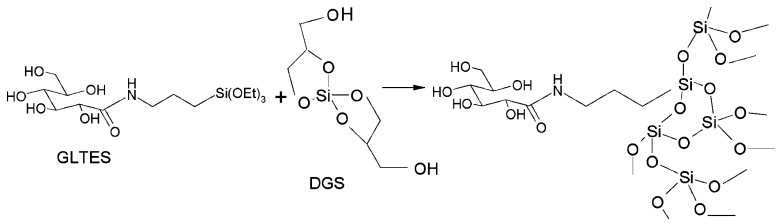
Communication

Ultrasensitive ATP Detection Using Firefly Luciferase Entrapped in Sugar-Modified Sol–Gel-Derived Silica

Jorge A. Cruz-Aguado, Yang Chen, Zheng Zhang, Nadine H. Elowe, Michael A. Brook, and John D. Brennan

J. Am. Chem. Soc., **2004**, 126 (22), 6878-6879• DOI: 10.1021/ja0490424 • Publication Date (Web): 14 May 2004



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 3 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Published on Web 05/14/2004

Ultrasensitive ATP Detection Using Firefly Luciferase Entrapped in Sugar-Modified Sol−Gel-Derived Silica

Jorge A. Cruz-Aguado,[†] Yang Chen,[†] Zheng Zhang,[†] Nadine H. Elowe,[‡] Michael A. Brook,[†] and John D. Brennan*,[†]

Department of Chemistry, McMaster University, 1280 Main Street West, Hamilton ON, Canada L8S 4M1, and HTS Lab, Department of Biochemistry, McMaster University, 1200 Main Street West, Hamilton ON, Canada L8N 3Z5

Received February 19, 2004; E-mail: brennanj@mcmaster.ca

ATP is present in all living cells; hence, the ATP level on a surface or in a medium is a useful indicator of biological contamination that is either of human or bacterial origin. A prevalent method for detection of ATP is via bioluminescence using the ATP-dependent luciferase−luciferin reaction.[1] In solution, detection limits of 10^{-15} g (ca. 1 amol) of ATP can be achieved, corresponding to a single bacterial cell.[2,3] However, when used in solution, luciferase is unstable and cannot be reused.[4] Most of the methods developed thus far to immobilize luciferase involve the covalent attachment of firefly luciferase (FL) to a solid support, which is generally accompanied by a substantial loss in enzyme activity.[5] Retention of high levels of enzyme activity in relation to solution was achieved by Filippova et al. through the immobilization of FL on film carriers.[6] However, the retention of FL activity in the membrane was only about 10% after several washes.

One approach that has been used to immobilize a number of proteins has been entrapment in inorganic glasses formed by the sol−gel processing method.[7] However, in cases where either low pH or high alcohol levels destabilize proteins, encapsulation can result in a substantial loss of biological function[8] and can cause significant structural changes in proteins immediately upon entrapment and as the materials age.[9] It is worth noting that active FL has not been entrapped in sol−gel-derived materials, although other bioluminescent proteins such as aequorin have been entrapped in high water content silica materials.[10]

In this work, we have prepared sol−gel precursors that are based on covalent linkage of D-gluconolactone or D-maltonolactone to (aminopropyl)triethoxysilane to form *N*-(3-triethoxysilylpropyl)-gluconamide (GLTES) or *N*-(3-triethoxysilylpropyl)maltonamide (MLTES).[11] Hydrolysis and condensation of these species, along with diglycerylsilane, results in a material that has nonhydrolyzable sugar moieties covalently bound into the silica network.

Figure 1 shows the relative activity of FL in materials derived from TEOS, evaporated TEOS (EvTEOS),[12] sodium silicate (SS),[13] and diglycerylsilane (DGS)[14] that contain either a soluble sugar (AllylGL) or covalently bound GLTES or MLTES (experimental procedures are provided in the Supporting Information). EvTEOS, SS, and DGS were chosen since they are all commonly used biocompatible precursors for sol−gel synthesis. TEOS was selected since it has been widely employed for protein entrapment. No FL activity was detected in any of the unmodified silica-derived materials, including DGS-derived glasses. However, there was substantial enzymatic activity observed for sugar-modified SS- and DGS-derived silica containing 10−20 mol % of GLTES or MLTES. Interestingly, adding GLTES to TEOS had no beneficial effect, showing that the ethanol liberated from TEOS denatures FL even when GLTES is present. Thus, the data show that both a biocompatible precursor and a covalently bound sugar are needed to achieve activity for FL. It must be noted that such materials are the first sol−gel-based bioencapsulates to show any appreciable FL activity. Similar increases in activity in the presence of GLTES and MLTES were also observed for entrapped urease and Factor Xa (see Supporting Information).

