# Thermodynamic Stabilization Mechanism of Block Copolymer Vesicles

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Vesicles can be prepared from a wide range of molecules, including block copolymers,[1,2] phospholipids,[3] synthetic surfactants,[4] and ionic block copolymer complexes.[5] In general, the equilibrium nature of small-molecule vesicles, as well as the partitioning of mixed species between the inner and outer surface, have received considerable attention.[6] Furthermore, the stabilization of small-molecule vesicles by attachment of polymer chains to the outer layer has also been explored.[7] For vesicles prepared from block copolymers, the situation is much less clear concerning their equilibrium status. In a recent paper from this group,[8] we have shown that vesicles may possibly be equilibrium structures since their sizes can be changed reversibly by changing the composition of the solvent mixture in which they have been prepared. Therefore, while some evidence favors the equilibrium nature of block copolymer vesicles under some experimental conditions, their equilibrium nature remains to be proven, specifically by elucidating the mechanism of thermodynamic stabilization. In the present paper, we elucidate the mechanism of thermodynamic stabilization of vesicles, and, in the process, describe a method of attachment of molecules preferentially to the outside or inside walls of the vesicles with a selectivity of ∼90% or better, or of attaching two different species, one to the inside and the other outside of the wall, in one step.

It should be noted that during the past few years, extensive efforts have been devoted to the study of polymeric nanoscale vesicles or hollow spheres. In addition to the early studies from this group,[1] and several subsequent investigations,[8] a number of other groups have been exploring this field by preparing vesicles or hollow nanospheres from a number of different polymers.[2,9] This high level of activity is due to the many potential applications of vesicles in such fields as microreactor chemistry, pharmacology, medicine, and cosmetics. For some of these applications, an understanding of the equilibrium nature of vesicles may be very useful.

In our early studies, vesicles in solution were shown to be a part of a continuum of morphologies of spheres, rods, vesicles, and in some cases also bicontinuous and hollow-rod structures, in which the morphology could be altered by any number of morphogenic factors such as block length, ion concentration, water concentration, etc.[1,8] This behavior is analogous to what is seen for block copolymer in bulk.[10] Once the aggregates have been prepared under dynamically active conditions, they can be frozen by either dropping the temperature to that of liquid nitrogen or by quenching the sample into excess water; this quenching process is analogous to what is done in studies of block copolymers in bulk where the equilibrated morphologies are quenched from a high temperature to a temperature at which no morphological changes take place over reasonable time scales. In our case, as long as the vesicles, prepared from molecules such as $PS_{310}$-*b*-$PAA_{52}$, are dissolved in the solvent system consisting of dioxane and water, with a water content between 28 and 40%, we suggested that they are equilibrium structures since their sizes depend on the water content and can be changed reversibly. After quenching, the isolated structures can be visualized by electron microscopy.

Our hypothesis for the thermodynamic stabilization of the vesicles is based on the segregation of the hydrophilic chains by block length between the inside and outside of the vesicles. The curvature of the vesicles needs to be stabilized if the vesicles are to be thermodynamically stable, and this curvature stabilization, according to this hypothesis, is accomplished by having the long hydrophilic chains segregate to the outside of vesicle, while the short hydrophilic chains segregate to the inside. Thus, the repulsion among corona chains outside is stronger than that inside the vesicles, and the curvature is maintained in a thermodynamically stable manner. Since even the best anionically prepared diblock copolymers have a finite chain length distribution, this segregation can provide a mechanism for thermodynamic stabilization under a range of conditions.

The proof of the above hypothesis is based on the incorporation of a fluorescent chromophore (pyrene) into the junction point between the hydrophobic and hydrophilic segments. Any vesicles synthesized from such a block copolymer will have the chromophore located at the hydrophobic/hydrophilic interface. If a quencher is added to a solution of such vesicles, any fluorescent species which is located on a surface which is exposed to a quencher, that is, the external vesicle surface or the surface of a spherical micelle, will experience efficient quenching, in contrast to the internal vesicle surface. We synthesized three different block copolymers containing such a fluorescent label, with identical hydrophobic lengths of 295 units of styrene ($PS_{295}$), but with three different hydrophilic lengths, that is, 12, 45, and 74 units of acrylic acid ($PAA_{12}$, etc). Each of these three polymers is labeled with pyrene (Py) molecules at the junction point. The polymers are denoted, for example, as $PS_{295}$-Py-*b*-$PAA_{12}$ for the chain containing 12 PAA units. In addition, an unlabeled block copolymer was prepared consisting of 300 units of styrene and 44 units of acrylic acid ($PS_{300}$-*b*-$PAA_{44}$). Turbidity versus water content was monitored for a solution prepared from the unlabeled polymer, and discontinuities were found at ∼8% (due to micellizaiton), 13% (the sphere to rod transition), and 35% (the rod to vesicle transition). Each of the labeled polymers was then mixed with the unlabeled polymer at a molar ratio 1:19, and vesicles were

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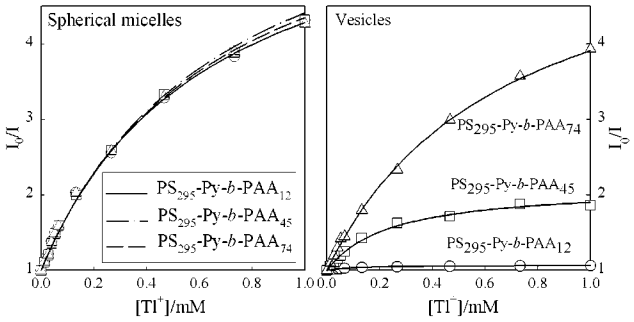


**Figure 1.** Steady-state fluorescence quenching by $Tl^+$ for spherical micelles and vesicles containing 5% of the labeled polymers.

prepared by dissolving the polymer in dioxane and adding water until a water content of 40%. The vesicles were then quenched by excess water and dialyzed against water. A transmission electron micrograph (TEM) of the vesicles prepared from the unlabeled polymer shows that the vesicles have average outside diameter (± standard deviation) of 98 ± 7 nm and an average wall thickness of 26 ± 3 nm (sample size = 100 vesicles). The other vesicles have average diameters (and wall thickness) of 97 ± 7 nm (26 ± 3 nm), 97 ± 8 nm (26 ± 3 nm), and 97 ± 7 nm (26.0 ± 3 nm), respectively.

