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Anisotropic Membranes with Carboxypeptidase G_1

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Abstract

Anisotropic polysulfone membranes were prepared with carboxypeptidase G_1 embedded in the polymer structure. The enzymatically active flat and hollow-fiber membranes were obtained by precipitating the polymer from solution in an organic mixture in which an aqueous solution of the enzyme had been dispersed. The process has been found to be particularly suitable for the immobilization of enzymes in anisotropic hollow fibers that exhibited no detectable enzyme leakage upon perfusion. The pH profiles measured with the enzyme in free solution and in the embedded form were similar. Kinetic parameters of multitubular enzyme reactors were investigated by measuring the rate of hydrolysis of glutamate from folic acid or methotrexate at different flow rates and substrate concentrations. The relatively slow mass transfer in such reactors was found to affect strongly the observed kinetics. The results of in vitro experiments with 5000 fiber reactors suggest that hollow fiber cartridges prepared with such membranes have clinical potential for the extracorporeal removal of methotrexate from blood.

Index Entries: Anisotropic membranes, with carboxypeptidase G_1; membranes, anisotropic; carboxypeptidase G_1, in anisotropic membranes.

Introduction

Membrane supported enzymes have become important tools in analytic work, medicine and industry (*1–3*). Entrapment of enzymes in cellulose triacetate fibers was successfully used to prepare reactors for industrial and biomedical applications

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(*4–7*). In recent years ultrafiltration membranes and hollow fibers have been extensively used for fabrication of enzyme reactors (*8–12*). Detailed account of theoretical analysis of such hollow fiber enzyme reactors is available in literature (*13–17*). Hollow fibers have also been used as extracorporeal shunts in hemodialysis (*18, 19*), for enzyme therapy (*20, 21*), and in artificial organs (*22*).

In a previous communication (*20*) we described the use of an enzyme Carboxypeptidase G_1 (CPG_1) and hollow fibers made of polysulfone by Amicon to prepare multitubular reactors for depletion of methotrexate (MTX) from blood. The enzyme hydrolyzes MTX, folic acid, and its derivatives to the corresponding pteroic acids. It has molecular weight of 92,000 daltons and the K_m values for folic acid and MTX are 1.1 and 6.7 μM, respectively (*23, 24*). The enzyme was adsorbed onto the polymer surface in the sponge region of hollow fibers and subsequently crosslinked with glutaraldehyde. Animal experiments in which these hollow fiber reactors were used as extracorporeal shunts in healthy dogs have indicated that the activity of such reactors was too low to bring about sufficient increase in the clearance of MTX (*20*).

In an attempt to prepare hollow fiber reactors having significantly higher CPG_1 activity than the previously used device, hollow fibers were spun by a novel technique that permitted simultaneous fabrication of anisotropic membranes and immobilization of CPG_1. In vitro investigation of the multitubular hollow-fiber enzyme reactors thus obtained showed properties superior to those of reactors containing the enzyme adsorbed on the polymer wall.

Materials

Enzyme

Carboxypeptidase γ_1 (γ-glutamylhydrolase EC: 3.4.22.12) isolated from a *Pseudomonas sp.* was purchased from New England Enzyme Center (Boston, MA). The enzyme was supplied in a solution of 15 m*M* sodium glutamate, pH 6.0, containing 0.1 m*M* $ZnCl_2$. The specific activity was 842 U/mg protein. One unit is defined as the amount of enzyme that catalyzes the hydrolysis of 1 μmol of MTX/min under standard assay conditions (*23*). Dihydrofolate reductase, DHFR (EC: 1.5.1.3) was obtained from Sigma (St. Louis, MO).

Reagents

Folic acid (pteroylglutamic acid) and MTX were obtained from Sigma and Lederle Laboratories (Pearl River, NJ) respectively. Crystalline bovine serum albumin (BSA) and ^{125}I-labeled BSA were obtained from ICN Pharmaceuticals (Cleveland, OH) and New England Nuclear (Boston, MA), respectively. Napthol blue black was supplied by Eastman Kodak (Rochester, NY). *N, N*-Dimethylformamide (DMF), *N*-methyl-2-pyrrolidone, *N,N*-dimethylacetamide, dimethyl sulfoxide, and methylene chloride, all ACS grade, were purchased from Fisher (Fair Lawn, NJ). Tris-Zn buffer, used in most experiments, contained 50 m*M* Tris-HCl, pH 7.3, 0.1 m*M* $ZnCl_2$, and 0.02% (w/v) sodium azide. Tris, sodium azide, and $ZnCl_2$ were purchased from Sigma.

