Investigations of the effects of mineral mesopores on the adsorption and preservation of organic matter

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ABSTRACT: Mineral mesopores (2-50 nm diameter) may protect organic matter (natural and pollutant) from microbial and fungal enzymatic degradation in soils and sediments. We tested the ‘mesopore protection hypothesis’ in a series of adsorption experiments, enzyme reactions and bacterial incubations with alumina and silica mineral analogues of varying porosity and amino acids and proteins. Small organic molecules (less than half the dimensions of the pores) adsorbed more strongly and desorbed less readily from internal-mesopore versus external mineral surfaces. Once sorbed, the degrees and rates of enzyme mediated degradation and bacterial utilization of mespore-sorbed molecules were found to be much lower than their externally-sorbed analogues. These results provide a potential mechanism for the selective sequestration and preservation of sedimentary organic matter and organic contaminants.

1 Introduction

Organic matter-mineral interactions may explain di-verse phenomena such as sequestration of pollutants and preservation of organic matter (OM) in soils and sediments. It is well known that mineral surface area may control mineral dissolution rates and mi-crobial interaction with mineral surfaces. However, surface morphology may also play an extremely im-portant role in microbial- and organic matter-mineral interactions. The observations of direct correlations between soil and sediment surface area and organic carbon content and bimodal rates of organic com-pound cycling within soils have led to the idea of a mineral surface-associated portion of the OM that is somehow protected from degradation. Because much of the surface of natural sediment and soil minerals is found within mesopores (2-50 nm diameter), it was proposed (Mayer, 1994) that OM sorbed within pores of this size may be protected from degradation by the exclusion of bacterial and fungal enzymes. We tested the viability of this protection mechanism with three sets of experiments;

1) a series of batch equilibrium adsorption/desorption experiments, reacting amino acid monomers, dimers, trimers and polymers (proteins) of varying size, charge and functionality with fabricated mineral analogues (silica and alumina) containing controlled and uniform mesoporosity,

2) experiments comparing the degradation of an amino acid, by the enzyme laccase while sorbed to nonporous versus mesoporous mineral surfaces, and

3) experiments comparing the growth rates of bacteria utilizing an amino acid substrate sorbed to nonporous versus mesoporous mineral surfaces.

2 METHODS

Amorphous mesoporous alumina and silica were synthesized using the methods of Komarneni et al. (1996) and Pauly & Pinnavaia (2001), respectively, to obtain powders with mean pore diameters of 8.2 and 4.0 nm, and specific surface areas of 242 and 962 m2g-1, respectively, as determined by N2 sorp-tometry. Nonporous alumina and silica powdered minerals (37 and 7.5 m2g-1, respectively) were cho-sen for the similarity of their surface chemistry to that of their mesoporous analogues.

Adsorption Experiments – Batch adsorption ex-periments were conducted by adding an aliquot of amino acid stock solution to centrifuge tubes con-taining a mineral suspension (3.2 x 10-3 m2 L-1; 0.02 M CaCl2 containing 200 mg L-1 HgCl2 was used as a background electrolyte solution). A pH of 5.7 was maintained by adding HCl or Ca(OH)2. After 5 days shaking, tubes were centrifuged, the supernatant re-moved, and replaced with OM-free background so-lution prior to a 5-day desorption period. The amount of compound sorbed or desorbed was calcu-lated as difference between the concentration in a control solution containing no mineral and that of the solution overlying each mineral. Amino acid and protein concentrations were measured on a fluoro-meter after the addition of a fluorescent derivative (Lindroth and Mopper, 1979).

Enzyme Degradation Experiments – Different amounts of nonporous and mesoporous alumina and silica with predetermined amounts of sorbed L-3-4-dihydroxyphenylalanine (L-DOPA), a model humic compound, was combined with an enzyme capable of degrading L-DOPA in an oxygen consuming re-action. The activity of the extracellular enzyme, laccase (from the fungi T. villosa, EC 1.10.3.2), was determined using a biological oxygen monitor mounted in a closed vessel. Oxygen concentration was recorded every 30 seconds after the introduction of 1 ul laccase solution (0.115 mg ml-1) through a side port by syringe to 5 ml aqueous solution (as above).

