Sol-gel materials as efficient enzyme protectors: Preserving the activity of phsophatases under extreme pH conditions

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Supporting Information

Experimental details

Chemicals: Tetramethoxysilane (TMOS), alkaline phosphatase (AlP, cat. no. P-3681), acid phosphatase (AcP, cat. no. P-0157), p-nitrophenyl phosphate (pNPP), dioctylsulfosuccinate sodium salt (AOT), cetyl-trimethylammoniumbromid (CTAB), bromocresol green, were all obtained from Sigma-Aldrich. Glycine buffers were prepared from glycine solutions (0.05 M; from BDH) containing 1 mM $MgCl_2$ with the desired volumes of 1.0 M NaOH or 1.0 M HCl.

Enzyme entrapment procedures: A mixture of 0.4 ml TMOS (0.003 mole) and 0.49 ml 2.5 mM HCl ($1.2x10^{-6}$ mole) was stirred for 30 minutes in a vial at 40 ^{O}C (the "two-step" method[1]). The resulting sol was cooled for 30 min at 4 ^{O}C. In a separate vial, 0.93 ml AlP (7.35 U/ml) was mixed with 0.04 ml of methanolic solution of the desired surfactant, the concentration of which was 100 mM, for 5 minutes (the final concentration of the surfactant in the gel was 2.15 mM. CTAB's CMC is 0.92 mM and that of AOT is 0.68 mM[2]). For entrapment without the surfactant, MeOH was added instead of the methanolic surfactant solution. The enzyme solution was then transferred to the ice-cooled sol solution and mixed briefly. From this mixture, 0.05 ml was transferred to polystyrene cuvettes and left to gel (2-3 minutes). Ten minutes later the gels were covered with 0.25 ml of a glycine-NaOH buffer solution (pH 9.5), sealed with Parafilm and left to age at 4 ^{O}C for 24 hours. One day after preparation, 0.05 ml of the supernatant buffer solution was transferred to cuvettes in order to determine the amount that was not entrapped (by checking the enzymatic activity of this solution, as described below). Residual un-entrapped enzyme was then removed by replacing the remaining buffer solution with 0.25 ml of fresh buffer and incubating again at 30 ^{O}C for 30 min. This rinse was also tested for enzymatic activity. The third rinse was found to be enzyme free, and these sol-gel materials – AlP@SG - were used for the further study. We refer to enzyme concentration within the gels the amount used for entrapment minus the various washings. For comparative purposes AcP was entrapped under a similar procedure, replacing the above buffer with a glycine-HCl buffer solution (pH 4.5). In this case, gelation occurred after about 2 h, resulting in AcP@SG.

Determination of reaction rates of the entrapped enzymes under extreme conditions: Prior to the kinetics measurement the AlP@SG samples were rinsed by replacing the buffer solution with fresh one (0.25 ml), leaving it for incubation at 30^{O}C for 30 min. The buffer solution was then replaced with 0.25 ml of 2.7 mM of the substrate

(pNPP) in the glycine solution (saturation concentration for enzyme activity in gel and in solution; data not shown). The pH of which (optimal, non-optimal and extreme) was brought to the desired value with the addition of NaOH or HCl solutions (keeping the pNPP concentration at 2.7 mM). The initial rate of the reaction (V, taken as the initial slope of the kinetic graph. In cases were a lag appeared due to diffusion the slope was calculated from the end of that lag) was determined by following the formation of the p-nitrophenol (pNP) absorption maximum at 405 nm (using HP-8453 spectrophotometer). The reported results are the mean of 3 to 6 experiments. As for AcP@SG, the pNPP concentration was kept the same for comparison purposes, (2.7 mM) and the reaction kinetics was measured similarly in solution by mixing 0.043 ml methanolic surfactant solution with 1.95 ml buffer solution containing 3.8 U/ml AlP (or AcP). Identical concentrations of the enzyme and surfactant were in the solid material.

Matrix stability under high pH values was tested, since sol-gel matrices are known to tend to decompose at these pH values. However, our formulation was found to result in a stable matrix, as indicated, for instance in the following experiment: A matrix containing 100 CTAB, bromocresol green and AlP was prepared as described above (the indicator concentration in the enzyme solution was 1%). The glycine buffer above the samples was replaced as described above, and 0.250 ml of buffer at pH 12.8 was added. The sample was then incubated for 60 min at $30^{O}C$. The supernatant buffer was collected again and tested for bromocresol green by measuring its typical absorbance at 615 nm. No absorbance was determined (at the limit of detection of the spectrometer), confirming the ability of the matrix to withstand the alkaline environment for that period of time.

Determination of the enzymatic activity constants: The activity parameters of entrapped AlP, namely the Michaelis-Menten (MM) constant, Km, the reaction rate, Vmax (both extracted from the Lineweaver-Burk (LB) representation of the MM equation), and the apparent turn-over-number (T.O.N., calculated by dividing the rate of reaction at substrate saturation by the concentration of the entrapped enzyme), were determined by using different pNPP concentrations, ranging from 0.022 to 2.7 mM in glycine buffer. AcP - which was used for comparison - did not reveal MM behavior.

References

1. Brinker, C. J.; Keefer, K. D.; Schaefer, D. W.; Ashley, C. S., *J. Non-Cryst. Solids* **1982,** 48, 47-64.

2. Rosen, M. J., *Surfactants and Interfacial Phenomena*. John Wiley & sons, Inc.: 1978.