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

Larisa F. Atyaksheva, Tamara I. Ibrahimzade and Ivan A. Kasyanov*

Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. Fax: +7 495 939 3570; e-mail: i.a.kasyanov@gmail.com

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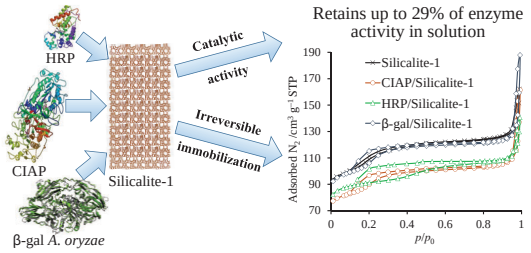
Microporous hydrophobic silicalite-1 was used as a carrier for immobilization of different enzymes, such as horseradish peroxidase, calf intestinal alkaline phosphatase and two β-galactosidases of different origin, to create heterogeneous biocatalytic systems. The peculiarities of enzyme adsorption on the surface of silicalite-1, as well as catalytic properties of the obtained systems compared to enzyme activity in solution and on the surface of other carriers, are discussed.



Keywords: horseradish peroxidase, alkaline phosphatase, β-galactosidase, immobilization, silicalite-1, adsorption isotherms, catalytic activity.

The adsorption of enzymes on zeolites has drawn certain attention as a facile way to create heterogeneous biocatalysts and biosensors.[1,2] Various silica materials and composites based on them can be used to create containers for delivery of drugs,[3] such as neuropeptides[4] or acyclovir.[5] In this regard, silicalite-1, a hydrophobic silica material with MFI zeolite structure,[6] is of high interest as a carrier for the adsorptive immobilization of enzymes. The results of studies on the adsorption of cytochrome C, lipase,[7] bovine serum albumin[8,9] and β-glucosidase[10] on silicalite-1 have been published. Although the pore size of this carrier is smaller than the size of enzyme molecules, the published data indicate a possible fixation of fragments of biomolecules in narrow carrier channels. It is also known that the catalytic activity of lipase and cytochrome C on the silicalite-1 surface is higher than that on the surface of other zeolites,[7] and an active biocatalyst, based on the β-glucosidase immobilized on the surface of silicalite-1, has already been reported.[10] Thus, the creation of new strongly bound enzyme–carrier systems based on silicalite-1, which retain their catalytic activity after immobilization, remains an urgent and significant scientific task.

In this work, the adsorption behavior of various enzymes on silicalite-1 was explored, the maximum adsorption values and catalytic activity of adsorption layers of enzymes of various molecular masses (M_R) and the nature of sites, such as horseradish peroxidase (HRP), calf intestinal alkaline phosphatase (CIAP), β-galactosidases (β-gal) from *Aspergillus oryzae* fungi and from bovine liver, were determined. Silicalite-1 was synthesized using published technique.[11] All enzymes were supplied by Sigma-Aldrich and were used as received. Adsorption was carried out in aqueous solutions at 280 K for two days. To study the reversibility of adsorption, a similar volume of water was added to heterogeneous samples and left in a refrigerator for two days, then the protein content was analyzed. It was shown that the adsorption of enzymes under the used conditions is practically irreversible. The maximum alkaline phosphatase desorption value from the surface of silicalite-1 was 7 wt% at large degrees of filling, whereas for other enzymes the desorption value did not exceed 3–4 wt%. The adsorbent characterization data, as well as the procedures of immobilization and determination of the catalytic activity of enzymes and the physicochemical characterization techniques of the systems obtained are given in Online Supplementary Materials.

The two-step adsorption isotherms of enzymes shown in Figure 1 may indicate several types of interaction in the enzyme–carrier system. After processing the adsorption isotherms in Langmuir coordinates, the two values of the maximum adsorption were obtained for each enzyme except for HRP (Table 1). In the case of HRP, the bend of the adsorption isotherm may also exist, but it lies beyond the interval of the studied enzyme concentrations. Previously, two-step adsorption isotherms have been obtained for adsorption of hemoglobin[5] and β-galactosidase[12] on silicas and aluminosilicates with a bimodal pore distribution. These two-step isotherms may be due to the texture features of the adsorbents.[13]

The adsorption data of the above enzymes on silicalite-1 have not been published yet. The isotherms of adsorption on silicalite-1 were obtained only for cytochrome C[7] and bovine serum albumin (BSA)[11] with the maximum adsorption values

