The Polarized Light-Induced Enzymatic Formation and Degradation of Biopolymers

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Summary: White, linearly polarized transformed starch amylopectin into amylose. Moreover, it activated α-amylolysis of starch, hydrolysis of xylane with xylanase, hydrolyses of chitin with chitinase, chitosan with chitosanase, and, interestingly, influenced production of cyclodextrins with cyclodextrin glycosyltransferase. Effect of duration of illumination of cyclodextrin glucosyltransferase with polarized light had certain effect upon the yield and isomer ration of three isomeric cyclodextrins. Application of the polarized light required 1–2 hour illumination of the enzymes in a small reaction vessel followed by admixture of so activated enzymes to a bioreactor. Further reactions did not require any illumination.

Keywords: chitin; chitosan; cyclodextrins; starch

Introduction

Polarized light has found several applications in therapy, for instance, in treatment of severe burns,[1] healing wounds,[2–4] and eczema psoriasis.[5] An increase in the immunological response in peripheral blood,[6] and, generally, biostimulation[7] were observed under the influence of exposure to polarized light. Single skin exposure to visible polarized light induced rapid modification of entire circulating blood.[8] Polarized light could also control certain physical phenomena such as reorientation of dopants in polymer films,[9] self focusing of light in the nematic phase of some liquid crystals.[10,11]

Two findings have stimulated our research. In 1928, Navez and Rubinstein [12] reported that polarized light accelerated amylolysis of starch and in 1936 Semmens[13] observed decomposition of starch in moonlight. The latter was considered to be the polarized light.

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Effect of White, Linearly Polarized Light on Solid, Enzyme-Free Starch

In first experiments, granular starches in aqueous suspension were subjected to the illumination with white linearly polarized light.[14–18] Prior to illumination enzymes regulating the growth of the polysaccharides inside granules were thermally deactivated. Figure 1 presents change in molecular weight distribution in granular potato starch after 5, 25 and 50 h illumination.

Evidently, as shown with size-exclusion chromatography (MALLS), the contribution from the high-molecular weight fraction – amylopectin decreased in favour of low-molecular amylose-like fraction. Qualitatively, similar changes in the molecular weight distribution patter was observed for starches of other botanical origin. Observed effect and its dynamics were dependent not only on crystallinity of starches (Table 1). As a rule maximum of linear polysaccharide is formed within about 50 hour illumination but increase in the molecular weight of that fraction did not rise linearly in the illumination time. Prolonged illumination time caused reduction of the molecular weight of amylose in

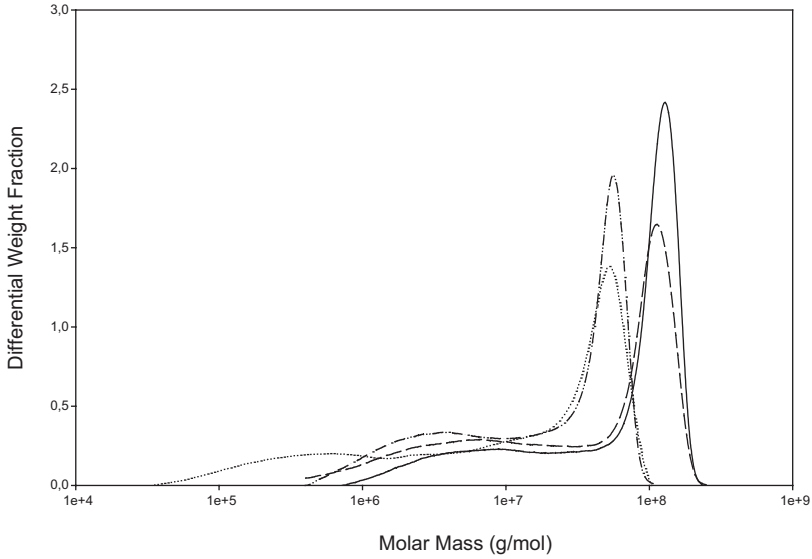

Figure 1.
The size-exclusion chromatographically recorded change in the molecular weight distribution in granular potato starch (—) illuminated with white, linearly polarized light for 5 (– – –), 25 (· · · ·) and 50 (— · · —) h.

Table 1.
Weight average molecular weights M_w and relative mass (m%) of the amylose structural units in native and illuminated starches.

