Loading of Bovine Serum Albumin into Hydrogels by an Electrophoretic Process and Its Potential Application to Protein Drugs

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INTRODUCTION

A variety of drug-loaded devices has been fabricated using techniques involving solvent casting (1,2), injection molding (3), compression molding (4–6), and microencapsulation (7–12). Drug loading into hydrogels can be achieved by equilibrating hydrogels in a drug-containing solution followed by drying (13–18) or by incorporating the drug during the preparation of hydrogels (19–22). In the former case, solvent removal from the gel can produce drug migration to the gel surface depending on the properties of the solvent and the drug (16). When this occurs, a significant portion of the drug is present on the gel surface and the drug distribution in the hydrogel is not homogeneous. If a drug is present during polymerization, biological activity could be compromised if its chemical structure and/or conformation is modified by chemical initiators, monomer constituents, or temperature increase during polymerization. Recombinant DNA technology has led to the production of a variety of protein drugs in large quantities (23). Incorporation of such protein drugs by swelling dried hydrogels in a drug-containing solution may be difficult and time-consuming due to the low diffusion coefficient of protein drugs into the hydrogel network. Synthesis of hydrogels in the presence of protein drugs may be impractical due to the possible detrimental effects that network formation may have on the properties of the protein. Apparently, an efficient loading process is needed for the practical application of hydrogels as protein drug delivery systems.

Protein drugs may be incorporated into hydrogels using electrophoresis. This approach utilizes an electrochemical gradient to transport proteins into hydrogels based on their net charge. Loading by an electrochemical gradient could be far less time-consuming and capable of loading large amounts of protein drugs into the hydrogel network. In this paper, we describe the experimental methods and preliminary data for the incorporation of a model protein drug, albumin, into hydrogels by electrophoresis.

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MATERIALS AND METHODS

Design of the Electrophoretic Loading Apparatus

The electrophoretic loading apparatus shown in Fig. 1A was constructed from polymethylmethacrylate. The donor and receptor compartments were separated by a hydrogel partition (Fig. 1B). The partition was made up of a fixed divider, a hydrogel slab, and a second divider that could be adjusted depending on the thickness of the gel slab. The adjustable divider was secured with four peripheral plastic bolts located in the donor compartment (black objects in Fig. 1A). The dimension of the partition window which exposes the gel to both compartments was 2 × 10.5 cm (shaded region in Fig. 1B). A constant voltage power supply (Bio-Rad, Model 250/2.5) was connected to platinum electrodes that run along the base of the donor and receptor compartments. An electrical gradient was generated across the face of the gel slab. The voltage could be adjusted from 0 to 250 V. Because of the current flow, negatively charged protein molecules migrate from the donor compartment to the receptor compartment through the gel.

Hydrogel Preparation

A single hydrogel formulation was used in this study since the emphasis of this work was on the process rather than the properties of the network. Polyacrylamide hydrogels were prepared by free radical polymerization using acrylamide (Bio-Rad) and *N,N'*-methylene-bis-acrylamide (Bio-Rad) at a concentration of 6.5% (w/v) and 3% (w/w) of the monomer, respectively. Ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine (TEMED) were used as initiators at a concentration 1.3% (w/w) of the monomer. Monomer solutions were cast polymerized between two glass plates at room temperature for 4 hr. The resulting gel slab was then cut into rectangular slabs and washed in distilled deionized water for 24 hr. The gels were then equilibrated at room temperature for 72 hr in phosphate-buffered saline (PBS; pH 7.2). The concentration of buffer and the ionic strength of PBS were 0.02 and 0.35 *M*, respectively. Gels were equilibrated in PBS since all protein loading experiments were run in PBS.

Protein Incorporation

Hydrogel slabs (3.5 × 13 × 0.3 cm) were inserted into the partition as shown in Fig. 1. All edges of the hydrogel partition were sealed with silicone grease to prevent the leakage of protein into the receptor compartment. Bovine serum albumin (BSA, Fraction V, Pentex; MW 66,296) was used as a model protein drug for all loading experiments. With an isoelectric point of approximately 4.8, BSA carries a net negative charge when solubilized in PBS. PBS was chosen for the loading experiments since phosphate buffer is commonly used in gel electrophoresis when the pH is to be buffered between 6 and 8 (24). Once the hydrogel partition was in place, 750 mL of a 1.5% (w/v) solution of BSA was added to the donor compartment and an equivalent volume of PBS was added to the receptor compartment. The exact volumes of BSA and PBS can be easily varied. The effect of current intensity on the degree of BSA incorporation was

