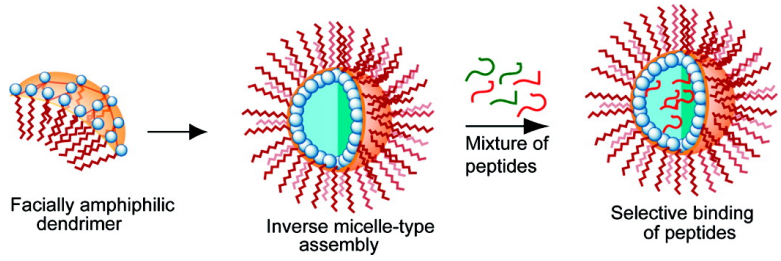
Article

Selective Peptide Binding Using Facially Amphiphilic Dendrimers

Andrea Gomez-Escudero, Malar A. Azagarsamy, Naresh Theddu, Richard W. Vachet, and S. Thayumanavan

J. Am. Chem. Soc., **2008**, 130 (33), 11156-11163 • DOI: 10.1021/ja803082v • Publication Date (Web): 29 July 2008



More About This Article

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

- Supporting Information
- 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

J|A|C|S
ARTICLES

Published on Web 07/29/2008

Selective Peptide Binding Using Facially Amphiphilic Dendrimers

Andrea Gomez-Escudero, Malar A. Azagarsamy, Naresh Theddu, Richard W. Vachet,* and S. Thayumanavan*

Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003

Received April 25, 2008; E-mail: rwvachet@chem.umass.edu; thai@chem.umass.edu

Abstract: Amphiphilic dendrimers, which contain both hydrophobic and hydrophilic groups in every repeat unit, exhibit environment-dependent assemblies both in hydrophilic solvent, water, and in lipophilic solvent, toluene. Upon investigating the status of these assemblies in a mixture of immiscible solvents, these dendrimers were found to be kinetically trapped in the solvent in which they are initially assembled. This property has been exploited to selectively extract peptides from aqueous solution into an organic phase, where the peptides bind to the interior functionalities of the dendritic inverse micelles. While the corresponding small molecule surfactant does not exhibit any selective binding toward peptides, all dendrons (G1−G3) are capable of this selective binding. We show that the inverse micelle-type assembly itself is crucial for the binding event and that the assembly formed by the G1 dendron has a greater capability for binding compared to the G2 or G3 dendrons. We have also shown that the average apparent pK_a of the carboxylic acid functionalities varies with generation, and this could be the reason for the observed differences in binding capacity.

Introduction

Dendrimers have attracted significant interest in supramolecular chemistry because of the high degree of control over their dimensions.[1] For example, the excellent control in molecular weights that can be achieved in these branched macromolecules provides a unique opportunity to carry out highly systematic structure−property relationship studies. Amphiphilic dendrimers have drawn particular interest because they offer micelle-like hydrophobic interiors for guest molecule binding within a globular macromolecule in an aqueous phase.[2] Similarly, inverse micelle-like amphiphilic dendrimers that have a hydrophobic exterior and hydrophilic interior have also been reported.[3] While the micellar dendrimers have been used for applications such as biomimetics,[4] drug delivery,[5] and gene delivery,[6] the utility of inverse micelle-like dendrimers has been limited.[3] In this paper, we describe the unique use of an inverse micelle-like dendrimer assembly for the selective extraction of peptides from aqueous solution. The systematic study outlined here delineates the necessary structural requirements for a dendrimer to effectively bind a peptide.

We have recently reported a unique class of amphiphilic dendrimers in which a hydrophilic functionality and a hydrophobic group are placed within each repeat unit.[7] The special feature of these molecules is that they form environment-dependent amphiphilic assemblies, i.e. micelle-like assemblies

(1) (a) Newkome, G. R.; Moorefield, C. N.; Vögtle, F. *Dendrimers and Dendrons: Concepts, Synthesis and Applications*, 2nd Ed.; Wiley-VCH: Weinheim, 2001. (b) Fréchet, J. M. J.; Tomalia, D. A. In *Dendrimers and Other Dendritic Polymers*; John Wiley & Sons: New York, 2001. (c) Grayson, S. M.; Fréchet, J. M. J. *Chem. Rev.* **2001**, *101*, 3819. (d) Fischer, M.; Vögtle, F. *Angew. Chem., Int. Ed.* **1999**, *38*, 885. (e) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665. (f) Moore, J. S. *Acc. Chem. Res.* **1997**, *30*, 402. (g) Caminade, A. M.; Majoral, J. P. *Acc. Chem. Res.* **2004**, *37*, 341. (h) Balzani, V.; Campagna, S.; Denti, G.; Juris, A.; Serroni, S.; Venturi, M. *Acc. Chem. Res.* **1998**, *31*, 26. (i) Astruc, D.; Chardac, F. *Chem. Rev.* **2001**, *101*, 2991. (j) Majoral, J. P.; Caminade, A. M. *Chem. Rev.* **1999**, *99*, 845. (k) Crespo, L.; Sanclimens, G.; Pons, M.; Giralt, E.; Royo, M.; Albericio, F. *Chem. Rev.* **2005**, *105*, 1663.

(2) (a) Diederich, F.; Felber, B. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4778. (b) Gorman, C. B.; Smith, J. C. *Acc. Chem. Res.* **2001**, *34*, 60. (c) Hawker, C. J.; Wooley, K. L.; Fréchet, J. M. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1287. (d) Newkome, G. R.; Moorefield, C. N.; Baker, G. R.; Johnson, A. L.; Behera, R. K. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1176. (e) Zeng, F.; Zimmerman, S. C. *Chem. Rev.* **1997**, *97*, 1681. (f) Cardona, C. M.; Mendoza, S.; Kaifer, A. E. *Chem. Soc. Rev.* **2000**, *29*, 37.

(3) (a) Stevelmans, S.; Hest, J. C. M.; Jansen, J. F. G. A.; Van Boxtel, D. A. F. J.; de Berg, E. M. M.; Meijer, E. W. *J. Am. Chem. Soc.* **1996**, *118*, 7398. (b) Chechik, V.; Zhao, M.; Crooks, R. M. *J. Am. Chem. Soc.* **1999**, *121*, 4910. (c) Balogh, L.; Tomalia, D. A. *J. Am. Chem. Soc.* **1998**, *120*, 7355. (d) Piotti, M. E.; Rivera, F., Jr.; Bond, R.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1999**, *121*, 9471.