Equipment

Light absorbance was measured with a Spectronic 2000 spectrophotometer, Bausch and Lomb (Rochester, NY), equipped with controlled temperature flow cell and kinetic accessory package. Radioactivity was measured with a Model 5320 Auto-gamma scintillation spectrometer (Packard, Wakefield, MA). Batch reaction with CPG_1 membranes were carried out by using a Model 346 shaker (Fisher). Perfusion with hollow-fiber enzyme reactors was conducted using a Servodyne peristaltic pump (Cole-Parmer, Chicago, IL) or a Model S-10K Blood Pump, (Sarns, Ann Arbor, MI).

Methods

Enzyme Activity in Free Solution

Activity of CPG_1 in free solution was measured at pH 7.3 by the procedure of McCullough et al. (*23*). The effective extinction coefficient for full conversion of 1 m*M* solution of folic acid or MTX to the corresponding pteroates was taken as 8.0 and 8.3 at 303 and 320 nm, respectively.

Methotrexate Assay

MTX levels were determined either by the classical assay method (*25*) based on the inhibition of DHFR, or by high performance liquid chromatography, HPLC (*26*). Separation and quantitative measurement of MTX and the corresponding pteroate was carried out by using a house built liquid chromatograph consisting of a model 100A pump (Beckman, Irvine, CA), a model 7010 injection valve (Rheodyne, Berkeley, CA), a model 770 variable wavelength detector (Kratos, Westwood, NJ), and a model SR-206 dual pen recorder (Heath Schlumberger, Benton Harbor, MI). The 150 × 4.6 mm column was packed with 5 μm Partisil ODS II (Whatman, Clifton, NJ).

Stability of CPG_1 in Organic Solvents

Fifty μL of Tris-Zn buffer containing 0.2 U CPG_1 and 1 mg BSA were added to 950 μL of organic solvent at 22°C. At specified times, aliquots (100 μL) were removed, diluted 10-fold in Tris-Zn buffer, and assayed for enzyme activity at 22°C by using the spectrophotometer (*23*).

Preparation of Enzymic Membranes

The casting solution for preparation of the enzyme membranes consisted of two parts of an aqueous solution containing 1800 U/mL of CPG_1 and 20 mg/mL BSA, and 98 parts of a polysulfone solution in DMF. Flat membranes were casted using the process of Michaels (*27, 28*). A thin layer of the casting solution was formed with a Gardner blade and the polymer was precipitated with water to yield a sheet of asymmetric membrane. Whereas the fabrication of flat membranes is a batch process, hollow fiber membranes are prepared in a continuous fashion. Enzymic

hollow-fiber membranes with an inner diameter of 190 μm and a wall thickness of 45 μm were spun from essentially the same casting solution by using the method and equipment described by Cross (*29, 30*). The membranes were extensively washed with water and subsequently soaked in a solution containing 10% (v/v) of glycerol in Tris-Zn buffer and then dried at 25°C. Membrane thickness was measured with a Model 22p-10 strain gage from Federal (Providence, RI), or a light microscope equipped with a micrometer.

Distribution of Enzyme in the Membrane

The membranes were stained (*31*) with a 0.025% (w/v) solution of napthol blue black in a 1 : 1 mixture of 5% (v/v) aqueous acetic acid and methanol for 30 min. Napthol blue black was chosen becasue it had negligible affinity to polysulfone membranes containing no embedded enzyme.

Enzymic Activity in CPG_l Membranes

Discs of the flat membranes 1.0 cm in diameter and eight pieces of hollow fibers, 1.25 cm long, were incubated at 22°C in 4 mL of folic acid solution in Tris-Zn buffer having substrate concentrations of 540 and 135 μ*M*, respectively. Shaking of the mixture was interrupted for 1 min in 30-min intervals and the absorbance of the solution was measured.

Preparation of Hollow-Fiber Cartridges

Cartridges containing hollow-fiber enzyme membraenes were fabricated by potting the ends of the fiber bundles in polymethacrylate housing by epoxy resin as described by Kohman and McAfee (*32*). Two sizes of reactors were prepared. The first contained 100 hollow fibers in a 9.5 cm long and 0.72 cm inner diameter housing, the second had 5000 hollow fibers in a 21.0 cm long and 4.45 cm inner diameter housing. The reactors were fitted with conical end pieces (*12*) in order to obtain uniform flow distribution at the inlet and outlet.