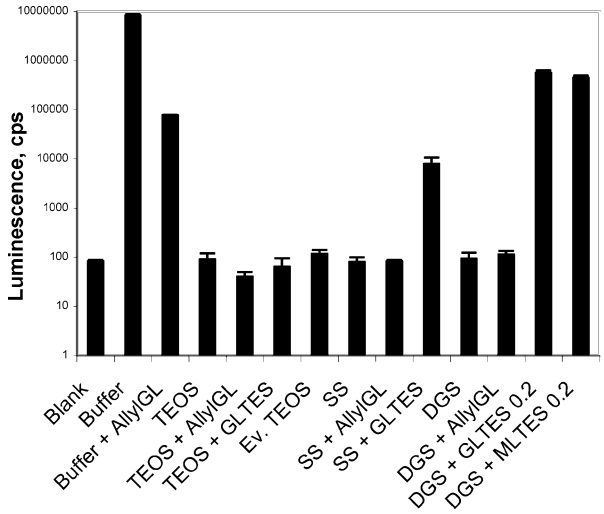


Figure 1. Relative activity of FL entrapped in a range of silica materials, showing significant activity only in sugar-derived silica glasses. All sugar-derived glasses contain 20 mol % of the sugarsilane.

The covalent incorporation of gluconamide or maltonamide into the silica matrix results in a material that shows less shrinkage (15% v/v for DGS/GLTES, 70% v/v for DGS, 85% for TEOS or SS after drying), larger pore diameters (7.3 nm for DGS/GLTES, 4.6 nm for DGS, 2.6 nm for TEOS or SS after drying), and higher enzyme activity than alkoxysilane- or DGS-derived materials.[11b,12] To demonstrate the importance of covalently tethering the sugar to the silica, assays were performed in TEOS-, SS-, and DGS-derived materials that had free allylGL (0.1 mole ratio); such materials did not show any enzymatic activity. The results show that only covalently tethered sugars lead to improved enzyme activity, indicating that it is not direct effects of the sugars on the enzyme that are most important, but rather the effect of the sugars on the structure of the material (i.e., degree of cross-linking and pore size) that result in improved FL activity. It is also likely that the coverage of the silica pore surface with nonhydrolyzable sugars may reduce adsorption of protein onto the silica surface, thus reducing the tendency for protein denaturation.

Figure 2 shows the variation in catalytic efficiency with the reuse for firefly luciferase entrapped in a 0.2 mol:mol GLTES/DGS gel.

[†] Department of Chemistry.
[‡] Department of Biochemistry.

10.1021/ja0490424 CCC: $27.50

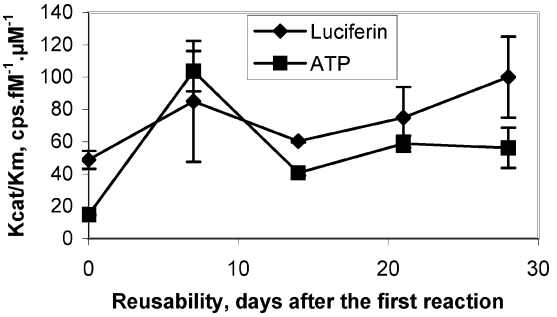


Figure 2. Variation in catalytic efficiency with reuse for firefly luciferase entrapped in a 0.2 mol:mol GLTES/DGS gel.

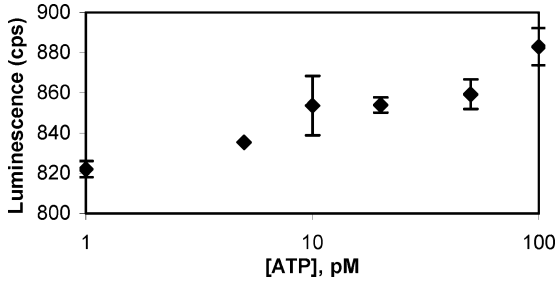


Figure 3. Sensitivity toward ATP of FL entrapped in 0.2 mol:mol GLTES/DGS. Errors represent RSD over three replicates. The value of the blank was 760 ± 5 cps.

The initial K_M values of the enzyme entrapped in 0.2 mol:mol GLTES/DGS materials in relation to luciferin and ATP were 4.9 ± 1.3 and 5.2 ± 0.4 μM, respectively, just slightly higher than the corresponding values in solution, 2.4 ± 0.2 and 3.4 ± 0.1 μM. This result shows that the accessibility of substrates to the enzyme in the sugar-modified sol−gel-derived silica was relatively high. Our results also demonstrate higher accessibility of the substrates to the enzyme in relation to the data reported for other immobilization methods. For example, the K_M values for luciferin and ATP in a system where the FL was covalently immobilized in agarose beads were 12.5 and 177.3 μM, respectively;[5f] in epoxy methacrylate the values were 89 and 6.6 μM;[5d] and in sepharose the values were 5.5 and 300 μM, respectively.[5a]

As shown in Figure 2, the kinetic parameters of the entrapped enzyme were highly stable after reusing the gel in catalytic reactions over five cycles. There was no enzymatic activity in the washing solution before the assay, which rules out leaching of the enzyme from the gel as the source of the activity. The increase in catalytic efficiency of the encapsulated enzyme after the first use is not fully understood, but has been observed previously for enzymes entrapped in silica.[14] It is possible that during the drying of the gel, part of the enzyme became highly adsorbed to the silica surface. The interaction with at least one of the substrates during the first catalytic cycle may change the conformation of the enzyme, releasing it to the void volume of the pores and making the protein available for both substrates in the second catalytic cycle. We cannot exclude the possibility that a fraction of the luciferin remained in the gel after the first catalytic cycle. After five catalytic cycles, both K_m and k_{cat} decreased, leading to an increase in K_m/k_{cat} for luciferin.