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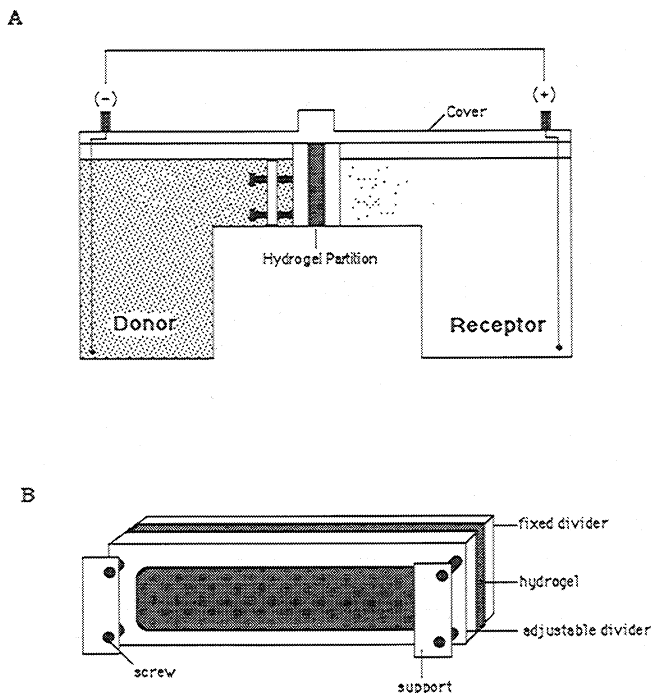

Fig. 1. (A) Side view of the electrophoretic loading apparatus. Bovine serum albumin located in the donor compartment was transported across a hydrogel partition to the receptor compartment by applying current across the gel slab. The hydrogel partition was secured by four peripheral plastic bolts located in the donor compartment (black objects). A constant voltage power supply (Bio-Rad, Model 250/2.5) was connected to platinum electrodes that run along the base of the donor and receptor compartments. An electrical gradient was generated across the face of the gel slab. (B) Front view of hydrogel partition. The partition was made up of a fixed divider located in the receptor compartment, a hydrogel slab, and an adjustable divider located in the donor compartment. The shaded region represents the hydrogel window which was exposed to both the donor and receptor compartments.

studied by varying the current from 0.3 to 1.1 amp. The loading period ranged from 15 to 60 min. BSA loading in the absence of current was used as a control. To compare the electrophoretic process with conventional loading techniques, gel slabs were immersed in a 1.5% (w/v) BSA solution for 72 hr at room temperature.

BSA Determination

The amount of BSA loaded into hydrogels was determined colorimetrically using Biuret's reagent (25,26). In the presence of protein, the absorbance maximum of Biuret's reagent undergoes a shift from 677 to 620 nm. The shift is thought to be due to the complexation of copper with primary amine groups on the protein (26). To determine the BSA concentration in hydrogels, gel slabs were removed from the loading apparatus, cut into three 0.9 × 1.2-cm rectangles, and immersed in Biuret's reagent for 5.5 hr at room temperature. Five and one-half hours was necessary in order to have complete penetration of the reagent into the gel and to achieve a uniform color change within the network following complexation with BSA. The gels were then placed in a disposable cuvette and the Biuret's reagent was added to the cuvette. Absorbance was measured at 620 nm. At least three experiments were run for each loading experiment, and in each experiment two measurements were taken at different positions on a gel. Blank samples were prepared by immersing plain hydrogels in Biuret's reagent for 5.5 hr at room temperature. The absorbance of the blank samples was used to eliminate background absorbance due to the hydrogel and the Biuret's reagent. The concentration of BSA within the hydrogel slab was determined by using an absorptivity of 0.147 cm^2 mg^{-1} for the Biuret's reagent following complexation with BSA. It was found that BSA–Biuret complexation inside the polyacrylamide hydrogels occurred equally as the complexation in the aqueous solution.

BSA Penetration Studies

The degree of BSA penetration into hydrogels was determined using the Biuret's reagent and a microscope. Immediately after the hydrogel was loaded with BSA, the gel slab was cut into 9 × 12-mm rectangles and immersed in a formaldehyde (20%, v/v)/glacial acetic acid (7%, v/v) solution for 1 hr to conjugate the BSA to the polyacrylamide gel (27). The BSA-fixed gel was then transferred to the Biuret's reagent, incubated for 5.5 hr, and placed under a microscope (10× magnification) to examine the cross section of the gel. The depth of BSA penetration into the gel was determined by measuring the thickness of the resulting color band. The measuring limit was 0.1 mm.

