COMMUNICATION

ChemComm
www.rsc.org/chemcomm

A new approach towards acid sensitive copolymer micelles for drug delivery

Elizabeth R. Gillies and Jean M. J. Fréchet*

Center for New Directions in Organic Synthesis†, Department of Chemistry, University of California, Berkeley, CA 94720, USA. E-mail: frechet@cchem.berkeley.edu; Fax: 5106433079; Tel: 5106433077

Received (in Cambridge, UK) 17th April 2003, Accepted 20th May 2003
First published as an Advance Article on the web 6th June 2003

A new micellar system capable of selective release of its contents under mildly acidic conditions is described.

In recent years, micelles formed from amphiphilic block copolymers have been receiving attention as potential drug carriers. The size of these nanocontainers, typically between 20 and 100 nm, has been shown to be effective not only in avoiding renal exclusion and reticuloendothelial uptake, but also enables them to be selectively targeted to certain tissues such as tumors due to their high vascular permeability.[1] In addition, it has been proposed that such assemblies may be taken up by cells *via* an endocytosis process.[2]

One important issue determining the effectiveness of a micellar drug carrier is the ability to control the time over which drug release takes place, or to possibly trigger the drug release at a specific location or time. The mildly acidic pH encountered in tumor and inflammatory tissues (pH ~ 6.8) as well as in the endosomal and lysosomal compartments of cells (pH ~ 5–6), provides a potential means of drug release upon arrival at the drug delivery target.[3] Thus far, there have been relatively few copolymer-based systems described that are capable of releasing their contents under mildly acidic conditions.[4] These systems have usually employed titratable groups as the basis for their pH sensitivity.

We present here a new approach for the development of acid sensitive copolymer micelles. This approach is based on the attachment of hydrophobic groups to one block of a diblock copolymer *via* an acid sensitive linkage. It is proposed that upon hydrolysis of the linkage, the hydrophobic block will become more hydrophilic, thus destabilizing the micelle and enabling escape of the drug from its encapsulating micellar compartment.

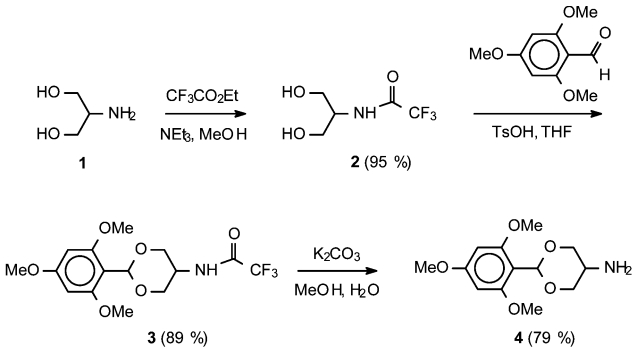
Cyclic benzylidene acetals were investigated as the acid sensitive linkages because they posses several favorable characteristics. First, they contain a hydrophobic aromatic ring that will contribute to micelle formation. In addition, a cyclic benzylidene acetal can mask the polarity of a copolymer bound 1,3 diol, thus affording a significant solubility change when the copolymer will later be hydrolyzed. The rate of hydrolysis of benzylidene acetals is generally proportional to the hydronium ion concentration and is expected to increase 250-fold as the pH is changed from 7.4 to 5.0.[5] Finally, the hydrolysis rate can be manipulated by introducing substituents to the aromatic ring.

This latter feature is particularly useful as cyclic acetals generally hydrolyze slowly,[5] and the introduction of electron-donating methoxy groups in the *ortho* and *para* positions relative to the acetal was necessary to obtain rapid hydrolysis at pH 5.0. It was also necessary to introduce a functional handle for attachment of the acetal to the copolymer. Therefore the trifunctional serinol was chosen to introduce the 1,3 diol. First, the amino group of serinol (**1**) was selectively protected as a trifluoroacetamide by reaction with ethyltrifluoroacetate in methanol to form **2** as shown in Scheme 1. Reaction with 2,4,6-trimethoxybenzaldehyde in dry THF in the presence of a catalytic amount of toluene sulfonic acid afforded the acetal **3**. The trfluoroacetamide group was then removed using K_2CO_3 in refluxing aqueous methanol providing the amine **4**.

Poly(ethylene oxide) (PEO) was chosen as the hydrophilic block because of its high water solubility, ability to provide steric stabilization, and because it tends to inhibit the surface adsorption of biological components.[6] Poly(aspartic acid) (P(Asp)) was chosen as the functionalizable core-forming block since it is a potentially biodegradable poly(amino acid) with a functional handle for attachment of the acid-labile acetal termini. A PEO-P(Asp) block copolymer **5** with approximately 20 aspartic acid units was prepared by a previously reported method using an amine terminated PEO (MW 8000) as an initiator.[7] An additional step, acylating the terminal amine was added to ensure that no crosslinking would occur during the amine coupling.