(4) (a) Dandliker, P. J.; Diederich, F.; Gisselbrecht, J.-P.; Louati, A.; Gross, M. *Angew. Chem., Int. Ed. Engl.* **1996**, *34*, 2725. (b) Weyermann, P.; Gisselbrecht, J. P.; Boudon, C.; Diederich, F.; Gross, M. *Angew. Chem., Int. Ed.* **1999**, *38*, 3215. (c) Jiang, D. L.; Aida, T. *Chem. Commun.* **1996**, 1523. (d) Javor, S.; Delort, E.; Darbre, T.; Reymond, J. -L. *J. Am. Chem. Soc.* **2007**, *129*, 13238. (e) Darbre, T.; Reymond, J.-L. *Acc. Chem. Res.* **2006**, *39*, 925, and references therein. (f) Hecht, S.; Fréchet, J. M. J. *Angew. Chem., Int. Ed.* **2001**, *40*, 74. (g) Bhyrappa, P.; Young, J. K.; Moore, J. S.; Suslick, K. S. *J. Am. Chem. Soc.* **1996**, *118*, 5708. (h) Castro, R.; Cuadrado, I.; Alonso, B.; Casado, C. M.; Moran, M.; Kaifer, A. E. *J. Am. Chem. Soc.* **1997**, *119*, 5760. (i) Peterca, M.; Percec, V.; Dulcey, A. E.; Nummelin, S.; Korey, S.; Ilies, M.; Heiney, P. A. *J. Am. Chem. Soc.* **2006**, *128*, 6713, and references therein. (j) Zhang, L.; Tam, J. P. *J. Am. Chem. Soc.* **1997**, *119*, 2363. (k) Kim, Y.; Zeng, F.; Zimmerman, S. C. *Chem.—Eur. J.* **1999**, *5*, 2133. (l) Kinberger, G. A.; Cai, W.; Goodman, M. *J. Am. Chem. Soc.* **2002**, *124*, 15162. (m) Love, C. S.; Hirst, A. R.; Chechik, V.; Smith, D. K.; Ashworth, I.; Brennan, C. *Langmuir* **2004**, *20*, 6580. (n) Twyman, L. J.; King, A. S. H.; Martin, I. K. *Chem. Soc. Rev.* **2002**, *31*, 69.

10.1021/ja803082v CCC: $40.75

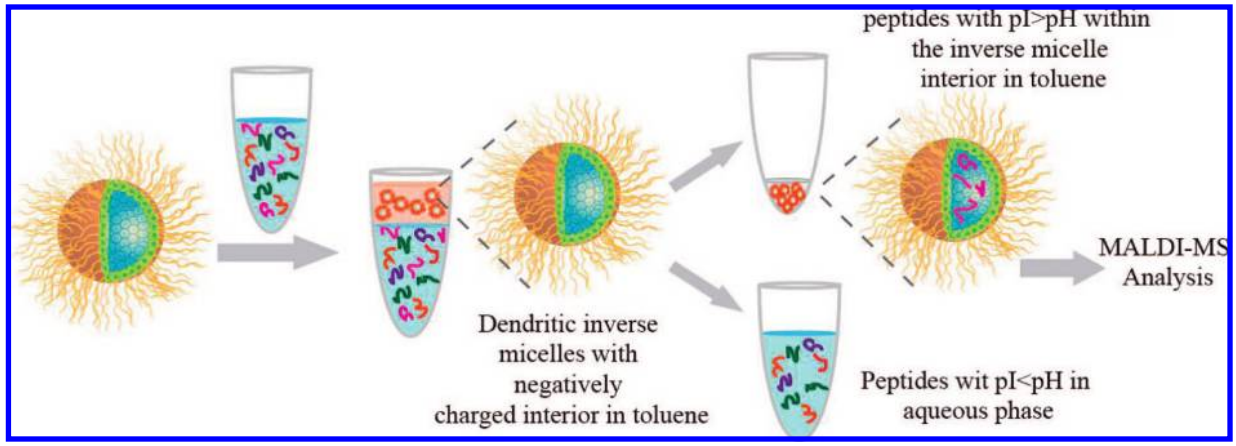


Figure 1. A schematic representation of the amphiphilic dendrons forming the inverse micelles that are capable of selectively extracting complementary peptides from the aqueous solution.

in water and inverse micelle-like assemblies in toluene, respectively. Since the inverse micelle-like assemblies of these molecules position their anionic carboxylate groups in the core of the assembly, we envisaged that these inverse micelles would be capable of sequestering positively charged guest molecules (Figure 1). Indeed, we have recently reported on such a possibility using an analogous amphiphilic homopolymer.[8] The versatility of this process has been utilized to selectively isolate peptide fragments from protein digests and thus reduce detection complexity.[9] We envision that this capability could impact important applications such as proteomics, as reducing the complexity of peptide mixtures will significantly impact protein identification.[10] To achieve such a lofty goal, however, it is necessary that we develop a fundamental understanding of the features that affect the ability of the macromolecular host to selectively bind complementary peptides. The exquisite control over the structure of dendrimers provides a unique opportunity to carry out such a systematic structure−property relationship study. More specifically, we seek to address questions such as: (*i*) what is the minimum number of repeat units needed for efficient peptide recognition? (*ii*) Are amphiphilic assemblies required or are simple anionic macromolecules dispersed in lipophilic solvents sufficient for binding? (*iii*) If assemblies are required, is it necessary that these assemblies be kinetically trapped in the assembled solvent? (*iv*) How does the arrangement and number of functional groups in the amphiphilic host affect the accessibility of the guest? (*v*) How does the protonation state of the host anionic groups influence the extent of guest binding?