Once the vesicles were prepared, $Tl^+$ was added to the solution of the vesicles in pure water (where the PS wall is below its Tg) at concentrations from 0 to 1 mM. If the hypothesis is correct, then the vesicles prepared with the labeled short hydrophilic diblock should experience minimum quenching, since those chains would be preferably segregated to the inside of the vesicles and be thus largely inaccessible to the quencher. By contrast, those with the longest hydrophilic block should experience maximum quenching since most of those chains will be on the outside of the vesicles. Those vesicles prepared with chains of intermediate length should experience an intermediate degree of quenching. Finally, spherical micelles prepared with labeled copolymers of any of the three hydrophilic block lengths should experience the same high degree of quenching since all the chromophores are on the outside. $Tl^+$ is assumed not to penetrate the glassy polystyrene wall of the vesicles.

The results of the steady-state quenching experiments are shown in Figure 1 plotted as $I_0/I$ versus the quencher concentration. $I_0$ is the fluorescence intensity without quencher, while $I$ is the steady-state fluorescence in the presence of quencher. $\phi$ and $K$ values were calculated from the Stern−Volmer equation:

$$I_0/(I_0 - I) = 1/\phi K[Tl^+] + (1/\phi)$$

which correlates the fluorescence intensity of a chromophore in the presence of a quencher to the accessibility of the chromophore to the quenching species. $\phi$ is the fraction of chromophores which can be quenched, and $K$ is the Stern−Volmer constant. The calculated values of $\phi$ and $K$ are given in Table 1. The lines in Figure 1 were calculated from the bracketed values of $\phi$ and $K$; those values, all of which are within one standard error, were chosen so as to optimize the fit. As can be seen from Figure 1, all three spherical micelle samples show $\phi$ values of ∼92%. By contrast, the vesicles prepared from the polymer mixture with the shortest labeled hydrophilic chain show an accessibility factor, $\phi$, of only ∼7%. Those with intermediate block length yield 53%, and those with the longest labeled hydrophilic chain yield 88%, only slightly below the values for spherical micelles. Those values are the percentages of pyrene molecules accessible to the $Tl^+$ ions in the solution. Clearly, the pyrene residues in the vesicles prepared from the longest labeled hydrophilic chains are ∼12 times more accessible to the quencher than the pyrene in the vesicles prepared from the shortest labeled hydrophilic chains, which proves that the longest chains are largely on the outside of the vesicles, while the shortest chains are on the inside. It should be recalled that the hydrophilic blocks in the shortest or longest of the three labeled samples also have a finite distribution of block lengths, so that not all of the chains would be segregated to either the inside or the outside. Even if the chains were monodisperse, some statistical segregation would still be expected on entropic grounds.

**Table 1.** Apparent $\phi$ and $K$ Values of the PS-*b*-PAA Micelle Solutions

| | spheres | | vesicles | |
|---|---|---|---|---|
| system | $\phi$ | $K$ ($mM^{-1}$) | $\phi$ | $K$ ($mM^{-1}$) |
| $PS_{300}$-*b*-$PAA_{44}$/ | 0.91 ± 0.07[a] | 9.5 ± 1.0 | 0.065 ± 0.003 | 9.0 ± 0.6 |
| $PS_{295}$-Py-*b*-$PAA_{12}$ | (0.85)[b] | (9.9) | (0.065) | (9.0) |
| $PS_{300}$-*b*-$PAA_{44}$/ | 0.92 ± 0.07 | 9.2 ± 0.9 | 0.53 ± 0.02 | 8.9 ± 0.4 |
| $PS_{295}$-Py-*b*-$PAA_{45}$ | (0.85) | (9.7) | (0.53) | (8.9) |
| $PS_{300}$-*b*-$PAA_{44}$/ | 0.91 ± 0.07 | 9.3 ± 0.9 | 0.88 ± 0.05 | 8.4 ± 0.6 |
| $PS_{295}$-Py-*b*-$PAA_{74}$ | (0.85) | (9.8) | (0.83) | (8.8) |

[a] Value± standard error. [b] The numbers in brackets were used to calculate the lines in Figure. They were chosen to give the best fit. All the values are within the error limits.

In summary, we have shown that the mechanism of thermodynamic stabilization of block copolymer vesicles consists of segregation of chains with short hydrophilic block lengths to the inside of the vesicles and of the long hydrophilic chains to the outside. This segregation increases the corona repulsion on the outside of the vesicle relative to that on the inside and provides thermodynamic stabilization of the curvature. This result is also consistent with the finding that it is easier to prepare block copolymer vesicles from polymers with long chains than it is from those with shorter chains, since the longer chain polymers contain a wider range of block lengths, even for the same polydispersity. From the practical point of view, this present finding allows us to attach substituents selectively to either the inside wall or the outside wall of a vesicle, which may have useful applications. One can even envisage the one-step self-assembly of vesicles in which one species is attached to the inside wall and another to the outside; this would be very difficult to achieve by any other technique.

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