J|A|C|S
COMMUNICATIONS

Published on Web 10/04/2002

A Novel Strategy for Encapsulation and Release of Proteins: Hydrogels and Microgels with Acid-Labile Acetal Cross-Linkers

Niren Murthy, Yi X. Thng, Stephany Schuck, Ming C. Xu, and Jean M. J. Fréchet*

Center for New Directions in Organic Synthesis, Department of Chemistry, University of California, Berkeley, California 94720-1460, and Lawrence Berkeley National Laboratory, Materials Sciences Division, Berkeley, California 94720

Received May 15, 2002

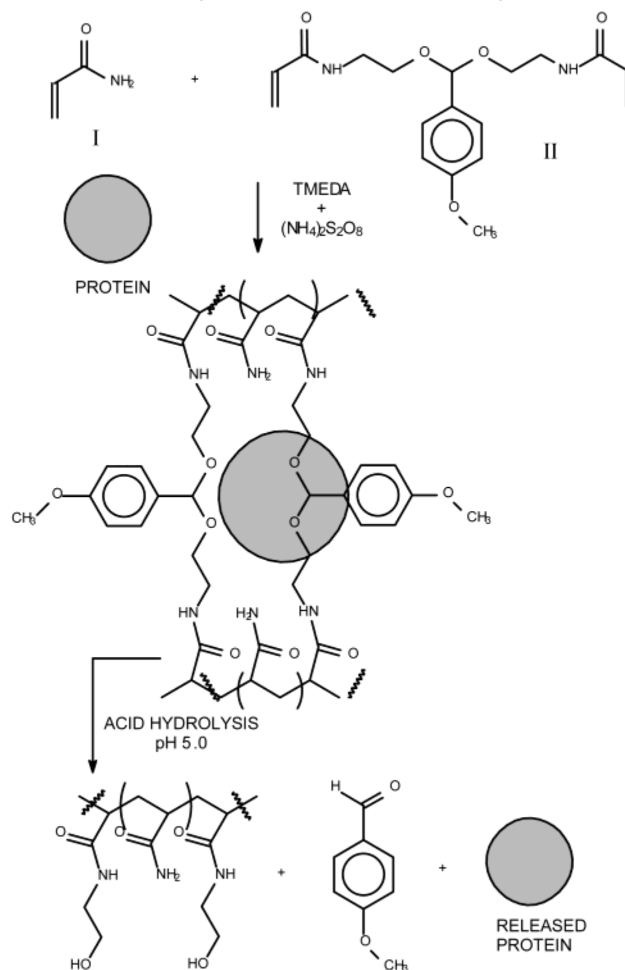
Hydrogels and microgels have been intensely investigated as protein delivery vehicles because of their excellent biocompatibility and hydrophilicity.[1] Numerous protein-loaded hydrogels and microgels have been synthesized and investigated. For example, hydrogels or microgels composed of acrylamide and methylene bisacrylamide, dextrans grafted with methacrylates, PEG-methacrylates, and PLGA-PEG-methacrylates have been synthesized and used to encapsulate proteins.[2] These hydrogels are usually cross-linked using ester, amide, or carbonate linkages that are most susceptible to degradation via base-catalyzed hydrolysis.

For drug delivery applications, it would be particularly useful to develop protein-loaded hydrogels and microgels that degrade by acid-catalyzed hydrolysis. There are numerous drug delivery targets that exist at acidic pHs, such as tumors, inflammatory tissues, and the phagolysosomes of antigen presenting cells.[3] Acid-degradable hydrogels and microgels designed to undergo degradation in these acidic tissues should therefore be capable of selective delivery of their therapeutic contents. At present, the only method available for engineering acid sensitivity in hydrogels is through the incorporation of cationic groups, which become protonated at acidic pHs and cause hydrogel swelling.[4] However, for protein delivery, it would be preferable to develop acid-sensitive hydrogels that were neutral, thus avoiding the potential toxicity of polycations and the complications of electrostatic interaction with proteins.[5]

In this communication, a new acetal cross-linker is synthesized and used to prepare acid-sensitive, acetal cross-linked, protein-loaded hydrogels and microgels (see Scheme 1). At acidic pHs, the pore size of the acetal cross-linked hydrogels increases, due to the hydrolysis of the acetal, and entrapped macromolecules diffuse out, whereas at neutral pHs the cross-linker remains largely intact, and the release of entrapped macromolecules is significantly slower.

