Block Copolymer Microdomains: A Novel Medium for Enzymatic Reactions

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Block copolymers exhibit the phenomenon of microdomain formation in pure states as well as in solutions. The microdomains vest the block copolymer assemblies with the intriguing characteristics of microheterogeneous media. We demonstrate that this microheterogeneity in hydrophobic–hydrophilic block copolymer systems can be exploited for immobilizing enzymes and to carry out enzymatic reactions. Examples involving cholesterol oxidase and horseradish peroxidase are provided here. The observed changes in the enzymatic activity in block copolymer microdomains from that in the aqueous media are interpreted in terms of the hydrophobicity of the reaction microenvironment. The block copolymer microdomains are simple to generate, well defined, and easily reproducible. Therefore, they hold significant potential as media for enzymatic biosynthetic reactions when the substrates or the reaction products are water insoluble.

Introduction

Block copolymers can be visualized as assemblies of homopolymers that are linked to one another by chemical bonds. For example, a diblock copolymer of type $[A_kB_m]$ consists of a block of k repeating units of kind A covalently bonded to a block of m repeating units of kind B. The two different types of blocks within the copolymer usually do not mix with one another. This behavior is a consequence of the high molecular weight of polymers, which results in a very small entropy of mixing that is insufficient in magnitude to offset the small but positive enthalpy of mixing. Consequently, at equilibrium, the dissimilar segments in block copolymers undergo separation into different phases. These segregated phases are referred to as microdomains (Reiss et al., 1985). The microdomains can be spherical, cylindrical, or lamellar in shape. Further, they can be disordered or ordered. These structural features of the microdomains are determined by the composition and molecular weight of the block copolymer as well as by the system conditions such as the temperature.

Microdomain formation occurs also in solutions of block copolymers (Tuzar and Kratochvil, 1976). For example, when an AB diblock copolymer is present in a solvent selective for the B block, the copolymer molecules spontaneously self-assemble to form microdomain structures characterized by a core region made up of the A blocks and a shell region made up of the B blocks and the solvent. Such a microdomain structure generated in block copolymer solutions is analogous to the micellar structures usually formed from low molecular weight surfactant or lipid molecules (Tanford, 1980).

A characteristic feature of microdomains in block copolymer assemblies, whether in pure state or in solution, is the large interfacial area between the segregated domains. Another distinguishing feature of the microdomains is their ability to solubilize chemical species that are compatible with the domains (Figure 1). Both these features contribute to the potential use of block copolymer

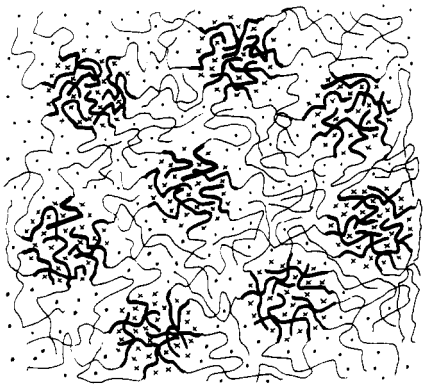

Figure 1. Schematic representation of the microdomain structure in block copolymer systems. A disordered spherical domain structure is illustrated. The heavy lines refer to hydrophilic domains, while the lighter lines refer to hydrophobic domains. The × symbols refer to water that could be solubilized, while the dots in the hydrophobic domains refer to hydrophobic substances. When enzymes are solubilized within the hydrophilic domains, they resemble enzymes dissolved in a polymer solution.

systems in various practical applications. For example, we showed earlier that block copolymer assemblies in water can selectively solubilize aromatic hydrocarbons in preference to aliphatic hydrocarbons (Nagarajan et al., 1986). This can be the basis for a chemical separation process. Here, we demonstrate the use of block copolymer microdomains as media for immobilizing enzymes and for carrying out enzymatic reactions.

Enzymatic reactions in aqueous media have been found to be inadequate for a number of reactions of commercial interest (Shield et al., 1986). In many cases, the reactants are sparingly soluble in water and their accessibility to the enzyme is limited by their very low concentrations. In other cases, the reaction product is sparingly soluble in water, thus saturating the water even in the early stages of the reaction; this inhibits the continued catalytic activity of the enzyme very early in the course of the reaction. To overcome these limitations, various enzymatic reaction media have been proposed such as two-phase dispersions of water and organic solvents (Antonini et al., 1981; Carrea, 1984), single-phase mixtures of water and organic solvents (Oyama and Kihara, 1984), pure organic solvents