The amine **4** was coupled to **5** to provide copolymer **6** as shown in Scheme 2, using a procedure similar to that developed by Kataoka *et al*.[7] Using both ^{1}H NMR and UV/vis spectroscopy it was determined that approximately 30% of the carboxylic acid groups had been coupled with **4**. Although this degree of coverage was not as high as had been hoped for, due to the relatively high density of carboxylic functional handles along the chain, and the high hydrophobicity of the acetal, this was deemed sufficient for testing our new concept. At mildly acidic pH, hydrolysis of the acetals is expected to occur, generating hydroxyl groups from the acetals as shown in copolymer **7** disrupting the micellar assembly of the copolymer **6** and triggering release of the contents of the micelle.

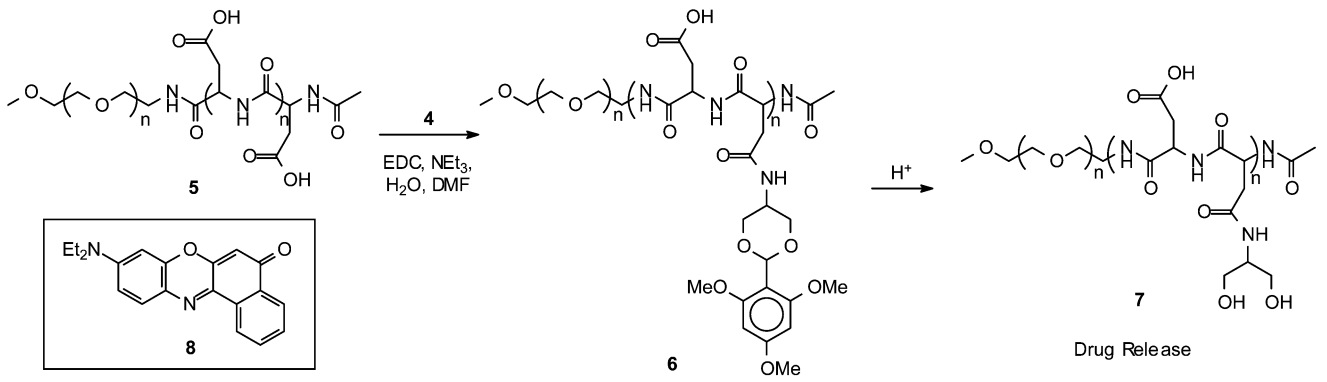
Micellization of copolymer **6** at the physiological pH of 7.4 was confirmed using both the model encapsulation of a hydrophobic dye and dynamic light scattering. Nile Red (**8**) was chosen as the hydrophobic dye since its fluorescence is negligible in aqueous solutions but is known to increase substantially in the hydrophobic environments found in some membranes or micelles.[8] Furthermore, its absorption λ_{max} is at 553 nm, far from interfering absorbances of the aromatic groups on the copolymer. By observing the increase in Nile Red fluorescence over a series of copolymer concentrations from 5 $\times$ 10^{-4} to 2 mg mL^{-1}, a critical micelle concentration of approximately 0.34 mg mL^{-1} was determined. Dynamic light scattering showed that the average diameter of the self-assembled micelles was 90 nm, and no large aggregates were observed.



Scheme 1

† The Center for New Directions in Organic Synthesis is supported by Bristol-Myers Squibb as Sponsoring Member

DOI: 10.1039/b304251k



Scheme 2

The hydrolysis rate of the acetal moieties in the micelles was measured at 37 °C at pH 5.0 and pH 7.4. Appearance of 2,4,6-trimethoxybenzaldehyde was readily detected by its absorbance at 292 nm. As shown in Fig. 1, the hydrolysis is rapid at pH 5.0, with a calculated half life of 60 min, while at pH 7.4 hydrolysis is slow with a half life on the order of days. The hydrolysis rate was not affected by incorporation of the acetal groups in the micelle as demonstrated by a comparison with the hydrolysis rate of a low MW model compound.

The fluorescence of Nile Red was studied as a function of time at pH 7.4 and pH 5.0 at 37 °C. The micelles were first equilibrated with Nile Red overnight in 10 mM pH 7.4 buffer, then the pH was adjusted to 5.0 by addition of a small volume of concentrated pH 5.0 acetate buffer. A pH 7.4 sample was adjusted to the same salt concentration, confirming that the change in salt concentration did not affect the fluorescence intensity. As shown in Fig. 2, the fluorescence of Nile Red decreased at pH 5.0 over a timescale similar to that of the acetal hydrolysis, while the fluorescence of the sample at pH 7.4 remained essentially constant over this time period. These observations are consistent with release of Nile Red from the micelle and the time dependence of these changes strongly suggests that they are indeed due to hydrolysis of the acetals, and not only to a titration effect such as the protonation of the residual carboxylic acids of **6**.