Figure 3 shows the use of the FL-DGS/GLTES system as a sensor with high sensitivity toward ATP detection. We were able to measure a concentration as little as 1 pM ATP, which means a quantity of 20 amol ATP in our detection volume of 20 μL (S/N = 3). This corresponds to the total quantity of ATP in about 20 cells. Similar values of ATP detection with immobilized FL have been reported elsewhere.[5d,6] However, this is the first time FL has been encapsulated in a sol−gel system in a reusable format, and hence, this work paves the way for further development of organically modified glasses with higher sensitivity and higher retention of enzyme activity, which may push detection limits to even lower levels.

Acknowledgment. We thank MDS-Sciex, NSERC, the Ontario Ministry of Energy, Science and Technology, the CFI, and OIT for support of this work. J.D.B. holds the Canada Research Chair in Bioanalytical Chemistry.

Supporting Information Available: Experimental procedures and figures of relative activity of FL, urease, and Factor Xa in materials prepared with a range of molar ratios of sugar/DGS and variation of kinetic constants of FL after reusing the entrapped enzyme a number of times. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Gorman, M. W.; Marble, D. R.; Ogimoto, K.; Feigi, E. O. *Luminescence* **2003**, *18*, 173. (b) Maeda, M. *J. Pharm. Biomed. Anal.* **2003**, *30*, 1725. (c) Miska, W.; Geiger, R. *J. Clin. Chem. Clin. Biochem.* **1987**, *25*, 23.
(2) Lundin, A. *VTT Symp.* **1999**, *193*, 337.
(3) (a) Adachi, Y.; Sugawara, M.; Taniguchi, K.; Umezawa, Y. *Anal. Chim. Acta* **1993**, *281*, 577. (b) Yamana, K.; Ohtani, Y.; Nakano, H.; Saito, I. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3429.
(4) Ribeiro, A. R.; Santos, R. M.; Rosario, L. M.; Gil, M. H. *J. Biolumin. Chemilumin.* **1998**, *13*, 371.
(5) (a) Ugarova, N. N.; Brovko, L. Y.; Kost, N. V. *Enz. Biochem. Biotechnol.* **1982**, *4*, 224. (b) Wienhausen, G. K.; Kricka, L. J.; Hinkley, J. E.; DeLuca, M. *Appl. Biochem. Biotechnol.* **1982**, *7*, 463. (c) Carrea, G.; Bovara, R.; Mazzola, G.; Girotti, S.; Roda, A.; Ghini, S. *Anal. Chem.* **1986**, *58*, 331. (d) Carrea, G.; Bovara, R.; Girotti, S.; Ferri, E.; Ghini, S.; Roda, A. *J. Biolumin. Chemilumin.* **1989**, *3*, 7. (e) Blum, L. J.; Coulet, P. R.; Gautheron, D. C. *Biotechnol. Bioeng.* **1985**, *27*, 232. (f) Eu, J.; Andrade, J. *Luminescence* **2001**, *16*, 57.
(6) Filippova, N. Yu.; Dukhovich, A. F.; Ugarova, N. N. *J. Biolumin. Chemilumin.* **1989**, *4*, 419.
(7) (a) Jin, W.; Brennan, J. D. *Anal. Chim. Acta* **2002**, *461*, 1. (b) Gill, I. *Chem. Mater.* **2001**, *13*, 3404.
(8) (a) Miller, J. M.; Dunn, B.; Valentine, J. S.; Zink, J. I. *J. Non-Cryst. Solids* **1996**, *220*, 279. (b) Dave, B. C.; Miller, J. M.; Dunn, B.; Valentine, J. S.; Zink, J. I. *J. Sol.-Gel Sci. Technol.* **1997**, *8*, 629.
(9) (a) Eggers, D. K.; Valentine J. S. *Protein Sci.* **2001**, *10*, 250. (b) Flora, K. K.; Brennan, J. D. *Chem. Mater.* **2001**, *13*, 4170.
(10) Blyth, D. J.; Poynter, S. J.; Russell, D. A. *Analyst* **1996**, *121*, 1975.
(11) (a) Tleugabulova, D.; Zhang, Z.; Chen, Y.; Brook, M. A.; Brennan, J. D. *Langmuir* **2004**, *20*, 848. (b) Brook, M. A.; Brennan, J. D.; Chen, D. Y. U. S. Provisional Patent 60/384,084, 2002.
(12) Ferrer, M. L.; del Monte, F.; Levy, D. *Chem. Mater.* **2002**, *14*, 3619.
(13) Bhatia, R. B.; Brinker, C. J.; Gupta, A. K.; Singh, A. K. *Chem. Mater.* **2000**, *12*, 2434.
(14) 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.

JA0490424