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Sol−Gel Materials as Efficient Enzyme Protectors: Preserving the Activity of Phosphatases under Extreme pH Conditions

Hagit Frenkel-Mullerad and David Avnir*

Contribution from the Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Received February 5, 2005; E-mail: david@chem.ch.huji.ac.il

Abstract: By entrapment in (surfactant modified) silica sol−gel matrixes, alkaline phosphatase (AlP) − naturally with optimum activity at pH 9.5 − was kept functioning at extreme acidic environments as low as pH 0.9, and acid phosphatase (AcP) − naturally with optimum activity at pH 4.5 − was kept functioning at extreme alkaline environments, up to pH 13.0. Propositions are offered as to the origin of the ability of the matrixes to provide such highly efficient protection and as to the origin of the synergetic enhancing effect when both the silica and the surfactants are used as a combined entrapping environment. It was found that the protectability of the enzymes against harsh pH values is dependent on the nature of the surfactant.

Background

As the name of the enzyme alkaline phosphatase (AlP) implies, its catalytic activity (hydrolysis of phosphoesters to phosphate and to the corresponding alcohol or phenolate) is optimal at basic pH values (9−10[1,2]). Here, we show that by utilizing the protective features of sol−gel materials, one can keep this alkaline enzyme active under extreme *acidic* conditions, going down the pH scale to as low as pH 0.9!, and that when, for comparison purposes, the acidic enzyme acid phosphatase (AcP, optimal performance at pH 4.5−6.0[2]) is entrapped in these materials, it is kept active under extreme *alkaline* conditions, as high as pH 13. Silica-based sol−gel materials, with and without surfactant modification, were the key to these unusually large effects, which amount to practical alteration of the classical phosphatases' properties.

We recall that sol−gel materials have proven in the last two decades to be versatile carriers of active dopants.[3−5] Diverse reactive functionalities have been introduced into these materials by either direct physical doping[6−8] or covalent attachment.[9−11] Of the various families of functional sol−gel materials that have been developed, one, which has progressed particularly fast, has been the family of sol−gel materials with biochemical and biological activities.[12−16] Enhanced stability of entrapped biomolecules,[17] ease of their heterogenization,[18] compatibility with opposing reagents,[19] the convenience of tailoring the chemical and physical properties as needed for specific bioapplications,[17] improved endurance of the entrapped proteins to denaturing thermal conditions,[17] to long-term storage conditions,[20,21] and to organic solvents[22,23] are but some of the reasons for this fast growth. Here, we show an extreme pH-protectability of enzymes provided both by silica sol−gel matrixes and through *synergism* between matrix and surfactant interactions, thus utilizing yet another observation, namely, that the properties of dopants can be tailored and modified by the coentrapment of surfactants within sol−gel materials.[24,25] Finally, we note that the interaction of enzymes with surfactants in solution was studied in various contexts, such as providing enzymes with hydrophobic working environments[26,27] and shifting the optimal pH for activity.[28,29]

Results and Discussion

The activity of AlP entrapped in three types of sol−gel matrixes is shown in Figure 1A, and in Figure 1B, it is compared to the activity in solution.

(1) Fernley, H. N. Mammalian Alkaline Phosphatases. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1971; Vol. IV, pp 417−447.
(2) McComb, R. B.; Bowers, G. N.; Posen, S. *Alkaline Phosphatase*; Plenum Press: New York, 1979.
(3) Ciriminna, R.; Pagliaro, M. *Chem.−Eur. J.* **2003**, *9*, 5067−5073.
(4) Dave, B. C.; Ottosson, J. E. A. *J. Sol-Gel Sci. Technol.* **2004**, *31*, 303−307.
(5) Barbe, C.; Bartlett, J.; Kong, L.; Finnie, K.; Lin, H. Q.; Larkin, M.; Calleja, S.; Bush, A.; Calleja, G. *Adv. Mater.* **2004**, *16*, 1959−1966.
(6) Gelman, F.; Blum, J.; Schumann, H.; Avnir, D. *J. Sol-Gel Sci. Technol.* **2003**, *26*, 43−46.
(7) Zhang, J.; Au, K. H.; Zhu, Z. Q.; O'shea, S. *Opt. Mater.* **2004**, *26*, 47−55.
(8) Gutierrez, J. A. R.; Dominguez, M. D. P.; Macias, J. M. P. *Anal. Chem. Acta* **2004**, *524*, 339−346.
(9) Brusatin, G.; Innocenzi, P.; Guglielmi, M.; Babonneau, F. *J. Sol-Gel Sci. Technol.* **2003**, *26*, 303−306.
(10) Frenkel-Mullerad, H.; Avnir, D. *Chem. Mater.* **2000**, *12*, 3754−3759.
(11) Fireman-Shoresh, S.; Avnir, D. *Langmuir* **2001**, *17*, 5958−5963.
(12) Wei, Y.; Dong, H.; Xu, J.; Feng, Q. *ChemPhysChem* **2002**, *9*, 802−807.
(13) Reetz, M. T.; Tielmann, P.; Wiesenhofer, W.; Konen, W.; Zonta, A. *Adv. Synth. Catal.* **2003**, *345*, 717−728.
(14) Gill, I. *Chem. Mater.* **2001**, *13*, 3404−4321.
(15) Jing, J. C.; Chuang, M. H.; Lan, E. H.; Dunn, B.; Gillman, P. L.; Smith, S. M. *J. Mater. Chem.* **2004**, *14*, 2311−2316.
(16) Nassif, N.; Bouvet, O.; Rager, M. N.; Roux, C.; Coradin, T.; Livage, J. *Nat. Mater.* **2002**, *1*, 42−44.
(17) Brennan, J. D.; Benjamin, D.; DiBattisa, E.; Gulcev, M. D. *Chem. Mater.* **2003**, *15*, 737−745.
(18) Braun, S.; Rappoport, S.; Zusman, R.; Avnir, D.; Ottolenghi, M. *Mater. Lett.* **1990**, *10*, 1−5.
(19) Gelman, F.; Blum, J.; Avnir, D. *New J. Chem.* **2003**, *27*, 205−207.
(20) Shtelzer, S.; Braun, S. *Biotechnol. Appl. Biochem.* **1994**, *19*, 293−305.
(21) Besanger, T. R.; Chen, Y.; Deisingh, A. K.; Hodgson, R.; Jin, W.; Mayer, S.; Brook, M. A.; Brennan, J. D. *Anal. Chem.* **2003**, *75*, 2382−2391.
(22) Li, J.; Tan, S. N.; Oh, J. T. *J. Electroanal. Chem.* **1998**, *448*, 69−77.
(23) vanUnen, D.; Engberson, J. F. J.; Reinhoudt, D. N. *Biotechnol. Bioeng.* **2001**, *75*, 154−158.
(24) Rottman, C.; Grader, G.; Hazan, Y. D.; Melchior, S.; Avnir, D. *J. Am. Chem. Soc.* **1999**, *121*, 8533−8543.
(25) Badjic, J. D.; Kostic, N. M. *J. Phys. Chem. B* **2001**, *105*, 7482−7489.
(26) Gladilin, A. K.; Levashov, A. V. *Biochemistry (Moscow)* **1998**, *63*, 345−356.