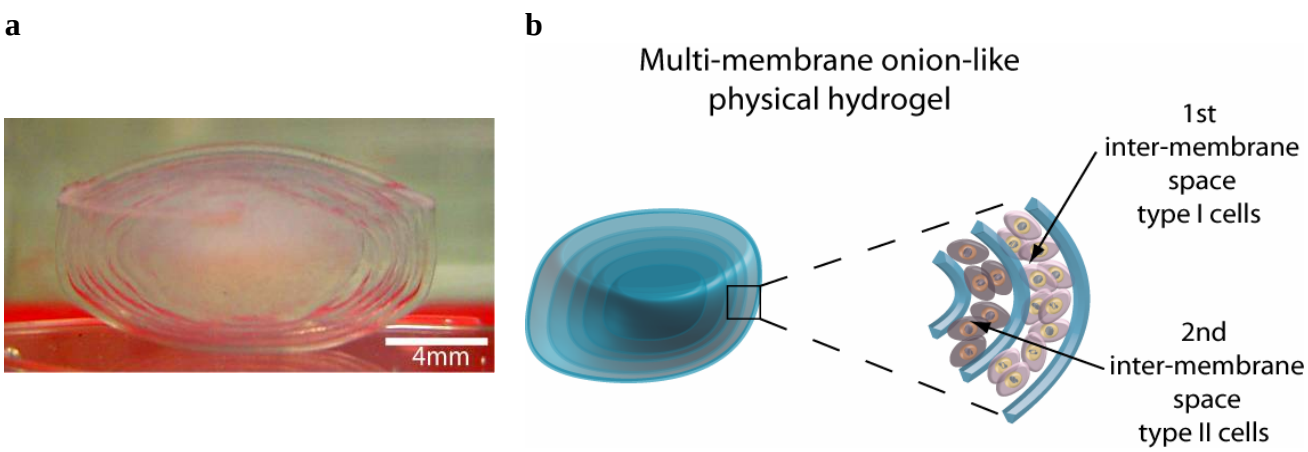

Figure 1. **a**, Multi-membrane onion-like materials composed of 7 membranes and gel part in center; **b,** Potential applications of multi-membrane onion-like structures for in vitro tissue regeneration of complex layered cellularized systems.

In the case of chitosan hydrogels, the control of the bio-resorption kinetics can be easily ensured by the possibility to process gels with either a chosen value or a blend of different value of the degree of acetylation (DA)[2].

In contrast to the “layer-by-layer” self-assembly process, our materials are only constituted of one polymer (chitosan) and water, moreover the membrane shape leads to the formation of an inter-membrane space which useful to do subsequent cells introductions (**Fig 1b**). This architecture was designed by a multi-step neutralisation (**Fig.2**) which leads polymer chains contraction in the non-neutralized gel of chitosan. This complex mechanism of neutralisation was used to generate multi-membrane onion-like architectures. During neutralization, there is modification of the balance between hydrophobic and hydrophilic interactions which induced a contraction of the neutralized gel and the formation of separated gel membranes. The neutralization step appears highly complex with several phenomena acting simultaneously in different ways, but the parameters controlling the base diffusion and the contraction of polymer chains appear to be the key factors of such multi-membrane structure.

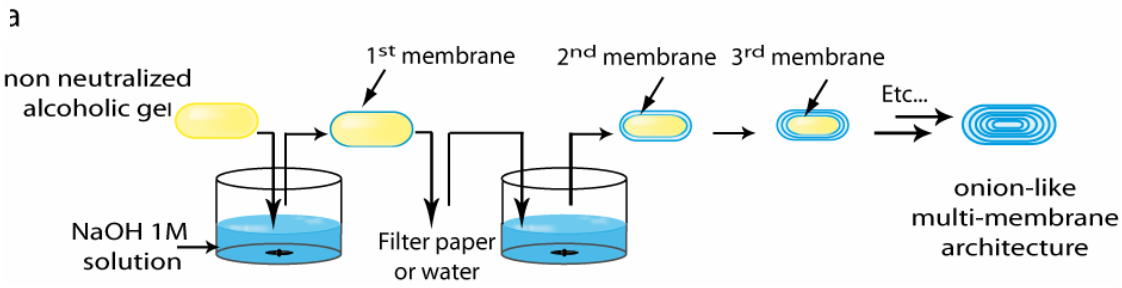

Figure 2. Multi-step neutralization process of the multi-membrane hydrogels.