Kinetics of Hollow-Fiber CPG_1 Reactors

The enzymatic activity of the small hollow-fiber reactors was measured by recirculating 20 mL of folic acid solution in Tris-Zn buffer. The substrate solution was continuously pumped from a well-stirred reservoir, through the flow cell of the spectrophotometer, the hollow fiber reactor, and subsequently returned to the reservoir. Absorbance change was monitored at 303 nm and recorded. The rate of reaction was calculated from the slope of the concentration versus time curve thus obtained.

Large hollow-fiber CPG_l reactors were evaluated in a similar fashion by recirculating 1 L of MTX solution at a flow rate of 340 mL/min. Samples drawn from the reservoir at various intervals were asaayed for MTX by the DHFR inhibition method (*25*) and by HPLC (*26*).

Results and Discussion

Stability of CPG_1 in Organic Solvents

The stability of CPG_1 in various organic solvents with potential use in membrane casting solution was evaluated (Table 1). The results show no loss of, but rather an increase in, enzymic activity when DMF was used. Therefore casting solutions were prepared with this solvent in the fabrication of all membranes described here.

Flat Membranes with CPG_1

Prior to attempting the fabrication of hollow fibers containing CPG_1, flat membranes were prepared to investigate the feasibility of the enzyme-inclusion technique and to optimize fabrication conditions. The rationale of this approach rests with the convenience of preparing relatively small quantities of flat membranes with the concomitantly low enzyme requirement, and with the similarity between the process of casting flat membranes and spinning hollow fibers.

Distribution of Protein in the Membrane

The concentration of total protein ranged from 40 to 80 μg/mL in the casting solution because we found that at these concentrations a uniform embedding of the enzyme in the polymer matrix resulted. In enzymatic membranes fabricated from casting solutions having protein concentrations greater than 80 μg/mL, the protein was present in large agglomerates that could be seen by the eye upon staining with napthol blue black. Such membranes containing large protein agglomerates showed a leakage of active enzyme, whereas no leakage of active enzyme was observed with membranes in which the protein was dispersed finely and uniformly.

Enzyme Aggolmeration in the Casting Solution

The present procedure for the preparation of casting solution calls for the addition of an aqueous solution of CPG_1 to DMF. Since it is likely that the enzyme forms a microprecipitate upon adding the enzyme solution to the organic solvent, filtration studies were conducted with DMF-resistant filters in order to estimate the size of the protein agglomerates. DMF containing the enzyme was filtered through a glass fiber filter (Whatman), nominal pore diameter 0.7 μm, and a teflon filter (Gore-Tex), nominal pore diameter 0.2 μm. The precipitate retained on filters was dissolved in Tris-Zn buffer and enzyme activity in this fraction and the filtrate was determined by the spectrophotometric method (*23*). We infer from the results shown in column A of Table 2 that the enzyme in the standard DMF preparation was essentially present in the form of 0.2–0.7 μm particles. In contradistinction, we found that mixing DMF with the aqueous CPG_1 solution in the reverse order, i.e, when DMF was added to the aqueous CPG_1 solution, yielded a turbid suspension, indicating that the enzyme precipitated as relatively large particles. Indeed, results of the filtration experiments shown in column B of Table 2 indicate that the particle size of enzyme precipitate in this hydro-organic preparation was greater than 0.7 μm because upon dissolving the material retained by the 0.2 and 0.7 μm

TABLE 1
Stability of CPG_1 in Organic Solvents at 22°C[a]

Solvent	Enzyme activity after incubation for 15 min	60 min
Tris-Zn buffer (control)	100	100
N,N-Dimethylformamide	121	131
N-Methyl-2-pyrrolidone	94.7	42.1
N,N-Dimethylacetamide	84.2	78.9
Dimethyl sulfoxide	47.4	0.0
Methylene chloride	0.0	0.0

[a]Activity was expressed as percentage of control. Details are given in the text.

filters in Tris-Zn buffer essentially all of the initial enzyme activity was recovered.