Bacterial Growth Experiments – Pseudomonas (P. putida and P. aeruginosa) inoculum was prepared in aqueous solution (as above) with the amino acid L-tyrosine. After incubation for one week at 32oC, cells were harvested and washed 3 times by centrifugation at 4500 rpm. The inoculums were di-luted to 103 cells ml-1 and 100 ml was added to planktonic (5 ml in round bottom glass tubes, shaken) and biofilm (petri dishes, not shaken) cul-tures. Both strains of Pseudomona were provided with either free L-tyrosine, mesoporous alumina-sorbed, or nonporous alumina-sorbed tyrosine (80 m2 equivalent mineral for each). Planktonic cell cul-tures were counted every second day by smearing a homogeneous sample of the culture (including min-eral powder) onto nutrient agar plates and counting colonies grown after 1-2 days (32oC). The relative development of biofilm, or more precisely attached cells, in unstirred cultures, was quantified by rinsing three times to remove unattached cells, adding 1% crystal violet dye, followed by extraction of the dye into ethanol and measurement of absorbance at 580 nm.

3 RESULTS

Adsorption Experiments – All amino acid monomers and polymers smaller than about one-half the mesopore diameter exhibited significantly greater adsorption (on a surface area normalized basis) to mesoporous alumina (8.2 nm mean pore diameter) and silica (3.4 nm mean pore diameter) versus nonporous phases (Fig. 1). Amino acid polymers (lysozyme, albumin, γ-globulin) of sizes approaching and larger than the mesopores, however, exhibited greater adsorption to the nonporous phases indicating their exclusion from the internal surfaces of the mesoporous minerals.

Further, evidence for enhanced retention of small organic compounds within mesopores was found in increased desorption hysteresis for mesoporous versus nonporous sorbent-sorbate pairs (e.g., Fig. 2) and decreased post-adsorption surface areas. Adsorption isotherms were successfully modeled using a hybrid Langmuir-Freundlich approach that provided additional binding parameters such as sorbent-sorbate affinity distribution and surface heterogeneity indices.

Capillary condensation, or ‘pore-filling’, is the mechanism proposed to explain the experimental observations. That is, a unique chemical environment exists within pores, due perhaps both to electric double layer overlap and water exclusion, such that multiple layers of sorbate may be more energetically favorable. Thus, adsorption is enhanced and desorption is inhibited.