The study of the physico-chemical parameters occurring during the membrane formation allowed us to propose a mechanism of neutralization.

Then, the influence of the neutralization conditions on the multi scale morphology was studied by X-ray scattering/diffraction and electronic microscopy. We report on the formation of the anhydrous allomorph of chitosan during the neutralization of alcohol gel of chitosan. The kinetics involved during the gel neutralization is shown to control the nanostructuration of the hydrogels and allowed us to highlight a transition between an isotropic amorphous to a fibrillar semi-crystalline morphology (**Fig. 3**). The morphology transition explains the evolution of the physical properties with the neutralizing conditions.

Moreover, the mechanical properties of the membranes of such biomaterials have been evaluated to compare with those of the living tissues. We performed traction experiments on membrane and determined the elastic modulus and the maximum strain as a function of the polymer and neutralizing bath concentration.

At a macroscopic scale, the physical structure and the mechanical properties obtained from this very simple gelation process opens perspectives in the range of complex cell cultures for multilayered tissue engineering.

After having introduced the concept of decoy of biological media, Montembault et al.[3] have shown that physical hydrogels only based on chitosan have very interesting biological properties on the chondrocyte behavior.

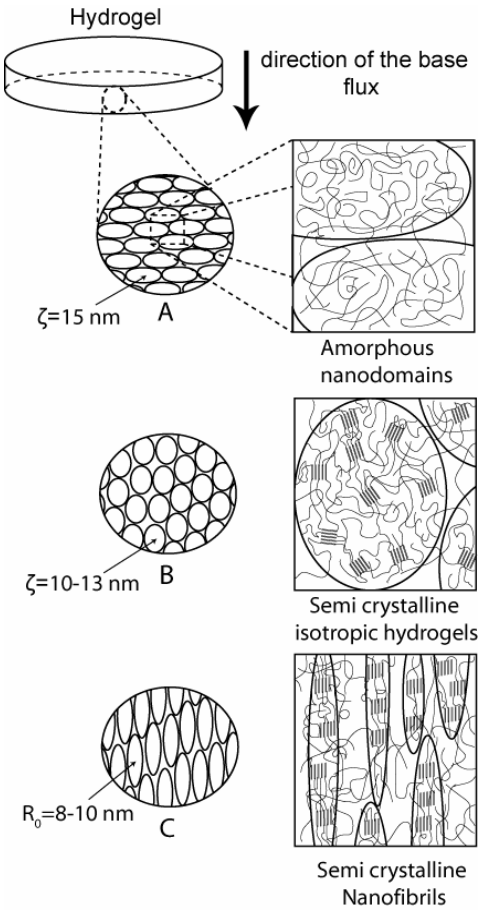

Figure 3. Schematic representation of the nanostructure transition of the hydrogel neutralized in different conditions. A, nanostructure of the hydrogel neutralized with the low base concentrations (C_{NaOH}= 1M; C_{NH4OH}= 1M); **B**, intermediate nanostructure of the hydrogel neutralized with C_{NaOH}= 2M and 4M; **C**, fibrillar structure of the hydrogel neutralized with C_{NH4OH}= 2M, 4M, 7M and C_{NaOH}= 7M.

Then, in order to evaluate the biological potential of these materials, we investigated the cells behaviour by introducing rabbit chondrocytes in several inter-membrane spaces of such biomaterials.

