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Biodegradation of random co-polypeptide hydrogels consisting of *N*-hydroxypropyl L-glutamine as one component

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Abstract

Two component random co-polypeptide hydrogels consisting of *N*-hydroxypropyl L-glutamine and L-alanine (Ala) or L-phenylalanine (Phe) were prepared by performing aminolysis reactions with 3-amino-1-propanol together with crosslinking reaction with 1,8-octamethylenediamine on hydrogels of the starting co-polymers consisted of γ-benzyl L-glutamate and Ala or Phe. The relationship between their bulk structure and properties was evaluated with regard to the swelling ratio in water (q), the rate of water vapor permeability (V_f), tensile properties, and enzymatic degradation behaviors of hydrogels in a pseudo-extracellular fluid (PECF). The tensile property of the hydrogels was highly dependent on q in PECF, and on the hydrophobicity of the side chains. A relationship was obtained between the V_f and q of hydrogels in PECF regardless of the differences in the nature of the side chains. Biodegradation of the hydrogels in vitro by bromelain indicated that degradation took place in bulk rather than on the surface, and that the rate of degradation was also highly dependent on q in the samples as well as on the hydrophobicity of the side chains of the samples.

Keywords: Biodegradation; Co-polypeptide hydrogel; Bromelain; Tensile property; Water vapor permeability

1. Introduction

Poly(α-amino acid)s and their co-polymers may be used in the biodegradable medical fields as temporary artificial skin substrates in burn therapy, temporary barriers to prevent adhesion between natural tissue planes that have been damaged by accidents or by surgery between the pericardium and the heart wall during open-heart surgery, polymer carriers for conjugates that are coupled to proteins for therapeutic use and drug delivery systems [1]. It has been shown that the rate of in vivo degradation of synthetic poly(α-amino acid)s can be controlled by varying the hydrophilicity of the side chain groups [2]. The degradation was attributed to cleavage of the poly(α-amino acid) chains by proteolytic enzymes, such as endopeptidase cathepsin B, that were released during acute and the chronic stages of the inflammatory response. Research about partial modification of side chains will be very useful in regards to the development of practical applications of poly(α-amino acid)s in biomedical material fields because they significantly influence in regards to the degradation of poly(α-amino acid)s by proteolytic enzymes. On the other hand, it is well known that co-polymer conformation and properties can be strongly influenced by the sequential distribution of the co-monomers in a co-polymer chain as well as co-polymer molar composition.

In this paper, we prepared two component random co-polypeptide hydrogels that were made up of

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Table 1
PECF ($NaHCO_3$, K_2HPO_4, NaCl, KCl)

Ion	Concentration (meq./l)	
	Physiological	PECF
Na	145	145
K	5	5
Cl	113	118
HCO_3	30	30
HPO_4	2	2

N-hydroxypropyl L-glutamine (HPG) and a hydrophobic amino acid, such as L-alanine (Ala) or L-phenylalanine (Phe), and we examined the relationship between their molecular structures and the hydrogels properties, such as the swelling ratio (q) in a pseudo-extracellular fluid (PECF) [3], the tensile properties in PECF, water vapor permeability (V_f), and enzymatic degradation behavior in vitro in the hydrogels in PECF in order to simulate in vivo polymer degradation from the perspective of their possible use as biomedical materials. The composition of the PECF is listed in Table 1. Bromelain was selected as a model protease in this study. It is a well characterized plant thiol endopeptidase [4–6] with a rather broad selectivity to substrates. It is closely related to cathepsin B, which is a thiol endopeptidase that has been isolated from mammalian spleen, liver and kidney, and is released by cell's response to inflammation [7]. The hydrolysis of poly(*N*-hydroxypropyl L-glutamine) (PHPG), as well as its co-polymers by papain or ficine has already been studied extensively [8–13] at various pH regions.

