International Journal of Pharmaceutics, 90 (1993) 95–104

IJP 02922

Research Papers

Diffusion characteristics of fibrin films

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(Received 21 January 1992)
(Modified version received 24 March 1992)
(Accepted 29 May 1992)

Key words: Fibrin film; Diffusion; Alkyl paraben; Macromolecule; Permeability

Summary

Fibrin films prepared by enzymatic conversion of fibrinogen using thrombin were evaluated as a biodegradable and biocompatible carrier for drug delivery. Permeabilities, partition coefficients and diffusion coefficients were employed to characterize the diffusion of various solutes through fibrin film. For a series of alkyl parabens, permeabilities decreased and partition coefficients into the films increased with increasing chain length of the alkyl group. On the other hand, there was no significant alteration of diffusion coefficients for propyl paraben permeating through fibrin films treated with glutaraldehyde for different intervals. However, permeabilities of various macromolecules decreased as the time interval of crosslinking increased. A linear correlation between the logarithm of permeability and the square of macromolecular radius was observed. Partition coefficients increased for all macromolecules examined with increasing extent of crosslinking as a result of introducing hydrophobic glutaraldehyde into fibrin film. Diffusion of macromolecules through free water regions within the films is a possible mechanism.

Introduction

Natural polymers are being increasingly studied for controlled release applications because of their biocompatibility and biodegradability. Various materials such as hyaluronic acid (Saettone et al., 1989; Hunt et al., 1990), collagen (Gilbert et al., 1988), fibrinogen (Miyazaki et al., 1986) and fibrin (Senderoff et al., 1991) have been tested as carriers for drug delivery systems. Nevertheless, recent advances in biotechnology have made the production and use of proteins and peptides as drugs feasible, but such materials present a challenging formulation and drug delivery problem (Cohen et al. 1991).

Previously (Ho et al., 1989, 1990a,b), we have developed and reported on fibrin films produced by the biochemical reaction between fibrinogen and thrombin as a delivery system. A fibrin based delivery system has several unique properties: (1) the fibrin polymer represents a natural, biodegradable and biocompatible matrix; (2) since the fibrin polymer is formed by an enzymatic process, it would be a better carrier for heat-labile drugs such as proteins; (3) the microstructure of the fibrin polymer formed in vitro is dependent on reaction conditions such as pH and ionic strength, suggesting that formulation parameters may be manipulated to control relative availability; (4)

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the chemical nature and physiologic role of fibrin suggest that a fibrin based drug delivery system will have bioadhesive properties. The specificity and mild conditions of the fibrinogen/thrombin reaction are such that reactive molecules such as proteins and peptides may be incorporated into a fibrin based delivery system without serious modification. Fibrin films could therefore be a potential carrier for protein drugs if fibrin is available in production scale at a reasonable cost.

As understanding of the controlling mechanisms of transport through fibrin polymers is important in the effective design of polymeric drug delivery devices. In this paper, the effects of crosslinking, polarity and molecular size of solutes on the diffusional properties of fibrin films have been examined and reported.

Materials and Methods

Materials

Bovine fibrinogen (type IV), thrombin, methylparaben, ethylparaben, propylparaben, butylparaben, lysozyme (Mol. Wt, 14500; radius, 1.58 nm; p*I*, 10.5; no., L-4631), carbonic anhydrase (Mol. Wt, 29000; radius, 2.04 nm; p*I*, 60% is 5.9 and 5.4; 40% is 5.2–7.5; no., C-2273), ovalbumin (Mol. Wt, 45000; radius, 2.38 nm; p*I*, 4.6; no., A-7642), bovine serum albumin (Mol. Wt, 66000; radius, 2.69 nm; p*I*, 4.7; no., A-7517) and Tris biochemical buffer were obtained from Sigma Chemical Co. and used without any further purification. A 25% glutaraldehyde aqueous solution and sodium azide were purchased from Merck Co., Inc..