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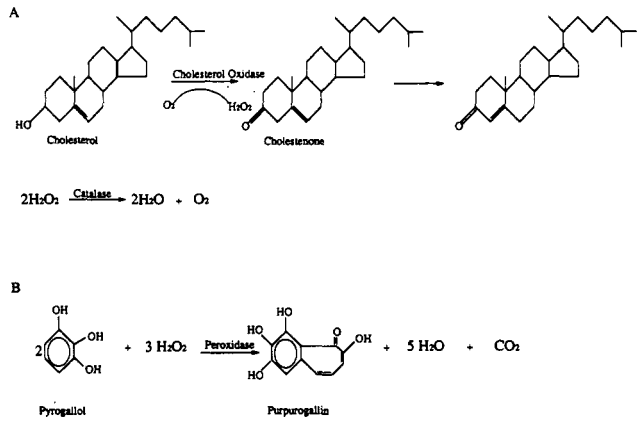

Figure 2. Schematic representation of the enzyme-catalyzed reactions investigated in this study.

(Zaks and Klibanov, 1984, 1985; Laane et al., 1987), reverse micelles (Martinek et al., 1977, 1982, 1986; Menger and Yamada, 1979; Wolf and Luisi, 1979; Luisi, 1985), and supercritical fluids (Randolph et al., 1988a,b; Hammond et al., 1985).

In this paper, we propose the use of a novel microheterogeneous system, namely, the microdomain structure of hydrophilic–hydrophobic block copolymers, as an effective enzymatic reaction medium. We show that enzymes solubilized within the hydrophilic domains retain their catalytic activity. The immobilized enzyme reacts with water-insoluble substrates that are solubilized within the hydrophobic domains acting as microreservoirs of substrates. It is possible to use the block copolymer microdomains profitably also in case of water-soluble substrates, if the reaction product is water insoluble. In this case the product is continuously removed from the hydrophilic domains by its transfer into the hydrophobic domains acting as microsinks; thus the problem of product inhibition is avoided.

Two enzymatic reactions (Figure 2) have been explored in this work. In the first example, the enzyme cholesterol oxidase is used to oxidize cholesterol to cholestenone. The coproduct hydrogen peroxide, which can inactivate the enzyme, is removed as soon as it is produced by the action of the enzyme catalase via a decomposition reaction. It may be noted that the poor aqueous solubility of cholesterol restricts the reaction in aqueous media from being of practical interest. This limitation is easily overcome through the use of block copolymer microdomains. This reaction is typical of various steroid conversions catalyzed by cholesterol oxidases and reductases, which can be of potential value to food and pharmaceutical industries. The advantage derived from the increased solubility of cholesterol in the block copolymer system (approximately 22 000 μM in the block copolymer liquid employed here and up to 50 000 μM in solutions of this block copolymer in cyclohexane, depending upon the amount of copolymer in the solution) compared to the aqueous-phase solubility of 4.7 μM, is obvious. The second enzymatic reaction involves horseradish peroxidase, which is used to catalyze the oxidation of pyrogallol to purpurogallin by hydrogen peroxide. In this case, the substrate has considerable water solubility while the reaction product has a lower water solubility. The dissolution of purpurogallin in the hydrophobic microdomains removes it from water as it forms and helps prevent product inhibition from affecting the catalytic activity. Horseradish peroxidase also catalyzes oxidation reactions involving a large number of water insoluble substrates such as substituted phenols and aromatic amines that are toxic and/or carcinogenic, and thus it is of potential significance to hazardous waste treatment (Klibanov and Morris, 1981; Klibanov, 1982). The present approach may be useful for these applications as well.

Experimental Methods

Materials. The block copolymer used in this study is a triblock copolymer of ethylene oxide and propylene oxide. It is distributed under the commercial name of Pluronics by BASF Corp. The block copolymer has the general structure $HO(EO)_x(PO)_y(EO)_zH$, where EO and PO refer to ethylene oxide and propylene oxide and x, y, and z refer to the numbers of monomeric units in the different blocks. Pluronics are available in a range of molecular weights and at various compositions of EO and PO. All the experiments discussed here have been carried out with Pluronic L61, which has a molecular weight of 2000 daltons and contains 10 wt % ethylene oxide. This block copolymer is a liquid at room temperature and is insoluble in water at 25 °C. One and ten weight percent aqueous solutions of this copolymer have cloud points of 24 and 17 °C, respectively. This block copolymer has low toxicity and is mild in terms of eye and skin irritation, and it is thus safer to use when compared against synthetic surfactants used in the formulation of analogous microheterogeneous structures such as reverse micelles. Laser light scattering measurements in solutions of the block copolymer with cyclohexane as solvent and in solvent-free block copolymer liquid indicated no microdomain structure to be present. This is expected on physicochemical grounds (Nagarajan and Ganesh, 1989a,b) because the low molecular weight of the block copolymer and the relative composition of the two blocks do not favor the formation of the microdomains. But on the addition of even traces of water, the scattering studies revealed the existence of microdomain structure. Thus, this block copolymer, either as a pure phase or as a solution in a nonpolar solvent, spontaneously generates the microdomains for hosting the enzyme on the simple addition of a buffered solution of the enzyme.