Results and Discussion

Assembly Properties of the Amphiphilic Dendrons. To address the questions mentioned above, we need to first understand the assembly characteristics of the facially amphiphilic dendrons in mixed solvents. Although we have previously demonstrated the micelle-like and inverse micelle-like container properties of the amphiphilic dendrons of the type shown in Chart 1, we have not investigated the critical aggregation concentrations of these molecules. Also, since these molecules are capable of forming assemblies in both aqueous solutions and lipophilic solvents, it is not immediately obvious that these dendrons would be capable of acting as inverse-micelle like containers in a heterogeneous solvent mixture. It is reasonable to hypothesize that it is necessary that these assemblies be kinetically trapped in the solvent that they are initially assembled to exhibit the selective extraction properties.[8b]

The critical aggregation concentration (CAC) of the dendrons in aqueous solutions has been determined using the ratio of peak intensities at 338 and 333 nm (I_{338}/I_{333}) from pyrene's excitation spectra.[11,7c] For this purpose, a number of I_{338}/I_{333} values have been obtained by varying the concentration of the dendrons (Figure 2a). When the concentration of the dendrons is low,

(5) (a) Duncan, R.; Izzo, L. *Adv. Drug Delivery Rev.* **2005**, *57*, 2215. (b) Gillies, E. R.; Fréchet, J. M. J. *Drug Discov. Today* **2005**, *10*, 35. (c) Liu, M.; Kono, K.; Fréchet, J. M. J. *J. Controlled Release* **2000**, *65*, 121. (d) Esfand, R.; Tomalia, D. A. *Drug Discovery Today* **2001**, *6*, 427. (e) Pistolis, G.; Malliaris, A.; Tsiourvas, D.; Paleos, C. M. *Chem.—Eur. J.* **1999**, *5*, 1440. (f) Twyman, L. J.; Beezer, A. E.; Esfand, R.; Hardy, M. J.; Mitchell, J. C. *Tetrahedron Lett.* **1999**, *40*, 1743. (g) Yoo, H.; Sazani, P.; Juliano, R. L. *Pharm. Res.* **1999**, *16*, 1799. (h) Aulenta, F.; Hayes, W.; Rannard, S. *Eur. Polym. J.* **2003**, *39*, 1741. (i) Cloninger, M. J. *Curr. Opin. Chem. Biol.* **2002**, *6*, 742. (j) Boas, U.; Heegaard, P. M. H. *Chem. Soc. Rev.* **2004**, *33*, 43. (k) Crampton, H. L.; Simanek, E. E. *Polym. Int.* **2007**, *56*, 489. (l) Stiriba, S. E.; Frey, H.; Haag, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 1329. (m) Florence, A. T. *Adv. Drug Delivery Rev.* **2005**, *57*, 2104. (n) D'Emanuele, A.; Attwood, D. *Adv. Drug Delivery Rev.* **2005**, *57*, 2147.

(6) (a) Kukowska-Latallo, J. F.; Bielinska, A. U.; Johnson, J.; Spindler, R.; Tomalia, D. A.; Baker, J. R., Jr *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 4897. (b) Dennig, J.; Duncan, E. *Rev. Mol. Biotechnol.* **2002**, *90*, 339. (c) Joester, D.; Losson, M.; Pugin, R.; Heinzelmann, H.; Walter, E.; Merkle, H. P.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1486. (d) Tang, M. X.; Redemann, C. T.; Szoka, F. C., Jr *Bioconjugate Chem.* **1996**, *7*, 703. (e) Radu, D. R.; Lai, C. Y.; Jeftinija, K.; Rowe, E. W.; Jeftinija, S.; Lin, V. S. Y. *J. Am. Chem. Soc.* **2004**, *126*, 13216. (f) Dufèsa, C.; Uchegbub, I. F.; Schätzlein, A. G. *Adv. Drug Delivery Rev.* **2005**, *57*, 2177. (g) Shah, D. S.; Sakthivel, T.; Toth, I.; Florence, A. T.; Wilderspin, A. F. *Int. J. Pharm.* **2000**, *208*, 41. (h) Loup, C.; Zanta, M. A.; Caminade, A. M.; Majoral, J. P.; Meunier, B. *Chem.—Eur. J.* **1999**, *5*, 3644.

(7) (a) Vutukuri, D. R.; Basu, S.; Thayumanavan, S. *J. Am. Chem. Soc.* **2004**, *126*, 15636. (b) Klaikherd, A.; Sandanaraj, B. S.; Vutukuri, D. R.; Thayumanavan, S. *J. Am. Chem. Soc.* **2006**, *128*, 9231. (c) Savariar, E. N.; Aathimanikandan, S. V.; Thayumanavan, S. *J. Am. Chem. Soc.* **2006**, *128*, 16224.

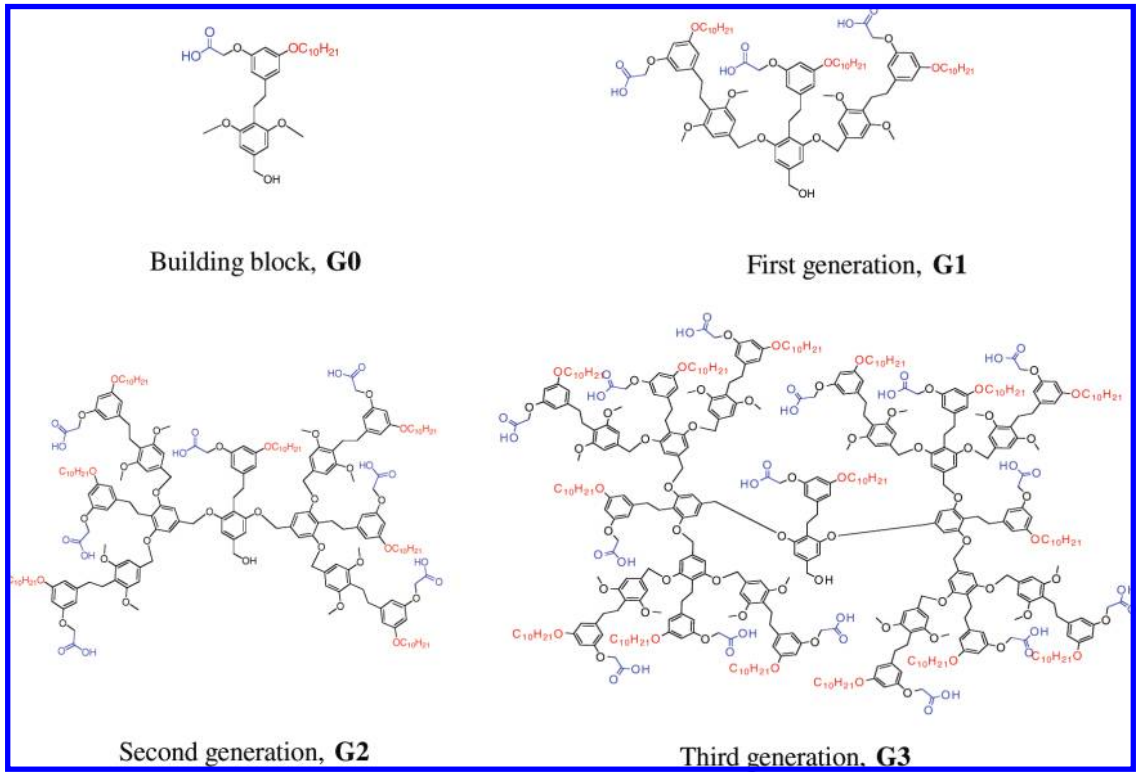
(8) (a) Basu, S.; Vutukuri, D. R.; Shyamroy, S.; Sandanaraj, B. S.; Thayumanavan, S. *J. Am. Chem. Soc.* **2004**, *126*, 9890. (b) Basu, S.; Vutukuri, D. R.; Thayumanavan, S. *J. Am. Chem. Soc.* **2005**, *127*, 16794.