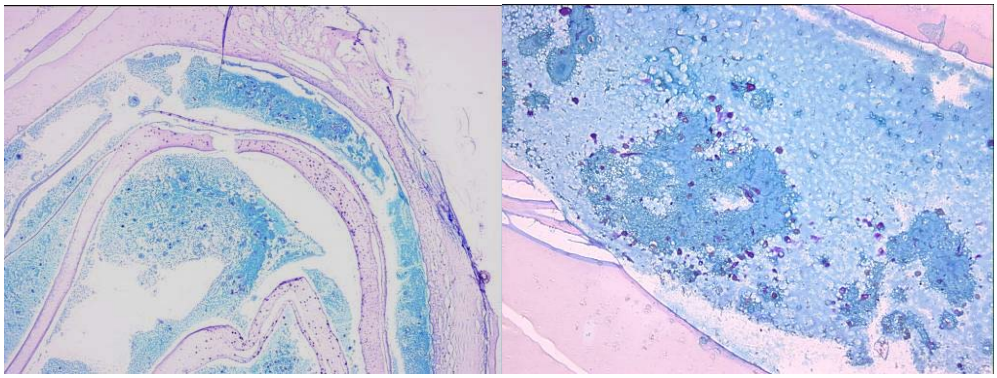

Figure 4. Histological morphology of rabbit chondrocytes embedded within multi-membrane hydrogels after 45 days of culture. Cells ($1x10^6$) were embedded in each inter-membrane space. After fixation and inclusion in paraffin, serial 5µm sections of the chondrocytes/hydrogel construct were colored with alcyan blue described in methods. Cpolymer of multi-membrane hydrogels: (a) 3; (b) 4 and (c) 5% (w/w). Symbols: Ag, cells aggregates; H, chitosan membrane hydrogel.

The first results showed that chondrocytes within multi-membrane hydrogels induced extra-cellular matrix production which was accumulated between the cells and hydrogels membrane (**Fig. 4**). Contrary to the scaffold concept, the cells and the extracellular matrixes do not penetrate the hydrogel membrane and only have surface interactions.

Thanks to the bioactivity and resorbability of chitosan; the adapted mechanical properties of such physicals hydrogels and this innovating architecture, the cells can differentied and proliferate within our multi-membrane system as an enclosed “bioreactor” witch simulates the natural environment of the body (osmotic pressure, molecule diffusion…) to produce biomolecules that are found in healthy tissues. This onion-like architecture could have a great potential for tissue engineering. Indeed the way of pluricellular culture allows considering the possibility of complex tissue regeneration (in vivo or in vitro) such as the bone-cartilage junction and could open the way of growing entire organs, as blood vessels, inter-vertebral discs, etc.

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Characterisation of Chitin and a Study of its Acid-Catalysed Hydrolysis

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Defended April 24th, 2007.
Supervisor: Professor Kjell M. Vårum Co-supervisors: Professors Olav Smidsrød and Kurt I. Draget

Chitin is one of the most abundant biopolymers in nature and the most widespread amino-polysaccharide. Chitin is used as raw material for the industrial production of chitin-derived products such as chitosans, derivatives of chitin/chitosan, oligosaccharides and glucosamine (GlcN). The main industrial source of raw material for the production of chitin are cuticles from various crustaceans, mainly crab and shrimp.

The chemical composition of shrimp shells from deep water shrimp harvested industrially in the Barents Sea was studied in relation to the use of shrimp shells as a raw material for chitin production. No clear seasonal variations were found in the content of the three main components of the shell (protein, minerals and chitin). Average chitin content was 18±2 % of the dry weight of the shrimp shells. No significant seasonal variation was found in the molecular weight of the chitin extracted from the shells using an optimised procedure for chitin extraction. This indicates the chitin producers can rely on shrimp shell waste as a stable raw material for chitin production throughout the year.

Concentrated and deuterated hydrochloric acid (DCl) was found to be a suitable solvent in order to characterise the chemical composition (F_A) of chitin by ^{1}H-NMR-spectroscopy. In chitin samples extracted from shrimp shell using 1 M NaOH at 95°C for 1-24 hours as the deproteinisation step, F_A was found to decrease linearly with time from 0.96 to 0.91 during the isolation procedure. Extrapolation to zero time suggests that chitin from shrimp shell has a F_A of 0.96, i.e. contains a small but significant fraction of de-*N*-acetylated units.