Sample and illumination time (min)	Amylose fraction	
	$M_w \times 10^3$	m%
Amarantus (native)	2.42	38.61
Amarantus 5	5.07	37.57
Amarantus 15	9.04	36.98
Amarantus 25	4.32	38.40
Amarantus 50	4.05	42.99
Sago (native)	4.56	50.79
Sago 5	4.44	57.64
Sago 15	4.28	50.65
Sago 25	5.73	53.96
Sago 50	6.10	53.47
Waxy corn (native)	2.26	37.0
Waxy corn 5	2.28	17.1
Waxy corn 15	2.28	19.9
Waxy corn 25	2.28	22.6
Waxy corn 50	2.27	23.2
Potato (native)	2.36	27.6
Potato 5	2.36	29.4
Potato 15	2.36	16.2
Potato 25	2.37	27.6
Potato 50	2.38	33.4
HylonV (native)	2.35	68.2
Hylon V 5	3.83	50.2
Hylon V 15	0.89	69.1
Hylon VII(native)	2,07	54,6
Hylon VII 25	5.53	55.9
Hylon VII 50	3.16	54.0

high-amylose Hylon starches. In every case, illumination reduced amount of amylopectin and increased the contribution from amylose, that is, such treatment produced effects usually available by means of genetic modifications of plants.

According to the recent model of the starch granule[19] crystalline parts of granule consist of ordered double helical amylopectin side chains clusters. Clusters of parallel amylopectin side chains could absorb energy of incoming polarized light with electrical vector parallel to the longer axis of the polysaccharide chains. Absorbed energy of polarized light could induce vibration of starch crystalline lattice, resulting in a bond cleavage. It led to induction of depolymerisation – repolimerisation reactions of starch polysaccharide chains as in Figure 2. In

Figure 2.
Proposed course of changes in starch polysaccharides on illumination with polarized light.

such manner, branched amylopectin turned into linear amylose-like polymer.

The effect of the polarized light upon the solid matter results also from better penetration of solids by such light.[20]

Activation of Enzymes with Polarized Light

The following enzymes have been activated with polarized light: chitinase,[21] chitosanase,[21] glucoamylase,[22] α-amylase,[22] β-xylanase,[23] and cyclodextrin glycosyltransferase (CGTase).[24] Then the effect of illumination was tested on chitin, chitosan, sago starch, beechwood xylan and cellulose, respectively.

Illumination was performed with white, linearly polarized light and, for comparison, with non-polarized light. The energy flux of the enzyme (2 ml) was 8 and 15 W/cm^2 for polarized and non-polarized light, respectively, for up to 5 hrs. Experiments were run on two ways: (i) enzyme was illuminated in a separate flask then introduced into reactor with a polysaccharide and reaction proceeded further in the dark and (ii) enzyme was illuminated in the reactor filled with an enzyme – polysaccharide combination. In every case regime (i) provided significantly better results than the (ii) regime.

Table 2 provides concentration of polysaccharides and enzymes in particular experiments.

As checked in case of α-amylase, polarized light changed the enzyme conformation.[22]

Illumination with white linearly polarized light stimulated chitinase and chitosanase in their degradation of chitin and chitosan, respectively. 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, increase in the rate of hydrolysis depended on the illumination time. It required up to 60 min exposure of the enzyme in solution free of polysaccharide.

Figure 3 presents the course of hydrolysis of chitin with chitinase prior and after its stimulation with polarized light.

There is a certain period of illumination providing superior stimulating effect. In case of chitinase it lasted 1 hour. Figure 4 presents the effect of illumination of chitosanase upon hydrolysis of chitosan to glucosamine.

Stimulation of chitinase was more efficient than the stimulation of chitosan.

The course of α-amylolysis of potato starch is presented in Figure 5

One could see that also illumination of α-amylase with nonpolarized light stimulated amylolysis, however, to a lesser extent that did it illumination with polarized light.

Figure 6 and 7 present kinetics of amylolysis of sago starch (Wah Chang International Group of Companies, Singapore) with α-amylase to maltose and with glucoamylase to D-glucose, respectively.

In both cases, illumination with polarized light resulted in acceleration of the enzymatic reaction and higher reaction yield.

Interesting is effect of illumination with polarized light upon xylanase.[23] The

Table 2.
Concentrations of polysaccharides and enzymes in particular experiments.