Methods

Preparation of fibrin films

Fibrin films were prepared using the procedure reported previously (Ho et al., 1990). Briefly, 0.1 ml of thrombin solution (0.5 unit/0.1 ml) was added to 1 g of the fibrinogen solution (60 mg/g), and then the mixture was immediately transferred to an acrylic round mold, which had been sealed with parafilm paper (American Can Co.) around the bottom. Once the film cured, the parafilm paper was peeled off carefully and the film attached to the mold was then dried in an Electric Dryer (Intech, Osaka, Japan) to a total water content of about 50%.

Hydration of fibrin films

An estimate of hydrated film thickness was required in the data analysis. The rate and extent of hydration for fibrin films were studied by immersing them in 25 ml of 0.05 M Tris buffer (pH 7.4). The weight of the hydrated fibrin film was measured at appropriate time intervals. The degree of hydration of the film was then calculated based on the percentage weight increase relative to the initial weight. Approx. 1h was needed to reach equilibrium and the extent of hydration was about 120% for untreated films. Therefore, fibrin films used in the following experiments were equilibrated in Tris buffer for at least 1 h. The thickness of hydrated fibrin films was measured using a thickness gauge (Teclock Corp., SMD-540); the mean of five measurements was used as an estimate of film thickness in the data analysis.

Crosslinking of fibrin films

The non-polar bifunctional agent glutaraldehyde was used to crosslink the fibrin films. After equilibrating with Tris buffer, fibrin films were soaked in 25 ml of 0.5% glutaraldehyde aqueous solution for either 10, 30 or 60 min. At the end of this period, treated fibrin films were washed three times with 10 ml of the same buffer solution for 30 s to remove unreacted glutaraldehyde. These fibrin films were stored in a desiccator at ambient temperature until use. There was no significant change in film thickness and width after crosslinking. It was concluded that the extent of hydration of treated films was similar to that of untreated ones.

Differential scanning calorimetry studies

The structural character of untreated and treated fibrin films was examined using a differential scanning calorimeter (DuPont, DSC-10). A heating rate of 10°C/min was used from 30-250°C in an atmosphere of nitrogen with the film sealed

in an aluminum pan. Indium was used as a calibration standard.

Permeation studies

Permeation studies were carried out for alkyl parabens and macromolecules at 37 and 25°C, respectively, in a horizontal type diffusion cell (obtained from Dong-Hong instruments Co., Ltd). After equilibration, a fibrin film with the appropriate size was clamped between the two compartments. The area available for permeation was around 0.8 cm^2. In the case of alkyl parabens, the donor compartment was filled with 4 ml of 0.05 M Tris buffer (pH 7.4) containing excess alkyl parabens, whereas the same volume of Tris buffer with no solute was added to the receiver side. The permeation of alkyl parabens through the fibrin films was followed by determining their concentrations in the receiver compartment as a function of time. Concentrations were determined by UV absorbance measurement at $\lambda = 295$ nm and comparison with a standard curve. To attain reproducibility, a constant stirring rate of 300 rpm (± 10 rpm) was used and fresh buffer was replaced after sampling to maintain a constant volume. An average of six samples was reported.

For macromolecules, 4 ml of 0.05 M Tris buffer (pH 7.4) having a concentration of 0.8, 0.4, 3.0 or 1.5 mg/ml for lysozyme, carbonic anhydrase, ovalbumin and bovine serum albumin, respectively, was placed in the donor cell. The Tris buffer contained 0.1% sodium azide as a preservative. The concentration change of macromolecules in the receiver compartment was determined using a gradient HPLC system: solution A, 0.05% trifluoroacetic acid/deionized water; solution B, 0.05% trifluoroactic acid/acetonitrile; mixed 32–68% B in A over 20 min. The flow rate was 1.5 ml/min. A Synchropak C_4 column (4.6 × 150 mm, 300 μm) was used and UV detection was at a wavelength of 220 nm. The HPLC system was validated. The retention times of lysozyme, bovine serum albumin, carbonic anhydrase and ovalbumin were determined at varying concentrations for 4.56, 6.76, 10.30 and 14.11 min, respectively, with acceptable coefficients of variation.

