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# Effects of hydrophilicity of crosslinker on membrane properties of poly(*N*-hydroxyethyl-L-glutamine) hydrogels

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## Summary

Poly(*N*-hydroxyethyl-L-glutamine) (PHEG) hydrogels were prepared by aminolysis of poly( $\gamma$ -benzyl L-glutamate) with 2-aminoethanol and hydrophobic or hydrophilic crosslinkers, and the effect of the hydrophobicity of the crosslinkers was evaluated. The swelling properties, tensile properties and enzymatic degradation behavior were studied in phosphate buffered saline (PBS). The swelling ratio and degradation rate of these hydrogels were highly dependent on the hydrophobicity of the crosslinkers, while tensile properties were dependent on the swelling ratio, but not on the hydrophobicity of the crosskinkers.

## Introduction

Polypeptides may be used in biodegradable medical product applications, such as temporary artificial skin substrates in burn therapy, temporary barriers to prevent adhesion between natural tissue planes that have been damaged by accidents or surgery and between the pericardium and the heart wall during open-heart surgery, polymer carriers for protein conjugates, and drug delivery systems [1].
Anderson et al. showed that the rate of *in vivo* degradation of synthetic polypeptide membranes could be controlled by varying the crosslink density of the membranes [2]. Hayashi et al. prepared copolypeptide membranes using synthesized copolypeptides, and investigated the membrane properties, such as the swelling ratio, the tensile properties, enzymatic degradation behavior, and water vapor permeability, from the viewpoint of applicability to biomedical materials [3-8]. It was shown that the swelling ratio of the hydrophilic membranes plays an important role in determining their properties. The tensile properties of the membranes are highly dependent on the swelling ratio of the membranes and on the hydrophobicity of side chains. A relationship was obtained between the rate of water vapor permeation and the swelling ratio of the membranes independent of side chain characteristics. The biodegradation of the membranes *in vitro* indicated that degradation takes place in the bulk rather than on the surface, and that the rate of degradation is also highly dependent on the

swelling ratio of the membranes as well as on the hydrophobicity of the side chains in the copolypeptides. The rate increased with the hydrophobicity of the membranes.
In our previous study, aimed at furthering the design of polypeptide hydrogels, we attempted to control the properties of polypeptide hydrogels by tailoring the characteristics of the crosslinking point [9]. A series of diamines with varying numbers of methylene groups were used as crosslinkers for preparing polypeptide hydrogels, whose membrane properties were investigated with respect to the chain length of the crosslinker. The swelling ratio and tensile properties were found to be highly dependent on the crosslinker chain length. This indicates that the membrane properties of hydrogels can be controlled by varying the nature of the crosslinking point.
In the present study, an investigation into the effect of crosslinker, two types of diamines having different hydrophobicity but the same chain length were used as crosslinkers for designing polypeptide hydrogels. It is known that poly(*N*-hydroxyethyl-L-glutamine) (PHEG), synthesized via aminolysis of poly( $\gamma$ -benzyl L-glutamate) (PBLG) with 2-aminoethanol, is biocompatible [6]. Thus, PHEG was selected as a model compound for biomedical materials. PHEG hydrogels were prepared by aminolysis of PBLG with 2-aminoethanol and hydrophobic or hydrophilic crosslinkers. The relationship between the crosslinker structure and membrane properties, such as swelling ratio, tensile properties, and enzymatic degradation behavior *in vitro*, of the hydrophilic membranes was investigated.