The enzyme cholesterol oxidase (EC 1.1.3.6) from *Nocardia erythropolis* was supplied by Boehringer Mannheim as a solution in ammonium sulfate. The enzyme catalase (EC 1.11.1.6) was obtained from Boehringer Mannheim in the form of a suspension. The enzyme horseradish peroxidase (EC 1.11.1.7) was obtained from Sigma in the form of a powder and had an RZ value of 1.4. The RZ value, defined as the ratio of the absorbance at 403 nm to the absorbance at 275 nm, specifies the ratio of the hemin to protein content in the enzyme. The cholesterol oxidase solution in ammonium sulfate was extensively dialyzed against buffers at appropriate pH values before its use in the catalytic studies. All other chemicals used as solvents and for preparing various buffers were obtained in reagent grade.

Immobilization of Enzymes in Block Copolymer Microdomains. The block copolymer was dissolved in cyclohexane, which is a good solvent for poly(propylene oxide), and this solution was used as the microheterogeneous system for the enzymatic reactions. The amount of block copolymer in the solution was changed between 15 and 100 vol %; thus the block copolymer in solution as well as the solvent-free block copolymer liquid were employed. A change in the amount of block copolymer in solution may give rise to changes in the dimensions of the microdomains as well as of domain morphologies. These changes remain to be explored in a future study. The enzyme in an aqueous buffer at the desired pH was added

to the block copolymer–solvent system. For studies involving cholesterol oxidase, the aqueous enzyme solution employed in all the experiments was prepared with 50 mM Tris-HCl buffer. In addition to the enzyme cholesterol oxidase, the enzyme catalase in aqueous buffer was also added for the purpose of destroying the undesirable coproduct hydrogen peroxide generated from the cholesterol oxidation reaction (Figure 2). For studies involving horseradish peroxidase, the aqueous enzyme solution employed in all the experiments was prepared from 25 mM potassium phosphate buffer at a pH of 7. The amount of the enzymes in the aqueous phase was typically 0.1 mg/cm^3. The enzyme solution was spontaneously solubilized in the poly(ethylene oxide) microdomains and no intensive mixing was needed. This immobilized enzyme system was used in the subsequent studies of enzymatic activity.

Determination of Activity of Cholesterol Oxidase. The substrate cholesterol was added to the block copolymer system containing the solubilized enzymes cholesterol oxidase and catalase. The overall reaction mixture had a volume of 3.19 cm^3 and contained 2 μg of cholesterol oxidase and 4 μg of catalase. The concentration of cholesterol based on the total system volume was 21.56 mM. This concentration corresponds to the solubility limit of cholesterol in a 16.7 vol % solution of the block copolymer in cyclohexane. Following the addition of cholesterol, the mixture was slightly shaken. The reaction was thus initiated. Initial velocities of enzymatic cholesterol oxidation were determined spectrophotometrically at 25 °C, by continuously recording the increase in absorbance at 240 nm, corresponding to the absorbance maximum of cholestenone. The activity was calculated on the basis of the specified molar absorption coefficient of cholestenone of 15.5 mM^{-1} cm^{-1}. The enzyme activity has been expressed in the figures in terms of units, where one unit represents 1 μmol of cholestenone formed/min at 25 °C. The activity of cholesterol oxidase in aqueous media was also determined in a similar manner. In this case, the aqueous phase was a 50 mM Tris-HCl buffer. It contained 2 μg of cholesterol oxidase and 4 μg of catalase. The substrate cholesterol was added to this aqueous phase. In order to promote the solubility of cholesterol in water, approximately 3% by volume of 1-propanol and 4 mg/cm^3 of the nonionic detergent Thesit were added as suggested by the enzyme supplier. The total volume of the reaction mixture was 3.19 cm^3 as before. The concentration of cholesterol in this reaction mixture was 0.323 mM, which was much larger than the aqueous-phase solubility, 4.7 μM, of cholesterol. On the addition of the substrate cholesterol, the reaction was initiated. The enzyme activity was determined via absorbance measurements as described above.