In another set of experiments the enzyme membrane was examined with the scanning electron microscope. This investigation did not reveal any detectable morphological differences with respect to polysulfone membranes prepared by the same casting procedure, but without protein in the casting solution.

We may assume, therefore, that precipitation of the polymer in the membrane casting procedure is accompanied by the entrapment of a portion of the protein agglomerates in the polymer matrix so that they become a part of the membrane. The rest of the enzyme is likely to be washed away in the water used for precipitation. As shown in the following section generally, about 20% of total protein is embedded in these membranes. This agrees with other findings that in the process of making such membranes various other additives are also entrapped at approximately the 20% level. It appears, therefore, that during the casting procedure the same fraction of substances present originally in the casting solution is embedded in the polymer matrix and this fraction (0.2) equals the volume fraction of the polymer proper in the membrane.

TABLE 2
Recovery of Enzyme Activity upon Membrane Filtration of CPG_1-Containing DMF–Water Mixtures Prepared by Adding Aqueous CPG_1 to DMF[a] or DMF to Aqueous

Membrane filter	Nominal diameter, μm	Enzyme activity in filtrate A, %	B, %
Glass fiber (Whatman)	0.7	100	2.0
Teflon (Gore-Tex)	0.2	6.3	1.0

[a]A 200 μL volume of aqueous CPG_1 was added to 2.3 mL DMF.
[b]A 2.3 mL volume of DMF was added to 200 μL of aqueous CPG_1 solution.

Activity of Flat Membranes with Embedded CPG_1

Substrate solutions having folic acid concentrations in the range from 135 to 675 μM were used in batch reactors to measure the activity of 1 cm diameter discs of flat membranes containing CPG_1 embedded in the polymer matrix. The measured rate of hydrolysis catalyzed by these membranes first increased with the concentration of folic acid and reached a saturation value of 540 μM. We concluded from this observation that, at folic acid concentrations higher than 540 μM, the kinetics of the enzymic reaction were not appreciably affected by the diffusion resistances, so that under these conditions the saturation rate, V_{max}, for the flat enzyme membrane was obtained.

To estimate the relative amounts of active enzymic protein and total protein incorporated in the enzymic membranes, a flat membrane was fabricated with both ^{125}I-BSA and active CPG_1 present in the casting solution. As shown in Table 3 the yields of embedded ^{125}I-BSA and of CPG_1 activity were 18.9 and 11.7% respectively. If the yield for entrapment of the enzyme protein having a molecular weight of 92,000 daltons is assumed to be similar to that of BSA (mw, 68,000), then we can conclude that 62% of the enzyme incorporated into the membrane is active.

Numerous attempts were made to increase the fraction of active enzyme in the membrane without success. For instance the addition of protecting agents such as glycerol or sorbitol and the substrate folic acid to the casting solution did not improve the recovery of CPG_1 activity.

The CPG_1 membranes were tested for leakage of active enzyme. Folic acid solutions that had previously been in contact with the membrane were incubated at 22°C in the dark from 1–7 d and monitored for absorbance changes over time. In all evaluations, no detectable enzyme was observed in the substrate solution.

Hollow Fiber Membranes with CPG_1

The enzymic activity of the CPG_1 fibers was measured by using folic acid solutions having concentrations in the range of 135–675 μM and eight pieces of 1.25 cm long hollow fibers in a stirred vessel. The rate of folate hydrolysis was measured by monitoring the absorbance of the supernatant and was found to be constant at all folic acid concentrations examined. Thus, the saturation rate was obtained already at a folic acid concentration of 135 μM so that no diffusion resistances af-

TABLE 3
Incorporation of CPG_1 and BSA in Polysulfone Membranes

	^{125}I-BSA			CPG_1 activity		
Membrane type	Offered, cpm	Found, cpm	Yield, %	Offered units	Found units	Yield, %
Flat membrane	3.4×10^5	6.6×10^4	18.9	0.54	0.063	11.7
Hollow-fiber cartridge	ND[a]	ND	ND	633.5	32.5	5.13

[a]Not determined.

fected the intrinsic kinetics of the reaction catalyzed by the hollow-fiber enzyme membrane. We recall that with the flat enzyme membranes a folic acid acid concentration of 540 μM was required to reach such a situation. The higher folic acid concentration needed to reach the saturation rate of reaction with flat membranes compared to that with hollow fibers can be explained by the relative thicknesses of the membranes. Flat membranes had a thickness of 101.6 μm, whereas the hollow fibers had a wall thickness of 45 μm. Clearly, a higher concentration of folic acid was required to overcome the effect of diffusion resistances in the relatively thicker flat membrane than in the hollow fibers.