2. Experimental

2.1. Synthesis of co-polypeptides

γ-Benzyl-L-glutamate *N*-carboxyanhydrides (γ-BLG-NCA) as well as L-alanine NCA (Ala-NCA) or L-phenylalanine NCA(Phe-NCA) were prepared by phosgenation of γ-benzyl L-glutamate (BLG), Ala, and Phe, respectively, in tetrahydrofuran (THF) and was purified by multiple recrystallization [14]. Solutions (0.1 M, ≈2 wt.%) of γ-BLG-NCA and L-Ala-NCA or L-Phe-NCA of various co-monomer ratios in 1:1 (v/v) mixture of dioxane/methylene dichloride were prepared and the polymerization was initiated with triethylamine (TEA) at a monomer:initiator ratio of 25:1. The co-polymerization reaction was followed by CO_2 evolution according to Patchornik and Shalitin's method [15]. The polymerization was stopped at about a 50 mol% conversion. All of the solvents that were used for the synthesis and the initiator were purified by the usual methods that have been described in the literature. The polymers that were formed were precipitated by adding four times the amount of the methanol in the volume, including 5 vol% of 0.1 N HCl to the polymer solution at 4°C. Then the precipitation products were washed with pure methanol and dried under reduced pressure at 50°C. The composition of these co-polymers was determined by amino acid analysis. The aminolysis reaction of the end group of the side chains of the γ-BLG residues was performed by 3-amino-1-propanol (P).

Fig. 1 illustrates a schematic diagram of hydrogel preparation. The completion of the aminolysis reaction was ascertained by using an UV spectra measurement. After the aminolysis reaction was complete, an aqueous polymer solution was dialyzed exhaustively against distilled water, filtered through a Millipore filter and lyophilized.

2.2. Molecular characterizations

The intrinsic viscosity [η] (dl/g) of the starting polymers was determined in dichloroacetic acid (DCA) at 25.0°C using an Ubbelohde-type viscometer. The experimentally determined values of the polymer yield (P), the compositions of the γ-BLG component at the initial monomer mixture (F_B), and in the co-polymer (cf_B), (together with the [η] value), are summarized in Table 2. The [η] of the water soluble samples after the aminolysis reaction was measured with an Ubbelohde-type viscometer at pH 7.4 and 37.0°C in PECF. The molecular weights (M_w) of the water soluble samples were analyzed by gel permeation chromatograph (GPC) on a Toyo-Soda high-speed liquid chromatograph HLC-803D equipped with a TSK-gel type G-4000SW, C-No. SW46A0015 at 25°C in PECF. The preparative data of these water soluble samples are summarized in Table 3. The chain conformation of these co-polymer molecules in PECF was examined by circular dichroism (CD) measurements (JASCO J-720 type). The experimental results for $[\theta_{222}]$ and the helical content (f_H) (which was calculated from an equation; $f_H = -[\theta_{222}]/40{,}000$ for the samples) are also listed in Table 3.

2.3. Preparation of hydrogels

After a co-polypeptide membrane of about 100 μm in thickness was cast from a chloroform solution, an aminolysis reaction of γ-BLG residue was performed by using 3-amino-1-propanol (P). The original co-polypeptide membrane was immersed in a mixture of (P) and a crosslinker, 1,8-octamethylenediamine (OMDA), with an appropriate composition at 58°C to 60°C for 24 h. After that, the hydrogel was washed with methanol, pure water, ethyl ether, and were stored in ethanol.