Determination of Activity of Horseradish Peroxidase. The substrate pyrogallol was added in required amounts to the block copolymer system containing the immobilized horseradish peroxidase. An excess of hydrogen peroxide was then added to the system to initiate the reaction. The overall reaction mixture had a volume of 3 cm^3 and contained 0.12 μg of horseradish peroxidase, 2.57 mM pyrogallol, and 4.28 mM hydrogen peroxide. Initial velocities of pyrogallol oxidation at 25 °C were measured spectrophotometrically by following the absorbance changes at 420 nm, which is characteristic of the reaction product purpurogallin. The enzyme activity was determined on the basis of the specified mass absorption coefficient of purpurogallin of 121 g^{-1} cm^{-1}. The measured activity has been expressed as units, where one unit represents 1 mg of purpurogallin formed/20 s. The activity of horseradish peroxidase in the aqueous medium was determined in a similar manner. In this case, the aqueous phase was a 25 mM potassium phosphate buffer at a pH of 7.0. The overall reaction mixture had a volume of 3 cm^3 and contained 0.12 μg of the enzyme peroxidase, 2.57 mM pyrogallol, and 4.28 mM hydrogen peroxide. The reaction was initiated on the addition of hydrogen peroxide and the enzyme activity was determined by absorbance measurements as described above.

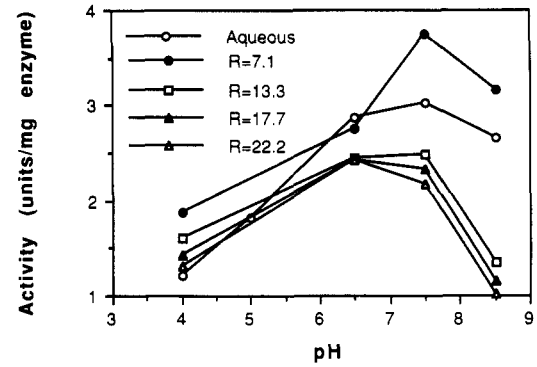

Figure 3. Activity of cholesterol oxidase in block copolymer microdomains as a function of R (the molar ratio of water to block copolymer) and pH. One unit represents 1 μmol of cholestenone formed/min at 25 °C. The concentration of the substrate cholesterol was kept constant for all experiments with block copolymer microdomains at 21.56 mM based on the overall reaction mixture volume. The concentration of cholesterol for the experiment in aqueous medium was 0.323 mM.

Results and Discussion

Effect of pH and Water Content on Cholesterol Oxidase Activity. The activity of cholesterol oxidase in block copolymer microdomains is shown in Figure 3 as a function of the water content in the microdomains as well as of pH. The water content is specified in terms of the ratio R between the moles of water in the hydrophilic domains and the moles of the block copolymer in the system. The microdomains in this study were those resulting in a 16.7 vol % solution of the block copolymer in cyclohexane. The concentration of the substrate cholesterol in the overall reaction mixture was 21.56 mM. Indeed, the local concentration of cholesterol in the hydrophilic domain would be much smaller when compared to this overall concentration, because of the preferential partitioning of cholesterol in the hydrophobic domain. The local concentration of cholesterol in the hydrophilic domain is expected to be comparable in magnitude to its aqueous-phase solubility of 4.7 μM. The activity data are reported in terms of units, where one unit is defined as the amount of enzyme that will oxidize 1 μmol of cholesterol to cholestenone in 1 min at 25 °C. Also shown for comparison is the activity in the aqueous medium. For determining the enzyme activity in the aqueous medium, a cholesterol concentration of 0.323 mM was employed. The amount of enzyme and the total reaction mixture volume were the same as for the experiments with block copolymer systems. The results show that enzymatic activity in microdomains is comparable to that in the aqueous medium. Indeed, if the very low local concentration of cholesterol in the hydrophilic microdomains (compared to the concentration of 0.323 mM in the aqueous medium experiments) is taken into account, the activity of the enzyme in the block copolymer microdomains can be viewed as being much superior to the activity of the enzyme in the aqueous medium. The enzymatic activity

