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Adsorption and catalytic properties of enzymes on the surface of silicalite-1

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Adsorbent

Silicalite-1, a carrier for adsorption of enzymes, was synthesized according to the procedure described earlier using tetraethyl orthosilicate as a source of silicon and tetrapropylammonium hydroxide as a structure-directing agent.[S1] The average size of the resulting particles is ~ 300 nm (Figure S1). Textural characteristics of the sample obtained are the following: S_{BET} = 370 m^2 g^{-1}, S_{micro} = 240 m^2 g^{-1}, V_{total} = 0.25 cm^3 g^{-1} and V_{micro} = 0.11 cm^3 g^{-1}. The average pore diameter of silicalite-1 is ~ 0.55 nm, which allows specific adsorption of enzymes predominantly on the outer surface of crystals.

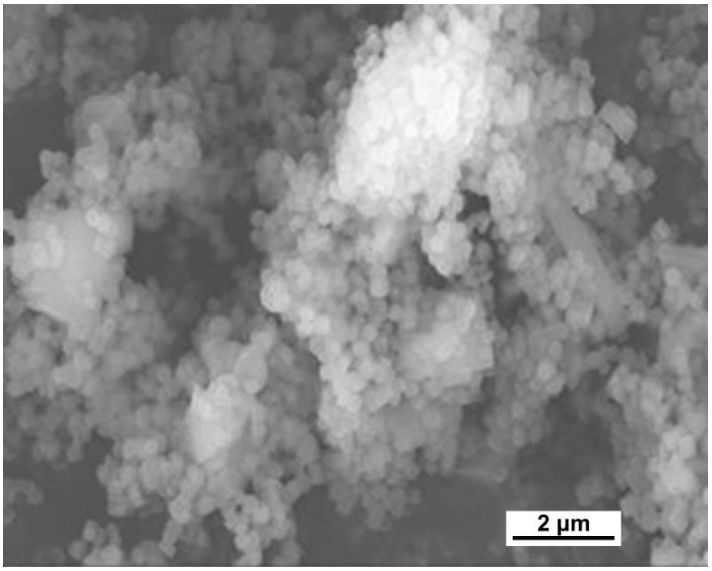

Figure S1 SEM image of the synthesized silicalite-1.

Determination of catalytic activity of the enzymes studied

The catalytic activity of enzymes was determined in solution and in adsorption layers on the surface of silicalite-1. The enzymatic reactions were carried out directly in measuring cuvettes with a thickness of 1 or 0.5 cm. To determine the activity of enzymes on the surface, 5–10 mg of yielded biocatalytic samples were used.

Peroxidase (HRP) activity was determined in the reaction of HRP oxidation of iodide anion in an acidic medium (pH 5.0, 0.1 M acetate buffer). The amount of resulting I_2 was analyzed on spectrophotometer at λ = 350 nm. The contribution of the non-enzymatic oxidation reaction of I^-

with hydrogen peroxide under these conditions is less than 2%. To determine the activity of β-galactosidase, a synthetic substrate *o*-nitrophenyl-β-D-galactopyranoside was used. The enzymatic reaction was carried out in 0.1 M phosphate buffer (pH 7.5). The *o*-nitrophenol formed as a reaction product was analyzed on spectrophotometer at λ = 420 nm. The alkaline phosphatase (CIAP) activity was determined in the hydrolysis of the disodium salt of *p*-nitrophenyl phosphate. The reaction was carried out in a 0.1 M buffer solution of Tris-HCl at pH 8.0. The *p*-nitrophenol formed in the reaction was analyzed on spectrophotometer at λ = 400 nm.

Determination of Michaelis-Menten equation parameters

To compare catalytic properties of enzymes in solution and in the adsorption layers the Michaelis constant (K_M) and the maximum rate (V_{max}) of the reaction were determined. For this, we found the dependence of the rate of the enzymatic reaction on the concentration of the substrate, and the experimental results obtained were processed in the Lineweaver-Burk plot (Figure S2). When determining the parameters of the Michaelis-Menten equation for HRP, the concentration of KI in the reaction mixture was constant (0.8 mM), and the concentration of hydrogen peroxide was changed from 1.5 to 30 μM. When determining K_M and V_{max} for β-galactosidases, *o*-nitrophenyl-β-D-galactopyranoside solutions were used in the concentration range of 0.1-2.5 mM. The concentration of disodium salt of *p*-nitrophenyl phosphate was varied in the range of 0.04-0.8 mM when determining the parameters of the Michaelis-Menten equation for CIAP.