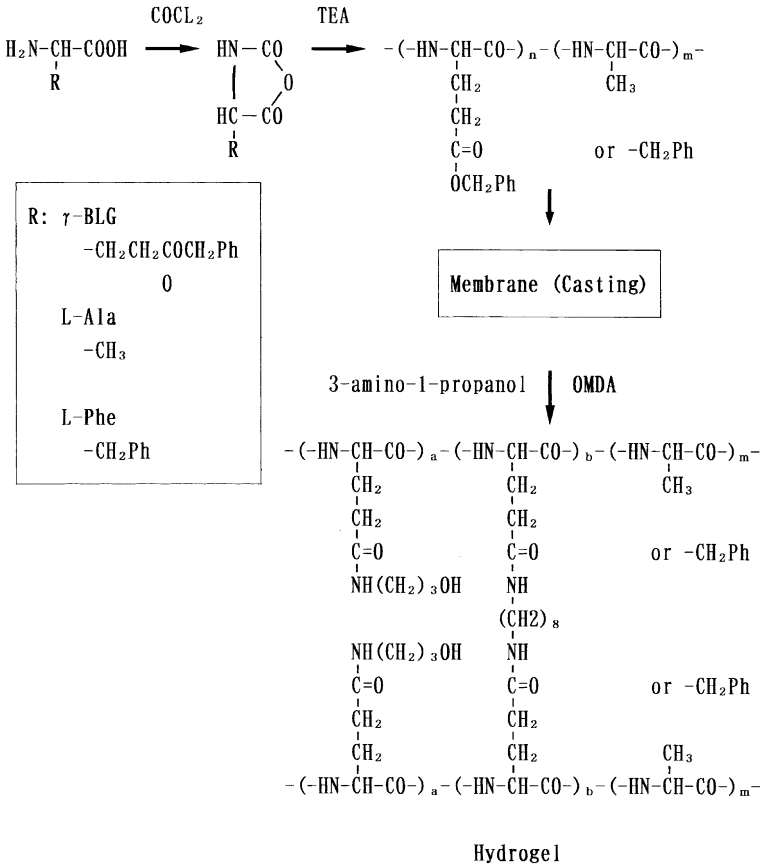

Fig. 1. Schematic diagram of the preparation of hydrogels.

Table 2
Co-polymerization data for starting co-polypeptides

Sample code	F_B (mol%)	cf_B (mol%)	P (%)	[η] (dl/g) (DCA, 25°C)
PBLG-1	100	100	79	1.25
BLG/Ala-1	80	85	48	1.14
BLG/Ala-2	60	68	46	1.02
BLG/Phe-1	80	87	49	1.20

PBLG: poly(γ-benzyl L-glutamate); BLG/Ala: co-poly(γ-benzyl L-glutamate/L-alanine); BLG/Phe: co-poly(γ-benzyl L-glutamate/L-phenylalanine).

2.4. Physical measurements of hydrogels

The swelling ratio q of the hydrogels in PECF was determined by equilibrating the hydrogel samples in PECF at 37.0°C. The hydrogel was removed, blotted in order to remove surface PECF, and was weighed until a constant weight was achieved. The hydrogel was then dried in a vacuum oven. q was defined as the ratio of the weight of PECF swelling hydrogels to that of the dried one. Table 4 lists the preparative data of hydrogels. The tensile properties of the hydrogels were measured

Table 3
Water soluble co-polypeptides prepared by aminolysis of the starting polymers[a]

Sample code	Starting polymer	HPG (mol%)	[η] (dl/g) (PECF, 37°C)	M_w	$-[\theta_{222}]$	f_H
PHPG-10	PBLG-1	100	0.420	83,500	500	0.01
HPG/Ala-10	BLG/Ala-1	85	0.435	87,500	1050	0.03
HPG/Ala-20	BLG/Ala-2	68	0.425	85,000	4200	0.11
HPG/Phe-10	BLG/Phe-1	87	0.450	91,000	7800	0.20

The last numeral "0" of the sample codes denotes that the aminolysis reaction of the end group of the side chains of the γ-BLG residues was performed by 3-amino-1-propanol without OMDA.

[a] PHPG: poly(*N*-hydroxypropyl L-glutamine); HPG/Ala: co-poly(*N*-hydroxypropyl L-glutamine/L-alanine); HPG/Phe: co-poly(*N*-hydroxypropyl L-glutamine/L-phenylalanine).

Table 4
Preparative data and swelling ratio (q) of hydrogels

Sample code	Starting polymer	HPG (mol%)	OMDA (mol%)	q (W_w/W_d)
PHPG-11	PBLG-1	100	1.0	14.9
PHPG-12			2.0	10.8
PHPG-13			3.0	8.4
PHPG-14			4.0	6.8
HPG/Ala-11	BLG/Ala-1	85	1.0	9.0
HPG/Ala-12			2.0	6.2
HPG/Ala-13			3.0	4.9
HPG/Ala-21	BLG/Ala-2	68	1.0	5.3
HPG/Ala-22			2.0	4.1
HPG/Ala-23			3.0	3.4
HPG/Phe-11	BLG/Phe-1	87	1.0	3.5
HPG/Phe-12			2.0	3.0
HPG/Phe-13			3.0	2.7

The last numeral of the sample codes denotes the mol% of OMDA in 3-amino-1-propanol on the aminolysis reaction of γ-BLG residue of the starting polymers.