(9) Combariza, M. Y.; Savariar, E. N.; Vutukuri, D. R.; Thayumanavan, S.; Vachet, R. W. *Anal. Chem.* **2007**, *79*, 7124.

(10) (a) Aebersold, R.; Goodlett, D. R. *Chem. Rev.* **2001**, *101*, 269. (b) Baldwin, M. A. *Mol. Cell. Proteomics* **2004**, *3*, 1.

(11) (a) Lavasanifar, A.; Samuel, J.; Kwon, G. S. *Colloids Surf., B* **2001**, *22*, 115. (b) Wilhelm, M.; Zhao, C. L.; Wang, Y.; Xu, R.; Winnik, M. A.; Mura, J. L.; Riess, G.; Croucher, M. D. *Macromolecules* **1991**, *24*, 1033.

Chart 1. Structures of the Amphiphilic Dendrons G0−G3



the I_{338}/I_{333} value is the same as that of pyrene in water. When the concentration of the dendrons increases, the red shift from 333 to 338 nm in the pyrene excitation spectra indicates the movement of pyrene into a more hydrophobic environment. This characteristic feature of pyrene excitation spectra is utilized to calculate the CAC values. In Figure 2b, the I_{338}/I_{333} values are plotted against log c, where c is the concentration of dendron. No change in the ratio of intensities is observed at low concentrations. As concentration increases, however, the curve becomes sigmoidal. The inflection point of this curve is taken as the CAC, as shown in Figure 2b.

The inverse micelle CACs of the dendrons were obtained by surface tension measurements using a tensiometer with water as the interface.[12] This was accomplished by means of an inverse drop method.[13] As the concentration of dendron is increased, the formation of the inverse micelles from their monomers becomes favored. At low concentrations, the slight change in the surface tension indicates that the monomers are still on the surface of the liquid. When the concentration is such that the inverse micelles are formed, the surface tension significantly decreases and reaches equilibrium (Figures 3, 4). Therefore, the breakpoint at which surface tension changes significantly is taken to be the CAC of the dendron. The CACs obtained for these dendrons are listed in Table 1.

For the dendrons to be optimal hosts in the toluene phase, the inverse micelles formed by these molecules should remain in the lipophilic phase when equilibrated in a biphasic mixture. Since these dendrons form assemblies in both water and toluene (micellar and inverse micellar assemblies, respectively), it is not obvious that this will be the case. There are two limiting possibilities for the amphiphilic dendrons when equilibrated with an immiscible biphasic mixture of water and toluene: (*i*) the dendrons are thermodynamically equilibrated between the two layers dictated by the distribution coefficient of the molecule, or (*ii*) the dendrons are kinetically trapped in the solvent in which they are initially assembled. The latter possibility would be useful for the selective extraction and binding of guest molecules by the dendrons.[8b] Amphiphilic dendrons provide a unique opportunity to systematically investigate the number of amphiphilic repeat units that are necessary for forming kinetically trapped assemblies and thus provide the opportunity for selective extraction of the guest molecules.

The possibilities were tested by a simple set of experiments where the dendrons were first allowed to form micelles in water. Then an equivalent volume of toluene was added to this solution and equilibrated. Following this, the immiscible phases were separated, and both solutions were analyzed for the presence of the dendrons by linear absorption and fluorescence spectroscopy. Both measurements indicate that there are no measurable amounts of the dendrons G1−G3 in toluene after 2 h of equilibration. Also, the absorbance of these dendrons in the aqueous solution did not change before and after equilibration. These results indicate that these assemblies are kinetically trapped in the solvents in which they are initially assembled. However, these could also simply mean that the distribution coefficient of the dendrons favor water significantly. To distinguish this possibility, we also carried out the experiments where the dendrons are initially assembled as inverse micelles in lipophilic solvents. When phase separated, the dendrons were found to be completely retained in toluene. These results suggest that these assemblies are indeed kinetically trapped in toluene. Interestingly, the small molecule surfactant (G0 dendron) does not seem to be fully trapped in the solvent in which it is initially assembled. It is also important to note that this molecule is not fully distributed between the solvents since the ratio of G0 in the two solvents is not identical when assembled in water vs when assembled in toluene. However, it is clear that there is some leakage of G0 across the solvent interface from the spectral

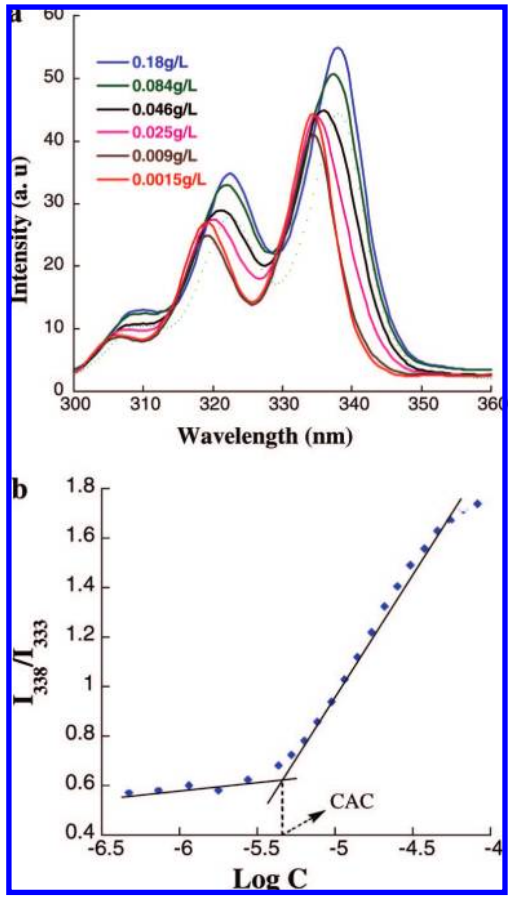


Figure 2. (a) Excitation spectra of pyrene at various concentrations of G2. (b) Plot of ratios of I_{338}/I_{333} from pyrene excitaion spectra vs concentration of G2 to estimate the critical aggregation concentration (CAC).

