Effect of Nonprotein Polymers on Water-Uptake Properties of Fish Protein-Based Hydrogel

G. V. N. RATHNA, SRINIVASAN DAMODARAN

University of Wisconsin–Madison, Department of Food Science, Madison, Wisconsin 53706

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ABSTRACT: The effects of nonprotein polymers on the water-swelling properties of fish protein-based hydrogel were studied. Inclusion of carboxymethyl cellulose (CMC), poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), or guar gum at a 2.5% (w/w) level in an 80% ethylenediaminetetraacetic dianhydide (EDTAD)-modified fish protein hydrogel (10% monomer concentration) significantly decreased the extent of water uptake of the hydrogel. Among these polymers, PVA exhibited the greatest inhibitory effect. The inhibitory effect of these polymers on the water uptake of fish protein hydrogel was apparently due to the thermodynamic incompatibility of these polymers with the fish protein gel network and the consequent effect on the extent of relaxation of the crosslinked polypeptide network. In contrast, inclusion of 60% EDTAD-modified soy protein up to a level of 40% of the total protein in the gel did not affect the extent of the equilibrium water uptake of the gel. At higher levels, however, soy protein also decreased the amount of water uptake by the gel. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 85: 45–51, 2002

INTRODUCTION

Crosslinked hydrophilic polymers with exceptional water-uptake properties are designated as hydrogels. The potential use for hydrogels in several industrial,[1–13] biomedical,[14–18] pharmaceutical,[19–24] and biotechnology[25] applications has been established. Although hydrogels made from synthetic polymers, such as polyacrylate and polymethacrylate, possess excellent water-absorbing properties, their toxicity and nonbiodegradability might pose long-term environmental problems. Hence, new approaches are needed to develop nontoxic biodegradable hydrogels from natural polymers such as proteins. Recently, we showed that chemical modification of proteins with a tetracaboxylic acid diahydride followed by crosslinking with glutaraldehyde results in a polyanionic hydrogel capable of binding a large amount of water.[26–29] The swelling capacity of these hydrogels could be manipulated by changing the extent of chemical modification, the degree of crosslinking, and the protein concentration at the time of crosslinking. However, we observed that the rate of swelling of these protein-based hydrogels was slower than that of hydrogels made from synthetic polymers. This was principally due to the presence of a significant amount of folded α-helix and β-sheet structures in protein monomers even after denaturation at pH 12 and modification with dianhydrides. To improve the rate of swelling, it is essential to minimize the folded secondary structure content of the polypeptide and increase the aperiodic (or random-coil) structure content. This was partially accomplished by treating the crosslinked hydrogel (without drying) with an organic solvent, such as ethanol.[30] The ethanol-

Correspondence to: S. Damodaran (sdamodar@facstaff.wisc.edu).

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treated hydrogels exhibited at least a twofold increase in the rate of swelling and about 100% increase in the extent of swelling at equilibrium compared with the control.

A homogeneous interpenetrating polymer network can be formed by crosslinking two different polymers. Interpenetration of the chains in the network can impart a combination of properties to the hydrogel. Generally, the two components in the network retain their individual properties if there is no chemical bonding between them. Interpenetrating polymer networks may exhibit strong mechanical properties in comparison with homopolymer networks.[31] There is no experimental evidence in the literature to indicate that globular proteins can form an interpenetrating gel network with other nonprotein polymers. Nevertheless, to elucidate if the swelling properties of protein-based hydrogels can be improved by the addition of other polymers, we studied the water-swelling property of binary polymer networks of chemically modified fish protein with modified soy protein, sodium carboxymethylcellulose, poly(vinyl alcohol), guar gum, and poly(ethylene glycol). We selected these polymers because they are water-soluble, hydrophilic, biodegradable, and nontoxic.[32–39]

EXPERIMENTAL

Materials

Walleye pike (fish) was obtained from a local fish farm. Soy protein isolate (Supro 620) was obtained from Protein Technologies International (St. Louis, MO). Ethylenediaminetetraacetic dianhydride (EDTAD), poly(vinyl alcohol) (PVA), sodium carboxymethylcellulose (NaCMA), and guar gum (GG) were from the Aldrich Chemical Co. (Milwaukee, WI) and picryl sulfonic acid (TNBS) and glutaraldehyde (50% aqueous solution) were obtained from the Sigma Chemical Co. (St. Louis, MO). Heat-sealable and water-wettable paper was procured from Bolmet Inc. (Dayville, CT). Poly(ethylene glycol) (PEG: molecular weight 20,000) was from Fisher Scientific (Pittsburgh, PA). All other chemicals were of analytical grade. Deionized water was used for the swelling studies.