The pH activity profile of the hollow fiber membrane with CPG_1 was evaluated in a stirred vessel and was compared to the pH profile obtained with CPG_1 in free solution. As seen from the results in Fig. 1, the effect of pH is about the same on the kinetics of both the soluble and membrane embedded enzyme.

Uniformity of axial distribution of CPG_1 activity in the fibers was investigated as follows. Fiber samples were taken randomly from each production run and analyzed for enzymic activity. Twenty-two samples from a total length of 90 m thus evaluated had the same measured enzyme activity viz., 6.9×10^{-3} U/cm^2 inner surface area. From this we concluded that the hollow-fiber enzyme membranes had uniform axial distribution of embedded CPG_1 on a scale of the sample lengths.

Stability of Hollow-Fiber Enzyme Membranes

The stability of the hollow-fiber enzyme membranes was tested under a variety of conditions and the results are shown in Table 4. Enzyme fibers were soaked in a solution containing 10% (v/v) of glycerol in Tris-Zn buffer, dried at 25°C, and thereafter stored at 4, 22, or 50°C. In addition to the study with such dry fibers, enzyme fibers were also incubated wet in Tris-Zn buffer at 37°C and were subse-

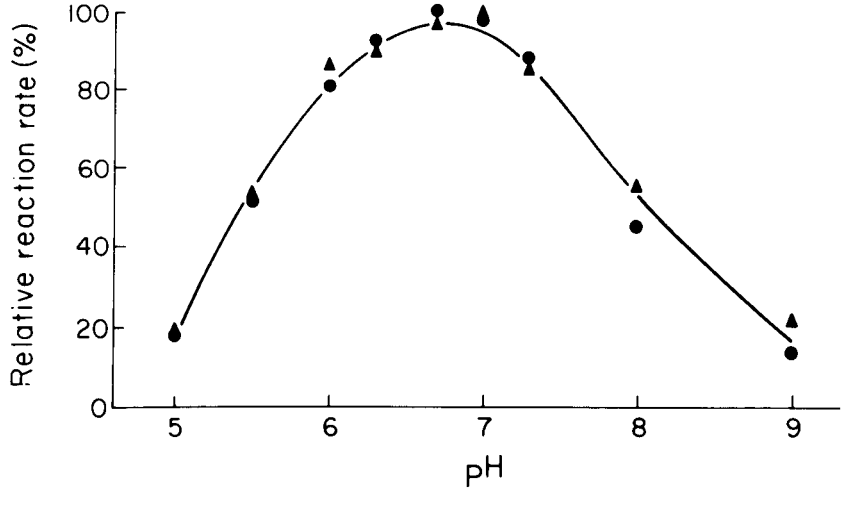

Fig. 1. pH-Activity profiles of free and embedded CPG_1 for folic acid hydrolysis at 22°C. The initial substrate concentration was 135 μM in Tris-Zn buffer containing 0.1M Na acetate: (●) enzyme in free solution: (▲) hollow-fiber embedded enzyme.

TABLE 4
Stability of Hollow-Fiber Enzyme Membranes[a]

Temperature, °C	Condition	Incubation time	% Activity
4	Dry	Initial (control)	100
4	Dry	9 months	100
22	Dry	9 d	97
37	Wet	2 h	97
37	Wet	4 h	97
37	Wet	6 h	90
37	Wet	18 h	50
50	Dry	1 h	54
50	Dry	2 h	25

[a]The enzymic membranes were exposed to various conditions and CPG_1 activity was measured as described in methods.

quently analyzed for enzymic activity. As seen in Table 4, storage at 4°C for 9 months did not result in any detectable decrease in hollow-fiber enzymic activity. Incubation at 37°C for up to 4 h did not result in any significant loss of activity, whereas incubation at 37°C for 18 h did result in a 50% decrease in the enzymic activity of the hollow fibers.