Partition coefficient measurements

Partition coefficients for alkyl parabens into the fibrin films were determined. Pieces of round fibrin film (volume: 0.022 ± 0.005 cm^3) were presoaked in 0.05 M Tris buffer (pH 7.4). The film was wiped dry and transferred to 2.5 ml of paraben-containing solution. The concentration for methyl-, ethyl- and propylparaben was 400 μg/ml, whereas butylparaben was 200 μg/ml. The films were allowed to equilibrate with the paraben-containing solution for 24 h at 37 °C. Equilibrium concentrations of alkyl parabens (C_{eq}) were determined using an HPLC method. The samples were injected directly into a Lichrospher C_{18} column (4.6 × 150 mm, 5 μm) using $MeOH/H_2O = 60:40$ as the mobile phase. The detection wavelength was 295 nm and the flow rate was 1 ml/min. Partition coefficients (K) were calculated based on the following equation:

$$K = (C_0/C_{eq} - 1) \cdot V/V_f \quad (1)$$

where C_0 is the initial concentration of solute, C_{eq} denotes the concentration of solute in the buffer after equilibrium, V and V_f are the volumes of buffer and fibrin film, respectively. An average of triplicates was reported.

In the measurement of partition coefficients for macromolecules, conditions similar to those above were employed except the concentrations of the macromolecules were the same as those used in the permeation experiments. The volume of protein solution was 0.6 ml and equilibration was followed for up to 72 h at 25°C. Samples were taken at appropriate intervals and assayed by the HPLC method described above. The partition coefficient at any time was calculated from the concentration measured using Eqn 1. There was no significant alteration in partition coefficients over a 5 day period.

Data analysis

It is assumed that the transport of solutes through these films occurs by simple diffusion and that the solute is neither irreversibly bound within the films nor degraded at any time during transport. For a slab geometry, the permeation

rate of diffusing substance at steady state can be described by the following equation.

$$\frac{dC_r}{dt} = \frac{A \cdot K \cdot D}{V_r \cdot h}(C_d - C_r) \quad (2)$$

where C_r and C_d are the concentrations of solutes in the receptor cell and donor cell, respectively, at time t, A, V_r and h represent the area available for diffusion, the volume of the receptor compartment and the thickness of the film, respectively, and K and D are the partition and diffusion coefficients. For the paraben derivatives, a saturated solution (C_d = solubility) was maintained in the donor compartment and non-sink conditions were maintained in the receptor compartment. After integration of Eqn 2, the following equation is obtained.

$$\ln(C_d - C_r) = -\frac{A \cdot K \cdot D}{V_r \cdot h}t + \ln C_d$$

After rearrangement,

$$\frac{V_r \cdot h}{A}\ln\left(1 - \frac{C_r}{C_d}\right) = -K \cdot D \cdot t \quad (3)$$

The term on the left-hand side of Eqn 3 is designated as U and the permeability P is defined as the product of partition and diffusion coefficient. Then;

$$U = -K \cdot D \cdot t = -P \cdot t \quad (4)$$

For the macromolecules, since the solute concentration in donor cell was not constant, a modification of Eqn 2 is necessary. At any time t, a mass balance of macromolecules in the diffusion cell yields:

$$C_d V_d = C_0 V_d - C_f V_f - C_r V_r$$

If equilibration between the film and the donor compartment is rapid enough, the partition coefficient (K) between the buffer in donor compartment and fibrin film is equal to C_f/C_d and may be substituted for C_f ($= K \cdot C_d$).

$$C_d V_d = C_0 V_d - K C_d V_f - C_r V_r$$

where C_0 is the initial concentration of the macromolecule; C_d, C_r and C_f are the concentration in the donor cell, receptor cell and fibrin film at time t, respectively; V_d, V_r and V_f are the volume of donor cell, receptor cell and fibrin film, respectively. Then,

$$C_d = \frac{C_0 - C_r \cdot V_r/V_d}{1 + K \cdot V_f/V_d} = \frac{C_0 - C_r \cdot V_r/V_d}{Q}$$