Methods

Isolation of Crude Fish Protein (FP)

Isolation of protein from fresh fish was carried out as described elsewhere.[29] Briefly, the fresh fish upon arrival was filleted, chopped, and blended with chilled deionized water at a meat-to-water ratio of 1:9 (w/w). The suspension was adjusted to pH 12 and stirred using a magnetic stirrer for 30 min. The suspension was filtered through a 0.5-mm sieve, and the filtrate was dialyzed against water and lyophilized.

Protein Determination

Because the modifying groups used in this study interfered with all colorimetric methods for determination of the protein concentration, the protein concentration was determined by the dry-weight method.[29] A weighed aliquot of a protein stock solution in deionized water was dried to a constant weight at 110°C in a vacuum oven. The protein concentration was expressed as % w/v.

Modification of Fish Protein (MFP) and Soy Protein (MSP)

Chemical modification of the lysine residues of the protein with EDTAD was carried out as reported elsewhere.[27,29] A 1% protein solution was prepared at pH 12 and incubated for 30 min at 65°C. The solution was cooled to room temperature and a calculated amount of EDTAD was added in incremental amounts with continuous stirring. After complete addition of EDTAD, the reaction mixture was stirred constantly for 3 h while maintaining the pH at 12. At the end of the reaction, the pH of the protein solution was adjusted to 4.5 to precipitate the protein. The suspension was centrifuged at 10,000 g for 15 min. The protein sediment was then washed with water at pH 4.5 and centrifuged. The final precipitate was then redissolved in water at pH 7.0 and lyophilized. The extent of acylation, that is, the percentage of lysyl residues modified with EDTAD, was determined by the trinitrobenzenesulfonic acid (TNBS) method.[40]

Preparation of Crosslinked Hydrogels of MFP and MSP

A 10% dispersion of the EDTAD-modified proteins was prepared as reported earlier.[30] The required amount of protein was dissolved in deionized water at pH 10 and mixed homogeneously with an egg beater for 15–20 min. Because of high viscosity, the 10% protein dispersion looked like a thick paste. To this was added a known amount of a 50% aqueous solution of glutaraldehyde (which was also preadjusted to pH 10) so that the ratio of

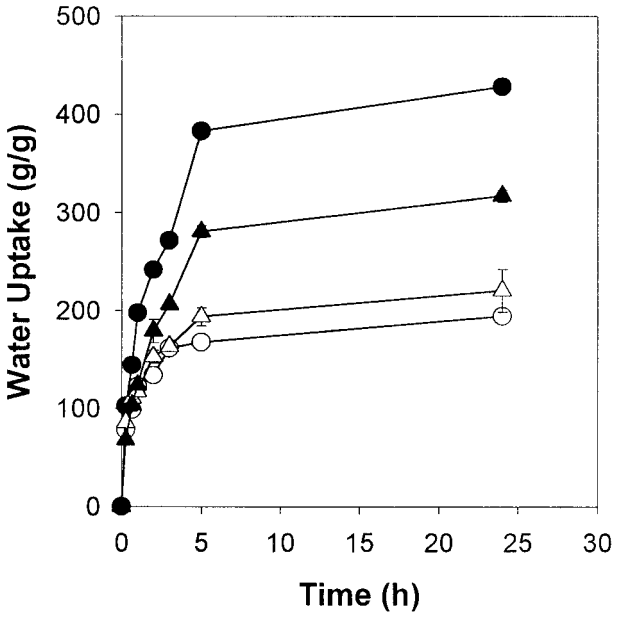

Figure 1 Rate of swelling of (circles) 80%EDTAD-modified FP hydrogel and (triangle) 60%EDTAD-modified SP hydrogel at 36°C (filled symbols) with and (open symbols) without ethanol treatment. The concentration of protein was 10% at the time of crosslinking with glutaraldehyde.

