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Effect of Illumination with the Visible Polarized and Nonpolarized Light on α-Amylolysis of Starches of Different Botanical Origin

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The α -amylolysis of potato, corn, waxy corn, and amaranthus starches with porcine pancreatic α -amylase upon illumination with visible polarized and nonpolarized light was studied. Samples were illuminated for 1 h either directly after blending the enzyme with starch or the enzyme solutions were illuminated for 1 h prior to the admixture of starch. Independently of the mode of illumination, no significant change in the hydrolysis kinetics was noted for corn, waxy corn, and amaranthus starches. The illumination of potato starch in the presence of α -amylase with polarized and nonpolarized light significantly accelerated the hydrolysis. In the first 5-h step the hydrolysis rate increased from 12.0 to 60.0 g kg⁻¹ h⁻¹. Preillumination of enzyme in solution resulted in an increase in the rate of hydrolysis to 151.6 and 131.4 g kg⁻¹ h⁻¹ after illumination with polarized and nonpolarized light, respectively. Circular dichroism spectra of α -amylase solutions stored in the dark and illuminated with visible polarized and nonpolarized light provided evaluation of the protein conformation, whereas exposure of enzyme solutions to the nonpolarized did not change the secondary structure of the protein. The illumination of the α -amylase solutions with polarized light significantly changed the amounts of α -helix and β -form vs unilluminated samples: 42.3% and 25.5% vs 36.6% and 30.2%, respectively.

KEYWORDS: α-Amylolysis; depolymerization; protein conformation; polarized light; repolymerization; starch

INTRODUCTION

Recently, visible, incoherent, polarized light (VIP) has found many applications in medicine and cosmetics. Medical cases being treated with polarized light include severe second degree burns (1, 2), wound healing (3), leg ulcers, psoriasis, egzema (4, 5), as well as cosmetic applications for fine lines and coarse pores on the face. The illumination of skin with polarized light led to the improvement of immunological response of blood (6). The biostimulating action of low-energy laser light, which is widely used in medicine, could be attributed to the polarization of laser light (7). The mechanism of the action of the polarized light on living organisms is complex and not fully recognized. Possibly, organized cell membrane lipid bilayers are the target for the polarized light. Absorption of the polarized light by these structures rearranges polar heads in the lipid bilayers, resulting in changes in their functions (8-10). However, the biological effect of the polarized light could be attributed to a stimulation of release of growth factors (11), the enhancement of specific enzymes involved in the cell regeneration, the activation of ATP production, as well as development of new blood vessels (12).

Reports on the action of the polarized light on plants are scarce and contradictory. An increase in the hydrolysis rate of starch after irradiation of the starch-diastase system with the ordinary and polarized light was reported (13).

On the other hand, degradation of starch took place in moonlight (14) as well as under illumination with artificial, polarized light under absence of hydrolyases (15, 16).

The 5–15-h illumination of granular corn (15) and potato (16) starches in aqueous suspensions with polarized light induced a degradation of starch polysaccharides. Polysaccharide chains from both amylose and amylopectin fractions of starch were degraded; however, a sharp decrease in the molecular weight of amylopectin molecules indicated that mainly polysaccharides from the amylopectin fraction of starch were sensitive to the polarized light. A prolonged illumination led to crosslinking of polysaccharide chains of amylopectin fractions with short polysaccharide molecules. The model of the starch granule (17) presents granule crystallites consisting of ordered double helical amylopectin side chain clusters. Such clusters might be capable of absorption of the light with electrical vector parallel to the longer axis of the double helical polysaccharide chains (18, 19). Therefore, induction of depolymerization-repolymerization reactions of the starch polysaccharides on their illumination with polarized light could be explained by the absorption of light energy by the clusters of the helical amylopectin side chains. Absorbed energy could generate vibrations in the crystalline lattice, resulting in bond cleavage. Illumination with

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polarized light of granular, waxy cornstarch initially led to a decrease in its intrinsic viscosity followed by a significant increase in the intrinsic viscosity after prolonged illumination (19). Similarly, the iodine-binding properties of waxy cornstarch illuminated with polarized light indicated initial degradation of polysaccharide chains followed by repolymerization on prolonged illumination. However, even prolonged illumination of isolated amorphous waxy corn amylopectin did not change the intrinsic viscosity and iodine-binding capacity. The results strongly confirmed the assumption that the crystalline regions of the starch granule were responsible for the absorption of the polarized light and induction of the depolymerizationrepolymerization reactions of the starch polysaccharide chains. Illumination of granular starches with polarized light slightly influenced their thermal properties. Only a prolonged illumination resulted in significant decrease in melting enthalpy of all starches. On the other hand, the hot paste viscosity of cornstarch significantly decreased within the first 25 h of illumination; then, on prolonged illumination, it returned into its original value (14, 15, 19). Similar, although much smaller, changes in hot paste viscosity were observed for waxy corn and potato starches. Illumination of all starches with polarized light significantly increased their swelling power and solubility and enhanced their susceptibility to enzymatic hydrolysis.

