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Note

Polarized light-stimulated enzymatic hydrolysis of chitin and chitosan

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ABSTRACT

Illumination with white linearly polarized light (WLPL) stimulated chitinase and chitosanase in their degradation of chitin and chitosan, respectively. Enzymes were illuminated at room temperature in separate vessels, then admixed in reactors containing polysaccharides. Hydrolysis of chitosan to glucosamine followed first order kinetics whereas hydrolysis of chitin to *N*-acetylglucosamine deviated from the first order kinetics. In both cases, an increase in the rate of hydrolysis depended on the illumination time. Efficient degradation required up to 60 min exposure of the enzyme to WLPL.

Chitin, a linear biopolymer of (1→4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose, is mainly used for production of chitosan and glucosamine.[1,2] For its functional properties such as water uptake, solubility, stability, coordination of metal ions and binding various anions, film forming, and antimicrobial properties, chitosan finds many applications, associated chiefly with food and pharmaceuticals.[1–5] Hydrolysis is, perhaps, the most common way of processing chitin and chitosan. There are several enzymes providing efficient hydrolysis of these polysaccharides. In case of chitosan, chitinase,[6] chitosanase, and lysozyme[7–9] are useful catalysts. Chitin is readily hydrolyzed with chitinases which belong to the glycoside hydrolase families 18 and 19.[10] Whereas family 19 chitinases primarily are found in plants and actinomycetes, family 18 chitinases occur in various organisms, including humans. For example, the soil bacterium *Serratia marcescens* produces three different family 18 chitinases.[11,12]

Recently a stimulating influence of white, linearly polarized light (WLPL) on all α-amylases in starch α-amylolysis,[13] xylanase in degradation of xylan,[14] cellulase in hydrolysis of cellulose,[15] and glucosyltransferase in production of cyclodextrins,[16] was observed. In this paper, the application of WLPL for the stimulation of chitinase and chitosanase in the degradation of chitin and chitosan, respectively, is demonstrated.

Figure 1 shows that WLPL-stimulated chitosanase in its degradation of chitosan. The glucosamine yield from chitosan was higher than that from the process with non-illuminated enzyme. The yield increased with the processing time. After 30 min illumination with WLPL, chitosanase performed approximately 13.5% better within the initial 10 min of the reaction. After 100 min, the yield of glucosamine increased up to 50%. Figure 1 also shows that 1 h illumination of chitosanase with WLPL was more beneficial. After the first 10 min of the reaction, the yield of glucosamine was approximately 36% higher than that with non-illuminated enzyme. After 100 min, the yield increased by approximately 77% in respect to the one achieved with non-activated enzyme.

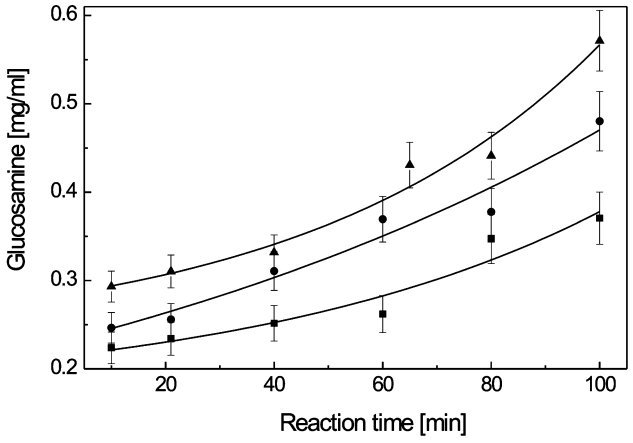

Figure 1. Course of degradation of chitosan with chitosanase: (■) with non-stimulated enzyme; (♦) with enzyme stimulated with WLPL for 30 min; (▲) with enzyme stimulated with WLPL for 60 min.

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As shown in Figure 2, rate of production of glucosamine from chitosan was higher when chitosanase was stimulated with WLPL, and the period of stimulation of the enzyme was fairly essential.

The $\log(1/c)$ versus time linear relationships with $R = 0.97–0.99$ provided rate constants for subsequent stages of the enzymatic reaction which appeared to be a first order process (Table 1). The 30 min activating illumination of the enzyme provided the peak efficiency (Fig. 2). Illumination of chitosanase in the solution together with chitosan did not improve hydrolysis of this polysaccharide to glucosamine.

Figure 3 presents the corresponding diagrams for WLPL-stimulated chitinase.