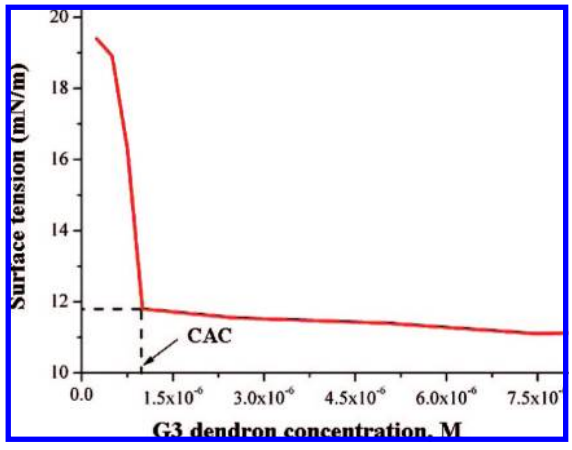


Figure 3. Data of the surface tension change with respect to the change in concentration of the dendrons.

data. A possible explanation for this behavior is that G0 does not form inverse micelles at the concentrations we were able to carry out these studies. To test this, we carried out the equilibration experiments with G1, G2, and G3 dendrons at concentrations below their CAC values. The results indicate that the higher generation dendrons do not readily equilibrate between the two phases to any detectable level even at concentrations below their CACs. These results suggest that G0's inability to form micelles may not be the only reason for its ability to partition between the two phases.

Given the stability characteristics of the inverse micelles, the ability of these dendrons to host molecules was then investigated by selectively extracting peptides from an aqueous solution. Since the core of the dendrimer inverse micelle contains negatively charged carboxylate functionalities, extraction of positively charged peptides was expected based on electrostatic complementarity. Aqueous solutions (pH = 7.0) containing a mixture of peptides were mixed with a toluene solution containing the dendrimer inverse micelles. After extraction, the aqueous and organic phases were separated and each were analyzed individually by MALDI-MS (Figure 5). The ion abundance ratios (K) from the analysis of each phase were then determined, where $K = I_{org}/I_{aq}$; I_{org} is the MALDI ion abundance in the organic phase and I_{aq} is the MALDI ion abundance in the aqueous phase. From Table 2, three results are noteworthy. First, as expected only peptides that are positively charged (i.e., isoelectric points above 7) are significantly extracted. Bradykinin, kinetensin, ACTH, and angiotensin I are extracted, but spinorphin, β-amyloid, and preproenkephalin are not (see also Figure 5). Second, the G0 dendron does not significantly extract any of the peptides, regardless of their charge. This result likely occurs because G0 does not completely remain in the organic phase, but it may also be due to G0's apparent inability to form inverse micelles. Third, extractions with the higher generation dendrons result in higher K values for the positively charged peptides. This result is probably due to the higher number of negatively charged groups in the higher generations that allow a greater number of peptides to be accommodated at a given dendron concentration. While the first observation is directly obvious from Figure 5 and Table 2, we were interested in investigating our second and third observations further.

First, we further investigated whether the higher generation dendrons can indeed accommodate higher concentrations of peptides. For this purpose, we measured the binding capacity of the different dendrons. Increasing concentrations of bradykinin were extracted using a constant dendron concentration of 1.0×10^{-4} M (Figure 6). The MALDI ion abundances of bradykinin that remained in the aqueous phase after extraction were plotted as a function of the initial bradykinin concentration to determine the maximum peptide concentration that each dendron could accommodate. Results from these experiments show that G1, G2, and G3 have peptide binding capacities of 4×10^{-7}, 6×10^{-7}, and 8×10^{-7} M, respectively, when present at a concentration of 1×10^{-4} M in the toluene. The increase in capacity for the higher generation dendrons qualitatively explains the greater K values obtained for the extractions involving the higher generation dendrons (Table 2).

Extractions with the higher generation dendrons result in higher K values because of their greater capacity, but are these increases commensurate with the increases in the number of carboxylate units that each of these dendrons have? To answer this, extractions of bradykinin were performed using the same concentration of carboxylate groups for each dendron generation. Note that G1 contains 3 carboxylate units, whereas G2 and G3 dendrons contain 7 and 15 carboxylate units each. Therefore, separate extractions were performed using a G1 concentration of 5.0×10^{-4} M, a G2 concentration of 2.0×10^{-4} M, and a G3 concentration of 1×10^{-4} M. Each of these solutions has an identical total carboxylate concentration of 1.5×10^{-3} M. Bradykinin solutions having concentrations of 1.0×10^{-6} M and 2.0×10^{-6} M were extracted, and the MALDI ion abundances of the peptide remaining in the aqueous phase are shown in Figure 6b. Clearly, the amount of bradykinin left in

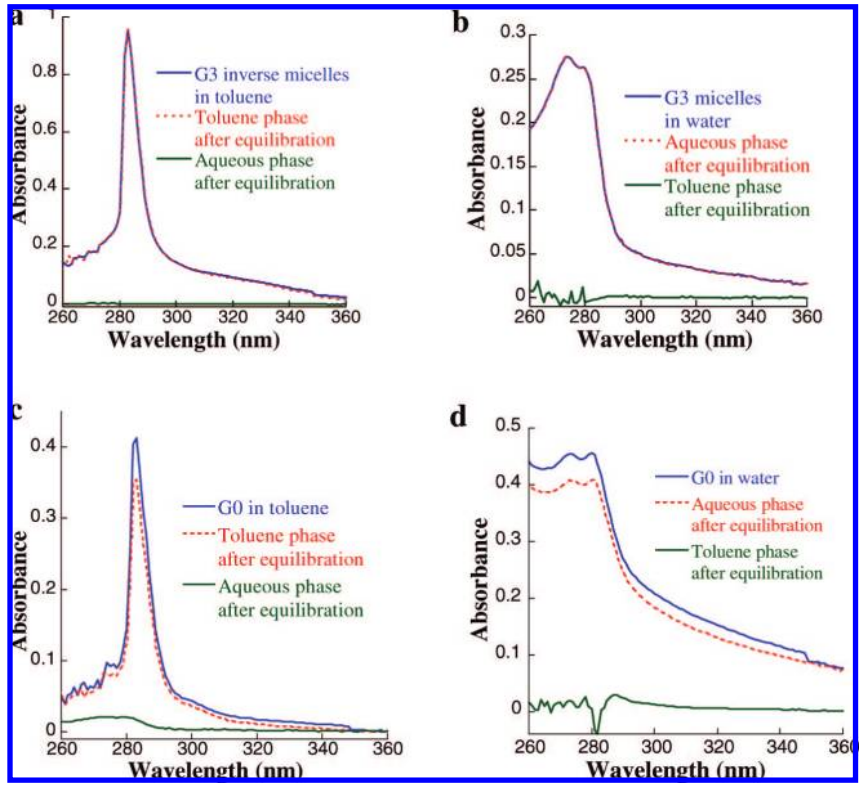


Figure 4. UV−vis experiments with G3 starting (a) (1×10^{-4} M) in toluene and (b) (5×10^{-5} M) in water; with G0 (1×10^{-4} M) starting (c) in toluene and (d) in water. Identical results are obtained for G1 and G2.