Enzyme	Concentration of polysaccharides [mg/ml]	Concentration of enzyme
Chitinase	1.0	43.75 UN/1g chitin
Chitosanase	1.0	43.75UN/1g chitosan
α-Amylase	1.5	140 U/1mg sago
Glucoamylase	1.0	250U/1 mg sago starch
Xylanase	2	0.25 U/1mg xylan
Cyclodextrin glycosyltransferase	15	0.004 ml/ml
	30	0.008 ml/ml

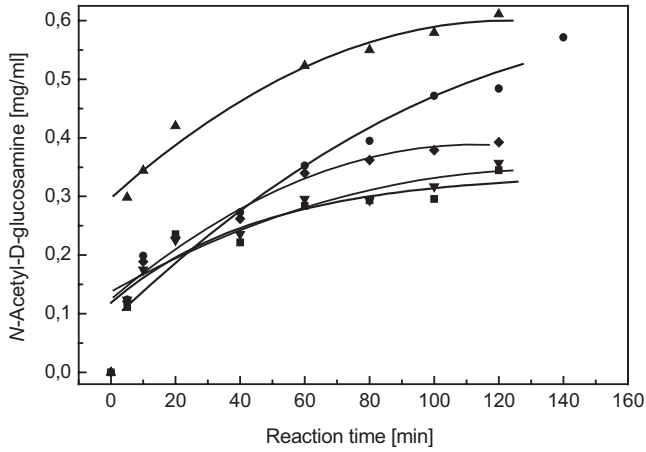

Figure 3.
Course of degradation of chitin with chitinase. ■: with non-stimulated enzyme; ●: with enzyme stimulated with polarized light for 30 min; ▲: with enzyme stimulated with polarized light for 60 min; ▼: with enzyme stimulated with polarized light for 60 min in course of the enzymatic reaction,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

illumination of xylanase with visible polarized light prior to its action increased its activity against beechwood xylan. For the dispersion of xylanase from *Thermomyces lanuginosus*, illumination with polarized light for a prolonged time significantly enhanced the activity of that enzyme even for a long-term reaction. The hydrolysis followed the first-order kinetic in the range of 0.056–0.111 min^{-1}. As seen in Figure 8, initially, the illuminated enzyme performed worse than that non-illuminated. Illumination of enzyme with either nonpolarized or polarized light provided higher yield of D-xylose, concentration of which after 1 hour process increased over that achieved with non-illuminated enzyme.

Thus far, in our research, cyclodextrin glucotransferase, CGTase, is the sole non-hydrolysing enzyme tested. Impact of

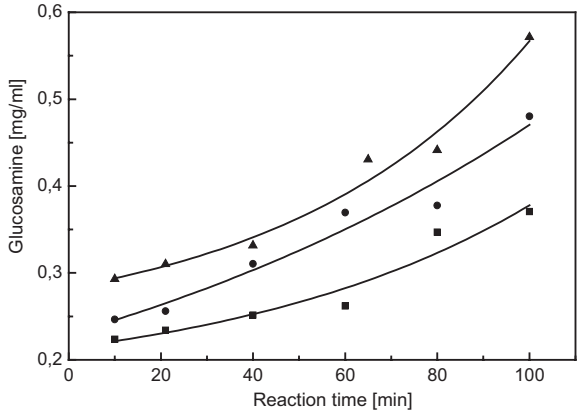

Figure 4.
Course of degradation of chitosan with chitosanase. ■: with non-stimulated enzyme; ◆: with enzyme stimulated with polarized light for 30 min; ▲: with enzyme stimulated with polarized light for 60 min.

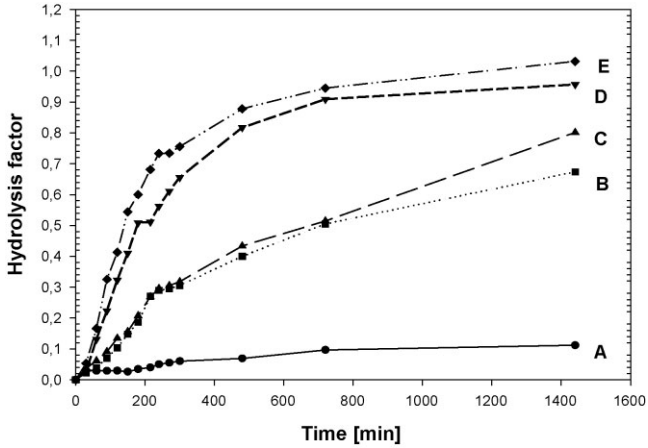

Figure 5.
α-Amylolysis kinetics of potato starch measured under following conditions: native kept in the dark (A), starch-amylase solution illuminated for 1hr with the visible non-polarized light (B), starch amylase solution illuminated for 1hr with the visible polarized light (C), amylase solution illuminated for 1hr with the visible non-polarized light prior to the starch addition (D), amylase solution illuminated for 1 hr with the visible polarized light prior to the starch addition (E).

enzyme illumination time upon concentrations of α-, β- and γ-cyclodextrins in the reaction mixture after 2h incubation is presented in Figure 9.