Kinetics of 100 Hollow-Fiber Enzymic Membrane Reactors

Kinetic parameters of cartridges containing 100 hollow-fiber enzyme membranes were evaluated using a recirculating system described above. Substrate solutions of 135 μ*M* folic acid in Tris-Zn at 22°C were recirculated at flow rates ranging from 4 to 70 mL/min. The rate of reaction was calculated from the slope of the concentration versus time plots. The measured rate of reaction at different flow rates as a function of concentration is illustrated in Fig. 2. At low flow rate the reaction is

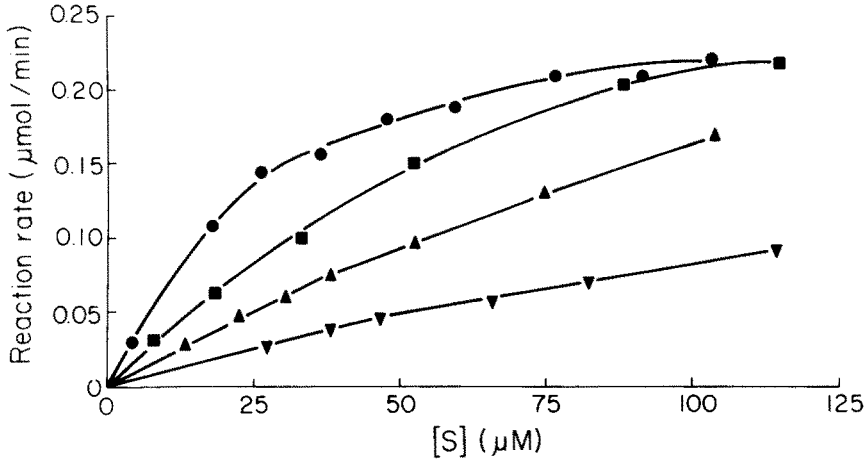

Fig. 2. Effect of folic acid concentration and flow rate on the rate of reaction in CPG_1 hollow-fiber reactor. A 100-fiber reactor, 9.5 cm in length and 0.72 cm inner diameter, was perfused with folic acid in Tris-Zn buffer pH 7.3 at 22°C: (●) 70 mL/min; (■) 30 mL/min; (▲) 10 mL/min; (▼) 4 mL/min.

pseudo-first order, as seen by the quasilinear plot. With increasing flow rate the reaction gradually approaches Michaelis-Menten kinetics, as seen by the rectangular hyperbolic plot. This behavior is readily explained by the relaxation of diffusion resistances in the heterogeneous catalytic system with increasing flow rate. For diffusion-controlled kinetics in tubular and multitubular wall reactors such as the hollow fiber cartridges, the observed reaction rate, $\dot{r}$, is determined by the mass transfer of substrate to the immobilized enzyme in the fiber wall when the diffusion resistance in the wall is negligible. At intermediate conversions the rate can be expressed (*34*) for a simple tube as:

$$\dot{r} = 2.86(\Pi DL)^{2/3}(\bar{S}_{1n})\, F^{1/3} \tag{1}$$

where D is the substrate diffusivity, L is the tube length, F is the volumetric flow rate through the tube, and $\bar{S}_{1n}$ is the log mean substrate concentration given by:

$$\bar{S}_{1n} = S_i - S_0/\ln(S_i/S_0) \tag{2}$$

where S_i is the inlet concentration and S_0 is the mixing cup concentration in the effluent.

For multitubular reactors containing n hollow fibers when the flow rate is F_t, the rate is given by:

$$\dot{r}/\bar{S}_{1n} = 2.86(\Pi DLn)^{2/3}(F_t)^{1/3} \tag{3}$$

From Eq. (2) it follows that for a diffusion-controlled reaction in a multitubular reactor having constant length and a fixed number of fibers, a plot of $\dot{r}/\bar{S}_{1n}$ vs $F_t^{1/3}$ yields a straight line with slope 2.86 $(\pi DLn)^{2/3}$. Such a plot for the data obtained at different initial concentrations of folic acid is shown in Fig. 3. It is seen that, at the lowest initial substrate concentration of 25 μM folic acid, the plot is linear, indicating that the reaction was bulk diffusion controlled. From the slope of this line and the pertinent dimensions of the reactor, the diffusivity of folic acid in water at 22°C and pH 7.3 is obtained as 3.5×10^{-6} cm^2/s, which is in close ageement with the value of 4.0×10^{-6}cm^2/s calculated from the Stokes-Einstein hydrodynamic model of the diffusivity.