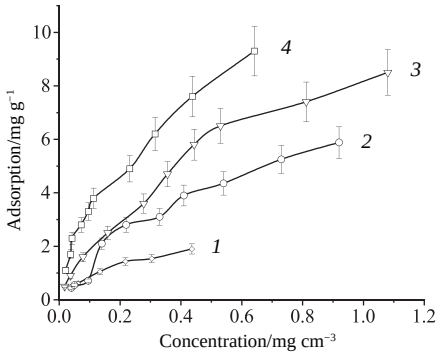


Figure 1 Adsorption isotherms of enzymes on the silicalite-1 surface: (*1*) HRP; (*2*) CIAP; β-galactosidases from (*3*) *Aspergillus oryzae* fungi and (*4*) bovine liver.

Table 1 Studied enzymes and values of their maximum adsorption on the silicalite-1.

Enzyme	M_R	Content of protein (wt%)	Maximal adsorption/ mg g^{-1}	
			a_1	a_2
Horseradish peroxidase (HRP)	44000 (monomer)	42[a]	3.0	
Calf-intestinal alkaline phosphatase (CIAP)	69000 × 2 (dimer)	25[b]	4.5	10.0
β-Galactosidase from *Aspergillus oryzae* fungi	100000 (monomer)	8[b]	5.5	13.5
β-Galactosidase from bovine liver	41000 (monomer)	95[a]	7.0	17.0

[a] According to Sigma-Aldrich data. [b] Determined by Bradford protein assay.

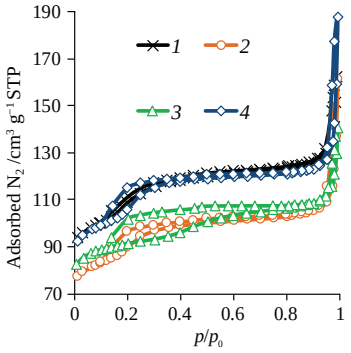


Figure 2 Low-temperature N_2 adsorption–desorption isotherms obtained on (*1*) silicalite-1 and on enzyme–carrier systems based on (*2*) CIAP, (*3*) HRP and (*4*) β-gal from *A. oryzae*.

of 64 and 90 mg g^{-1}, respectively. Adsorption of β-gal from *A. oryzae* on several silica supports has been studied previously, and the following maximum adsorption values were obtained: 13.5 mg g^{-1} (silochrome), 5.7 mg g^{-1} (SBA-15 type silica), 15.2 and 96 mg g^{-1} (biporous silica).[12] CIAP adsorption isotherms were obtained on several natural minerals[14] and microcrystalline cellulose, the maximum adsorption values of two bacterial phosphatase mutants on cellulose being 30 and 43 mg g^{-1}.[15] HRP adsorption isotherms were obtained on various silica adsorbents, including mesoporous molecular sieves.[16] The magnitude of adsorption is usually small owing to the formation of linear associates of HRP molecules on the carrier surface.[13]

Table 2 shows the sites of enzyme molecules ($S_{\text{single unit}}$) on the surface of silicalite-1 calculated from the maximum adsorption value and the external surface of the carrier (130 m^2 g^{-1}), as well as from the change of the external surface of the sample as a result of enzyme adsorption. The values obtained exceed those calculated from the geometric dimensions of the molecules by more than an order of magnitude. For example, the theoretical site of HRP molecule is 20 nm^2, and that of β-gal from *A. oryzae* is not more than 150 nm^2, while on the surface of silicalite-1 these values become ~ 2900–3200 and ~ 1000–1300 nm^2, respectively. Such large values of the sites of enzyme molecules are caused to a greater extent by the properties of the protein rather than the properties of the adsorbent, since there is a multicenter binding of proteins to the surface of the carrier. For example, the minimum value of the β-gal from *A. oryzae* molecule site on the surface of various silica adsorbents is 1700 nm^2.[12] The values of the areas of two enzymes with close molecular weights: HRP (M_R = 44000) and β-gal from bovine liver (M_R = 41000), differ from each other by a factor of 3–5. This discrepancy can be explained by the formation of linear associates of peroxidase molecules on the surface of the adsorbent.[13]

To clarify the mechanisms of interaction of enzyme molecules with a carrier, the isotherms of low-temperature nitrogen adsorption–desorption on silicalite-1 were explored together with those after adsorption of enzyme molecules on the carrier. The results obtained coincide with the information about conformation of adsorbed enzymes that could be provided by IR spectroscopy. It was shown earlier, that in the case of adsorption of BSA on silicalite-1 the content of α-helices and β-layers is reduced by more than 10%.[11] As a result of such disorder of the secondary structure, a considerable number of free functional groups in the side chains emerged. However, the IR data itself could not give an insight into the interactions between enzyme molecules and the carrier, so the nitrogen adsorption was applied.