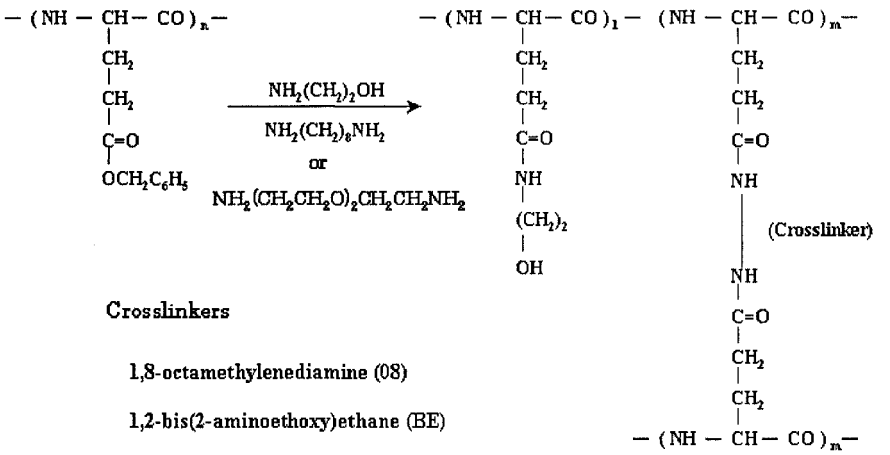
## Experimental

### *Materials*

The parent polymer, poly( $\gamma$ -benzyl L-glutamate) (PBLG) was kindly supplied by Ajinomoto Co., Ltd., Tokyo, Japan. The molecular weight of PBLG was determined to be 380,000 by viscometry in dichloroacetic acid [10].
Hydrophilic membranes, PHEG-x (x: crosslinker's code), were prepared by the aminolysis of PBLG membranes cast from a chloroform solution [11]. PBLG membranes of *ca.* 150 $\mu$ m thickness were immersed in a solution of varying proportions of 2-aminoethanol, and the crosslinking agent 1, 8-octamethylenediamine (08) (hydrophobic crosslinker) or 1, 2-bis(2-aminoethoxy)ethane (BE) (hydrophilic crosslinker) at 56°C. The aminolytic reaction was complete after 20 h. The debenzylation of $\gamma$ -BLG was confirmed by the disappearance of the absorption at 700 and 750 $cm^{-1}$ in IR spectra. The absence of the $\gamma$ -benzyl ester group was confirmed by the disappearance of absorption due to ester groups at 1730 $cm^{-1}$ in IR spectra. Scheme 1 schematizes the preparation of PHEG membranes.

### *Swelling ratio of hydrogels*

Swelling ratio q was determined by equilibrating the membrane in PBS at 37.0℃. The membrane was then blotted to remove surface PBS and weighed until a constant weight was reached. q was defined as the ratio of the swelling weight to the dry weight of the crosslinked membrane.




Scheme 1. General synthesis procedure for crosslinked poly(*N*-hydroxyethyl-L-glutamine) membranes.

*Tensile Properties of hydrogels*

Once they sufficiently swelled in PBS at 37.0℃, the tensile properties of the hydrogels were measured in PBS with AGS-50 ND (Shimazu. Co.). All hydrogels were tested at the elongation rate of 300% per minute. Mechanical parameters, such as Young's modulus (E), tensile strength ( $\sigma_B$) and strain at break point ( $\varepsilon_B$), were determined on the basis of the obtained stress-strain curves.

*Biodegradation of hydrogels* in vitro

*In vitro* enzymatic degradation studies were carried out by using papain [12]. The enzyme solution was prepared by standard techniques at 37.0℃. PHEG hydrogels were exposed to a 0.1 mg/ml papain solution in PBS at pH 7.4 and 37.0℃ with 5 mM cystein as an activator. PHEG hydrogels were removed singly from the enzyme solution after an appropriate time interval. After degradation, PHEG hydrogels were washed in distilled water and methanol, and then dried *in vacuo*. The ratio of the dry weight after and before degradation (Wd,t/Wd) and the swelling ratios after degradation (q) were determined.

## Results and Discussion

The degree of swelling in a solvent is controlled by the interaction energy between solvent molecules and polymer segments. This energy is regarded as the elastic energy (crosslink density) of a swollen polymer. When the degree of swelling q is relatively large, it is given by the following equation according to the rubber elasticity theory [13]:

$$q^{5/3}=(vMc/V_1)(1-2Mc/M)^{-1}(1/2-\chi_1) \quad (1)$$

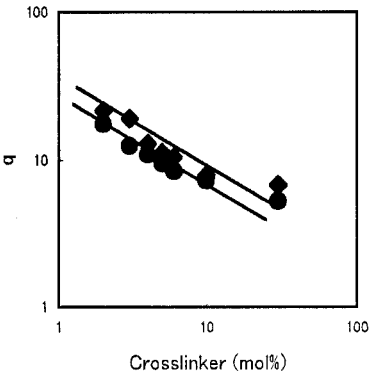



Figure 1. The swelling ratio (q) of hydrophilic membranes in PBS as a function of the molar percent of crosslinker: (●) PHEG-08 and (◆) PHEG-BE.