(let $Q = 1 + K \cdot V_f/V_d$)

Substituting C_d into Eqn 2 and integrating, then

$$\frac{-V_r \cdot h}{A \cdot \left(\dfrac{V_r/V_d + Q}{Q}\right)}\ln\left(1 - (V_r/V_d + Q)\frac{C_r}{C_0}\right)$$

$$= K \cdot D \cdot t$$

In the permeation studies, V_d and V_r are equal. Then,

$$\frac{-V_r \cdot h}{A \cdot \left(\dfrac{1+Q}{Q}\right)}\ln\left(1 - (1+Q)\frac{C_r}{C_0}\right) = K \cdot D \cdot t \quad (5)$$

When K is very large, which is the case for lysozyme, a significant amount of lysozyme will be retained on/in the film at equilibrium. A correction Q in the equation is necessary. If the term on the left-hand side of Eqn 5 is designated as U', then

$$U' = K \cdot D \cdot t = P \cdot t \quad (6)$$

If $K \cdot V_f/V_d \ll 1$, which is the case for macromolecules with low K values, or if the volume of the fibrin film is very small, Q can be approximated to unity (for example, carbonic anhydrase, ovalbumin and bovine serum albumin). Then, Eqn 5 can be simplified to:

$$\frac{-V_r \cdot h}{2A}\ln\left(1 - 2\frac{C_r}{C_0}\right) = K \cdot D \cdot t \quad (7)$$

This equation has been used in a study of the diffusion of macromolecules through collagen

films (Gilbert et al., 1988). If the term on the left-hand side of Eqn 7 is designated as U'', then

$$U'' = K \cdot D \cdot t = P \cdot t \quad (8)$$

The slopes of the plots according to Eqn 4, 6 or 8 (U, U' or U'' vs t) give the permeability P ($= K \cdot D$). Using the partition coefficients determined above, diffusion coefficients were then calculated with back substitution.

Results and Discussion

Fibrin is a nontoxic, flexible and absorbable material. A fibrin film can be prepared simply by clotting a solution of fibrinogen with thrombin. A method for preparing a fibrin film without using high temperature has been developed. It was thought that the introduction of chemical crosslinking would further modify the network and flexibility of fibrin films, in turn affecting the diffusion of solutes. These were accomplished by treating fibrin films with a glutaraldehyde aqueous solution for different intervals. The treated and untreated fibrin films were first analyzed with a differential scanning calorimeter. The corresponding spectrum of untreated fibrin film shows a transition temperature at approx. 206°C, which probably reflects its decomposition temperature. Using shifts in this temperature as an indicator of structure in treated fibrin film, no significant differences among treated and untreated fibrin films were noted. Therefore, it was of interest to examine the effect of the crosslinking on fibrin film structure using the transport behavior of solutes.

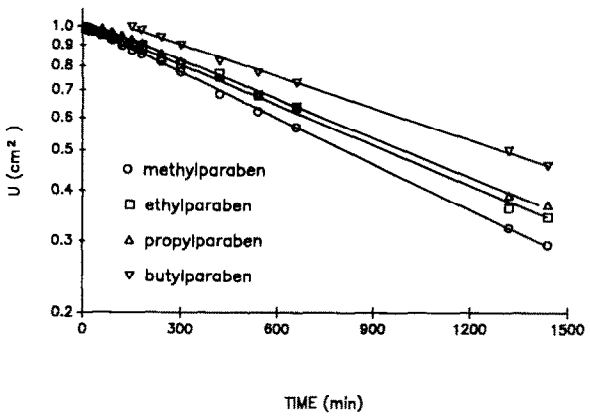

Fig. 1. The variability of permeability coefficients for alkyl parabens through untreated fibrin films.