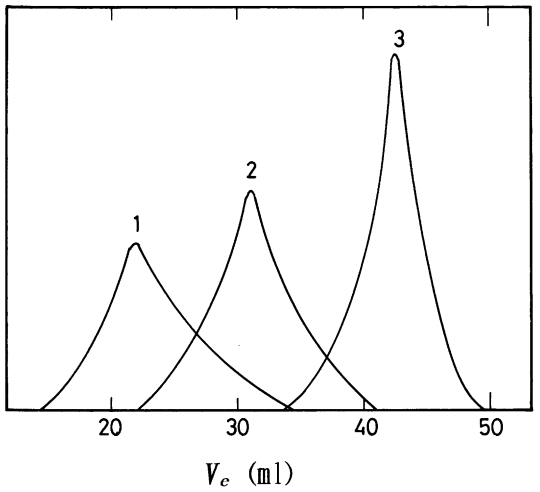

Fig. 2. GPC elution curves for reaction products of HPG/Ala-10 at pH 7.4 in PECF by bromelain: $[E] = 2.5 \times 10^{-5}$ M; (1) original, (2) 45 min of digestion, and (3) 240 min of digestion.

by a Tensilon UTM-II-20 (Toyo-Boldwin Co., Tokyo, Japan) using a standard technique in PECF at 25.0°C. All of the samples were tested at an elongation rate of 40% per min. Water vapor permeation through the hydrogels was measured with a cylindrical glass cell at 37.0°C [16]. The exposed sample area was 12.57 cm^2.

2.5. Biodegradation of hydrogels in vitro

Enzymatic degradation studies in vitro were performed by using bromelain (EC 3.4.22.4, from Pineapple Stem, No. B-2252, Sigma), which was obtained from Amano Co. Ltd. (Nagoya, Japan) and was used without further purification. A series of the crosslinked hydrogels were exposed to PECF at pH 7.4 and 37°C. The hydrogels were removed from the enzyme solution at appropriate time intervals, weighed, and were then vacuum dried at 60°C until a constant weight was achieved.

3. Results and discussion

3.1. Type of degradation

In order to determine whether random degradation of the main chain of a polypeptide is dominant in a reaction with bromelain, a GPC analyses of partially degraded water soluble polypeptides was performed by using a Toyo-Soda high-speed liquid chromatograph at 25°C in PECF.

Fig. 2 illustrates the GPC curves for the co-poly (*N*-hydroxypropyl L-glutamine/L-alanine) [HPG/Ala-10] sample, as an example. From Fig. 2, one can see that HPG/Ala-10 is primarily degraded by a random main chain cleavage as is the case for the degradation of [PHPG] homopolymer by endopeptidases such as papain or ficin. These have been researched previously by us. The similar behavior was observed by Miller [17] previously in the case of the degradation of poly(L-glutamic acid).

3.2. Swelling ratio of hydrogels in PECF

Swelling ratio q in a solvent is determined by the interaction energy between the solvent molecules and the polymer segments as well as the elastic energy (crosslink density) for a solvent-swollen polymer [18]. The precise crosslink density has not been determined because of uncertainty in regards to the relative reactivities of 3-amino-1-propanol and OMDA, and also because estimation of the fraction of the reacted diamine molecules from effective crosslinks is difficult. The effects of OMDA concentration in the reaction on the q of the crosslinked hydrogels in PECF is shown in Fig. 3. q in PECF decreases with an increase in the OMDA molar concentration in the reaction mixture.

When q is significant in size, it is given by the following equation according to rubber elasticity theory [18].

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

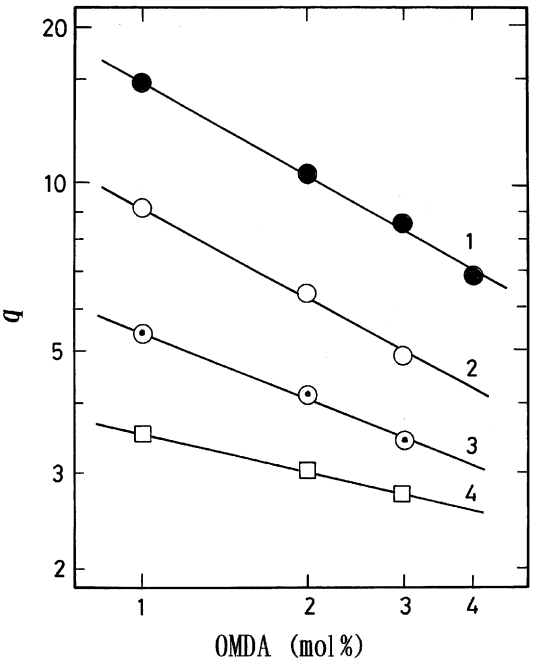

Fig. 3. Swelling ratio (q) of hydrogels at 25°C as a function of the mol% of OMDA: (1) (●) PHPG-1, (2) (○) HPG/Ala-1, (3) (⊙) HPG/Ala-2, and (4) (□) HPG/Phe-1.

