

Does adsorption of polycations affect the biodegradation rate of cellulose particles?

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MATERIALS AND METHODS

Materials

Sodium hydrogen phosphate dodecahydrate $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, sodium dihydrogen phosphate $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (all chemically pure grade) were used as received, and 10^{-2} M buffer solutions were prepared by weighing. Concentrated hydrochloric acid HCl, sodium hydroxide NaOH and sodium chloride (all chemically pure grade) were also used without additional purification.

Cationic poly-L-lysine hydrobromide (PL) (Sigma-Aldrich), degree of polymerization equal to 350; cationic kaustamin (OAO" BSK", Russia), degree of polymerization equal to 80 as determined via static light scattering as described below.

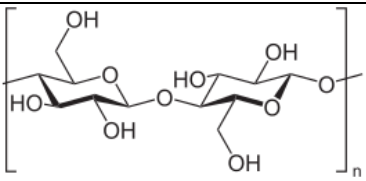
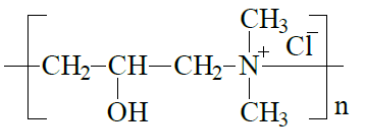
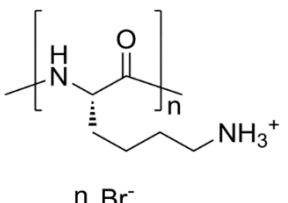
Hydrolyzed crystalline cellulose was used for the preparation of model particles.

Cellulase from *Aspergillus niger* (Sigma-Aldrich).

Water soluble barley β -1,4-glucan (Sigma-Aldrich).

The structures of the polymers are presented in **Table S1**.

Table S1. Structure of the polymers.

Cellulose	
Kaustamin	
Polylysine hydrobromide (PL)	

To prepare solutions, double-distilled water was used, which was additionally treated by passing through a Milli-Q system (Millipore) equipped with ion-exchange and adsorption columns and a 0.22 μm polyethersulfone membrane filter to remove organic/inorganic particles and bacteria. The conductivity of purified water was 0.8 $\mu\text{S}/\text{cm}$.

Methods

The hydrodynamic diameter of cellulose particles and cellulose-polycation complexes was determined by means of dynamic light scattering (DLS), at a fixed scattering angle (90°) in a constant-temperature cell using a Brookhaven Zeta Plus instrument (USA). Software provided by the manufacturer was employed to calculate the diameter values (error $\pm 7\%$).

Electrophoretic mobility (EPM) of cellulose particles and cellulose-polycation complexes was determined via microelectrophoresis using a Brookhaven Zeta Plus instrument (USA) with the corresponding software (error $\pm 3\text{-}5\%$).

General Procedures

Determining the degree of crystallinity

The crystalline structure of enzymatically hydrolyzed cellulose was investigated by wide-angle X-ray diffraction (XRD) analysis. The sample was dried and crushed into a fine powder before analysis. The obtained diffractogram (Figure S1) revealed characteristic crystalline peaks, with the main peak at $2\theta = 26^\circ$. Based on the position of these peaks and analysis of literature data [S1], it can be concluded that cellulose crystallizes in the monoclinic 1β form. The ratio of amorphous and crystalline phases in the polymer was quantitatively estimated using XRD deconvolution method. This method comprises mathematical treatment to deconvolute the diffraction data into separate peaks associated with crystalline planes and amorphous cellulose according to Bragg's law. The calculated degree of crystallinity is 64%.

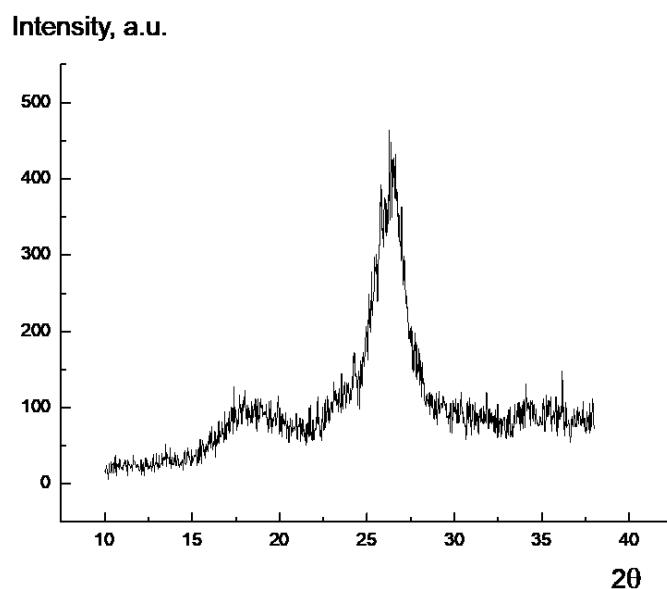


Figure S1. X-ray diffractogram of crystalline cellulose with a hydrolysis degree by cellulases of 35–41%.