Table 1. CAC Values (in M) for the Dendrons in Toluene and Water

generation	G0[a]	G1	G2	G3
toluene	-	5.0×10^{-5}	2.5×10^{-6}	1.0×10^{-6}
water	-	1.5×10^{-5}	4.8×10^{-6}	1.9×10^{-6}

[a] It was not possible to determine the CAC values for G0 in either water or toluene.

the aqueous phase increases as the generation increases. These results indicate that at a given concentration of carboxylate groups, the ability of the dendrons to host the positively charged peptide decreases as the dendron generation increases. The likely reason for this is that the percentage of carboxylate groups in the higher generation dendrons that are accessible to the positively charged peptide is lower. This could be due to any combination of the following three reasons: (*i*) under the experimental conditions used, a greater percentage of the carboxylate groups are protonated in the higher generations; (*ii*) steric crowding in the higher generations decreases the flexibility of these dendrons, making them less capable of arranging their carboxylate groups to accommodate the positively charged peptides; (*iii*) in a higher generation dendron such as G3, the relative positioning of 15 carboxylate units is completely predetermined by the dendritic backbone structure. In contrast, in a lower generation dendron, such as G1, only three carboxylate units are restricted by the covalent backbone structure and the 15 carboxylate units can be more optimally presented by the noncovalent organization of five dendrons. Therefore, the lower generation dendron should be better able to accommodate the complementary structure of the guest molecule, i.e., the positive charges of the peptide molecules.

All three reasons suggested above are provisional, because we do not have an unambiguous way of distinguishing the possibilities. Nonetheless, we could test the extent of protonation of the carboxylate units in different generations of dendrons. We examined this by analyzing the guest capacity of the G1, G2, and G3 dendrons as a function of aqueous solution pH. We assumed that the pH of the aqueous solution is also likely to dictate the pH of the hydrated interiors of the inverse micelles where the binding takes place.[9] Plotting the capacity values for each dendron as a function of pH is instructive, because it indicates the average protonation state of the interior carboxylates, which likely controls the ability of the dendrons to host positively charged guest molecules. In essence, these data provide an effective pK_a for each dendrimer inverse-micelle assembly, and Figure 7 makes it clear that the lower generations have lower effective pK_a values. In light of these effective pK_a values, the data in Figure 6b can now be understood. G1 is able to accommodate more positive charges per carboxylate in its interior than G2 and G3 because at a pH of 7.0 a greater percentage of carboxylic acids are deprotonated. This is understandable once again because the G1 dendron has only 3 carboxylate units that are tied-up covalently in the otherwise noncovalent assembly, relative to 7 and 15 carboxylate units in G2 and G3 dendrons. Therefore, the influence of a carboxylic acid functionality on a neighboring one's pK_a will be relatively small with G1.

It is also interesting to test whether the inverse micelle organization of the dendrons itself is crucial, or if a molecule that bears a charge but is soluble in organic solvent is sufficient for these molecules to host peptide guest molecules. For this, extraction experiments were performed above and below the CAC values of certain dendrons. To control the effect of carboxylate concentration on the extraction, constant carboxylate concentrations were chosen when comparing two dendrons. In one experiment, for example, bradykinin extractions using a G1

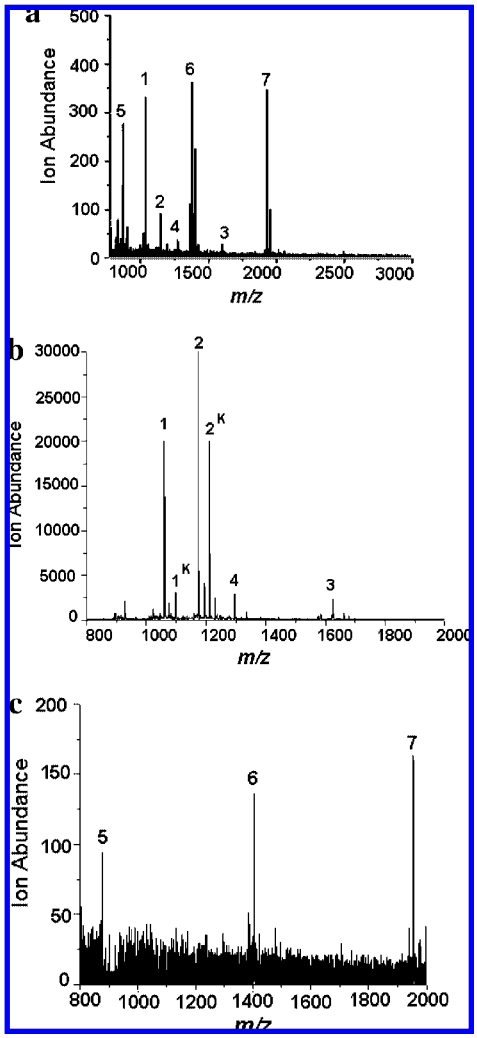


Figure 5. (a) MALDI-MS of the mixture of the peptides in the aqueous solution before extraction; (b) MALDI-MS of the toluene phase after the extraction using the G2 dendron. Note the predominant presence of the positively charged peptides; (c) MALDI-MS of the aqueous phase after extraction. Note the disappearance of the positively charged peptides, while the negatively charged peptides are still present in solution. 1^K and 2^K refer to ions $(M + K)^+$ of the respective peptide (see Table 2).

concentration of 2.5×10^{-5} M, which is below its CAC, were compared to extractions using a G2 concentration of 1.1×10^{-5} M and a G3 concentration of 5.0×10^{-6} M, both of which are above the CAC values for these dendrons. In these experiments, the carboxylate concentration is approximately 7.5×10^{-5} M for all dendron solutions. Even though G1 had the best extraction capabilities when all the dendrimers were above their CACs, it had the poorest extraction capability in this experiment. This is because among the three dendrons G1 is below its CAC, while G2 and G3 are above their CACs. Both organic phase data (Figure 8a) and aqueous phase data (Figure 8b) demonstrate that the inverse micelle formation is important for efficient extraction.