Kinetic Studies with Large Hollow-Fiber CPG$_1$ Reactors

Kinetics of reactors containing 5000 hollow fibers with embedded CPG$_1$ were evaluated using MTX as substrate and the recirculating system described above. A 1-L volume of 100 μM MTX in Tris-Zn at pH 7.3 and 37°C was recirculated through the reactor at a volumetric flow rate of 340 mL/min and samples were analyzed by both DHFR kinetic assay (*25*) and HPLC (*26*). A typical chromatogram for the separation of MTX and 2,4-diamino,^{10}N-methylpteroic acid is shown in Fig. 4. The resulting time course of reaction shown in Fig. 5. The data in Fig. 4 demonstrate that the DHFR inhibition assay is not a suitable analytical technique to follow the time course of reaction since it yields, when the conversion reaches 99%, an apparently constant MTX concentration that is much higher than the values obtained by HPLC, which can be considered a reliable technique. This discrepancy is caused by the inhibition of DHFR by the product 2,4-diamino, ^{10}N-methylpteroic acid, which becomes commensurate to that by the substrate at

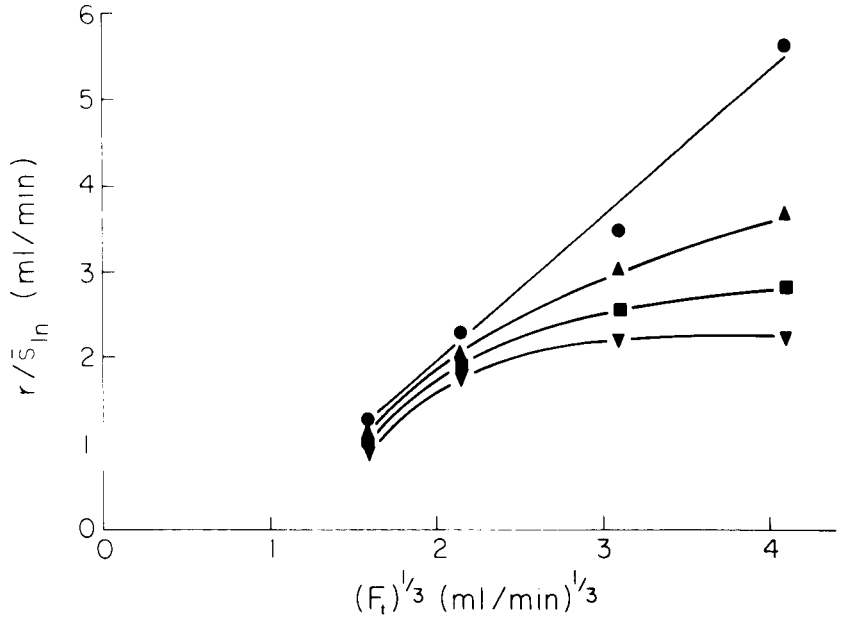

Fig. 3. Effect of flow rate on folic acid hydrolysis in a multitubular CPG_1 reactor having a contact area of 41.7 cm^2. Experimental conditions were described in Fig. 2. The individual plots were obtained at different initial substrate concentrations: (●) 25 μ*M*, (▲) 50 μ*M*, (■) 75 μ*M* and (▼) 100 μ*M* folic acid.

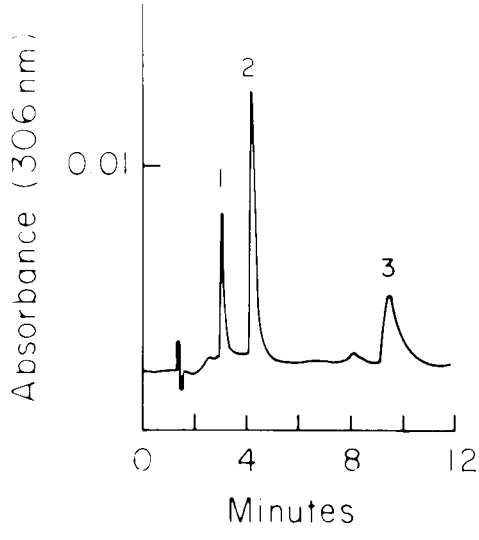

Fig. 4. Chromatogram for separation of MTX (2)and 2,4-diamino,^{10}*N*-methylpteroic acid (3). 3,4-Dihydroxyphenylacetic acid (1) was used as internal standard. The mobile phase contained 14% (v/v) acetonitrile in 0.1*M* sodium phosphate buffer, pH = 3.0. The flow rate was 0.8 mL/min.