The ratio of amorphous and crystalline phases in the polymer was quantitatively estimated using XRD deconvolution method [S2]. This method decomposes the scattering curve into crystalline reflections and an amorphous halo in the form of Gaussian curves using the PeakFit program.

The degree of crystallinity of cellulose was calculated as the ratio of the scattering contribution from the crystalline phase to the total scattering intensity according to the following equation:

$$\alpha = \frac{(S_{\text{sum}} - S_{\text{am}})}{S_{\text{sum}}} \times 100\% ,$$

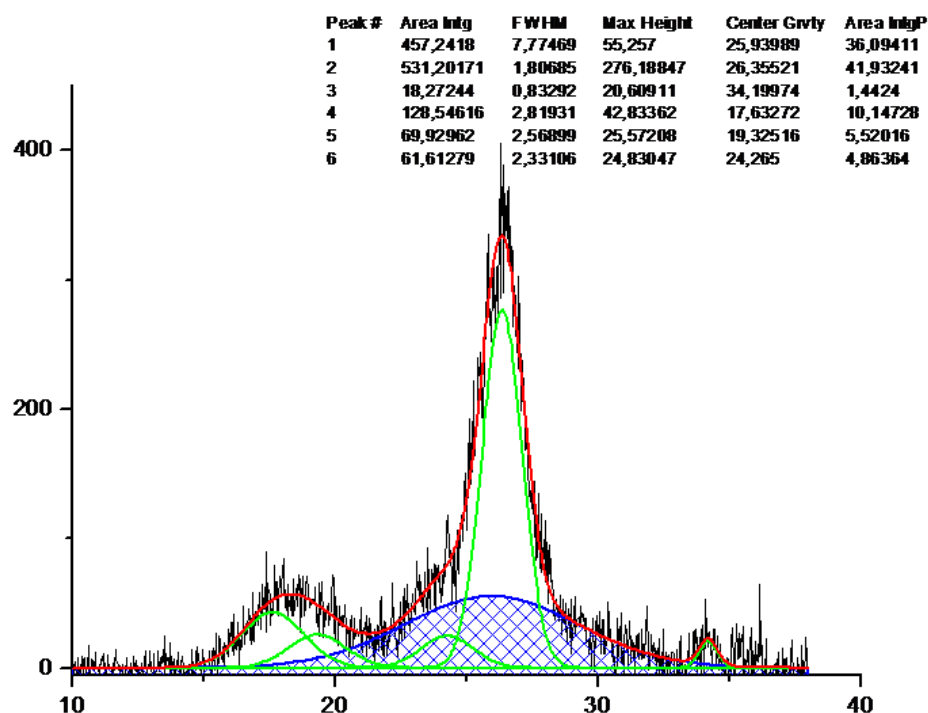
where S_{am} — peak area of the amorphous halo, and S_{sum} — total area under the integral scattering curve.

Figure S2 shows the dependence of the X-ray scattering intensity for cellulose on the scattering angle (2θ) (red curve). The results of processing the scattering curve using the PeakFit program are also presented in Figure S2. The blue curve refers to the amorphous phase, the green curves to the crystalline phase. The peak positions, the half-width at half-maximum (FWHM) value and the normalized area of each peak are also presented. It should be noted that for the cellulose sample, three most intense peaks are observed at 2θ equal to 26.4, 17.6 and 19.3°. Based on the position of these peaks and analysis of literature data [S1], it can be concluded that cellulose crystallizes in the monoclinic 1β form.

It also follows from Figure S2 that the scattering curve of cellulose is decomposed into 6 Gaussian curves: 5 curves have a relatively small FWHM value of 0.83 – 2.82, they can be attributed to the crystalline

phase, one curve is characterized by a high FWHM value of 7.75, it can be attributed to the amorphous halo. From these data, the degree of crystallinity of the cellulose was calculated as 64% using the above formula.

Intensity, a.u.



20

Figure S2. X-ray scattering intensity (I) versus scattering angle (2θ) for a cellulose sample (red curve). Scattering curves processed using PeakFit. The blue curve corresponds to the amorphous phase, the green curves to the crystalline phase.

Preparation of cellulose particles

Cellulose particles were obtained according to the following technique. A suspension of hydrolyzed crystalline cellulose was prepared in double-distilled water at concentration of 4 mg/mL. The mixture was stirred for 10 minutes. The resulting suspension was filtered through a syringe filter with pore size of 450 nm to remove large aggregates. The particle concentration in the suspension was 0.02 mg/mL. The size of freshly prepared cellulose particles remained within a 200 ± 20 nm range.