When CIAP and β-galactosidase are adsorbed on silicalite-1 (Figure 2), the slope of the linear isotherms decreases in the relative pressure p/p_0 regions of 0.1–0.2 and 0.3–0.9. Thus, it can be concluded from the nitrogen adsorption data, that the surface of the enzyme–silicalite-1 system becomes more uniform. In addition, in the region of relative pressures p/p_0 = 0.1–0.25 a distinct hysteresis appears, indicating the absorption of nitrogen inside the globules of enzymes and the blocking of pores of silicalite-1 with a diameter of ~2 nm. Since the size of CIAP molecule is larger than that of β-galactosidase, the difference in the adsorption isotherms of the immobilized systems is more significant with the same amounts of enzymes introduced.

A sample of HRP on silicalite-1 has a wide hysteresis in the p/p_0 range of 0.1–0.6, which confirms the previously made assumption on the formation of enzyme associates with various lengths.

All studied enzymes are catalytically active on the surface of silicalite-1, however, their activity decreases by a factor of 4–9. At the same time, the Michaelis constant characterizing the affinity of the enzyme and the substrate increases by a factor of 2–7 (Table 2). The decrease in the catalytic activity of the adsorbed enzymes and the increase in the Michaelis constant are

Table 2 Adsorption data and catalytic activity of the enzymes.

Enzyme	Adsorption value/ mg g^{-1}	ΔS_{outer}/m^2 g^{-1}	$S_{\text{single unit}}$[a]/nm^2	$S_{\text{single unit}}$[b]/nm^2	K_M/mM		V_{max}/μmol s^{-1} mg^{-1}		V_{ads}/V_{sol}
					In solution	Adsorbed on silicalite-1	In solution	Adsorbed on silicalite-1	
HRP	1.9	75–80	2900–3100	3170	0.11	0.35	2.05	0.31	0.15
CIAP (dimer)	5.0	60–70	2600–3000	2800	0.31	2.13	2.20	0.25	0.11
β-gal from *A. oryzae*	4.0	25–30	1050–1250	1270	1.50	2.80	0.40	0.12	0.29
β-gal from bovine liver	1.0	10–15	675–1000	650	0.80	1.50	0.04	0.01	0.24

[a] Calculated from the change of the carrier's outer surface area after adsorption. [b] Calculated from the maximum adsorption values and outer surface area of silicalite-1.

owing to changes in the conformation of the protein globules upon adsorption, the formation of inactive associates and diffusion difficulties. Interaction of protein molecules on the surface may also significantly contribute to the deactivation of the catalyst in the adsorption layer.

On the surface of other silica adsorbents, enzyme activity decreases to a greater extent than on silicalite-1. Thus, β-gal from *A. oryzae* retains 14% of its activity after adsorption on silochrome and 22% in adsorption layers on biporous silica[12] compared to 29% of activity retained on silicalite-1 obtained herein. The data obtained in this work show that HRP retains 15% of its activity on the surface of silicalite-1. According to the published results, this enzyme retains only 10% of its solution activity being adsorbed on the surface of silochrome, 14% on SBA-15 carrier[16] and completely loses its catalytic activity when immobilized on cellulose.[17]

Thus, we have shown that the immobilization of enzymes on the surface of silicalite-1 does reveal some peculiarities. The adsorption isotherms are bended giving two values of maximum adsorption. The obtained results indicate the irreversible binding of protein molecules with the surface of silicalite-1 with 11–29% retention of the enzyme activity. In the case of HRP and β-gal from *A. oryzae*, the activity of the obtained biocatalytic systems is higher than in the case of other silica adsorbents described previously. The data on blocking the pores of silicalite-1 during the immobilization of enzymes were obtained for the first time by low-temperature nitrogen adsorption.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.09.033.

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