where M_c 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 χ_1 isthe interaction parameter. Factor $(1-2M_c/M)$ expresses the correction for network imperfections that results from chain ends. For a very high molecular weight polymer chain, it is reduced to unity. As for the effective crosslink density, f_c is proportional to the value of M_0/M_c, where M_0 is the molecular weight of the repeating unit (monomeric unit), Eq. (1) may be simplified as

$$q^{5/3} = (vM_0)(1/2-\chi_1)V_1 f_c \quad (2)$$

Thus, $\log q$ will be in linear relationship with $\log f_c$ with a slope that is $-3/5$. Fig. 3 illustrates the effects of the OMDA concentration in the reaction mixture on the q of the crosslinked hydrogels. q decreases with an increase in the OMDA molar concentration in the reaction mixture. The slope of the log–log plots in PHPG-1 and HPG/Ala-1 series (which exist in a random coil conformation) has a value of $-3/5$ as was predicted in Eq. (2). Effective crosslink density f_c is proportional to the crosslinker OMDA concentration (mol%) in the reaction mixture. On the other hand, HPG/Ala-2 and copoly(*N*-hydroxypropyl L-glutamine/L-phenylalanine) [HPG/Phe-1] series which exist in an interrupted α-helix conformation with a lower q cannot be understood according to rubber elasticity theory because the slope is less than $-3/5$. This suggests that the theory given in Eq. (1) can only be applied to hydrogels which contain random coil regions that have enough segmental mobility.

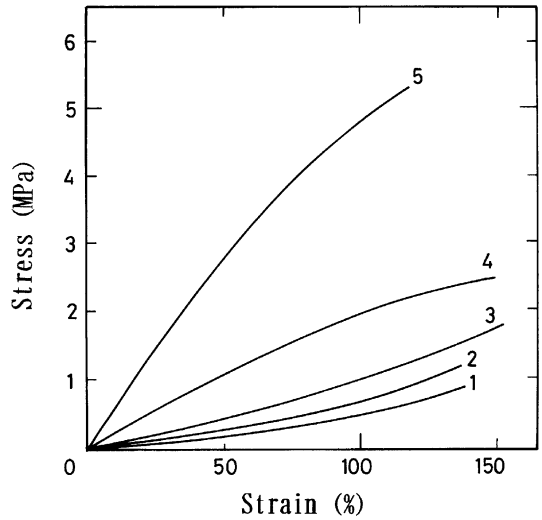

Fig. 4. Stress–strain curves of hydrogels at 25°C in PECF: (1) PHPG-12, (2) PHPG-13, (3) HPG/Ala-11, (4) HPG/Ala-21, and (5) HPG/Phe-11.

3.3. Tensile properties of hydrogels in PECF

The tensile properties of hydrogels are highly dependent on the q in PECF. Furthermore, elastomeric materials are highly suited to biomedical applications, such as materials for artificial organs and reconstructive prosthesis. Fig. 4 illustrates the stress–strain curves of some of the hydrogels in PECF at 25°C. Table 5 lists the experimental findings of Young's modulus E at an elongation of 1%, tensile strength σ_B and elongation ε_B

Table 5
Mechanical properties of hydrogels at 25.0°C in PECF

Sample code	q (W_w/W_d)	E (MPa)	σ_B (MPa)	ε_B (%)
PHPG-12	10.8	0.20	0.87	138
PHPG-13	8.4	0.46	1.25	137
PHPG-14	6.2	0.75	1.29	120
HPG/Ala-11	9.0	0.84	1.70	152
HPG/Ala-12	6.2	1.12	2.05	150
HPG/Ala-13	4.9	1.40	2.45	146
HPG/Ala-21	5.3	2.25	2.50	149
HPG/Ala-22	4.1	3.16	3.75	140
HPG/Ala-23	3.4	4.20	4.40	142
HPG/Phe-11	3.5	6.80	5.25	118
HPG/Phe-12	2.1	8.80	7.65	105