Permeation studies were conducted first for a series of alkyl parabens with different polarity. Eqn 4, derived under Data analysis, is suitable for analyzing the data in terms of experimental conditions. Figs. 1 and 2 illustrate the validity of the relationship with $r^2 > 0.99$. From the slope of the regression lines, permeabilities (P) of the alkyl parabens through untreated and treated fibrin films were determined. The results are listed in Tables 1 and 2. They also show the values of partition (K) and diffusion coefficients (D); the latter was calculated using the relationship of $D = P/K$. As indicated in Table 1, the permeabilities decrease, whereas partition coefficients increase, with increasing chain length of the alkyl parabens. The increase of partition coefficient

TABLE 1

Permeability of the fibrin films to alkyl parabens at pH 7.4 (Tris buffer) and 37 °C

Parabens	Methyl	Ethyl	Propyl	Butyl
Mol. wt	152.2	166.2	180.2	194.2
Solubility (μg/ml)	3702.3 ± 54.1	1 514.9 ± 168.5	665.7 ± 27.5	387.9 ± 22.7
Permeability (cm^2/s)($\times 10^6$)	4.1553 ± 0.5327	3.4321 ± 0.2833	3.0457 ± 0.2707	2.7215 ± 0.1293
Partition coefficient	0.9135 ± 0.0690	1.2100 ± 0.0970	1.7200 ± 0.0720	1.8800 ± 0.0930
Diffusion coefficient (cm^2/s)($\times 10^6$)	4.5766 ± 0.3670	2.8607 ± 0.2381	1.7769 ± 0.0744	1.4485 ± 0.0698
Mutual diffusion coefficient (M.D.C.)	1	0.6251	0.3883	0.3165
Theoretical M.D.C.	1	0.9261	0.8646	0.8126

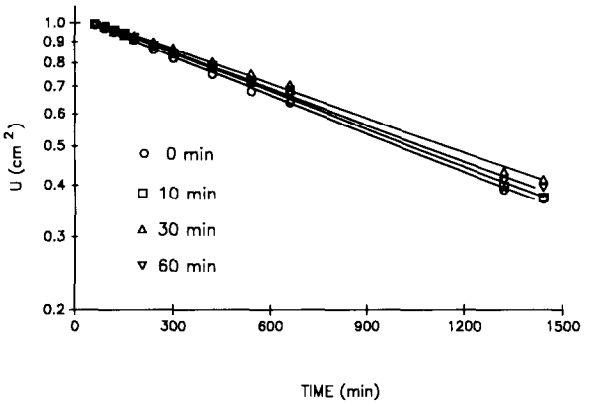

Fig. 2. The variability of permeability coefficients for propylparaben through fibrin films treated with glutaraldehyde for different intervals.

with increasing chain length of the alkyl group is explainable by the hydrophobic nature of the alkyl chain, and may imply a partial hydrophobic structure of the fibrin film itself. The results indicate that the more hydrophobic compounds partition preferentially into the fibrin film, but with a corresponding decrease in permeability. It suggests that diffusion of the parabens with longer alkyl chains is more hindered.

Wilke and Chang suggested an equation to estimate the diffusion coefficient in solution. In this equation, the diffusion coefficient of the solute varies inversely with the solute molar volume at the normal boiling point (V_b) raised to the power of 0.6 (Hunt et al., 1990). V_b was estimated based on structural contributions, and then the diffusion coefficient of alkyl parabens in solution was calculated (Liley et al., 1973). The ratio of the diffusion coefficient of any paraben derivatives in solution to that of methylparaben in solution is designated as the theoretical mutual diffusion coefficient (theoretical M.D.C.). Some decrease in the diffusion coefficient with increasing alkyl chain length is expected due to the increase of molecular weight. However, Table 1 shows that the decrease in mutual diffusion coefficients within the film is greater than the decrease expected in solution values. The difference suggests that the diffusion of parabens within the film is retarded not only by molecular weight effects, but also by some additional interactions with the film. These interactions could be attributed mainly to hydrophobic interactions with the polymer chain, or could be a reflection of steric hindrance or tortuosity of the diffusion path (Hunt et al., 1990). The extent of interaction seems to parallel the increase in alkyl chain length resulting in a decrease in diffusion coefficient with increasing chain length. Similar results have been reported in a study of hyaluronic acid films (Hunt et al., 1990).