The time-dependent increase in the α -amylolysis of illuminated potato and waxy corn starches was noted. For potato starch samples, the amylolysis rate of potato starch illuminated for 50 h increased by several orders (20). Such effects were reported only for starch preilluminated with the polarized light. Now, α -amylolysis of starch of different botanical origin on the simultaneous polarized light illuminated α -amylase was used in this study.

MATERIALS AND METHODS

Enzymatic Digestion. α -Amylase from porcine pancreas (EC.3.2.1.1, Merck, Germany) was reconstituted in 20 mM phosphate buffer, pH 6.5–7.0, containing 2 mM NaCl and 0.25 mM CaCl₂ to obtain a stock solution with an enzyme activity of 250 units/mL. Potato, corn, waxy corn, and amaranthus starch samples were exactly weighed in Erlenmeyer flasks and suspended in the phosphate buffer (38 mL). The enzyme stock solution aliquots (2 mL) were added to obtain a final concentration of starch of 1 mg/mL and a final enzyme activity of 12.5 units per mg of substrate. The solution was stirred with a magnetic stirrer and incubated at 37 °C. Immediately after lending, the starch solutions were illuminated for 1 h with either polarized or nonpolarized light. In separate experiments, the α -amylase solution was illuminated for 1 h followed by admixture of a controlled amount of starch.

After terminating of illumination, aliquots (2 mL) were collected within different time intervals and the amount of soluble starch was determined with the 3,5-dinitrosalicylic acid method (21). Starch digestibility was calculated as the amount of starch hydrolyzed to the total dry substrate ratio.

Illumination Conditions. Either the α -amylase solution or the solution containing enzyme and starch was illuminated from a 30-cm distance with the KB 502 slit illuminator (Kabid, Chorzów, Poland) equipped with a 150-W xenon arc lamp XBO 150 (Oriel, England). The HN 22 linear, polarizing filter (Polaroid), with a glass filter cutting out wavelengths below 500 nm, was mounted between the slit illuminator and the sample. In experiments involving nonpolarized light, the HN 22 filter was removed. The light source emitted continuous intensity in the visible range. Its energy flux at the place of the samples was 8 mW/cm² with the presence of polarizing filter and 15 mW/cm² without the HN 22 filter, as checked by a YSI radiometer. Samples of starches kept in the dark under the same conditions as illuminated samples served as control.



Figure 1. α -Amylolysis kinetics (porcine pancreatic α -amylase, 12 U/mg of starch at 37 °C) of potato starch measured under the following conditions: native kept in the dark (**A**), starch–amylase solution illuminated for 1 h with visible nonpolarized light (**B**), starch amylase solution illuminated for 1 h with visible nonpolarized light (**C**), amylase solution illuminated for 1 h with visible nonpolarized light prior to the starch addition (**D**), and amylase solution illuminated for 1 h with visible nonpolarized light prior to the starch addition (**E**).

Circular Dichroism Spectra. Circular dichroism spectra of the α -amylase solutions (c = 0.2 mg/mL) kept in the dark and illuminated for 1 h with visible and visible linearly polarized light were recorded in a 0.5 mm cell using a JASCO J-710 (Jasco, Japan) spectrophotometer. Evaluation of the protein conformation in the samples kept in the dark and illuminated with visible and linearly polarized light was performed using CDFIT software and standard poly-lysine curves (22, 23).

Statistics. Statistics was performed with Microcal Origin 6.0. All experiments were run in triplicates. One-way analysis of variance (Anova) at the $P \leq 0.05$ level was performed to determine the illumination effect.