The enzyme illuminated with WLPL for 1 h provided over 100% increase in the *N*-acetylglucosamine yield during 2 h digestion, whereas non-polarized light only slightly stimulated the enzyme enhancing the yield of *N*-acetylglucosamine hardly by approximately 30% under identical conditions of digestion.

Enzymes degrading carbohydrate polymers can involve three fundamentally different mechanisms.[11] They are (i) a multiple-chain mechanism, where the enzyme–substrate complex dissociates after each reaction; (ii) a single-chain mechanism, where the enzyme remains associated with the substrate until every cleavage-plaint linkage in the chain hydrolyzes; and (iii) a multiple attack mechanism, where a given average number of attacks were performed after the formation of the enzyme-substrate complex.[6] As it can be seen in Figure 3, the degradation of chitin follows two rate laws. The relatively rapid degradation is most likely due to the hydrolysis of easily accessible chitin strands in the beginning of the process, while the slower one is most likely due to hydrolysis of less accessible chitin strands. Only 60 min stimulation of chitinase resulted in acceleration of the hydrolysis in the first stage. Moreover, this stage gave 100% higher yield of *N*-acetylglucosamine compared to the yields of all other degradations. The rate of the second stage only slightly increased comparing to the process with non-stimulated enzyme. In contrast to this, the enzyme stimulated with WLPL only for 30 min performed in the first stage similarly as non-stimulated one, but the second stage of degradation was considerably faster.

In our former study[13] on stimulation of α-amylase for degradation of amylose, evidence was presented that WLPL evoked conformational changes in this enzyme. Therefore, one might assume that also chitosanase and chitinase underwent conformational changes on illumination with WLPL. The data in Figure 3 suggests that the selected conformation depended on the illumination time. After 60 min stimulation, chitinase performed better with the high molecular chitin whereas after 30 min stimulated better already partly degraded chitin.

One can see from Figure 3 that successful stimulation of the enzyme required illumination in a separate vessel prior to the enzyme's combination with chitin in a bioreactor. There might be two reasons for such behaviour: (i) an interaction between the polysaccharide and the enzyme inhibited assuming a right conformation on illumination with WLPL and (ii) the polysaccharide absorbed part of WLPL energy. The second possibility was indicated in our former papers showing that WLPL caused depolymerization of starch[17–20] and cellulose.[21]

Table 1
Rate constants of the enzymatic degradation of chitosan and chitin with WLPL-stimulated chitosanase and chitinase, respectively

Enzyme stimulation	Rate constant		
	Chitosan	Chitin	
	$k \times 10^{-3}$ (min^{-1})	$k_1 \times 10^{-2}$ ($mg\ mL^{-1}\ min^{-1}$)	$k_2 \times 10^{-3}$ ($mg\ mL^{-1}\ min^{-1}$)
Non-illuminated	2.5 ± 0.1	1.9 ± 0.1	1.1 ± 0.1
Illuminated 30 min	3.1 ± 0.2	2.0 ± 0.1	2.7 ± 0.1
Illuminated 60 min	3.1 ± 0.2	3.8 ± 0.2[a] 1.8 ± 0.2[b] 2.0 ± 0.1[c]	1.9 ± 0.2[a] 1.3 ± 0.1[b] 1.8 ± 0.2[c]

[a] The enzyme was illuminated in the absence of the polysaccharide.
[b] The enzyme was illuminated together with the polysaccharide that is in course of the enzymatic reaction.
[c] The enzyme was illuminated with non-polarized light in the absence of the polysaccharide.

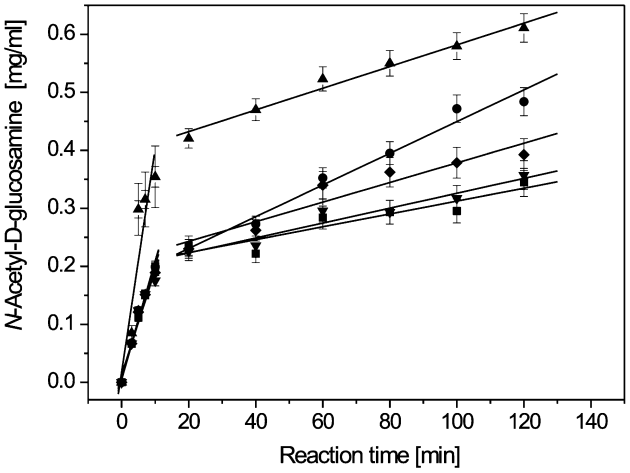

Figure 3. Course of degradation of chitin with chitinase: (■) with non-stimulated enzyme; (●) with enzyme stimulated with WLPL for 30 min; (▲) with enzyme stimulated with WLPL for 60 min; (▼) with enzyme stimulated with WLPL for 60 min in course of the enzymatic reaction, that is in the presence of chitin; (♦) with enzyme stimulated with non-polarized light under the absence of chitin.