Table 2 lists the permeability of propylparaben through fibrin films treated with glutaraldehyde for different intervals. There is a slight difference in permeability between treated and untreated fibrin films, but not among treated fibrin films. The partition coefficients reported here show the same trends. Since glutaraldehyde is a nonpolar crosslinker, the hydrophobicity of fibrin films would increase as the time interval of glutaraldehyde treatment increased. However, there was no significant alteration of diffusion coefficients of propyl paraben for untreated and treated fibrin films. Actually, treated fibrin films would be expected to show a more rigid nature and entanglement of the chains than untreated ones, resulting in smaller values of diffusion coefficients. Nevertheless, the diffusion coefficient of propyl paraben seems to be relatively insensitive to the change of rigidity of fibrin films. It may be that the molecular size of propylparaben is too small to be af-

TABLE 2

Permeability of glutaraldehyde-treated fibrin films to propylparaben at pH 7.4 (Tris buffer) and 37°C

	Untreated	10 min	30 min	60 min
Permeability (cm^2/s)($\times 10^6$)	3.0457 ± 0.2707	3.6162 ± 0.2219	3.4959 ± 0.1063	3.4335 ± 0.1544
Partition coefficient	1.7200 ± 0.0720	1.9300 ± 0.0207	1.9009 ± 0.0480	1.9215 ± 0.0489
Diffusion coefficient (cm^2/s)($\times 10^6$)	1.7769 ± 0.0744	1.8745 ± 0.0198	1.8695 ± 0.0316	1.7913 ± 0.0451

fected by the change in the rigidity of treated fibrin films.

Experiments were conducted to determine the effect of molecular size on the permeation of solutes through untreated and treated fibrin films. Macromolecules varying in molecular size were used, including lysozyme, carbonic anhydrase, ovalbumin and bovine serum albumin. The permeabilities of macromolecules through untreated and treated fibrin films were obtained from the slopes of the plot in Fig. 3 based on Eqn 6 or 8. Table 3 summarizes the results with the values of permeability (P), partition (K) and diffusion coefficients (D). For given treatment times, the permeability decreases with increasing protein size for same treatment of fibrin films. Only lysozyme, the smallest among the macromolecules examined, was permeable through fibrin films crosslinked with 0.5% glutaraldehyde aqueous solution for 1 h. A plot of the logarithm of permeability through untreated and treated fibrin films vs the square of macromolecular radius is shown in Fig. 4. The linear correlation supports

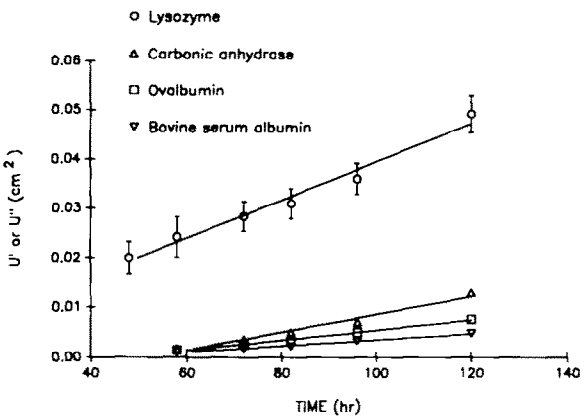

Fig. 3. The permeation of various proteins through untreated fibrin films. The slopes are equal to permeability for lysozyme (○), carbonic anhydrase (△), ovalbumin (□) and bovine serum albumin (▽).

the hypothesis that the size of the macromolecule is probably the determining factor for permeation. However, the permeability is defined as the product of the partition coefficient and the diffusion coefficient. It would be more appropriate to

TABLE 3

Permeability (P), partition (K) and diffusion (D) coefficients for various proteins across fibrin films crosslinked with glutaraldehyde for different intervals