RESULTS AND DISCUSSION

α-Amylolysis of potato, corn, waxy corn, and amaranthus starches was studied under the following conditions: (i) solutions of α -amylase and starch were illuminated for 1 h with the polarized and nonpolarized white light, respectively, and (ii) starches were admixed into the 1-h preilluminated α -amylase solutions. Either polarized or nonpolarized light was used. For comparison, for all starches kinetics of the α -amylolysis was also performed in darkness. The hydrolysis kinetics measured in the dark and under various illumination conditions for potato starch is presented in Figure 1. Hydrolysis kinetics curves for native corn, waxy corn, and amaranthus starches digested in α -amylase solutions illuminated for 1 h with polarized light and starches hydrolyzed in the solution of α -amylase preilluminated for 1 h with visible polarized light are shown on Figure 2. The hydrolysis kinetics curves for samples of starches illuminated with the white nonpolarized light and curves for samples kept in the dark were identical (P < 0.05).

Independently of illumination conditions, for all starches a classical two-stage hydrolysis was observed. The rate and final extent of hydrolysis for native starches kept in the dark increased in the order potato < corn < waxy corn < amaranthus. In the case of corn, waxy corn, and amaranthus starches, either illumination of the reaction mixture or preillumination of the enzyme solution had no effect on the hydrolysis kinetics (**Figure 2**).



Figure 2. Hydrolysis kinetics (porcine pancreatic α -amylase, 12 U/mg of starch at 37 °C) of native: corn (A), waxy corn (C), amaranthus (E) starches digested in α -amylase solutions illuminated for 1 h with polarized light and corn (B), waxy corn (D), and amaranthus (F) starches hydrolyzed in the amylase solution preilluminated for 1 h with the visible polarized light.

Table 1. Hydrolysis Rates and Final Extent of Hydrolysis Values for α -Amylolysis of Potato Starch (12.5 U of Enzyme per mg of Starch, 37 °C)

	hydrolysis ^{b,c}	
sample ^a	rate (g kg h ⁻¹)	final extent (%)
dark NPLI PLI PNPLI PPLI	$\begin{array}{c} 12.0 \pm 1.2 \text{ A} \\ 60.6 \pm 2.9 \text{ B} \\ 62.3 \pm 2.5 \text{ B} \\ 131.4 \pm 4.1 \text{ C} \\ 151.6 \pm 3.9 \text{ D} \end{array}$	$\begin{array}{c} 11.2 \pm 2.0 \text{ A} \\ 67.0 \pm 3.0 \text{ B} \\ 80.1 \pm 3.1 \text{C} \\ 95.7 \pm 2.7 \text{ D} \\ 100.3 \pm 2.1 \text{ E} \end{array}$

^{*a*} Measured under the following conditions: kept in the dark (dark); illuminated for 1 h with visible nonpolarized light (NPLI); or illuminated for 1 h with visible polarized light (PLI), as well as measured after the preillumination of the enzyme solution for 1 h either with visible nonpolarized light (PNLI) or visible polarized light (PPLI). ^{*b*} Means of three independent experiments ± standard deviation. ^{*c*} Means within columns with different indices are statistically different with *P* < 0.05.

The final extent of hydrolysis and rate of hydrolysis for the first 5-h stage of amylolysis for potato starch are presented in **Table 1**. Illumination of the reaction mixture or preillumination of the α -amylase solution with polarized and nonpolarized visible light had a remarkable effect on the hydrolysis kinetics. Illumination (1 h) of the reaction mixture, regardless of the radiation used, led to a significant increase in the hydrolysis rate of the first stage of reaction (62.4 and 12.0 g kg⁻¹ h⁻¹ for the control sample). A rise in the final extent of the hydrolysis was also observed for illuminated samples. However, it was higher for the samples illuminated with the polarized light than for the samples illuminated with the nonpolarized light (80.1 and 67.0%, respectively).

When α -amylase solution was preilluminated for 1 h prior to addition of potato starch, the effect of illumination on the hydrolysis kinetics was more pronounced. A further, radiationdependent increase in the hydrolysis rate and final extent of hydrolysis was observed. For potato starch samples illuminated with polarized light, the hydrolysis rate (151.6 g kg⁻¹ h⁻¹) and final hydrolysis extent (100.3 ± 1.7%) were higher than for samples preilluminated with the nonpolarized visible light (131.4 g kg⁻¹ h⁻¹ and 95.7 ± 1.4%, respectively).



Figure 3. Circular dichroism spectra of α -amylase solution (0.2 mg mL⁻¹) kept in the dark (—); illuminated for 1 h with the visible polarized light (----); and illuminated for 1 h with the visible nonpolarized light (----)

Granule size (24), crystalline organization (25), amylose– amylopectin ratio (26), and the possibility of the formation of lipid–amylose complexes (27, 28) are the main factors influencing susceptibility of granular starches to amylolysis. Susceptibility of cereal starches to α -amylolysis is much higher than that of potato starch (29, 30). Under conditions applied in our work, the hydrolysis rate measured for cereal starches, especially waxy corn and amaranthus, was much higher than that for potato starch.