In the period of the yield increase, the following rate constants were found for the formation of three cyclodextrins: $k_\alpha = 1{,}1 \cdot 10^{-3}$, $k_\beta = 1{,}4 \cdot 10^{-3}$ and $k_\gamma = 0{,}05 \cdot 10^{-3}$.

Illumination of enzyme for 1 and 2 h led to increase in overall yield of reaction as compared with reaction catalyzed by non-illuminated enzyme. One exception in this trend was noted in the case of γ-cyclodextrin formed in the reaction catalyzed by enzyme illuminated for 1h. In this case, concentration of γ-cyclodextrin (0.00231 g/cm^3) in the reaction mixture was significantly lower than in the case of reaction catalyzed by non-illuminated enzyme (0.00253 g/cm^3). Prolonged illumination

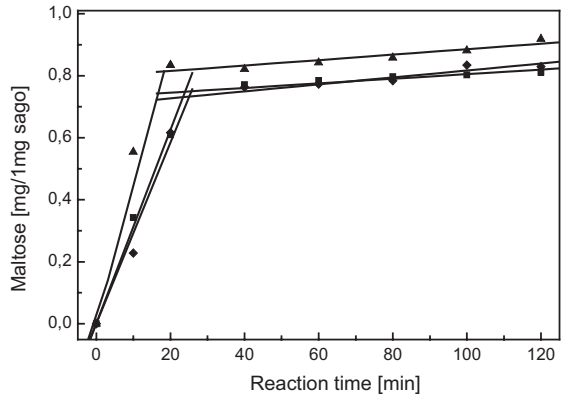

Figure 6.
Kinetics of hydrolysis of sago starch with α-amylase to maltose. ▲- enzyme stimulated with polarized light ($k_1 = 4.5 \times 10^{-2}$, $k_2 = 0.9 \times 10^{-3}$); ◆- enzyme stimulated with nonpolarized light ($k_1 = 2.9 \times 10^{-2}$, $k_2 = 1.1 \times 10^{-3}$); ■ – enzyme non-stimulated ($k_1 = 3.2 \times 10^{-2}$, $k_2 = 0.7 \times 10^{-3}$).

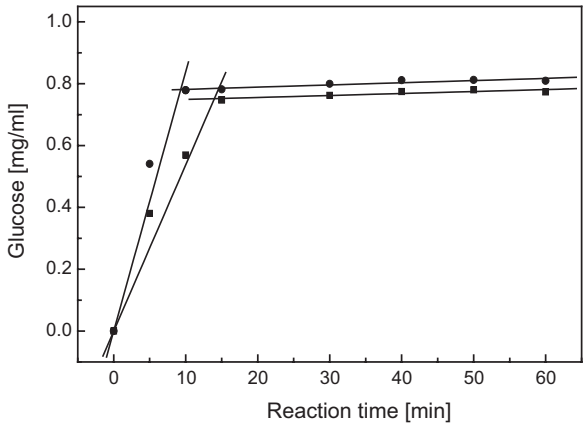

Figure 7.
Kinetics of hydrolysis of sago starch with glucoamylase to glucose. ● - enzyme stimulated with polarized ligh ($k_1 = 8.4 \times 10^{-2}$, $k_2 = 0.7 \times 10^{-3}$); ■ – enzyme non-stimulated ($k_1 = 5.4 \times 10^{-2}$, $k_2 = 0.6 \times 10^{-3}$).

time (5h) caused significant decrease in the yield of CD production in comparison with yields obtained in reactions catalyzed by enzyme illuminated for 2 and 5 h. It is worth to note that, although illumination of enzyme for 2 h led to increase in the concentration of all three cyclodextrins formed in the reaction mixture, an increase in the α-cyclodextrin concentration was most pronounced. This resulted in the increase in the α-cyclodextrin/β-cyclodextrin concentration ratio from 0.78 after 1 h illumination to 0.93 for reaction catalysed by enzyme illuminated for 2h.

Data presented above show that ratio of cyclodextrins formed in reaction mixture could be controlled by the enzyme illumination time. However, under reaction conditions applied ($c_s = 0.02$ g/cm^3; $c_e = 0.004$ cm^3/cm^3) concentration of β-cyclodextrin was always higher than concentration of α-cyclodextrin formed in the reaction mixture, regardless of enzyme illumination time.