Benzaldehyde acetals are particularly well suited for this application because their acid lability can be manipulated by introducing substituents in the *para* position, and it should therefore be possible to engineer the hydrolysis kinetics of these gels for a wide range of applications.[6] Therefore, the bisacrylamide acetal cross-linker (II) with a *p*-methoxy substituent was chosen as the acid-degradable linkage to ensure that microgels derived from it hydrolyzed rapidly within the pH 5.0 environment that is encountered in phagolysosomes. Compound II was synthesized in two steps with an overall yield of 40%. Reaction of *N*-(2-hydroxyethyl)-2,2,2-trifluoroacetamide (4 equiv) with *p*-methoxybenzaldehyde in dry THF in the presence of a catalytic amount of *p*-toluenesulfonic acid afforded 70% yield of the acetal with two trifluoroacetamide groups. This intermediate was then deprotected with 6 N NaOH, and the product was allowed to react with acryloyl chloride to afford the desired compound, II, in 60% yield.

Scheme 1. Acid-Degradable Protein-Loaded Hydrogel[a]



[a] Proteins are encapsulated by copolymerizing acrylamide (I) with the bisacrylamide acetal cross-linker (II). Acidic hydrolysis of the hydrogel results in protein release.

The hydrolysis of II was measured at pH 5.0 and at pH 7.4. At pH 5.0, hydrolysis is rapid, with a half-life of 5.5 min, whereas at pH 7.4, the half-life is 24 h. The acceleration of the hydrolysis kinetics of this cross-linker from pH 7.4 to pH 5.0 is expected because the rate of hydrolysis of benzaldehyde acetals is proportional to the hydronium ion concentration, which increases 250-fold between pH 7.4 and pH 5.0.[6] The second-order hydrolysis rate constant of this cross-linker is 5610 min^{-1} mol^{-1}.

* To whom correspondence should be addressed. Phone: (510) 643-3077. Fax: 643-3079. E-mail: frechet@cchem.berkeley.edu.

10.1021/ja026925r CCC: $22.00 © 2002 American Chemical Society

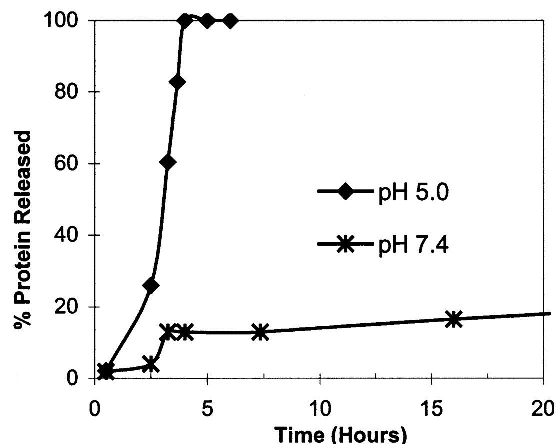


Figure 1. pH-dependent release of FITC-Albumin from acid-degradable hydrogels (data points are an average of three experiments).

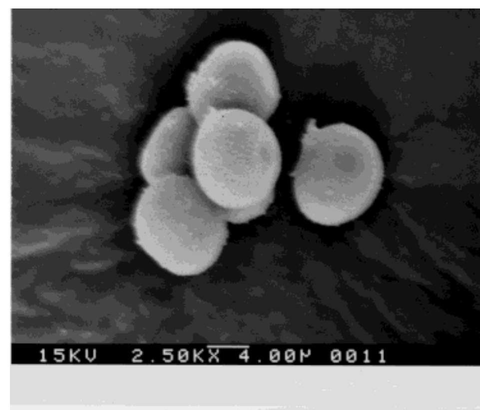


Figure 2. SEM image of microparticles synthesized by polymerization of acrylamide (I) with the bisacrylamide acetal cross-linker (II).

Protein-loaded hydrogels were prepared using II, acrylamide (I), and a fluorescently labeled protein (FITC-Bovine Serum Albumin (BSA)). Encapsulation was carried out by copolymerizing II (60 mg/mL) with I (200 mg/mL) in the presence of FITC-BSA (1 mg/mL) in PBS buffer. This hydrogel was washed with PBS (pH 8.5) buffer; analysis of the washing solution demonstrated essentially quantitative gel entrapment of the FITC-BSA, suggesting that the pore size of this gel, at this cross-linking ratio, is smaller than 3.48 nm (the Stokes radius of bovine albumin).[2c] As expected from the molecular design of the gel, the rate of protein release was found to be pH dependent. At pH 5.0, the acetal cross-links hydrolyze, rapidly transforming the gel into a soluble polymer, and the encapsulated protein is completely released within 2 h (see Figure 1). Gel electrophoresis of the released protein confirmed that it was not significantly modified by either the encapsulation or the hydrolysis procedures (see Supporting Information). At pH 7.4, release of the entrapped protein is significantly slower as only 5% of the encapsulated protein is released after 2 h, and 96 h was required for the hydrogel to completely release its contents (Figure 1). The equilibrium water content, at this cross-linking ratio, is 5.1 mL of water per gram of gel.