where Mc is the molecular weight per crosslinked unit, M is the primary molecular weight, v is the specific volume of polymer, $V_1$ is the molar volume of solvent and $\chi_1$ is the interaction parameter. The factor (1 − 2Mc/M) corrects for network imperfections resulting from chain ends. It reduces to unity for high molecular weight polymer chains. Equation 1 then simplifies to:

$$q^{5/3}=(vMc/V_1)(1/2-\chi_1) \qquad (2)$$

But $f_c$, the effective crosslink density, is given by $f_c = K/Mc$, where K is a constant. Substituting this expression into Eq. 2, we obtain:

$$\log q=-3/5\log f_c+3/5\log[(vK/V_1)(1/2-\chi_1)] \qquad (3)$$

Thus, logq varies linearly with $\log f_c$, with a slope of −3/5.

The effect of crosslinker concentration in the reaction mixture on q of the crosslinked hydrogels is shown in Figure 1. q decreases with molar concentration of crosslinker in the reaction mixture. The slope of the log-log plots for the hydrogels is −3/5 as predicted by Eq. 3. The effective crosslink density is proportional to the crosslinker concentration in the reaction mixture. q values for PHEG-BE membranes were higher than those for PHEG-08 membranes. At a roughly fixed molar concentration of crosslinker, q depends on hydrophobicity of the crosslinker in the membranes: it decreases with increasing crosslinker hydrohobicity.

The tensile properties of hydrophilic membranes are highly dependent on the swelling ratio q in PBS [3]. In general, elastomeric membranes are highly suited to biomedical applications [6]. Figure 2 illustrates the stress-strain curves of hydrophilic membranes in PBS. While a higher q value membrane (q=11.4) gave higher elongation, a lower q value membrane (q=6.1) gave higher strength in the PHEG-08 system. Similar trends were observed in the PHEG-BE system. These results indicate that tensile properties are highly dependent on the swelling ratio of hydrophilic membranes and independent of the hydrophobicity of crosslinker at a constant chain length.

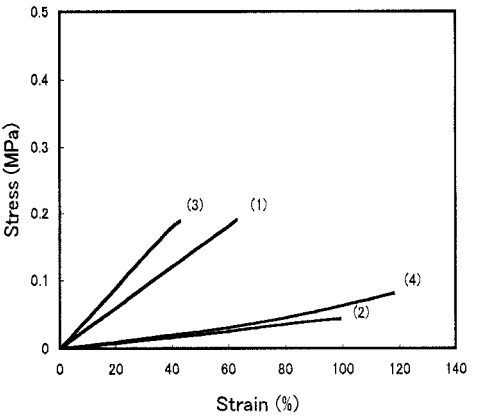



Figure 2. Stress-strain behavior of PHEG membranes: (1) PHEG-08 (q=6.1), (2) PHEG-08 (q=11.4), (3) PHEG-BE (q=6.6), and (4) PHEG-BE (q=11.4).

Numerous proteases may be present at a wound site [14]. These proteases classified according to the structure of their active site. Enzymes of inflammatory response that are likely to degrade poly( $\alpha$ -amino acid)s include endopeptidase cathepsin B and exopeptidases carboxypeptidase and leucine amino peptidase [15]. In the present investigation, the plant thiol endopeptidase papain was selected as a commercially available analogue of cathepsin B.

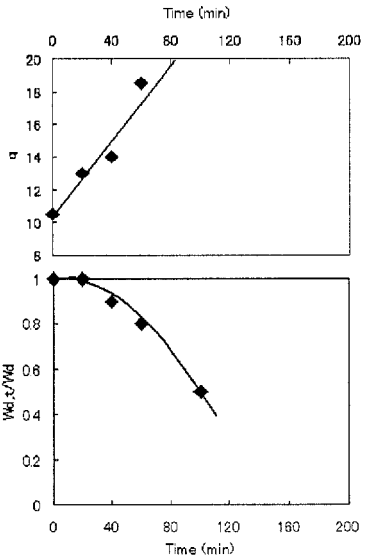



Figure 3. Dry weight ratio ($W_{d,t}/W_d$) and swelling ratio (q) in PBS for the PHEG-BE membrane as a function of papain digestion time (min) at 37℃ and pH 7.4.