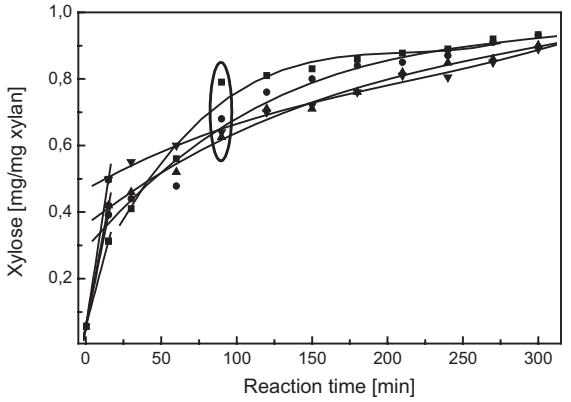

Figure 8.
Kinetics of hydrolysis of beechwood xylan with non-illuminated xylanase (▼) ($k_1 = 3.0 \times 10^{-2}$, $k_2 = 1.8 \times 10^{-3}$, $k_3 = 1.0 \times 10^{-3}$); xylanase 1 h illuminated with non-polarized light (▲) ($k_1 = 2.4 \times 10^{-2}$, $k_2 = 2.6 \times 10^{-3}$, $k_3 = 1.1 \times 10^{-3}$); xylanase 1 h illuminated with polarized light (●) ($k_1 = 2.2 \times 10^{-2}$, $k_2 = 3.6 \times 10^{-3}$, $k_3 = 0.9 \times 10^{-3}$); and xylanase 2 h illuminated with polarized light (■) ($k_1 = 1.7 \times 10^{-2}$, $k_2 = 6.2 \times 10^{-3}$, $k_3 = 0.7 \times 10^{-3}$).

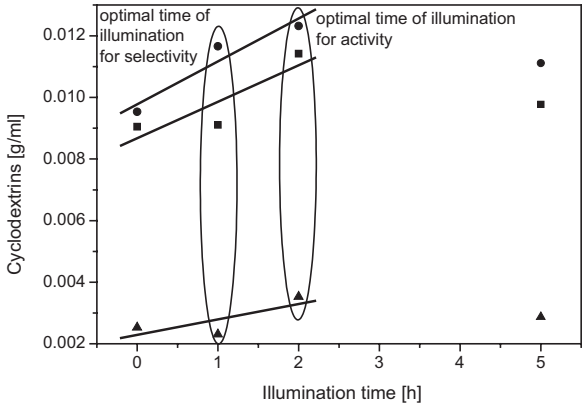

Figure 9.
Impact of CGTase ($c_e = 0.008$ cm^3/cm^3) illumination time with polarized light upon concentration of α- (■), β- ◆) and γ- (▲) cyclodextrins formed in the reaction mixture after 2h of incubation of sago starch ($c_s = 0.03$g/cm^3) with non-illuminated and 1, 2 and 5h illuminated enzyme.

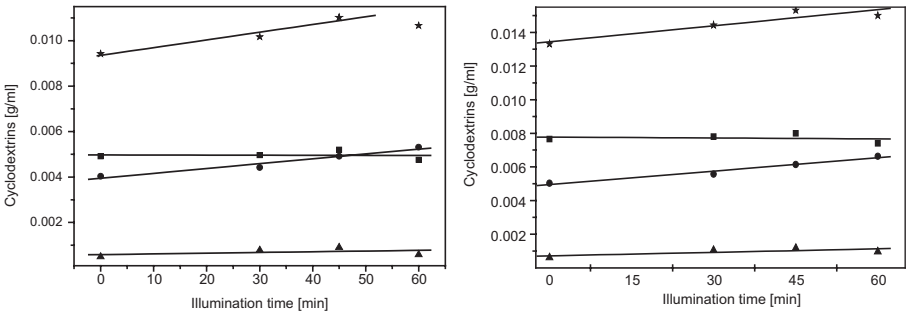

Figure 10.
Effect of the storage of illuminated CGTase for 3 months in the dark Left: kinetics measured directly after introduction of illuminated enzyme to the reaction mixture ■ - α-cyclodextrin, $k = 4.4 \times 10^{-7}$; ● - β-cyclodextrin, $k = 2.2 \times 10^{-5}$; ▲ - γ-cyclodextrin, $k = 3.2 \times 10^{-6}$ (* - $k_{total} = 3.4 \times 10^{-5}$); right: kinetics measured after applying stored enzyme. Rate constants, $kx10^{-5}$, are 0.18, 2.7, 0.7, for α-, β-, and γ-cyclodextrins, respectively. (* - $k_{total} = 3.2 \times 10^{-5}$).

Stimulation of enzyme by means of illumination with polarized light is a long lasting process. Figure 10 shows a decrease in the illuminated enzyme activity after 3 months storage in the dark, temperature 3–4 °C.

One could see that the storage had an impact solely upon the rate of formation of cyclodextrins, particularly upon formation of α-cyclodextrin.

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