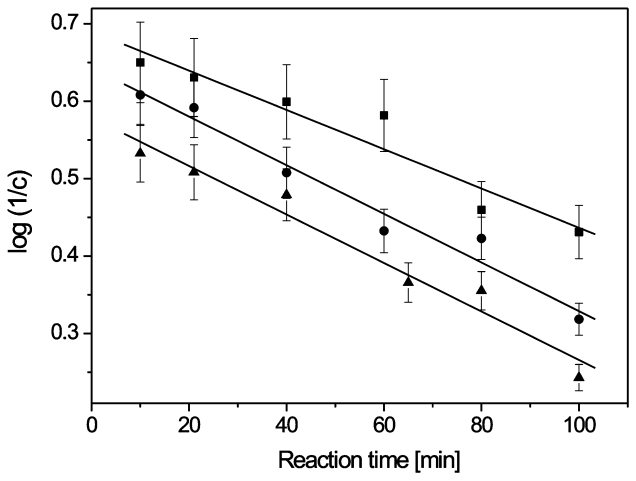

Figure 2. Overall rate constants, *k*, for degradation of chitosan with non-stimulated enzyme (■), with enzyme stimulated with WLPL for 30 min (♦), and with enzyme stimulated with WLPL for 60 min (▲).

1. Experimental

1.1. Illumination

The enzyme [either chitosanase from *Streptomyces* sp. in buffered aqueous glycerol solution, ≥15 units/mg protein (E1%)

(Chitosan *N*-acetylglucosaminohydrolase; EC 3.2.1.132) (C0794 Sigma–Aldrich, Poznan, Poland) or chitinase from *Serratia marcescens*, lyophilized powder, ≥10 units/g solid (chitodextrinase poly(1,4-β-[2-acetamido-2-deoxy-D-glucoside]) glycanohydrolase; EC 3.2.1.14) (C1650 Sigma–Aldrich, Poznan, Poland)] was dissolved in acetate buffer 0.1 M pH 5.5 (10 mL). Enzyme solution (2 mL) was placed in a glass cell and illuminated from the 30 cm distance with a KB 502 slit illuminator (Kabid, Chorzów, Poland) equipped with 150 W xenon arc (XBO 150, Oriel, Maidston, UK). An HN 22 linear polarizing filter (Polaroid, Waltham MA, USA) with a glass filter cutting off wavelengths below 500 nm was mounted between the slit illuminator and the sample. In the experiments involving non-polarized light, the HN 22 filter was removed. The light source emitted continuous radiation in the visible range. Its energy flux at the position of the sample was 8 mW/cm^2 as checked by YSI radiometer (Yellow Spring OH, USA).

Samples were illuminated at 4 °C (chitosanase) and 20 °C (chitinase) for both 30 and 60 min. Control non-illuminated samples were stored in the dark under the same conditions as the illuminated samples.

1.2. Enzymatic reaction

Either chitin [from crab shells, suitable for analysis of chitinase, purified powder (Sigma–Aldrich, Poznan, Poland)] or chitosan [high molecular weight deacetylated in >80% (Sigma–Aldrich, Poznan, Poland)] was suspended in acetate buffer 0.1M pH 5.5 (32.0 mL, chitin and chitosan concentration was 1 mg/1 mL) and heated at 85–90 °C for 15 min. Chitin and chitosan solutions were cooled to room temperature, then placed in a 37 °C water bath. Enzyme solutions (1.4 mL) were added to the chitin and chitosan solutions.

After enzyme addition, samples were incubated with mild agitation at 37 °C. Aliquots of the reaction mixture (4.0 mL) were taken after 0, 10, 20, 40, 60, 80, and 100 min for reducing sugar determination.

For comparison, enzymatic reactions were also illuminated with non-polarized light under identical conditions as those described for experiments with WLPL. All reactions were run in duplicate.

1.3. Reducing sugar determination

3,5-Dinitrosalicylic acid (DNS) in alkaline sodium potassium tartrate was used as the reagent for reducing sugars according to Southgate.[22] Absorbance at 540 nm was recorded. Calibration curve was prepared using D-(+Glucosamine hydrochloride) (Sigma–Aldrich Poznan, Poland) and *N*-acetyl-D-glucosamine (Sigma–Aldrich, Poznan, Poland) were used as standards of substrates.

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