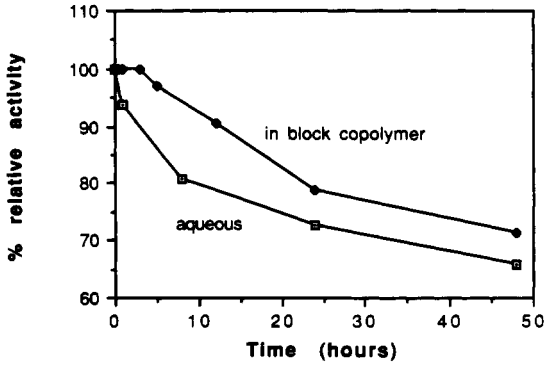

Figure 4. Percent relative activity of cholesterol in block copolymer microdomains and in aqueous solution at 25 °C, as a function of time. The activity is relative with respect to that at time zero when the enzyme is added to the reaction mixture. The microdomain experiments are those performed with a 16.7 vol % solution of block copolymer in cyclohexane with $R = 7.1$. The substrate concentrations are identical with those in Figure 3 for the microdomain and the aqueous medium experiments.

increases with decreasing water content ratio R in the microdomains. Further, a pH optimum can be discerned that depends somewhat upon the value of the water content ratio R in the microdomains. This pH optimum is practically identical with that in the aqueous medium. Intuitively, this may be expected, given the nonionic nature of the block copolymer studied.

Stability of Cholesterol Oxidase in Block Copolymer Microdomains. An important factor in the search for an enzymatic reaction medium is the stability of the enzyme in the immobilization medium. The stability was examined at 25 °C and for the optimal conditions identified in Figure 3, namely, for a pH value of 7.5 and a R value of 7.1. The microdomains in this case (as also in Figure 3) are those resulting from a 16.7 vol % solution of the block copolymer in cyclohexane. At time zero, the enzyme cholesterol oxidase was introduced into the block copolymer–solvent system. The substrate cholesterol was added to this solution at defined intervals of time. The catalytic activity of the enzyme was determined as specified earlier. The concentration of cholesterol used was 21.56 mM on the overall reaction mixture volume of 3.19 cm^3, while the amounts of enzymes in that volume were 2 μg of cholesterol oxidase and 4 μg of catalase. The activity of the enzyme at various times in the microdomains is compared against that in the aqueous medium at the same time in Figure 4. As mentioned earlier, the cholesterol concentration employed for the aqueous phase measurements was 0.323 mM. The relative activity (with respect to the activity at time zero) of the enzyme in the block copolymer microdomains is found to be better than that in the aqueous medium. The activity in the microdomains is retained over extended periods of time.

Effect of Copolymer Concentration on Cholesterol Oxidase Activity. An obvious feature of interest in the present study is the possibility of utilizing the block copolymer liquid, without any solvent (such as cyclohexane), as the enzymatic reaction medium. This would make the immobilization medium a very simple one since it would be based on a single-component system. Also, other advantages may possibly be derived, such as easier product recovery. One may note that when the amount of the copolymer in the solution is modified, changes may occur in the dimensions of the hydrophobic and hydrophilic microdomains as well as in the nature of the domain morphologies. These changes can modify the microenvironment of the enzyme and thus potentially give rise to altered intrinsic enzymatic activities.

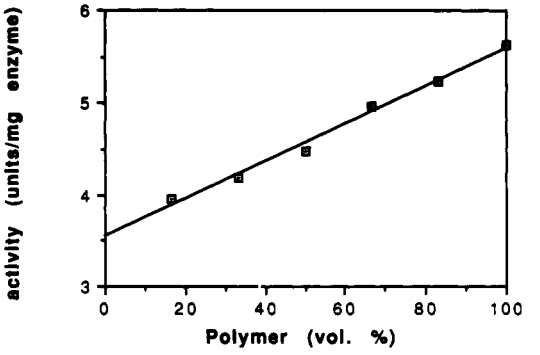

Figure 5. Activity of cholesterol oxidase as a function of the volume percent of block copolymer in cyclohexane. The substrate concentration and the water content ratio R are identical with those in Figure 3.

In this study, enzyme activity measurements were carried out as a function of the volume fraction of copolymer in solution at a pH of 7.5 and with a water content ratio R of 7.1 (at the optimal point from Figure 3). The concentrations of the enzyme and of the substrate cholesterol based on the overall reaction volume were kept constant, identical with those employed in Figure 3. The measured enzyme activity data are given in Figure 5 as a function of the volume percent copolymer in solution, including at 100%, when no solvent is present and the microdomains are formed in the copolymer itself. Interestingly, the activity is found to increase with an increase in the volume percent of copolymer in solution, with the highest activity exhibited in the solvent-free block copolymer systems.