Complexation of cellulose particles with polycations

Polycation-particle complexes were prepared by mixing the cellulose particle suspension with polycation solution (kaustamin/PL) in double-distilled water.

The interaction of particles with polycations was investigated using microelectrophoresis by controlling the EPM of cellulose particles. For this, the suspension of cellulose particles was titrated with suspension of polycation. The addition of each polycation to cellulose resulted in neutralization of the particle charge with further slight surface recharge, indicating the binding of the polycation to the negatively charged surface (Figure S3).

It was shown [S3] that polycations complete bind until concentration that slightly exceeded the value needed for complete neutralization of the cellulose particle charge.

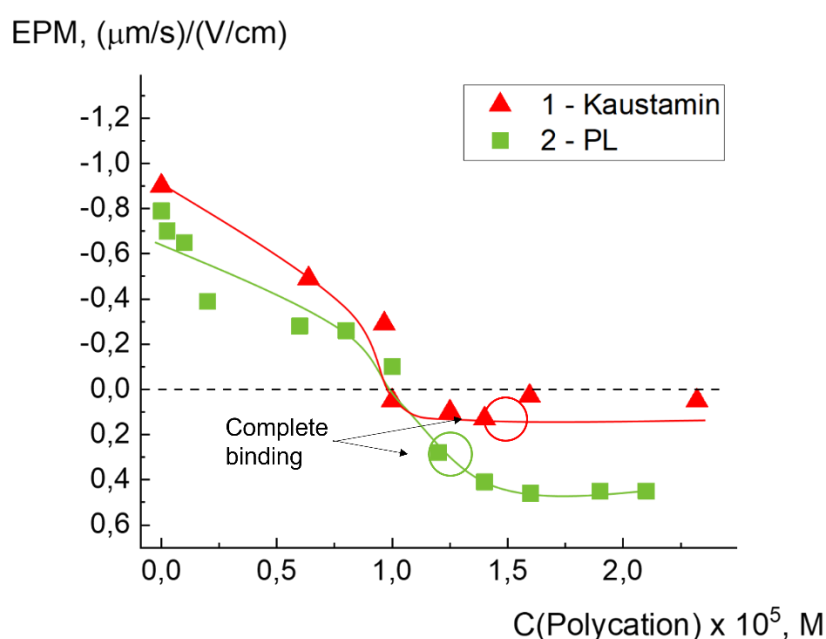


Figure S3. EPM of cellulose particle-polycation complexes vs. polycation concentration: 1 – kaustamin, 2 – PL. Cellulose particle concentration: 4.0×10^{-3} mg/mL.

Estimation of surface coverage by adsorbed polycations

To estimate the degree of surface coverage by adsorbed polycations, a simplified geometric model was used assuming that both cellulose particles and polycations have spherical shape upon adsorption.

The total surface area of cellulose particles was calculated based on their hydrodynamic diameter (~ 200 nm), number concentration and sphericity. The number of cellulose particles per unit volume was estimated from mass concentration ($C_m = 2.0 \times 10^{-2}$ mg/mL), cellulose density ($\rho \sim 1.6$ g/cm³) and average particle volume.

For this calculation, a sample volume of 1 mL was assumed as all derived quantities are proportional to volume and are eliminated in the final ratio. First, the total mass of cellulose in 1 mL is:

$$m_{total} = C_m$$

Then the total volume of cellulose material is:

$$V_{total} = \frac{m_{total}}{\rho}$$

The volume of one spherical particle ($d = 200$ nm) is:

$$V_{particle} = \frac{\pi d^3}{6}$$

The number of particles is:

$$N_{cellulose} = \frac{V_{total}}{V_{particle}}$$

The surface area of one particle (sphere) is:

$$A_{particle} = \pi d^2$$

Finally, the total surface area available for adsorption is:

$$A_{total} = N_{cellulose} \times A_{particle}$$

A similar approach was applied to estimate the number of adsorbed polycation globules N_{poly} , using their mass concentration (C_m), density ρ and diameter (d) (values given in Table S2). The only difference in the model is that each globule is assumed to occupy an area on the surface equal to d^2 corresponding to square packing. Thus, the total covered area is:

$$A_{covered} = N_{poly} \times d^2$$

The total area covered by all adsorbed polycation globules was then compared to the total available surface area of cellulose particles. The ratio of these areas provides an approximate estimation of surface coverage. The surface coverage ratio is estimated as:

$$\theta = \frac{A_{covered}}{A_{particle}}$$

The results of the surface coverage estimation are presented in Table S2.

Table S2. Estimated surface coverage of cellulose particles in polycation complexes.