protein to glutaraldehyde in the final mixture was about 1:0.035 (w/w). The mixture was mixed uniformly for about 15 min using an egg beater and allowed to cure overnight at room temperature. The cured gel was divided into two equal parts and one part was dried in an oven at 35°C. The other portion was suspended in ethanol for 3 h, during which time ethanol was changed at least twice. The ethanol treatment caused both denaturation of protein and dehydration of the crosslinked gel. At the end of the ethanol treatment, the gel was in the form of dried particles. The particles were further dried in an oven at 35°C for 2 h to remove ethanol and any residual moisture. Unmodified protein control gels were prepared in the same manner. After complete drying, the gels were ground to a particle size less than 1.0 mm and used for the swelling studies.

Preparation of Hydrogels of Interpenetrating Polymer Networks

The MFP–MSP interpenetrating hydrogels were prepared by mixing calculated amounts of 61% EDTAD-modified soy protein (SP) and 80% EDTAD-modified FP to a final protein concentration of 10% (w/v) in water at pH 10. The mixture was mixed homogeneously with an egg beater for 15–20min and then crosslinked using glutaraldehyde. Ethanol treatment and drying were performed in the same manner as described earlier. Interpenetrating hydrogels of 80% EDTAD-modified FP with other polymers, such as NaCMC, GG, PEG, and PVA, were also prepared as follows: To a 7.5% (w/w) dispersion of 80% EDTAD-modified FP in water, 2.5% (w/w) of the polymer was added; then it was mixed thoroughly and crosslinked by adding glutaraldehyde. In all these cases, the modified FP concentration was 7.5% and the concentration of the other polymer was 2.5% (w/w). The ethanol treatment and drying conditions of the crosslinked and cured gels were the same as described above.

Swelling Kinetics

The swelling properties of the hydrogels at 36°C were studied as described elsewhere. A weighed amount of the dried gel samples were taken in triplicate, in heat-sealable pouches, and allowed to swell in deionized water. At specific time intervals, the bags were removed and centrifuged at

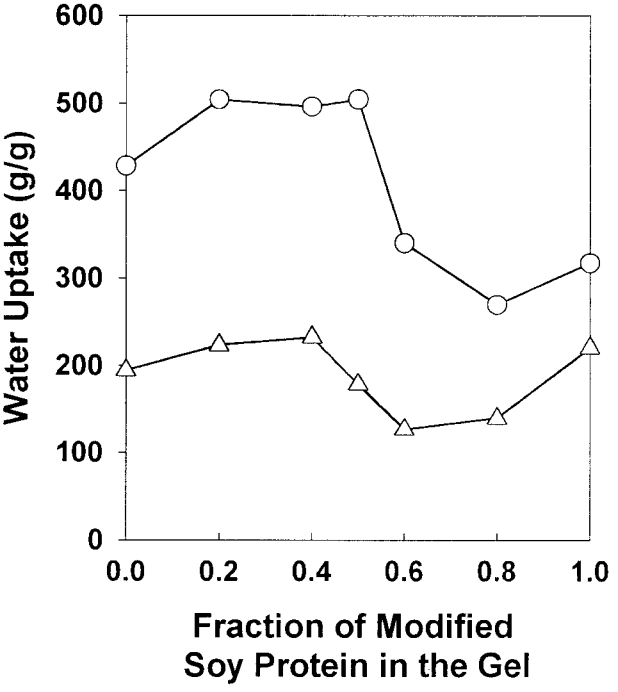

Figure 2 Equilibrium water uptake of mixed hydrogels of 80%EDTAD-modified FP and 60%EDTAD-modified SP at various ratios. The total concentration of protein was 10% at the time of crosslinking: (○) ethanol-treated; (△) without ethanol treatment.