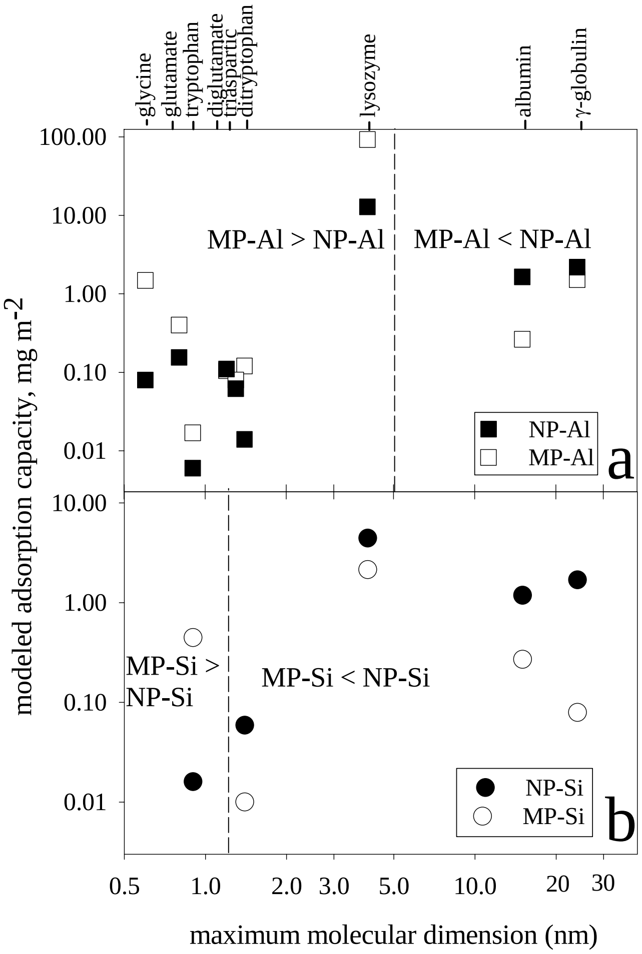

Figure 1. Maximum adsorption capacity of (a) nonporous (NP-Al) versus mesoporous (MP-Al) alumina and (b) nonporous (NP-Si) versus mesoporous (MP-Si) silica.

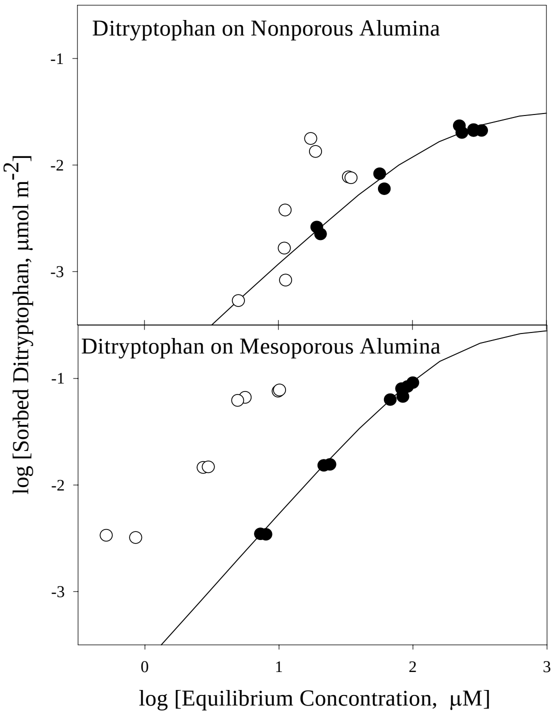

Figure 2. Adsorption (closed circles)/desorption (open circle) isotherms for ditryptophan on nonporous and mesoporous alumina. Line is Langmuir-Freundlich modeled isotherm.

Enzyme Degradation Experiments – Conversion of dissolved free L-DOPA to a quinone product (see Fig. 1 inset) was well-modeled by the Michaelis-Menton kinetic equation and yielded a V_{max} of 1.06 µmol O_2 consumed min^{-1} (equivalent to conversion of 2.12 µmol L-DOPA min^{-1}). Mineral-sorbed L-DOPA, however, was less available to laccase-mediated conversion. The maximum observed conversion rates of mineral-sorbed L-DOPA (on NP-Si) was 0.8 µmol L-DOPA min^{-1} and graphs of laccase activity versus substrate concentration (Fig. 3) were indicative of enzyme inhibition processes, probably due to mineral adsorption of laccase. Further, both the rate and the total amount of laccase-mediated conversion of nonporous alumina and silica-sorbed L-DOPA were greater than that of L-DOPA sorbed to mesoporous mineral analogues. For similar initial L-DOPA concentrations, initial laccase activity was 5 to 27 times greater and total laccase activity was 4 to 36 times greater for nonporous versus mesoporous alumina-sorbed L-DOPA.

Bacterial Growth Experiments – Cultures of the *P. putida* and *P. aeruginosa* were found to increase in number of cells (from 10^3 to 10^{5-7}) over the first two days of incubation and maintain constant cell numbers over the course of the following eight days when supplied with free dissolved L-tyrosine.

