Systematic Study of Proteins Confined in Nanoporous SBA-15 with a Controlled Pore Size

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ABSTRACT

When protein molecules are strongly adsorbed on a heterogeneous surface, their activity in catalyzing chemical conversions can be altered with respect to free protein molecules in solution, depending on the geometrical and chemical structure of the surface. For example, on silica nanoparticles and carbon nanotubes, effects of convex surface curvature on protein stability and activity were noted. If proteins are similar in size to the pore diameter of mesoporous materials, adsorption on their surface leads to confinement effects that could also alter their activity. By using monodisperse SBA-15 mesoporous silica particles with high surface area, and carefully controlled unique pore diameter in the range of 5-11 nm, in which large amounts of proteins are strongly adsorbed from buffered solutions, effects of local curvature and protein-surface interactions could be studied in a consistent way.

Our studies of the catalytic activity of adsorbed lysozyme (Lz) and myoglobin (Mb) on SBA-15 with carefully controlled surface geometries illustrate that confinement of mesopores can affect the catalytic activity of the adsorbed protein. A decrease in catalytic activity of the same adsorbed proteins on SBA-15, of which the pore surface is modified with hydrophobic moieties, but which has a similar pore size to the original SBA-15, for which a remarkable increase in activity was noted, reveals that local surface chemistry also affects catalytic activity.

Various methods were used to analyze changes in structural conformation of free and adsorbed protein molecules on mesoporous SBA-15 with different pore sizes and surface properties, such as: a study of the effect of reaction temperature, activity ratio analysis, and liquid-phase FTIR spectroscopy using the second derivative of the protein amide I band. Mathematical modeling complemented the experimental studies. From these studies, the conformation of the adsorbed protein appears to be strongly correlated with the pore size, hence the curvature of the mesoporous support. The thermodynamics of protein adsorption were analyzed by using isothermal titration calorimetry (ITC). A negative value of the Gibbs free energy change for both Lz/SBA-15 and Mb/SBA-15 suggests that protein adsorption on mesoporous SBA-15 is spontaneous and thermodynamically favored. Distinct binding affinities for lysozyme adsorption on

mesoporous SBA-15 suggest that two different binding sites on mesoporous SBA-15 are available for protein adsorption. Strong binding attraction between lysozyme and SBA-15 suggests that the local surface of the nanopores provides an environment advantageous for binding interaction with lysozyme molecules. Differences in binding enthalpy and entropy for lysozyme and myoglobin adsorption on SBA-15 with different pore diameters are associated to the effect of surface curvature on the conformation of the adsorbed protein on SBA-15. Correlations between pore size/surface curvature, and protein binding enthalpy, entropy and secondary structure perturbation of the adsorbed protein reflect the underlying fundamental interactions between the protein and the surface of mesoporous SBA-15.

Catalytic activity of proteins can be significantly enhanced by confining the protein in a mesoporous support with a pore size similar to the protein size. The concave, negative pore curvature of SBA-15 with hydrophilic surface property has a remarkable confinement effect, higher than that noted for protein adsorption on silica nanoparticles or carbon nanotubes, which have a convex, positive surface curvature.