New methods were developed for the determination of the intrinsic viscosity and the molecular weight of chitin dissolved in alkali. Chitin samples of different molecular weights (produced by heterogeneous acid hydrolysis) were dissolved in alkali (2.77 M NaOH), and their determined molecular weights (from light scattering) were related to intrinsic viscosity by the Mark-Houwink-Sakurada equation, which was found to be $[\eta] = 0.10\ M_W^{0.68}$ (ml/g). Our study of the solution properties showed that alkali is a good solvent to chitin and that chitin molecules behave as random coils in this solvent. Alkali is an attractive alternative to previously described solvents to chitin, where aggregates and a more extended chain conformation have been observed.

^{1}H-NMR-spectroscopy was used to study de-*N*-acetylation of the chitin monomer *N*-acetylglucosamine (GlcNAc) and depolymerisation of the chitin dimer (GlcNAc- GlcNAc) in DCl. A glucofuranosyl oxazolinium ion was found to exist in equilibrium with GlcNAc in acid concentrations above 6 M. The H-1 resonances from the oxazolinium ion can be used to quantify the amount of GlcNAc in a sample of chitin oligomers in DCl. The reaction rate constants for hydrolysis of the glycosidic linkage of the chitin dimer (k_{glyc}) and the *N*-acetyl linkage of the monomer GlcNAc (k_{acetyl}) were determined as a function of acid concentration (3-12 M) and temperature (25-35°C). The two rate constants were found to be similar at the lowest acid

concentration (3 M), while k_{glyc} was much higher than k_{acetyl} at the highest acid concentration (12 M). Activation energies of the de-*N*-acetylation and depolymerization reactions were similar at all acid concentrations. This implies that the acid concentration but not the temperature can be used to control k_{glyc} relative to k_{acetyl} during acid hydrolysis of chitin.

The kinetics of hydrolysis of chitin and chitosan tetramer ($GlcNAc_4$ and $GlcN_4$) in concentrated HCl was studied using gel filtration to determine the amounts of tetramer, trimer, dimer and monomer as a function of time. A new theoretical model for the kinetics of depolymerisation of a tetramer was developed. The model uses two different rate constants for the hydrolysis of the glycosidic bonds in the oligomers, assuming that glycosidic bond next to one of the end residues are hydrolysed faster than the two other glycosidic linkages. The two rate constants were estimated by fitting model data to experimental results. The results show that hydrolysis of the tetramers is a nonrandom process as glycosidic bonds next to one of the end residues are hydrolysed 2.5 and 2.0 times faster as compared to the other glycosidic linkages in the fully *N*-acetylated and fully *N*-deacetylated tetramer, respectively. From previous results on other oligomers and the reaction mechanism, it is likely that the glycosidic bond that is hydrolysed fastest is the one next to the nonreducing end. Rate constants for hydrolysis of glycosidic linkages of a fully *N*-acetylated oligomer were found to be 50 times higher as compared to the glycosidic linkages in a fully de-*N*-acetylated oligomer.

A new theoretical model was developed to simulate the kinetics of hydrolysis of chitin in concentrated acid. The model uses three different rate constants; two for the hydrolysis of glycosidic linkages following an *N*-acetylated or a de-*N*-acetylated sugar unit (k_{glycA} and k_{glycD}, respectively) and one for the de-*N*-acetylation reaction (k_{acetyl}). The three rate constants were estimated by fitting model data to experimental results from ^{1}H- NMR of chitin hydrolysed in concentrated DCl. The rate constant k_{glycA} was found to be 50 times higher as compared to k_{acetyl} and 110 times higher than the rate constant k_{glycD}. ^{1}H-NMR-spectra of chitin in concentrated DCl (40°C) were obtained as a function of time until the samples were quantitatively hydrolysed to the monomer glucosamine. Results show that the initial phase of the reaction involves mainly depolymerisation of chitin chains, resulting in that almost 90% (molar fraction) of the chitin is converted to the monomer GlcNAc which is then de-*N*-acetylated to glucosamine. Our model predicts (in comparison with our previous results) that the yield of GlcNAc is drastically lowered upon a decrease in the concentration of acid, and/or the F_A of the chitin starting material.