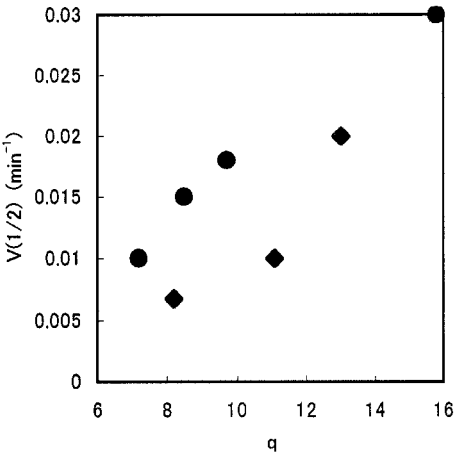



Figure 4. The rate of papain digestion V(1/2) ($min^{-1}$) as a function of the swelling ratio (q): (●) PHEG-08 and (◆) PHEG-BE.

Pre-weighed PHEG-BE membranes were exposed to papain. The results are illustrated in Figure 3. Although papain is a macromolecule with a molecular weight of about 21,000, the polymer network clearly does not exclude the papain molecule [5]. Degradation of PHEG membrane was measured by changes in the swelling ratio q. A sharp increase in q was observed, which strongly pointed towards bulk-, rather than surface-degradation. Geometrical considerations confirm that the enzyme could easily have accessed the membrane bulk. As shown in Figure 3, a sharp increase was observed in the q value of PHEG membranes during papain digestion, while weight loss occurred a bit more gradually. An endopeptidase must make two incisions in a chain segment to produce a soluble fragment, but a single cleavage will decrease the effective crosslink density, resulting in an increased q value. Thus, the initial effect of papain is to decrease the effective crosslink density without producing soluble materials.

It has been known that the rate of degradation is dependent on the hydrophobicity of side chain groups and increases with hydrophobicity [3-5]. Figure 4 summarizes the rate of papain digestion V(1/2) ($min^{-1}$) as a function of the swelling ratio q in PBS. V(1/2) is defined as the reciprocal of the time required for the sample weight to be reduced to one-half of its initial value. It is clearly shown that PHEG-08 degrades more rapidly than PHEG-BE at roughly the same q. The q value was proportional to the crosslinker concentration in the reaction mixture, and the slope of PHEG-08 was in agreement with that of PHEG-BE (Figure 1). This indicates that the crosslink effect of 1,8-octamethylenediamine to PBLG membrane is similar to that of 1,2-bis(2-aminoethoxy)ethane. 1,8-octamethylenediamine and 1,2-bis(2-aminoethoxy)ethane are hydrophobic and hydrophilic crosslinkers, respectively. Based on these results, the crosslink effect may be assumed to involve chemical bonds, rather than detachable physical bonds formed by hydrophobic interactions. This result and those shown in Figure 4 indicate that the rate of papain digestion is clearly dependent on the crosslink density of the membranes, as well as on the hydrophobicity of the environment. Changes in crosslinker properties led to effects similar to those caused by changes in

side chain properties in the degradation rate of hydrogel. This indicates that the hydrophobicity of the crosslinker is effective in varying the degradation rate of hydrogels.

## Conclusions

The effective crosslink density was shown to be proportional to the crosslinker content of the reaction mixture. q was dependent on the hydrophilicity of the crosslinker. The tensile properties of hydrophilic membranes depended on q in PBS. Biodegradation of these hydrophilic membranes by papain *in vitro* indicated that the degradation could be occurring in the bulk, rather than on the surface. The degradation rate of the sample was also highly dependent on q and the hydrophilicity of crosslinker of the hydrophilic membranes. These results indicate that the membrane properties of hydrogels depend on the hydrophobicity of the crosslinker. Moreover, these results suggest that crosslinker selection is key in designing polypeptide hydrogels.

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