at a breakage point with q values for hydrogels in PECF. Fig. 4 shows that the tensile behavior of these hydrogels can be classified into two types: skin type and elastomer type, according to the shape of the stress–strain curve. The skin-type hydrogels have concave behavior with a low Young's modulus (as is typical for human skin), while the elastomer-type hydrogels have sigmoidal behavior with the transition in the low strain region. Skin-type behavior can be accounted for by entropy elasticity without stress relaxation, creeping and hysteresis, while elastomer-type behavior may include the contribution of energy elasticity with stress relaxation, creeping and hysteresis. The differences in the shape of the stress–strain curves between the two examples was previously discussed in detail [19,20]. A hydrogel which exists in an interrupted α-helix conformation, such as HPG/Ala-21 and HPG/Phe-11, shows elastomer-type behavior, while those which contain random coil conformation, such as PHPG-12, PHPG-13, and HPG/Ala-11, show skin-type behavior. The hydrophobic nature of L-Ala or L-Phe affects the mechanical properties of hydrogels, giving higher values for the tensile parameters.

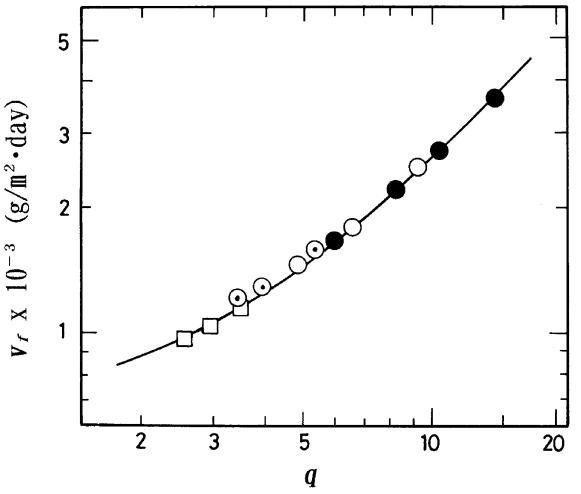

Fig. 5. Rate of water vapor permeation (V_f) of hydrogels as a function of swelling ratio (q) at pH 7.4 and 37.0°C in PECF: (●) PHPG-1, (○) HPG/Ala-1, (⊙) HPG/Ala-2, and (□) HPG/Phe-1 series.

3.4. Water vapor permeability of hydrogels

Number of synthetic polymeric hydrogels have been studied in regards to the treatment for burns [21–23]. For example, the formulation of a crosslinked polymer in the form of hydrogel appears to encourage cellular migration into grafts and vascularization [21]. At least two skin functions are essential for survival: the first is that skin keeps most bacteria out of the body; the second is that skin prevents tissue and organ water from evaporating. Massive infection and severe fluid loss are major threats to a patient's survival. Thus, to achieve effective wound closure, an appropriate rate of water vapor permeability V_f (g/m^2 day) must be maintained through the hydrogels. If the value of V_f is too low, water will accumulate at the interface between woundbed and impermeable graft and edema will result. Consequently, optimization of the water vapor permeation rate (one that is probably close to a human physiological level of about $V_f = 500$ (g/m^2 day) [24]) is a necessary factor in an appropriate design. As the result, in order to maintain the ability to wet the woundbed suitably, an inner skin substitute hydrogel should exhibit a V_f value that is higher than 500 (g/m^2 day).

Fig. 5 illustrates the relation between the V_f (g/m^2 day) of PECF and q for hydrogels at 37.0°C. Also, the V_f value is highly dependent on the q value in PECF. Fig. 5 shows that HPG/Phe and HPG/Ala hydrogels are more effective than PHPG hydrogels in an appropriate q region for use as artificial skin substrates, when a common relationship can be obtained between V_f and q of hydrogels regardless of the differences in the architecture of the polymer chains in a higher q region.