Because of such a high hydrolysis rate, any observation of the enhancement effect of illumination on the hydrolysis of original samples was impossible. Similarly, the digestibility of potato starch was strongly enhanced by illumination of granular starch with polarized light, whereas in the case of waxy cornstarch, only prolonged illumination with polarized light produced small changes in digestibility (20).

Differences in rates of enzymatic hydrolysis of polarizedlight-illuminated tuber (B-type) and cereal starches (A-type) could be attributed to differences in the structure of crystalline parts of the granules. According to Imberty et al. (31), double helices of both A- and B-types are identical, but the mode of the packing of the helices and the water content are different in the two polymorphs. Moreover, potato and other tuber starches contain a significant amount of monophosphate esters covalently bound to polysaccharide chains of the amylopectin fraction (32-34). Polysaccharide chains containing phosphate groups and surrounded by a higher number of water molecules could be more susceptible to electrical polarizability and, therefore, more prone to interactions with polarized light.

Under conditions applied in this study, illumination time was too short to induce structural changes in the starch granule. Therefore, enhancement of the amylolysis rate by the polarized light could be attributed to structural changes in the α -amylase backbone. Circular dichroism spectroscopy is a convenient tool for monitoring changes in the protein conformation (23). CD spectra of diluted solutions of α -amylase before and after illumination with visible polarized and nonpolarized light are presented in **Figure 3**. **Table 2** contains results of an evaluation of the protein conformation obtained from those spectra and standard poly-lysine curves using CDFIT software (22, 23). The data strongly suggests that illumination of α -amylase with polarized light induced conformational changes in the protein molecule.

Table 2. Evaluation of the Protein Conformation in the α -Amylase Samples Kept in the Dark and Preilluminated with Visible Nonpolarized and Polarized Light^a

conformation	native (%)	illuminated	
		nonpolarized light (%)	polarized light (%)
α -helix β -form	42.3 ± 1.5 25.5 + 1.2	40.9 ± 1.0 26.7 ± 1.4	36.6 ± 1.5 30.2 ± 1.2
random coil	32.2 ± 1.2	32.4 ± 1.3	32.4 ± 1.3

^a Means of three independent measurements ± standard deviation.

Up to date, a single report on enhancement of enzyme activity by visible, both polarized and nonpolarized, light was published by Navez and Rubenstein in 1928 (13). However, those authors did not address the possible mechanism of the observed results. More recent reports indicated the polarized-light-induced reorientation of the lipid bilayer in the cell membrane (9, 10). Fenyo (7, 8) suggested that active enzymes incorporate in the cell membranes and, therefore, a part of the light energy absorbed by the lipid bilayer could be transferred to the enzyme. It would decrease the activation energy of the enzyme reactions, possibly, via conformational changes in the protein structure.

Assumption that such structural changes in the enzyme molecule are beneficial for its activity could explain the very high hydrolysis rate of potato starch amylolysis, observed for starch treated with α -amylase preilluminated with polarized light.

All macromolecular structures prone to the illumination with polarized light i.e., the lipid bilayer of the cell membrane (9, 10), crystaline parts of starch granule (14, 15, 19, 20), and a variety of liquid crystals (35-37), show similiar architecture, regardless of their origin. They consist of clusters of parallely oriented long chains, which are electrically polarizable along their longer axis. One could assume that polarized light with its electric vector parallel to the longer axis of clusters is absorbed by such structures, inducing a chain reorientation. If even a part of α -amylase molecules in solution is oriented parallel to them, such clusters will absorb light with the electrical vector parallel to their longer chains. This means that even nonpolarized light could be partially absorbed by oriented α -amylase-producing changes in its conformation. Such effect could explain a significant increase in the rate of α -amylolysis of potato starch after illumination with nonpolarized light.

Conformational changes in the α -amylase molecule in the presence of starch could be inherited due to a strong interaction between protein and the starch granule. This could rationalize the observation that the hydrolysis rates for illuminated solutions containing starch and α -amylase were significantly lower than the rates observed for amylolysis induced by preilluminated enzyme.

Significant enhancement of the rate of α -amylolysis upon illumination with both polarized and nonpolarized light of starchamylase mixtures or preillumination of α -amylase solution could be of great importance for industrial processes in which potato starch is transformed into maltodextrins or high maltose syrup.

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