Protein	Time [a] (min)	P (cm^2/s)($\times 10^8$)	K	D (cm^2/s)($\times 10^8$)
Lysozyme	0	10.77 ± 1.41	15.99 ± 0.04	0.67 ± 0.09
	10	14.96 ± 0.09	27.43 ± 1.56	0.55 ± 0.03
	30	10.72 ± 1.42	32.46 ± 1.56	0.33 ± 0.04
	60	5.15 ± 0.12	38.14 ± 1.96	0.14 ± 0.01
Carbonic anhydrase	0	4.88 ± 0.28	0.29 ± 0.06	17.12 ± 0.98
	10	5.92 ± 0.42	0.43 ± 0.05	13.73 ± 0.98
	30	3.62 ± 0.98	0.51 ± 0.08	7.16 ± 1.93
	60	N.D. [b]	0.92 ± 0.06	
Ovalbumin	0	2.78 ± 0.35	0.35 ± 0.03	7.97 ± 1.00
	10	3.34 ± 0.16	0.59 ± 0.03	5.68 ± 0.28
	30	1.50 ± 0.06	0.68 ± 0.08	2.20 ± 0.09
	60	N.D.	1.01 ± 0.25	–
Bovine serum albumin	0	1.71 ± 0.29	0.25 ± 0.02	6.82 ± 1.16
	10	1.63 ± 0.07	0.37 ± 0.02	4.19 ± 0.47
	30	0.84 ± 0.16	0.42 ± 0.06	1.99 ± 0.38
	60	N.D.	0.56 ± 0.08	–

[a] Time intervals for treatment with glutaraldehyde solution.

[b] Not detectable after 168 h.

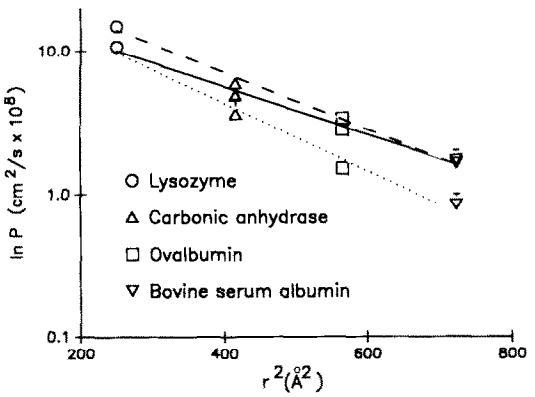

Fig. 4. Relationship between permeability and the square of protein radius. Results are shown for permeation of various proteins through fibrin films crosslinked with glutaraldehyde for different intervals [untreated (———), 10 min (— — —) and 30 min (- - - - -)].

examine the separate effects of partitioning and diffusion behavior to elucidate the dominant mechanism.

As indicated in Table 3, the partition coefficients of the four macromolecules increase with increasing the time of treating the fibrin film with glutaraldehyde. As explained above, the introduction of glutaraldehyde as a result of crosslinking probably increases the hydrophobicity of fibrin film. However, there is no clear correlation between partition coefficient and molecular size as obtained in the study of Gilbert et al. (1988). In the current study, lysozyme has the largest partition coefficient, whereas ovalbumin has the least. If lysozyme is ignored, the magnititude of the partition coefficients is comparable to the elution sequence in HPLC analysis using a gradient solvent system. Since the stationary phase of a C_4 column is nonpolar and the polarity of macromolecules is the major mechanism for the separation, the seqence of elution is expected to parallel the hydrophobicity of macromolecules as indicated by the equation below (Henry et al., 1976; Unger et al.,1978). Therefore, the association of the elution sequence with the order of partition coefficient is realistic.

$$\log K = a \cdot \log K' + b$$

where K' is capacity factor, and both a and b are constants. However, the partition coefficient for lysozyme, which has the shortest retention time is much larger than would be predicted by this relationship. It is interesting to note that the isoelectric point of lysozyme is 10.5 and that of fibrin is 3.2. Therefore, lysozyme is positively charged and fibrin negatively charged in pH 7.4 Tris buffer. Lysozyme may be adsorbed onto the fibrin film through charge interactions, resulting in partition coefficient that is several-fold larger than the rest of macromolecules. In all cases, equilibrium was reached within 12 hours. This suggests that equilibration is fast enough to support the assumptions made when deriving Eqns 6 and 8.