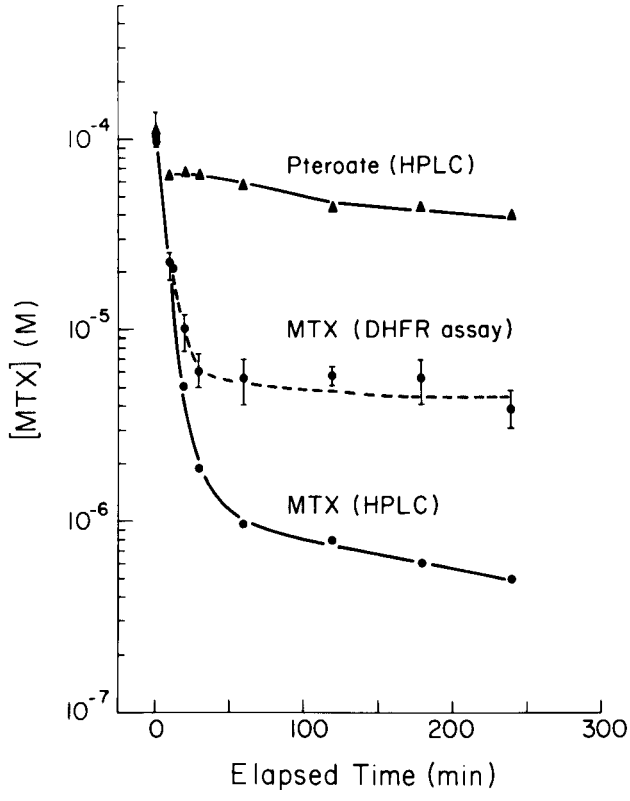

Fig. 5. Time course of MTX degradation by a 0.46-m^2 hollow-fiber CPG_1 reactor in the recirculating system at 37°C. The initial reaction mixture (1 L) contained 100 μM MTX in Tris-Zn buffer, pH 7.3. The flow rate was 340 mL/min.

high conversions. It is seen that the 5000 fiber CPG_1 reactor reduced the initial MTX concentration by two decades within 1 h. Detailed analyses of the data indicate that initially the reaction is kinetically controlled, but that as the substrate concentration decreases it becomes bulk diffusion controlled in agreement with the observations made with the small CPG_1 reactor. However, the kinetics of the reaction change again as the MTX concentration decreases below the micromolar level. This deviation from first-order kinetics observed after 30 min is currently under investigation.

Conclusions

A novel method has been developed for embedding of CPG_1 into the polysulfone matrix during the fabrication of asymmetric, semipermeable membranes. The practicality of the approach was initially demonstrated with flat membranes and thereafter the method was applied to the fabrication of hollow fiber membranes with CPG_1 embedded in the fiber walls.

Kinetic parameters of the CPG_1 membranes were evaluated by monitoring the enzyme catalyzed hydrolysis of folic acid or MTX in both stirred batch and

recirculating multitubular reactors. No detectable enzyme leakage was observed in any of the evaluations of hollow fibers with embedded CPG_1. The pH profile of the fiber-entrapped CPG_1 was found to be similar to that of the enzyme in solution. The overall kinetics of the 100 hollow-fiber enzyme reactors ranged from bulk diffusion-limited kinetics at low substrate concentrations (25 μM folic acid) to kinetic limitations at higher substrate concentrations (100 μM folic acid). Theoretical analysis of the bulk diffusion-controlled kinetics yielded a calculated value of 3.5×10^{-6} cm^2/s for the diffusivity of folic acid at 22°C. The hollow-fiber enzyme membranes were stable when stored dry at 4°C for at least 9 months, and when incubated in Tris-Zn buffer at 37°C for 4 h.

In a recirculating system of 1 L capacity, large hollow-fiber CPG_1 reactors containing 5000 fibers with a contact area of 0.46 m^2 reduced the initial MTX concentration of 100 μM MTX to less than 2 μM within 30 min at a flow rate of 340 mL/min. Further studies to evaluate the kinetic behaviour of such heterogeneous CPG_1 reactors at low concentrations of MTX are in progress. Neverthless, the data presented here suggest that sufficiently large multitubular CPG_1 reactors have potential therapeutic value in cancer treatment by MTX rescue regimen.

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