RESULTS

Protein Incorporation

The incorporation of BSA into hydrogels was studied as a function of time and current intensity (Fig. 2). A six- to ninefold increase in BSA concentration was observed for a current of 0.3 amp compared to control samples when the loading time increased from 30 min to 2 hr (data not shown).

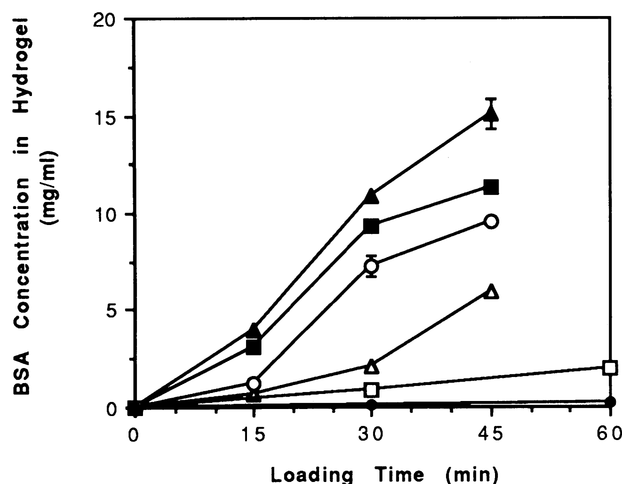

Fig. 2. The BSA concentration within the hydrogel slab as a function of loading time and current intensity. The loading currents were 0.3 amp (□), 0.5 amp (△), 0.7 amp (○), 0.9 amp (■), 1.1 amp (▲), and no current (●) (n = 3). Error bars represent the standard deviation of the mean. An absence of error bars indicates that the standard deviation of the data is smaller than the size of the symbol.

BSA loading by electrophoresis resulted in values ranging from 0.90 mg/mL after 30 min to 5.11 mg/mL after 2 hr, while loading in the absence of current ranged from 0.16 to 0.55 mg/mL. BSA loading for 72 hr in the absence of current resulted in only a moderate BSA content of 2.87 mg/mL. Clearly, loading efficiency was significantly increased by applying current. It should be noted, however, that a 0.3-amp current increased the temperature of the loading medium from 25 to 34°C after 2 hr.

The effect of current intensity on the degree of BSA incorporation is also shown in Fig. 2. BSA loading for 15 min resulted in a 12-fold increase, from 0.32 to 3.91 mg/mL, as the current increased from 0.3 to 1.1 amp. A similar relationship was observed with the 30-min loading and 45-min loading periods. The temperature of the loading medium also increased as a function of current intensity. As the current increased from 0.3 to 1.1 amp, the temperature increased from 25 to 45°C after a 15-min loading period, from 28 to 57°C after a 30-min loading period, and from 30 to 68°C after a 45-min loading period. The data presented in Fig. 2 suggest that the degree of BSA incorporation can be readily manipulated as a function of loading time and current intensity. As a result, larger quantities of protein can be incorporated in shorter loading periods by electrophoresis than with conventional methods of protein incorporation.

Penetration Studies

The movement of BSA molecules through the hydrogel was examined by monitoring the penetration front. Figure 3 shows the migration of BSA into a hydrogel at 30-min (Fig. 3A), 1-hr (Fig. 3B), and 2-hr (Fig. 3C) time points under a current of 0.3 amp. The BSA penetration front was clearly defined and the depth of penetration could be readily measured. Figure 3 shows that complete penetration through the gel occurred after 2 hr of loading. The penetration front was also observed in the absence of current, but the band intensity was much lower due to the lower amount of BSA that migrated into the gel. Figure 4 shows that the depth of BSA penetration into the hydrogel was significantly greater in the presence of current than that in the absence of current. With a current of 0.3 amp, the penetration distance increased from 0.9 to 3 mm as the loading time increased from 30 min to 2 hr. In the absence of current, the penetration distance increased from 0.5 to 1.6 mm. In all penetration studies, a penetration depth of 3 mm indicates that the BSA had moved completely through the hydrogel. The penetration distance also varied as a function of current (Fig. 4). After 15 min of loading, the depth of penetration increased from 0.2 to 1.7 mm as the current intensity increased from 0.3 to 1.1 amp. Complete penetration through the gel was observed after 30 min of loading if the current level was 0.7 amp or greater.