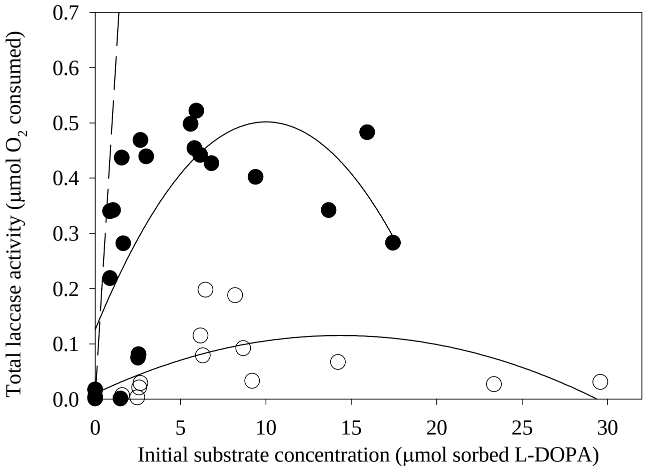

Figure 3. Total activity of laccase-mediated degradation of L-DOPA sorbed to nonporous (closed circles) and mesoporous (open circles) alumina. Data fit to a quadratic function. Dashed line represents stoichiometric enzyme-substrate reaction.

Cell-numbers decreased over the incubation period, however, when supplied with equivalent amounts of mineral-sorbed tyrosine. After six days of incubation, there were three orders of magnitude more *P. putida* cells and one order of magnitude more *P. aeruginosa* cells when grown on nonporous alumina-sorbed tyrosine versus mesoprous alumina-sorbed tyrosine (using equivalent amounts of mineral surface area and sorbed tyrosine). Unstirred incubations of both *P. putida* and *P. aeruginosa* with alumina resulted in attached cells (biofilm) that were not observed in the absence of mineral powders. Greater cell attachment occurred when mineral-sorbed versus free tyrosine was present (40 and 22 times more attached *P. putida* and *P. aeruginosa*, respectively). Secondly, greater *P. putida* and *P. aeruginosa* attachment occurred on nonporous and mesoporous minerals with versus without sorbed tyrosine (Figure 4). Lastly, greater cell attachment occurred on nonporous versus mesoporous alumina (11 and 27 times more attached *P. putida* and *P. aeruginosa*, respectively; Figure 4).

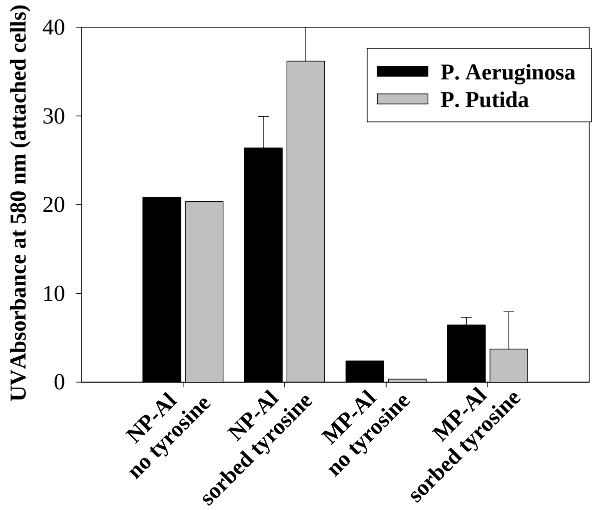

Figure 4. Attached *Pseudomonas* cells in unstirred incubations of nonporous (NP-Al) and mesoporous (MP-Al) alumina with and without sorbed tyrosine.

4 CONCLUSION

This series of experiments establishes both the viability and the probability of the mesopore protection mechanism for natural organic matter and organic contaminant preservation in sediments and soils. The adsorption experiments show that organic molecules as large as small proteins may be adsorbed within mineral mesopores and held strongly. That is, desorption is inhibited due to the presence of unique forces within mesopores such as capillary filling and the creation of a hydrophobic nanoenvironment. Additionally, we have experimentally demonstrated a decreased susceptibility of mineral-adsorbed organic compounds both to enzyme-mediated degradation and to bacterial utilization. This effect is enhanced many times by the presence of mineral mesoporosity. It is, therefore, reasonable to conclude that this protection mechanism occurs in natural systems. The extent and type of organic matter that may be sequestered within mineral mesopores over long periods of time, however, will be dependant upon the types of mineral surfaces and sizes of mineral mesopores actually present in natural soils and sediments. Future work will focus on examining mesopore adsorption and protection processes using natural assemblages of organic compounds and mineral surfaces.

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