Summary

We have shown that the facially amphiphilic dendrons, which exhibit environment-dependent formation of micelles or inverse micelles, are kinetically trapped in the solvents in which they are initially assembled. We have taken advantage of this character and the charged interiors of the inverse micelles to

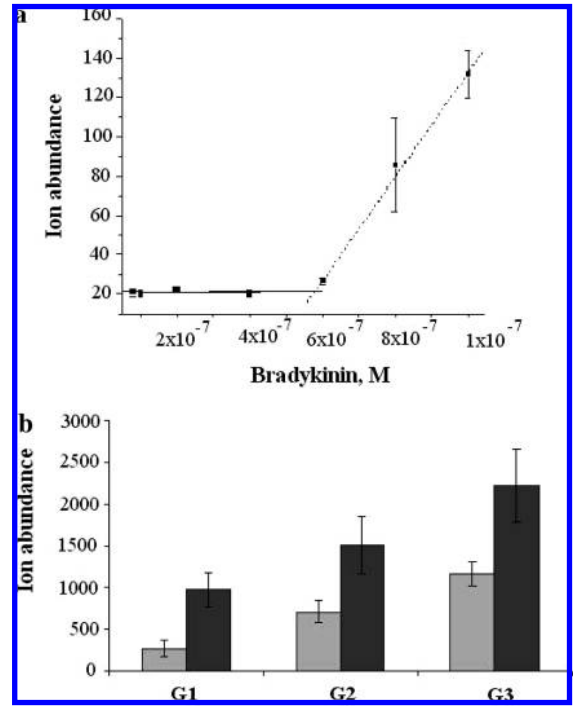


Figure 6. (a) Peptide ion abundance in the aqueous phase after extraction of bradykinin using a G2 dendron at pH 6.0. The maximum peptide capacity was determined from the intersection of two lines, one drawn at low peptide concentrations where all the peptide is extracted and one drawn at higher concentrations where increasing amounts of the peptide remain in the aqueous phase. (b) MALDI-MS ion abundances of bradykinin left behind in the aqueous phase after extraction of 1×10^{-6} M (light gray) and 2×10^{-6} M (dark gray) solutions of the peptide at pH 7.0 using 5×10^{-4} M of G1, 2×10^{-4} M of G2, and 1×10^{-4} M of G3 in toluene.

selectively extract peptides based on their isoelectric points. While the small molecule surfactant (G0) is not capable of such a selective extraction, G1−G3 dendrons are efficient in performing the task. At a given molar ratio, the G3 dendron exhibits a greater capacity for extracting peptides compared to the G1 and G2 dendrons. Trends in which higher generation dendrons exhibit a greater capacity to perform a task have been often called the dendritic effect. At first glance, our observations seem to be an example of such a dendritic effect, but closer examination of the dendrons on a per functionality basis shows that the trend is reversed. We have hypothesized that this could be either due to an increase in average pK_a of the carboxylates with increasing generation or due to the nonavailability of functional groups for binding due to the greater number of carboxylate units with fixed positions in the higher generation dendrons. This unique observation could be viewed as an "inverse dendritic effect". Finally, we have also shown that the inverse micellar organization of the dendrons is crucial for the selective binding of the peptides. For example, selective separation of peptides from mixtures of peptides could be ultimately useful in protein detection, since protein detection in mass spectrometry is achieved through digestion followed by detection of peptides.[10] Enhanced abilities in protein detection are likely to impact areas such as proteomics and pathogen detection.[14]

(12) (a) Hunter, R. J. *Foundations of Colloid Science*; Oxford University Press: Oxford, 1987; Vol. 1. (b) Yamauchi, K.; Onoue, Y.; Tsujimoto, T.; Kinoshita, M. *Colloids Surf., B* **1997**, *10*, 35.
(13) Malzert, A.; Boury, F.; Saulnier, P.; Ivanova, T.; Panaiotov, I.; Benoit, J. P.; Proust, J. E. *J. Colloid Interface Sci.* **2003**, *259*, 398–407.

Table 2. Ion Abundance Ratios of Various Peptides, Having an Initial Aqueous Solution Concentration of 1×10^{-7} M after Extraction with Different Generation Dendrons

peptide in Figure 5	peptide (pI)[a]	sequence	*K* values for different dendrons generations[b]			
			G0	G1	G2	G3
1	bradykinin (12.5)	RPPGFSPFR	1.6 ± 0.6	160 ± 30	200 ± 30	480 ± 40
2	kinetensin (11.1)	IARRHPYFL	6 ± 2	170 ± 25	260 ± 40	460 ± 40
3	ACTH (9.3)	SYSMEHFRWGKPV	1.4 ± 0.4	100 ± 40	120 ± 10	110 ± 10
4	angiotensin I (7.7)	DRVYIHPFHL	1.5 ± 0.5	38 ± 8	66 ± 8	80 ± 20
5	spinorphin (6)	LVVYPWT	1.1 ± 0.3	1.9 ± 0.6	4.4 ± 0.9	3.9 ± 0.6
6	β-amyloid (4.1)	EDVGSNKGAIIGLM	0.3 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.1 ± 0.1
7	preproenkephalin (3.6)	SSEVAGEGDGDSMGHEDLY	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1

[a] Isoelectric points were calculated using the program http://bioweb.pasteur.fr/docs/EMBOSS/pepstats.html. [b] Note that the volume difference between the organic (30 μL) and aqueous phases (1000 μL) that are ultimately analyzed by MALDI-MS means that equal partitioning between the two phases should result in *K* values close to 33 (i.e., 1000/30).

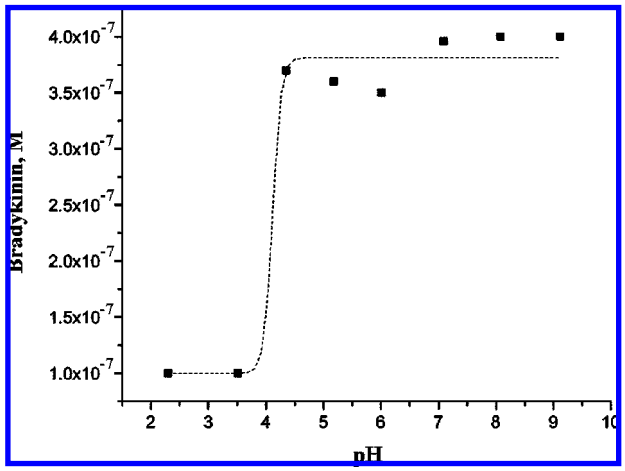


Figure 7. Peptide capacities at different pH values after extracting with 1×10^{-4} M solutions of the G1 dendron. The peptide capacities at each pH were determined in the same manner as that for Figure 6a. The inflection points reveal that the effective pK_a for the G1 is 4.4. Similarly, the effective pK_a values for the G2 and G3 dendrons are 5.6 and 6.2, respectively.