Glutaraldehyde crosslinking is via a bridging mechanism involving ϵ-amino groups of two lysine residues on peptides (Royer, 1983). The crosslinking density can be controlled by varying the length of time that fibrin films remain in the glutaraldehyde aqueous solution. Table 3 shows that the diffusion coefficients of four macromolecules decrease as the extent of crosslinking of fibrin films increases. Lysozyme shows the smallest value of the diffusion coefficient, whereas carbonic anhydrase the largest, for fibrin films treated with glutaraldehyde for the same length of time. Obviously, increasing the extent of crosslinking results in a rigid structure in fibrin films. More hindrance would be encountered by macromolecules within the films having higher extent of crosslinking. Therefore, the decrease in the diffusion coefficient for macromolecules except lysozyme is probably due to their molecular size.

According to the free volume theory of Yasuda (1971), the diffusion coefficient of a solute in a hydrated film is related to the volume available for diffusion within the film (V_{fw}), the degree of hydration of the film (H), and the size of the solute. The free volume relationship can be expressed by the following:

$$\ln(D/D_0) \propto -(Br^2/V_{fw}) \cdot (1/H - 1)$$

where D_0 denotes the solute self-diffusion coefficient, Br^2 is proportional to the solute cross-sec-

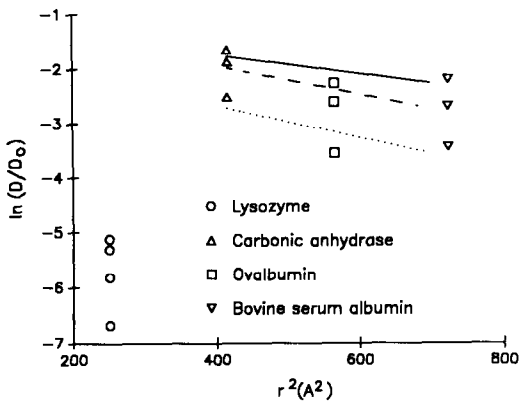

Fig. 5. Relationship between diffusion coefficients and the square of protein radius. Results are shown for various proteins through fibrin films crosslinked with glutaraldehyde for different intervals [untreated (———), 10 min (— — —) and 30 min (- - - - -)].

tional area (πr^2) and V_{fw} is the free volume of water in the fibrin film. As explained under Experimental, the extent of hydration is similar among untreated and treated films. The validity of free volume theory was tested by plotting $\ln(D/D_0)$ vs r^2, which is shown in Fig. 5. With the exception of lysozyme, there is a linear relationship between diffusivity and solute size for the other three macromolecules. The linear correlation suggests that the diffusion of macromolecules is through the free water region in the film. Specific interactions, such as charge attraction, will further retard the diffusion of macromolecules. As a result, lysozyme shows a larger deviation of diffusion coefficients from that predicted by molecular size. Therefore, both diffusivity and partition coefficient play an important role in controlling the permeation of macromolecules through fibrin films, whether crosslinked or not.

Conclusion

The unique properties of the fibrin film make it a viable system for drug delivery. Biodegradation of the fibrin film follows natural, physiologic process making it ideal as a surgically implantable or injectable delivery device. As indicated by the transport behavior of parabens, fibrin films may not be a suitable carrier for molecules like parabens, since they are too small in size to be regulated by changing crosslinking density. However, the permeation of proteins through fibrin films is dependent mainly on their molecular size and partition coefficient. The crosslinking of fibrin films further modifies the permeation rate of macromolecules. Therefore, a desired release rate can be engineered into the drug delivery systems by controlling these factors.

Acknowledgements

The authors wish to express their thanks to Professor Chin-Wen Lin, Department of Animal Husbandry, National Taiwan University for encouragement. We would like to express our sincere thanks for the financial support from the National Science Council, R.O.C. (NSC 80-0412-B-038-17), and Mr Jenh's Pharmaceutical Academic Foundation.

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