For the data reported in Figure 5, the water content ratio R and the concentration of cholesterol based on the total volume of the reaction mixture are held constant. However, in this reaction volume, the total amount of water and hence the volume of the hydrophilic domains increases as the amount of copolymer in the solution is increased (because of constant R). Since cholesterol is only sparingly soluble in water, it is essentially present in the hydrophobic domains. Therefore, the increase in the total amount of water in the reaction mixture will not cause any perceptible change in the concentration of the cholesterol in the hydrophilic domains. Thus, the observed increase in enzymatic activity must result from intrinsic variations in the nature of the microenvironment of the enzyme rather than due to any changes in the substrate concentration in the hydrophilic domains.

Absorption Spectra of Cholesterol Oxidase. Changes in the microenvironment of the enzyme are qualitatively reflected by the absorption spectra of the enzyme as shown in Figure 6. Absorption in the range 180–220 nm arises from electronic transitions in the polypeptide backbone itself and is therefore sensitive to the backbone conformation (Mathews and van Holde, 1990). A change in the conformation causes the microenvironment of all groups contributing to the absorption to change, thus resulting in some variation in the extinction coefficients as well. In Figure 6, one can observe that the characteristic absorbance peak is shifted from 210 nm in aqueous media to 220 nm in block copolymer microdomains. When the spectra for different block copolymer systems are compared, one finds that the absorbance intensity increases with a decrease in water content of the hydrophilic domain. This indicates interactions between the enzyme and the block copolymer as the water content ratio R is decreased and the consequent exposure of additional chromophores of the enzyme, which accounts

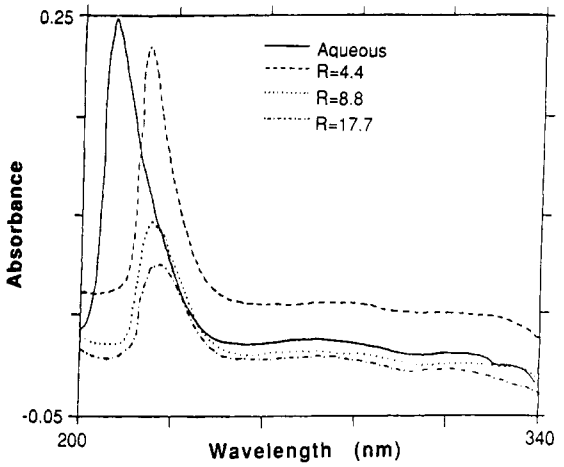

Figure 6. Absorption spectrum of cholesterol oxidase in aqueous solution and in block copolymer microdomains at various R values. The microdomains are those occurring in a 16.7 vol % solution of the block copolymer. The aqueous phase in all the experiments is 50 mM Tris-HCl buffer at a pH of 7.5. The concentration of enzyme based on the total volume in all experiments is 37.7 $\mu g/cm^3$.

for the increased absorbance intensity. As a result, the increase in the absorbance intensity with decreasing R can be attributed to a corresponding increase in the hydrophobicity of the enzyme's microenvironment. An observation that is apparently contradictory is the large absorbance intensity in the hydrophilic bulk water environment as shown by the spectrum for the aqueous medium. This contradiction is resolved by noting that the extinction coefficients in the water medium and in the block copolymer microdomains are different from one another, and hence the absorbance intensities cannot be compared for these two systems. With this recognition, the observed increase in enzymatic activity in block copolymer systems with decreasing R (Figure 3) can be interpreted as implying that the enzymatic activity of cholesterol oxidase is enhanced by increased hydrophobicity of the enzyme's microenvironment. This inference linking enzyme activity to the hydrophobicity of the microenvironment can be applied to the experimental data shown in Figure 5. There one observes an increase in enzyme activity with increasing copolymer concentration. This suggests that an increase in the hydrophobicity of the enzyme's microenvironment results from an increase in the copolymer concentration. Thus we find that either a decrease in R or an increase in the amount of copolymer in solution increases the hydrophobicity of the microenvironment and contributes to increased activity of cholesterol oxidase.

Effect of Water Content on Horseradish Peroxidase Activity. The activity of horseradish peroxidase in block copolymer microdomains at a pH of 7 has been determined for various amounts of the water content ratio R in the reaction mixture. The concentrations based on the overall reaction volume were 2.57 mM pyrogallol, 4.28 mM hydrogen peroxide, and 0.04 $\mu g/cm^3$ peroxidase enzyme. The enzymatic activity is represented in terms of units, where one unit is defined as the amount of enzyme that will form 1 mg of purpurogallin from pyrogallol in 20 s at a pH of 7 and at 25 °C. The measured activity of horseradish peroxidase is shown in Figure 7 as a function of the water content ratio R. The activity is much smaller than in the aqueous medium, where the activity as specified by the enzyme supplier is of the order of 200 units/mg of enzyme. The activity in the block copolymer microdomains increases substantially with increasing amount of water in the hydrophilic domain.