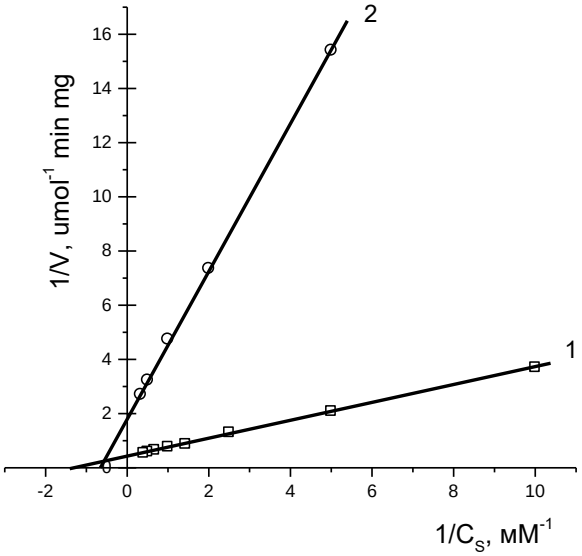

Figure S2 Determination of parameters of Michaelis-Menten equation for β-gal from bovine liver: 1 – enzyme in solution; 2 – enzyme on the silicalite-1 surface.

Adsorption measurements

The enzymes were adsorbed from aqueous solutions at 280 K during two days. A portion of the carrier weighing 50-100 mg was placed in glass cups, after that 5-10 cm^3 of solution with different content of the enzyme were added. The HRP concentration in the initial solutions was varied within 0.03–0.5 mg cm^{-3}, the concentration of β-gal from bovine liver was changed in the range of 0.02–0.8 mg cm^{-3}, β-gal *A.oryzae* – within 0.03-1.25 mg cm^{-3} and CIAP concentration was varied from 0.15 to 1.1 mg cm^{-3}. The amount of enzyme adsorbed was determined from the difference in the concentration of the enzyme in the initial and contact solutions. To determine the concentration of enzymes in solutions, the spectrophotometric method was applied. To determine the concentration of β-gal and CIAP, the optical density of the solutions was measured at $\lambda = 280$ nm using a GENESIS-5 spectrophotometer with a 0.5 cm cuvette. A calibration plot was preliminarily drawn using solutions of bovine serum albumin of a known concentration. When analyzing HRP, an analytical band of 400 nm (Soret band) was used. The measurements were performed on a Jenway 6310 spectrophotometer.

After the adsorption was complete, the heterogeneous samples were separated from the solution and washed several times with small portions of water, then 5 cm^3 of water were added and left in the refrigerator to check the reversibility of adsorption. After a time, comparable with the adsorption time, the concentration of protein in the contact solution was determined and its mass, which was desorbed from the surface of silicalite-1, was calculated.

Characterization techniques

X-ray diffraction (XRD) pattern of silicalite-1 obtained was recorded on a Bruker D2 PHASER diffractometer (CuKα radiation) in an angular range of $5° < 2\theta < 50°$. The diffraction pattern was further processed using the Bruker DIFFRAC.EVA software package. The phases were identified according to the ICDD PDF2 database. Electron microscope images of silicalite-1 were recorded on a JEOL JEM 2010 transmission electron microscope using a 200-keV electron beam. Sorption/desorption isotherms of nitrogen were measured at 77 K using an automated porosimeter Micromeritics ASAP 2020 with preliminary evacuation of the samples at 623 K. In the case of immobilized enzymes, pretreatment temperature before the analysis did not exceed 323 K in order to avoid destruction and desorption of enzymes. Micropore volumes (V_{micro}) were determined using *t*-plot method. The total sorbed volumes (V_{total}), including adsorption in the micropores and mesopores and on the external surface, were calculated from the amount of nitrogen adsorbed at relative pressure p/p_0 of 0.95 before the onset of interparticle condensation.

References

S1 A. Tavolaro, P. Tavolaro and E. Drioli, *Colloids Surf., B*, 2007, **55**, 67.