3.5. Biodegradation of hydrogels in vitro

Williams [25] has shown that numerous proteases may be present at a wound site. Inflammatory response enzymes that are likely to degrade poly(α-amino acid)s include endopeptidase cathepsin B, the exopeptidases carboxypeptidase and leucine aminopeptidase [26]. A plant thiol endopeptidase bromelain was selected as a commercially available analog of cathepsin B in the present study. Although bromelain is a general thiol endopeptidase, it has preferences in regards to peptide bonds where the amino acid residue of carbonyl group is arginine, lysine, or glutamine and where this amino acid is joined on either side by amino acids with hydrophobic side chains. Pre-weighed crosslinked hydrogels were exposed to 0.100 mg of bromelain in 1 ml of PECF at 37.0°C. The results that were obtained with a HPG/Ala-11 hydrogel is illustrated in Fig. 6. The degradation of the HPG/Ala-11 was measured by changes in its bulk property as well as in q (Fig. 6). It is shown in Fig. 6 that, an immediate increase in the swelling ratio of the HPG/Ala-11 hydrogel while weight loss was observed later in the course of the bromelain digestion. For an endopeptidase, one must make two incisions in a chain segment in order to produce a soluble fragment, while a single incision will decrease the effective crosslink density.

Next, it is important to know the exact change in the remaining tensile strength of the hydrogels that are under enzymatic digestion. Fig. 7 illustrates the relative tensile strength ($\sigma_{r,B}/\sigma_{0,B}$) and the dry weight ratio (W_r/W_0) for HPG/Ala-11 hydrogel as a function of the bromelain digestion time at 37.0°C and pH 7.4 in PECF.

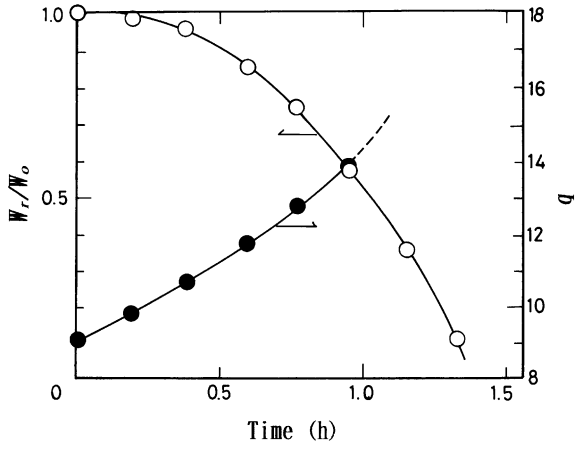

Fig. 6. Dry weight ratio (W_r/W_0) and q for HPG/Ala-11 hydrogel as a function of bromelain digestion time (h) at pH 7.4 and 37.0°C.

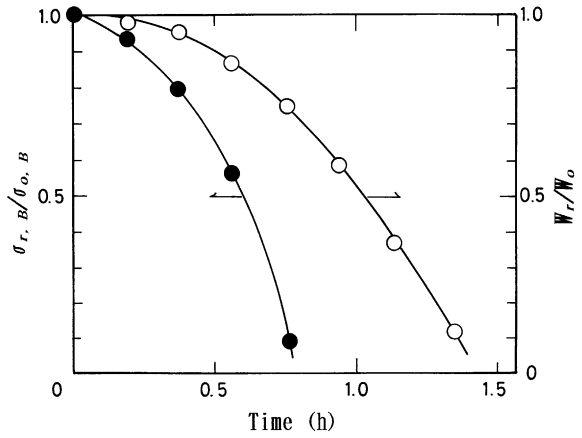

Fig. 7. Dry weight ratio (W_r/W_0) and relative tensile strength at break ($\sigma_{r,B}/\sigma_{0,B}$) of HPG/Ala-11 hydrogel as a function of bromelain digestion time (h) at pH 7.4 and 37.0°C.

It is clear that the tensile strength of the hydrogel decreased faster as compared to that of the mass of the bromelain digestion, suggesting that the crosslink cleavage occurs under enzymatic digestion.

Fig. 8 summarizes the bromelain digestion rate $V_{1/2}$ (h^{-1}) as a function of q of the PECF in these hydrogels. $V_{1/2}$ is defined as the reciprocal of the time required for the sample weight to be reduced to one-half its initial value. It is also clear that the rate of bromelain digestion for HPG/Ala and HPG/Phe groups is higher than that for PHPG series at the same q order. The effects of hydrophobic co-monomers may be important in explaining the faster digestion of hydrogels with higher hydrophobicity.