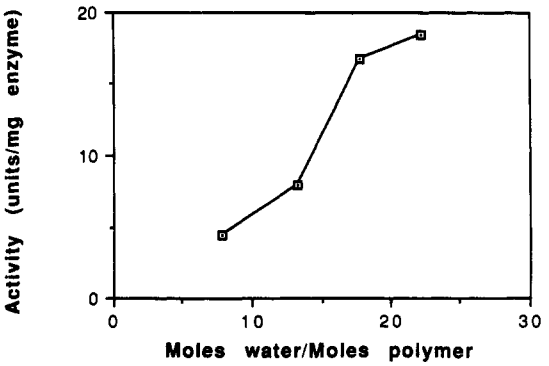

Figure 7. Activity of horseradish peroxidase in block copolymer microdomains as a function of the water content ratio R. One unit represents 1 mg of purpurogallin formed from pyrogallol in 20 s at a pH of 7.5 and at 25 °C. The microdomains are those formed in a 16.7 vol % solution of the block copolymer.

In the above experiments, the total amount of the substrate pyrogallol and the oxidizing agent hydrogen peroxide are kept constant while the ratio R is increased. An increase in R implies an increasing total amount of water. Since pyrogallol and hydrogen peroxide are both highly water soluble, the concentrations of these two reactants in the hydrophilic domains decrease with increasing R. Such decreased substrate concentrations should lead to a decrease in the measured enzyme activity rather than the observed increase in the enzyme activity shown in Figure 7. Evidently, the intrinsic enzyme activity increases substantially with increasing R, more than compensating the decrease mandated by lowered substrate concentrations. This behavior is in direct contrast to that exhibited by cholesterol oxidase, wherein the enzyme activity decreased with increasing value of R.

Influence of Copolymer Concentration on Peroxidase Activity. To explore the possibility of using the block copolymer without any solvent as the reaction medium, the enzymatic activity was determined as a function of the copolymer concentration. The experimental conditions were chosen to correspond to the optimal condition indicated in Figure 7, namely, a pH of 7 and a water content ratio R of 22.2. The concentrations of pyrogallol, hydrogen peroxide, and the enzyme peroxidase based on the total reaction volume were all kept identical with those employed in Figure 7. The activity of the enzyme in concentrated block copolymer solutions including the solvent-free block copolymer liquid is shown in Figure 8. The activity is found to decrease with increasing copolymer concentration. One may note that the total amounts of pyrogallol and hydrogen peroxide are held constant in these activity measurements along with the water content ratio R. Consequently, the increase in copolymer concentration accompanies an increase in the total volume of water and thus of the hydrophilic domains and proportionately decreases the concentrations therein of the water-soluble substrates pyrogallol and hydrogen peroxide. The observed decrease in the activity of peroxidase with increasing copolymer concentration is partly due to this decrease in the substrate concentrations in the hydrophilic domains.

The above dilution effect is accounted for by carrying out measurements of enzyme activity while holding constant the ratio between the reactants (pyrogallol and hydrogen peroxide) and the total amount of water in the microdomains. This would correspond to having a constant concentration of the reactants in the hydrophilic

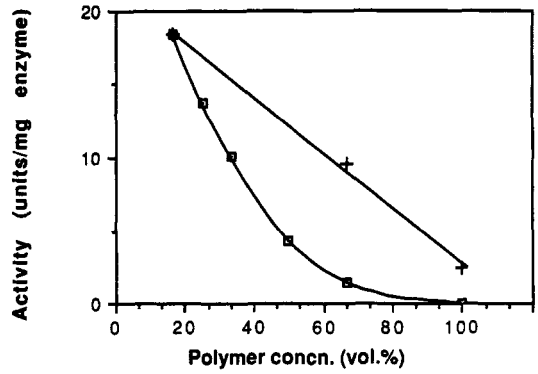

Figure 8. Activity of horseradish peroxidase in block copolymer microdomains as a function of the volume percent of the block copolymer in cyclohexane. The square symbols refer to activity data when the concentrations of the substrates based on the total reaction volume were held constant, while the plus symbols refer to activity data when the concentrations of the substrates in the hydrophilic microdomains were held constant.

domains for the limiting assumption of all reactants being present in the hydrophilic domains. These experimental results are also shown in Figure 8. The difference between the two lines on this figure can be attributed to the activity decrease caused by the dilution of the substrates. The activity data, even after accounting for the dilution effect, show that the activity of the enzyme decreases with increasing copolymer concentration. This decrease should reflect a change in the intrinsic activity of the enzyme originating from probable microenvironmental changes induced by increasing copolymer concentration. This behavior of horseradish peroxidase is also in direct contrast to that exhibited by cholesterol oxidase under equivalent conditions of increasing copolymer concentration.