	Kaustamin $d = 4$ nm, $\rho = 1,15$ g/cm ³		PL $d = 45$ nm, $\rho = 1,156$ g/cm ³	
	Complex +	Complex -	Complex +	Complex -
Concentration, mg/mL	1.0×10^{-2}	5.2×10^{-3}	1.25×10^{-2}	6.3×10^{-3}
θ	11.08	5.76	1.22	0.62

Degradation of particles at low surface coverage ($\theta = 0.1$)

To evaluate the effect of surface coverage on the protective effect, degradation experiments were performed at polycation concentrations corresponding to estimated surface coverage of $\theta = 0.1$ (calculated using the geometric model described above).

As shown in Figure S4, even at low estimated coverage enzymatic degradation of cellulose particles is strongly suppressed over 24 h. For PL complex, the size remains nearly constant (curve 2). For kaustamin complex, a slight size reduction is observed (curve 1), in contrast to unmodified cellulose particles, which degrade completely within 2 h (curve 3). These results show that the protective effect appears even at low surface coverage.

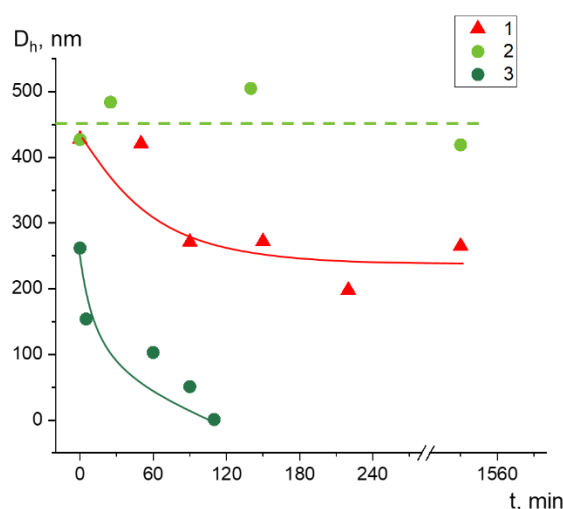


Figure S4. Time-dependent changes in average hydrodynamic diameter (D_h) of cellulose particle complexes in the presence of *A. niger* cellulases at estimated surface coverage $\theta = 0.1$: (1) kaustamin, (2) PL, (3) cellulose particles. Cellulose particle concentration 2.0×10^{-2} mg/mL, enzyme concentration 1.5×10^{-4} mg/mL. $C_{\text{kaustamin}} = 9.0 \times 10^{-5}$ mg/mL, $C_{\text{PL}} = 1.0 \times 10^{-3}$ mg/mL. Conditions: 37 °C, pH 5.5 (double-distilled water).

Determination of cellulase activity

The activity of *A. niger* cellulases preparation in the presence and absence of the polycations in the reaction mixture was evaluated using β -1,4-glucan as a substrate (with concentration 5 mg/mL). The activity was determined by measuring of accumulation of rate of reducing sugars using Nelson-Somogyi method [S4]. Enzymatic reaction was conducted during 10 min at pH 5.0 and 50 °C. Activity was expressed in International Units (IU). One units corresponded to the quantity of enzyme liberated 1 μ mol of reducing sugars (in glucose equivalent) per minute [S5]. The total activity of *A. niger* dry cellulases preparation toward β -1,4-glucan was 25000 IU/g.

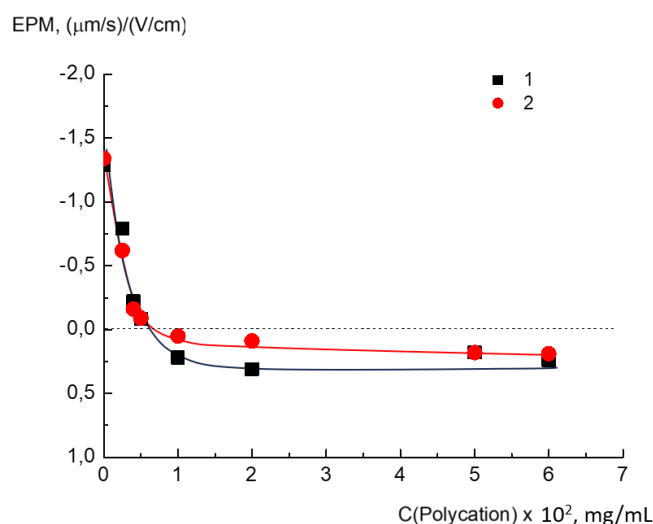


Figure S5. EPM of cellulases-polycation complexes vs. polycation concentration: 1 – PL, 2 – kaustamin. *A. niger* cellulases concentration: 1.5×10^{-4} mg/mL. Double-distilled water (pH 5.5)

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