214 g in a clinical centrifuge equipped with sample holders containing plastic wire mesh for proper drainage of the expelled water to the bottom of the holder. The weight of swollen gel was determined immediately. Appropriate controls for the wet weight of the pouch were included. The wet pouch with the swollen gel was dried in a oven at 104°C to a constant weight. The final dry weight of the gel was determined by subtracting the dry weight of an equivalent empty pouch treated in the same manner. The water uptake was expressed as gram water absorbed per gram dry gel.

RESULTS AND DISCUSSION

The lysine content of SP isolate and FP isolate was about 4.4 and 9.0 residues, respectively, per 10,000 molecular weight. Acylation of 61% of the lysine residues in SP isolate required a protein-to-EDTAD ratio of 1:0.09 and acylation of 80% of lysine residues in FP isolate required an FP-to-EDTAD ratio of 1:0.2. Under the reaction conditions employed, reaction of EDTAD with the proteins incorporated about three carboxyl groups for each lysine residue modified.[26]

Figure 1 shows the swelling behavior of modified SP and FP with and without ethanol treatment. With no ethanol treatment, both the MSP and MFP took up about 200 g water per g of dry gel at equilibrium. In the case of ethanol-treated samples, the equilibrium water uptake of the SP gel was about 320 g/g and that of the FP gel was about 425 g/g after 24 h. It has been shown that improvement in the rate and extent of swelling of these gels after ethanol treatment was due to ethanol-induced denaturation/unfolding of the polypeptide chains in the gel network.[30] Denaturation of polypeptide chains *in situ* in a gel matrix apparently prevents them from refolding upon removal of the denaturant; this apparently increases the flexibility of the protein chains and the relaxation rate of the gel network as water diffuses into the network.

Figure 2 shows the equilibrium water-uptake properties of hydrogels prepared using mixtures of 80% EDTAD-modified FP and 61% EDTAD-modified SP at various weight ratios. The total protein content of all these gels at the time of crosslinking was 10% (w/v). The water uptake of the mixed protein gel increased slightly initially as the fraction of the MSP in the gel was increased from 0 to 0.4 and then decreased at higher levels. It is not apparent if the reduction in water uptake at a higher fraction of SP in the gel is attributable to the interaction of SP with FP in the network. Nevertheless, the data suggest that mixed protein hydrogels of FP and SP with high water-uptake properties can be obtained by including SP up to 40% of the total protein in the FP-based hydrogel.

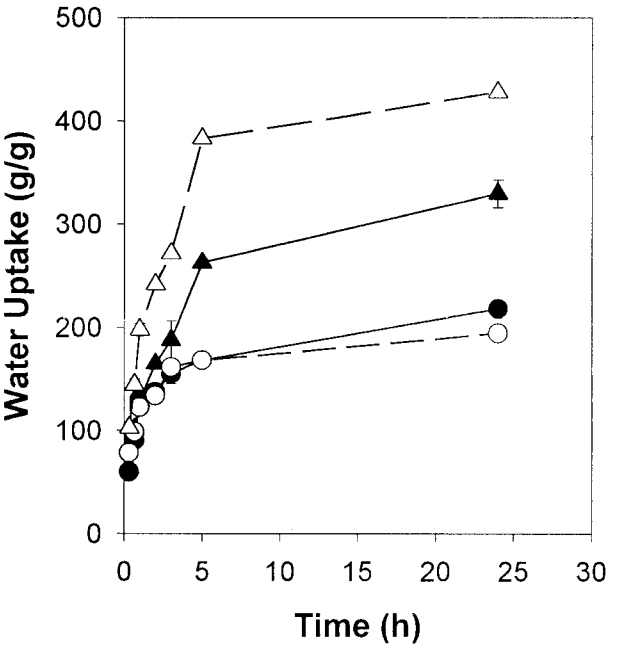

Figure 3 Rate of swelling of mixed hydrogels of 80%EDTAD-modified FP and CMC (▲) with and (●) without ethanol treatment. The concentration of FP and CMC was 7.5 and 2.5%, respectively, at the time of crosslinking. The swelling behavior of 80%EDTAD-modified FP alone (△) with and (○) without ethanol treatment is shown for comparison.