Absorption Spectra of Immobilized Horseradish Peroxidase. The absorption spectra of the enzyme in the aqueous medium and in the block copolymer microdomains are shown in Figure 9 for various values of R. The absorbance maximum is shifted from 409 nm in the aqueous medium to 420 nm in the block copolymer microdomains. When the spectra for block copolymer solutions with different R values are compared, one finds that the absorbance intensity increases with decreasing water content R. As mentioned earlier, the increase in the absorbance intensity may be attributed to increasing hydrophobicity of the enzyme's microenvironment. Therefore, the observed increase in the activity of horseradish peroxidase with increasing R suggests that the activity of this enzyme is enhanced by a more hydrophilic microenvironment. Since we had inferred earlier that an increase in the amount of the copolymer in solution increases the hydrophobicity of the enzyme's microenvironment, we should expect to find the activity of horseradish peroxidase decreases with increasing amounts of copolymer. Indeed, such a result was seen in Figure 8, demonstrating consistency between the absorption spectral data and the activity data measured as a function of R and of the copolymer concentration.

Conclusions

We have shown that block copolymer microdomains can serve as effective microheterogeneous media for carrying out enzymatic biosynthetic reactions. The observed activities of cholesterol oxidase and horseradish peroxidase make the block copolymer microdomains interesting systems for further exploration involving various types of enzymes. The illustrative studies with the two enzyme systems suggest that if the hydrophobicity of the microenvironment is increased, the activity of cholesterol oxidase is enhanced while that of horseradish peroxidase is diminished. We can conveniently manipulate the nature of the enzyme's microenvironment because the block copolymer microdomains are equilibrium entities, are easily reproduced, and possess well-defined geometrical characteristics depending upon the molecular weight, block composition, temperature, and the type and amount of solvent present. Further, substantial amounts of water-insoluble substrates could be solubilized within the hydrophobic domains, thus eliminating the substrate availability from limiting the rate of practical enzymatic reactions. The substrates and the reaction products can easily be removed from the block copolymer microdomains by altering the system temperature, which significantly affects the polymer solubility behavior. The closest analogue of the block copolymer microdomains are the reverse micelle systems formed from conventional low molecular weight surfactants. Here, one requires not only a surfactant and a solvent but often an alcohol cosurfactant as well to form the reverse micelles. In comparison, one can potentially work with the single-component system of pure block copolymers. By choosing the block copolymer molecular weight to be not too large, one can ensure that the diffusional resistances within the microdomains are similar to those that would occur in low molecular weight solvents. These resistances can be easily manipulated, if necessary, by the addition of solvents or by small changes in temperature. For these reasons, the block copolymer microdomains hold significant potential as microheterogeneous media for enzymatic reactions.

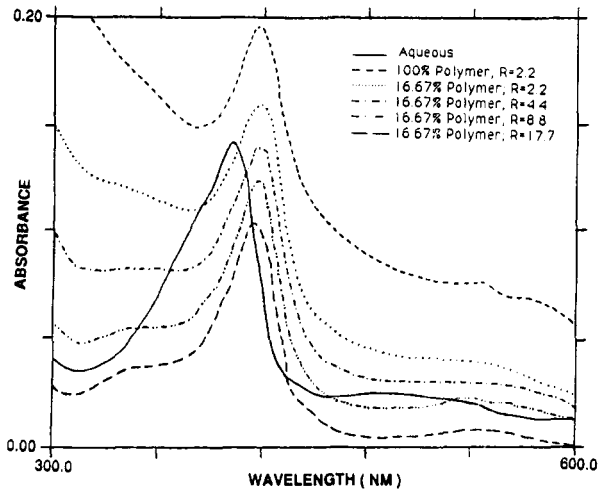

Figure 9. Absorption spectra of horseradish peroxidase in the aqueous solution and in block copolymer microdomains. The aqueous phase used in all the experiments is a 25 mM potassium phosphate buffer at a pH of 7.0. The microdomains employed are those formed in a 16.7 vol % solution of the block copolymer. The amount of enzyme present in all experiments is 82.4 $\mu g/cm^3$.

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