In summary, a new approach to the preparation of acid-sensitive micelles has been developed. This approach involves the conjugation of a highly sensitive cyclic acetal to a block copolymer in such a way that hydrolysis of the acetals reveals a more polar diol moiety, designed to disrupt the micellar assembly, triggering release of the micellar contents. This new system shows promise for the selective release of drugs in acidic environments such as those found in tumor tissue or cellular compartments such as endosomes or lysosomes. We believe

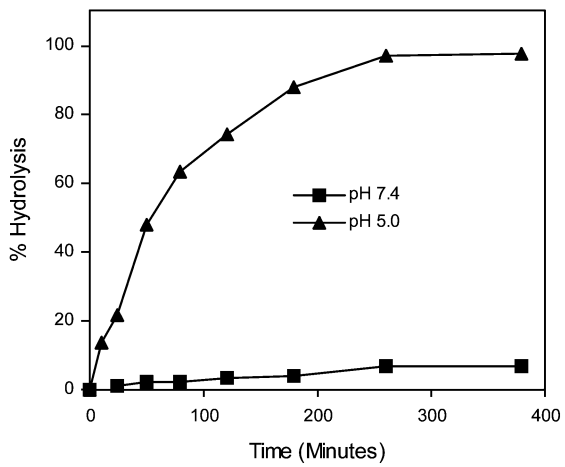


Fig. 1 pH-Dependent hydrolysis of acetals on copolymer **6**.

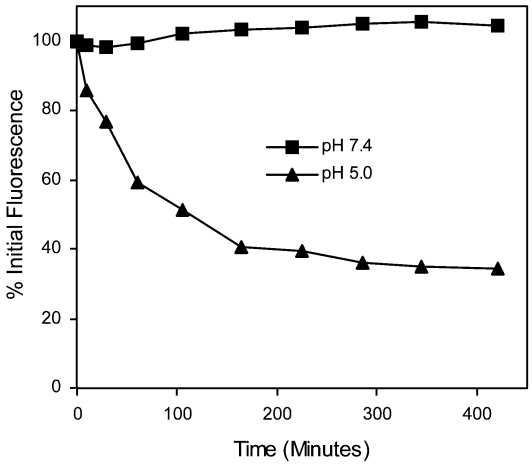


Fig. 2 pH-Dependent Nile Red fluorescence in micelles of copolymer **6**.

that the application of this system in drug delivery has the potential to be quite versatile since many different drugs or block copolymers could be used.

We thank the National Institute of Health (GM 65361 and EB 002047) for support of this research. We also thank T Rappl and N Balsara for assistance in dynamic light scattering experiments.

Notes and references

1 (*a*) For recent reviews see: K. Kataoka, A. Harada and Y. Nagasaki, *Adv. Drug Delivery Rev.*, 2001, **47**, 113; (*b*) A. Lavasanifar, J. Samuel and G. S. Kwon, *Adv. Drug Delivery Rev.*, 2002, **54**, 169; (*c*) A. V. Kabanov, E. V. Batrakova and V. Y. Alakhov, *J. Controlled Release*, 2002, **82**, 189.

2 (*a*) L. Luo, J. Tam, D. Maysinger and A. Eisenberg, *Bioconjugate Chem.*, 2002, **13**, 1259; (*b*) A. V. Kabanov, V. I. Slepnev, L. E. Kuznetsova, E. V. Batrakova, V. Y. Alakhov, N. S. Melik-Nubarov and P. G. Sveshnikov, *Biochem. Int.*, 1992, **26**, 1035.

3 (*a*) G. Helmlinger, A. Sckell, M. Dellian, N. S. Forbes and R. K. Jain, *Clin. Cancer Res.*, 2002, **8**, 1284; (*b*) S. Trevani, G. Andonegui, M. Giordano, D. Lopez, R. Gamberale, F. Minucci and J. R. Geffner, *J. Immunol.*, 1999, **162**, 2661.

4 (*a*) J.-C. Leroux, E. Roux, D. L. Le Garrec, K. Hong and D. C. Drummond, *J. Controlled Release*, 2001, **72**, 71; (*b*) J. Taillefer, M.-C. Jones, N. Brasseur, J. E. Van Lier and J.-C. Leroux, *J. Pharm. Sci.*, 2000, **89**, 52; (*c*) K. Na, E. S. Lee and Y. H. Bae, *J. Controlled Release*, 2003, **87**, 3.

5 T. Fife and L. Jao, *J. Org. Chem.*, 1965, **30**, 1492.

6 (*a*) S. E. Dunn, S. S. Brindley, M. C. Davis and L. Davies, *Pharm. Res.*, 1994, **11**, 1016; (*b*) R. Gref, Y. Minamitake, M. T. Paracchia, V. Trubeskoy, V. Torchilin and R. Langer, *Science*, 1994, **263**, 1600; (*c*) D. L. Elbert and J. A. Hubbell, *Ann. Rev. Mater. Sci.*, 1996, **26**, 365.

7 M. Yokoyama, G. S. Kwon, T. Okano, Y. Sakurai, T. Seto and K. Kataoka, *Bioconjugate Chem.*, 1992, **3**, 295.

8 (*a*) P. Greenspan, E. P. Mayer and S. D. Fowler, *J. Cell Biol.*, 1985, **100**, 965; (*b*) M. M. G. Krishna, *J. Phys. Chem. A.*, 1999, **103**, 3589.