The bisacrylamide acetal cross-linker was also used to synthesize acid-degradable micron-sized hydrogels (microgels). Micron-sized materials that decompose under acidic conditions are of great interest because they can induce a colloid osmotic disruption of phagosomes and deliver macromolecules into the cytoplasm of antigen presenting cells.[7]

Microgel particles were prepared by inverse microemulsion polymerization using I and II as co-monomers. Given the unusual combination of monomers, several different polymerization procedures involving different combinations of organic phases and blends of surfactant had to be tried.[2a,8] Inverse emulsion polymerizations with toluene/chloroform as the continuous phase and pluronic F-68 as the surfactant were unsuccessful, likely due to the high solubility of II in toluene/chloroform. However, particles could be obtained using hexane (4.0 g) as the continuous phase, dioctyl sulfosuccinate (0.506 g) and Brij 30 (0.16 g) as the surfactants, and an aqueous phase (5 mL, pH 8.4, 100 mM phosphate buffer) containing 40 mg (0.5 mmol) of acrylamide and 22 mg (0.061 mmol) of II. An SEM image of these particles is shown in Figure 2; the particle size varies between 1 and 10 μm, and the particles are therefore of a size appropriate for their phagocytosis by antigen presenting cells.

The protein-loaded hydrogels and microgels based on acid cleavable acetal cross-linkers are expected to release their contents under the mild acidic conditions found in lysosomes, tumors, and sites of inflammation. As a result, such cross-linkers should find applications as lysosomal escape promoters and more generally in the areas of tumor drug delivery, biomaterial coatings, and in the development of vaccine and DNA delivery systems.

Acknowledgment. The Center for New Directions in Organic Synthesis is supported by Bristol-Myers Squibb as Sponsoring Member and Novartis as a Supporting Member. Financial support of this research by the Biomolecular Program of the E. O. Lawrence Berkeley National Laboratory (Department of Energy, Basic Energy Sciences) is acknowledged with thanks. We would also like to thank Dr. Thomas Rohr for help with SEM images and Amish Patel for help with the protein gels.

Supporting Information Available: Experimental details of the synthesis of II, hydrogel synthesis, and protein release studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Park, K.; Shalaby, W. S.; Park, H. *Biodegradable Hydrogels for Drug Delivery*; Technomic Publishing Co.: Lancaster, PA, 1993.
(2) (a) Ekman, B.; Lofter, C.; Sjoholm, I. *Biochemistry* **1976**, *15*, 5115–5120. (b) Sawhney, A.; Pathak, C.; Hubbell, J. *Macromolecules* **1993**, *26*, 581–587. (c) Sanxia, L.; Anseth, K. S. *Macromolecules* **2000**, *33*, 2509–2515. (d) Dijk-Wolthius, W. N. E.; et al. *Macromolecules* **1997**, *30*, 4639–4645.
(3) (a) Helmlinger, G.; Sckell, A.; Dellian, M.; Forbes, N. S.; Rakesh, K. Jain. *Clin. Cancer Res.* **2002**, *8*, 1284–1291. (b) Trevani, S.; Andonegui, G.; Giordano, M.; Lopez, D.; Gamberale, R.; Minucci, F.; Geffner, J. R. *J. Immunol.* **1999**, *162*, 4849–57.
(4) Park, T. G. *Biomaterials* **1999**, *20*, 517–521. Kim, I. Y.; et al. *J. Appl. Polym. Sci.* **2002**, *85*, 2661–2666.
(5) Sassi, A. P.; Shaw, A. J.; Han, S. M.; Blanch, H. W.; Prausnitz, J. M. *Polymer* **1996**, *11*, 2151–2164.
(6) Fife, T.; Jao, L. *J. Org. Chem.* **1965**, *30*, 1492–1495.
(7) Lynn, D. M.; Mansoor, A.; Langer, R. *Angew. Chem., Int Ed.* **2001**, *40*, 1707–1710.
(8) Daubreese, C.; Grandfils, R.; Jerome, P. H.; Teyssie, P.; Goethals, P.; Schacht, E. *J. Pharm. Pharmacol.* **1993**, *45*, 1018–1023.

JA026925R