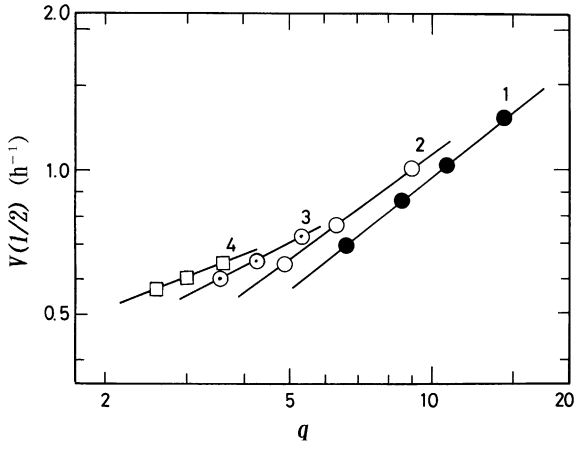

Fig. 8. Rate of bromelain digestion $V_{1/2}$ (h^{-1}) of hydrogels at pH 7.4 and 37.0°C in PECF as a function of q values: (1) (●) PHPG-1, (2) (○) HPG/Ala-1, (3) (⊙) HPG/Ala-2, and (4) (□) HPG/Phe-1 series.

4. Conclusions

In conclusion, the swelling ratio of hydrogels played an important role in determining their properties. The tensile properties of hydrogels were highly dependent upon the swelling ratio of the hydrogels and on the hydrophobicity of the side chains, whose behavior was typical of an elastomer. A relationship was obtained between the rate of water vapor permeation and the swelling ratio of hydrogels in spite of the differences in the nature of the side chains. The biodegradation of hydrogels in vitro by bromelain indicated that degradation took place in bulk rather than on the surface, and that the rate of degradation was also highly dependent on the swelling ratio of samples as well as on the hydrophobicity of the side chains in co-polymers, This rate increased with an increase in the hydrophobicity of the hydrogels.

References

[1] Anderson JM, Spilizewski KL, Hiltner A. In: Williams DF, editor. Biocompatibility of tissue analogs. Boca Raton, FL: CRC Press; 1985. p. 68.
[2] Anderson JM, Gibbons DF, Martin RL, Hiltner A, Woods R. J Biomed Mater Res Symp 1974;5:197.
[3] Homsey CA. J Biomed Mater Res 1971;4:341.
[4] Murachi T. Nippon Kagaku Zasshi 1967;88:899 [in Japanese].
[5] Murachi T. Meth Enzymol 1976;45:475.
[6] Ida S, Sasaki M, Ota S. J Biochem 1973;73:377.
[7] Otto K. In: Barnett AJ, Dingle JT, editors. Tissue proteases. New York: Elsevier; 1971. p. 1.
[8] Dickinson HR, Hiltner A. J Biomed Mater Res 1981; 15:591.

[9] Hayashi T, Tabata Y, Nakajima A. Polym J 1985;17:463.
[10] Hayashi T, Nakanishi E, Nakajima A. Polym J 1987; 19:1025.
[11] Hayashi T. Prog Polym Sci 1994;19:663.
[12] Miyachi Y, Jokei K, Oka M, Hayashi T. J Biomater Sci Polym Ed 1966;7:805.
[13] Hayashi T, Oka M, Miyachi Y. Macromol Symp 1997;123:163.
[14] Hayashi T, Nakajima A. Bull Inst Chem Res Kyoto Univ 1977;55:150.
[15] Patchornik A, Shalitin Y. Bull Res Counc Isr Sect A Chem 1956;5:300.
[16] Hayashi T, Iwatsuki M, Oya M. J Appl Polym Sci 1990;39:1803.
[17] Miller WG. J Am Chem Soc 1961;83:259.
[18] Flory PJ. In: Principles of polymer chemistry. Ithaca, NY: Cornell University Press; 1953. p. 576.
[19] Nakanishi E, Hamada K, Sugiyama E, Hibi S, Hayashi T. Polym J 1991;23:1053.
[20] Nakanishi E, Sugiyama E, Hibi S, Maeda H, Hayashi T. Polym J 1991;23:983.
[21] Hubacek J, Kliment K, Dusek J, Hubacek J. J Biomed Mater Res 1967;1:387.
[22] Schmolka IR. J Biomed Mater Res 1972;6:571.
[23] Guldalian J, Jelenko C, Galloway D, McKnight JT. J Trauma 1973;13:32.
[24] Yannas IV, Burke JF. J Biomed Mater Res 1980;14: 65.
[25] Williams DF. J Bioeng 1977;1:279.
[26] Salthouse TN. J Biomed Mater Res 1976;10:197.