Thus, the technique developed here has the potential to expand the repertoire for dendritic architectures in biological sciences.

Experimental Section

Reagents. Bradykinin, kinetensin, human angiotensin I, human ACTH, spinorphin, β-amyloid (22−35), and preproenkephalin were purchased from the American Peptide Company. Toluene, trifluoroacetic acid (TFA), α-cyano-hydroxycinnamic acid (α-CHCA), and Tris/Tris-HCl were acquired from Sigma-Aldrich (St. Louis, MO). The water used in all the experiments was obtained from a Milli-Q water purification system (Millipore, Bedford, MA). Tetrahydrofuran (THF) was obtained from Fisher Scientific (Pittsburgh, PA) and purified by distillation. Dendrons were prepared following the procedures previously reported from our laboratories, and the synthetic details will be reported elsewhere.[7a,c]

Inverse Micelle Preparation. Solutions of the inverse micelles were prepared at a 1×10^{-4} M concentration by dissolving an appropriate amount of the dendron in toluene, adding 2 mol equiv of water per equivalent of carboxylate group into the organic phase, and sonicating the mixture for 2 h.[15]

Extraction Procedure. The liquid−liquid extraction procedure involved mixing 200 μL of a toluene solution of inverse micelle dendrons with 1 mL of an aqueous solution of peptide(s) buffered to pH ~7.0 with 50 mM Tris/TrisHCl. For the extractions at different pH values, a stock solution of 50 mM Tris pH ~10 was prepared and HCl (1.2 M) was added until the pH of the solution reached the desired value. Unless otherwise stated, the dendron concentration that was used was 1×10^{-4} M. The mixture was vigorously vortexed for 120 min and then centrifuged at 12 000 rpm for 60 min to separate the resulting emulsion into separate organic and aqueous phases. The organic layer was recovered and dried overnight to obtain a solid residue. To the residue 10 μL of THF were added to resolubilize the extracted sample. Finally, 20 μL of an α-CHCA matrix solution (45 mg of α-CHCA in 70:30:0.3 THF/H_2O/TFA) were added to the sample for a final volume of 30 μL. A 1 μL aliquot of the sample/matrix mixture was then spotted on a stainless-steel target, and the solvent was allowed to evaporate before MALDI-MS analysis. The aqueous phase (~1 mL) remaining after extraction was also examined by MALDI-MS. Equal amounts (5 μL) of the aqueous phase and the matrix solution (45 mg of α-CHCA in 70:30:0.3 THF/H_2O/TFA) were mixed, and 1 μL of the resulting solution was spotted on a stainless-steel target for MALDI-MS analysis.

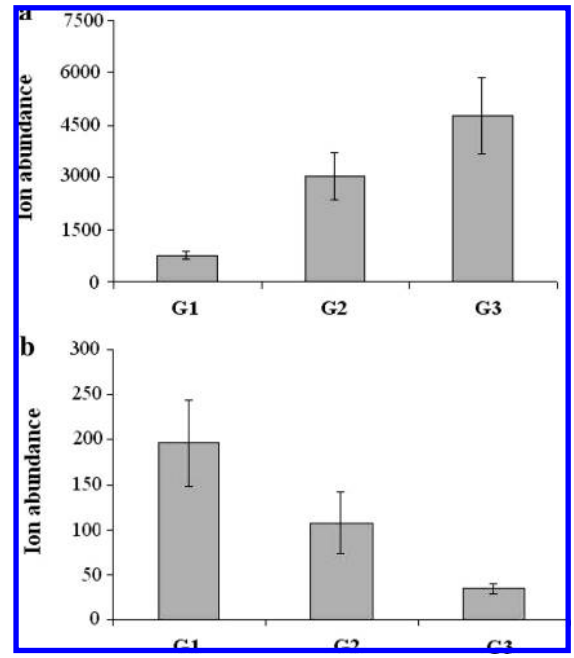


Figure 8. (A) MALDI ion abundances of bradykinin in the organic phase after extraction of an aqueous solution of 1×10^{-8} M bradykinin at pH 7.0 using a 2.5×10^{-5} M solution of G1, a 1×10^{-5} M solution of G2, and a 5×10^{-6} M solution of G3. (B) MALDI ion abundances of bradykinin in the aqueous phase after extraction of an aqueous solution of 1×10^{-8} M bradykinin at pH 7.0 using a 2.5×10^{-5} M solution of G1, a 1×10^{-5} M solution of G2, and a 5×10^{-6} M solution of G3.

(14) Fenselau, C.; Demirev, P. A. *Mass Spectrom. Rev.* **2001**, *20*, 157–171.

(15) The solution of the dendrons can be stored and used after 2 months of initial dissolution. For consistency in the results, these have to be sonicated first for at least 30 min.

Instrumentation. Surface tension measurements on the inverse micelles in toluene were obtained in a Dataphysics Contact Angle System 15 Plus tensiometer using water as the interface. This was accomplished by means of an inverse drop method in which a drop of toluene was formed with a 0.51 mm U needle that was placed into an optical glass bowl containing the water phase. The axial symmetric shape (Laplacian-Young profile) of the drop was analyzed by use of a video camera connected to a microcomputer. Analyzing the resulting droplet profile with the Laplace equation gave the interfacial tension, surface area, and drop volume in real time. Movements of the syringe piston were controlled by a stepping motor connected to a microcomputer. It was thus possible to compress or expand the surface of the drop within a few percent. The surface tensions of different concentrations of dendrons were studied at the toluene−water interface.

A Bruker Reflex III time-of-flight mass spectrometer was used to perform MALDI-MS analyses. All mass spectra were acquired in the reflectron mode and represent an average of 70 shots acquired at 37% laser power. The accelerating voltage was set to 20 kV.

Acknowledgment. We thank NIMGS of the NIH (GM-65255, ST) and the Office of Naval Research (N000140510501, RWV) for support of this work. We also thank Dr. K.N. Jayakumar for his assistance with developing the methodology for the dendrimers reported here.

JA803082V