The effects of incorporation of various nonprotein hydrophilic polymers at a 2.5% (w/w) level in the 80%-EDTAD-modified FP hydrogel (7.5% w/w protein at the time of crosslinking) are shown in Figures 3–6. Because CMC is anionic, we anticipated an increase in water uptake in the interpenetrating mixed polymer hydrogel containing CMC. However, as shown in Figure 3, the mixed FP–CMC hydrogel prepared with no ethanol treatment showed no improvement in water-uptake ability over that of the control without CMC. The ethanol-treated FP–CMC hydrogel absorbed more water than did the gel with no ethanol treatment, but the water uptake was significantly lower than was that of the ethanol-treated control

with no CMC. The data suggest that the interaction of CMC with the polypeptide chains[40] in the network apparently promotes segment–segment interaction between polypeptide segments within the network and inhibits the extent of their relaxation as water penetrates into the gel network. It should be noted that CMC in the hydrogel is not crosslinked to the protein by glutaraldehyde and, therefore, the polymer network exists only between protein chains.

Figure 4 shows the effect of PEG on the swelling behavior of the FP hydrogel. The rate and extent of swelling decreased both in the ethanol-treated and ethanol-untreated gels when 2.5% w/w PEG was included in the gel. The extent of reduction in water uptake was significantly greater in the presence of PEG than in the presence of CMC. This is partly because of the fact that, unlike CMC, PEG is not ionic and its effect on the structural state of polypeptide chains in the gel network might be more detrimental than that of CMC. Inclusion of GG (Fig. 5) and PVA (Fig. 6) also significantly decreased the water uptake of the FP hydrogel. Among these polymers, PVA exhibited the highest inhibitory effect on the water uptake. For instance, the improvement in water uptake facilitated by the ethanol treatment is completely offset by the addition of 2.5% PVA. GG contains about 10% protein covalently linked to the polysaccharide backbone. Therefore, it is expected that it will be covalently attached to FP during treatment with glutaraldehyde. This does not seem to impart a greater water-uptake ability to the FP hydrogel. It is likely that an increase in the crosslinking density[36,37] or the effect of the polysaccharide on the structural state of the polypeptides in the network might be involved in inhibiting the water-uptake property of the hydrogel.

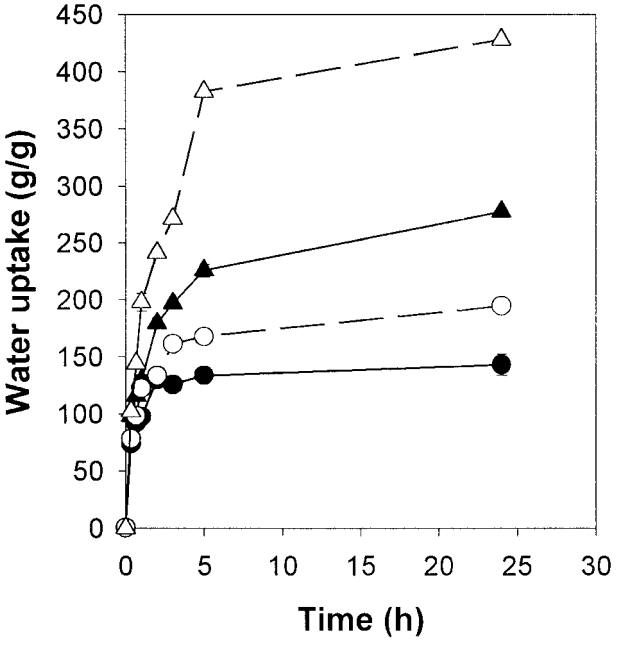

Figure 4 Rate of swelling of mixed hydrogels of 80%EDTAD-modified FP and PEG (▲) with and (●) without ethanol treatment. The concentration of FP and PEG was 7.5 and 2.5%, respectively, at the time of crosslinking. The swelling behavior of 80%EDTAD-modified FP alone (△) with and (○) without ethanol treatment is shown for comparison.

