Chemistry SELECT

Supporting Information

Ionic Liquid-Based Periodic Mesoporous Organosilica: An Innovative Matrix for Enzyme Immobilization

Mitra Khademy, Babak Karimi, and Shekufeh Zareian*

Experimental Section

Large-pore SBA-15 and PMO-IL (containing 10 mol% of IL) were synthesized by previous procedures with a few changes [1]. All enzyme assays were done in 3 min and at 37°C and activity measurement was done by dinitrosalicylic acid method (Bernfeld, 1955) [2]. For enzyme immobilization, the enzyme solution was prepared in 25 mM Tris-HCl, pH 7.5. The concentration of enzyme solution was optimized at 0.5 mg/ml. 10 mg silica support was mixed with 1 ml of the enzyme solution at 4°C on a magnetic stirrer. Leaching and loading of protein was measured by UV-absorbance at 280 nm. Free and immobilized enzyme activity was calculated in different concentrations of starch solution, from 0.1 to 1.5 g/dl in monobasic sodium phosphate buffer 20 mM, pH 6.9. Kinetics parameters were obtained by GraphPad Prism 6.07. For thermal stability samples were incubated in 25 mM Tris-HCl buffer solution, pH 7.5, at 70°C and 80°C for 60 min with 10 min intervals. For pH stability free and immobilized enzymes were incubated for 40 min at room temperature in different pH conditions, pH 3.0, 4.0, 5.0, 6.0, 7.0 and 7.6, which were made by mixing citric acid and sodium phosphate buffers. Enzyme activity was obtained afterwards. For recyclability measurements 1 ml immobilized enzyme was added to 1 ml of starch 1% (w/v) and incubated for 25 min at 37°C. 50 µl was collected for activity measurement, and the rest of enzyme-substrate mixture was washed with buffer Tris-HCl to get rid of substrate and product. Remaining immobilized enzyme used for the next cycle. This process was repeated 6 rounds for α-amylase@PMO-IL. It is worth noting that for each sample, enzyme leaching during incubation time was measured and subtracted from immobilized amount. Enzyme activity was normalized based on final enzyme amount.

[1] aR. B. Bedford, P. Braunstein, M. Veith, C. S. Cazin, Synthesis **2005**, 2005, 622-626; bB. Karimi, D. Elhamifar, J. H. Clark, A. J. Hunt, Chem. Eur. J. **2010**, 16, 8047-8053; cB. Karimi, D. Elhamifar, O. Yari, M. Khorasani, H. Vali, J. H. Clark, A. J. Hunt, Chem. Eur. J. **2012**, 18, 13520-13530.

[2] P. Bernfeld, in Methods in Enzymology, Vol. Volume 1, Academic Press, **1955**, pp. 149-158.