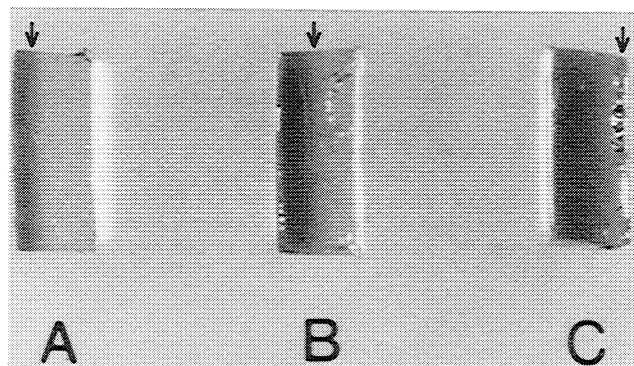

Fig. 3. Penetration of BSA through hydrogels under a current of 0.3 amp. The movement of BSA through the gel was monitored at 0.5-hr (A), 1-hr (B), and 2-hr (C) loading times. The arrow denotes the position of the BSA penetration front as it migrates through the gel. Complete penetration of BSA through the 3-mm-thick gel was observed after 2 hr of loading (C).

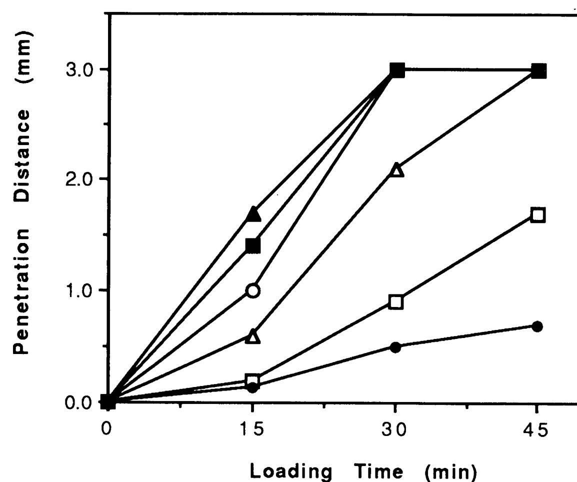

Fig. 4. Penetration distance of BSA in hydrogels as a function of loading time at various current intensities. The loading currents were 0.3 amp (□), 0.5 amp (△), 0.7 amp (○), 0.9 amp (■), 1.1 amp (▲), and no current (●). A penetration distance of 3 mm indicates that BSA had moved completely through the hydrogel ($n = 3$). The absence of error bars indicates that the standard deviation of the mean is smaller than the size of the symbol.

DISCUSSION

In the presence of electric current, BSA readily migrated into polyacrylamide hydrogels. The electrochemical gradient generated across the face of the gel slab resulted in significantly higher amounts of loaded BSA compared to controls loaded in the absence of current (Fig. 2). The higher loading efficiency of the electrophoretic process was best illustrated by the nearly twofold increase in absorbance when current was applied for only 2 hr, as opposed to the 72-hr loading in the absence of current. Furthermore, the extent of BSA incorporation into hydrogels could also be manipulated by varying the current intensity across the gel (Fig. 2). It was also observed that temperature increases were most significant at current levels exceeding 0.3 amp. Consequently, it is possible that the rise in temperature may have contributed to BSA transport through the gel due to temperature-dependent increases in the diffusion coefficient. Such heat generation, however, needs to be minimized either by using lower loading currents or by modifying the device to dissipate the heat. In the latter case, the temperature may be controlled by immersing the entire device in circulating cold water as in protein separation by electrophoresis. This problem will be addressed in future experiments.

Previous studies from our lab have shown that freeze-drying can be utilized to incorporate water-soluble drugs into

hydrogels (28). Dextromethorphan hydrobromide was loaded into enzyme-digestible hydrogels by swelling the gels in a drug-containing solution followed by freeze drying. Through the freeze-drying process, the drug was uniformly dispersed in the hydrogel since drug migration arising from solvent removal was minimized by entrapping the drug within the network upon sublimation. By combining the electrophoretic loading process with freeze-drying, it may be possible to produce dry, uniformly dispersed protein drug delivery systems that maintain biological activity while possessing unique release properties. Since the rate of protein release from the hydrogel can be slow, it may be controlled by using biodegradable hydrogels (29). This study is currently under way.

In summary, the potential use of electrophoresis to incorporate protein drugs into hydrogels is promising. For controlled delivery of protein drugs, however, we have to address other pertinent questions, such as the effects of loading conditions, solvent removal techniques, and polymer–protein interactions on the biological activity and conformational changes of the protein drugs. These points will be addressed in future studies.

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