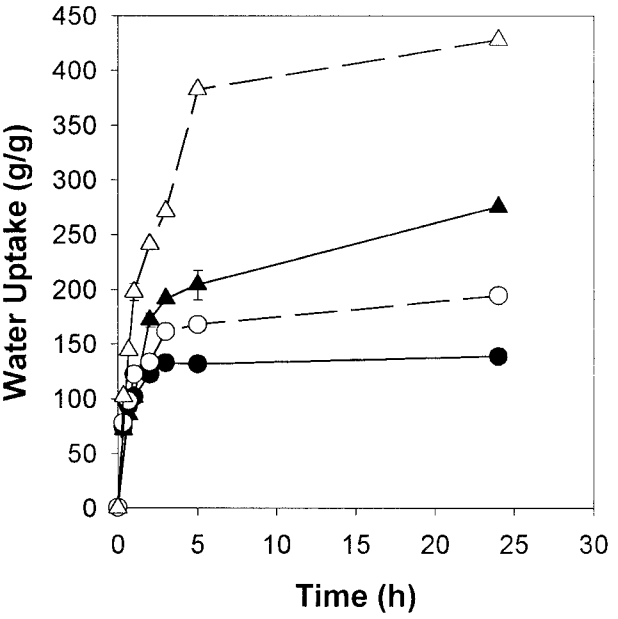

Figure 5 Rate of swelling of mixed hydrogels of 80%EDTAD-modified FP and GG (▲) with and (●) without ethanol treatment. The concentration of FP and GG was 7.5 and 2.5%, respectively, at the time of crosslinking. The swelling behavior of 80%EDTAD-modified FP alone (△) with and (○) without ethanol treatment is shown for comparison.

CONCLUSIONS

With the exception of 60%EDTAD-modified SP, incorporation of other polymers, namely, CMC, PVA, PEG, and GG, significantly decreased the water-uptake properties of FP hydrogel. This might be related to the thermodynamic incompatibility of mixing of these polymers with FP. This incompatibility may inhibit the extent of relax-

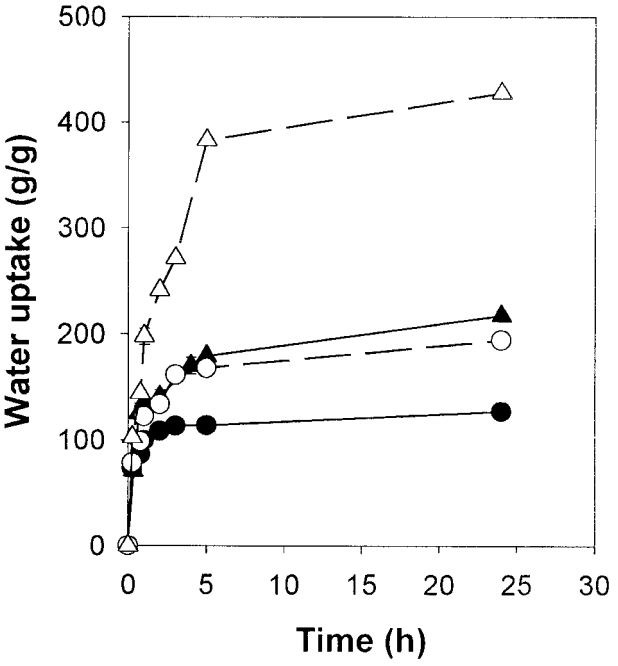

Figure 6 Rate of swelling of mixed hydrogels of 80%EDTAD-modified FP and PVA (▲) with and (●) without ethanol treatment. The concentration of FP and PVA was 7.5 and 2.5%, respectively, at the time of crosslinking. The swelling behavior of 80%EDTAD-modified FP alone (△) with and (○) without ethanol treatment is shown for comparison.

ation of the crosslinked protein network during swelling in water. Inclusion of 60% EDTAD SP up to 40% of the protein in the FP hydrogel does not affect the equilibrium swelling property of the gel. At high levels, however, SP markedly decreased the water-uptake ability of the FP hydrogel.

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