Forces Involved in Mixed Pork Myofibrillar Protein and Calcium Alginate Gels†

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Calcium alginate (CA) gels containing pork myofibrillar protein (MP) were prepared at pH 5.2–6.2, at 25 and 92 °C. Molecular interactions in CA/MP composite gels were examined by determining the effects of 5 M urea, 0.5 M NaCl, 0.5 M NaSCN, 10% propylene glycol, and 0.2 M 2-mercaptoethanol on gel strength. CA gel strength was increased by MP at pH 5.2–5.5 and destabilized above pH 5.5. Heating at 92 °C generally promoted CA gelation. CA/MP gels were markedly destabilized by NaCl and NaSCN and, to a lesser extent, by urea and propylene glycol but were slightly stabilized by 2-mercaptoethanol at 92 °C. These results suggest that electrostatic interactions and hydrogen bonds were the major forces involved in the CA/MP gel systems.

INTRODUCTION

Alginate is an anionic polysaccharide composed of guluronic and mannuronic acids. Because of its excellent gelation and water-holding properties, alginate is used in many food products such as breading mix, dairy foods, sauces, and processed meats (McDowell, 1975; Abd El-Baki et al., 1981). Recently, we reported on the use of calcium alginate as a meat binding agent in restructured meats (Means and Schmidt, 1986; Means et al., 1987). Products manufactured using the alginate gelling system have acceptable binding strength in both raw and cooked states, allowing for marketing of these products in fresh refrigerated form. The mechanisms of binding of the fabricated meat, however, are not fully understood.

Interactions between proteins and polysaccharide gums are considered to be the key to the texture of gum-containing foods (Morris, 1973). Understanding the mechanisms of gum–protein interactions during gelation is important for achieving desirable binding characteristics in restructured meat products and for controlling processing conditions. There are several reports on the nature of gel formation by polysaccharide gums (i.e., carrageenan, pectin, and xanthan) and proteins; induction of this phenomenon in aqueous solutions and by heat is the most widely studied (Imeson et al., 1977; Stainsby, 1980; Tolstoguzov, 1986; Bernal et al., 1987). These studies suggest that electrostatic forces were of primary importance for protein–gum interactions in model systems.

In an alginate-added meat system, protein molecules could interact with alginate to form a composite gel. Since alginate carries negatively charged carboxyl groups at meat pH (5.5–5.6), interaction with positively charged amino groups of proteins can be expected. Furthermore, hydroxyl groups in alginate may interact with side-chain polar amino acids or peptide bonds via hydrogen bonding, imparting additional binding strength. However, there is minimal information on the possible participation of these physical/chemical forces and their relative importance in restructured meat products. When restructured meat is subjected to cooking methods such as grilling, some parts of the meat may be heated above 92 °C. At this temperature muscle proteins would be fully denatured, which may cause additional alginate–protein interactions. Recently, Ensor et al. (1991) reported a destabilizing effect of calcium alginate on beef muscle proteins as studied by differential scanning calorimetry. The mechanisms of alginate–protein interaction and thermally induced protein–protein aggregation were not determined.

The purpose of this research was to investigate the nature of interactions involved in calcium alginate model gel systems containing isolated pork myofibrillar proteins. In particular, various molecular bond disrupting reagents (i.e., urea, NaCl, propylene glycol, NaSCN, and a sulfhydryl reagent) were incorporated into the gelling system to elucidate the mechanism of alginate binding in restructured pork products.

MATERIALS AND METHODS

Myofibrillar Protein Extraction. Fresh refrigerated (2 °C, 2 days post-mortem) pork loin was obtained from the University of Kentucky Meat Laboratory. A crude myofibrillar protein (MP) extract was obtained from trimmed and ground pork loin using the extraction procedure outlined by Bernal et al. (1987) with the following modifications. Only 200 g of muscle/700 mL of Guba-Straub solution (0.3 M KCl, 0.1 M K_2HPO_4, 0.1 M KH_2PO_4, pH 6.5, 4 °C) was mixed. The mixture was homogenized at 4 °C for 15 min in a Waring Blendor and allowed to settle at 4 °C overnight. The suspension was centrifuged the next day (10000*g*, 1 h, 2 °C) to collect the precipitate. Concentration of protein in the initial meat sample and in the extract was measured according to the Kjeldahl method (AOAC, 1985). A fresh crude MP extract was prepared for each experiment.

Preparation of Gels. Gels composed of 1% (w/v) sodium alginate (Manugel, DMB; Kelco, Division of Merck and Co. Inc., Chicago, IL) and 1% (w/v) $CaCl_2$ (J. T. Baker Chemical Co., Phillipsburg, NJ) with or without 2.5% (w/v) MP in distilled water were prepared in 250-mL beakers (6.0-cm diameter).

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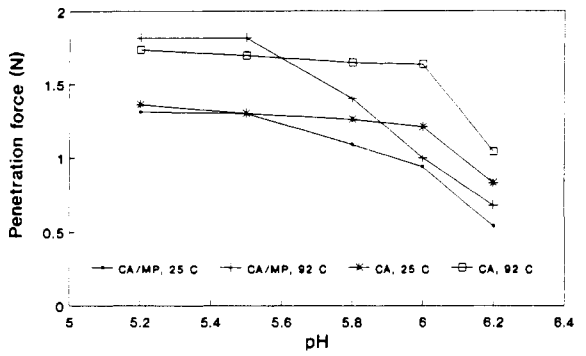

Figure 1. Relationship between pH and strength of 1% calcium alginate (CA) gels with or without 2.5% myofibrillar protein (MP).

Preliminary studies indicated 2.5% MP to be the minimum concentration for protein effect to be detectable in this gel system. Because each gel was made of 200 mL of solution, the gel size was 6.0 cm (diameter) × 6.5 cm (length). In the first experiment the gels were set at pH 5.2, 5.5, 5.8, 6.0, and 6.2, at 25 °C. Initially distilled water was adjusted to the desired pH using 2 N NaOH or 2 N HCl, and the ingredients were added and mixed for 20 s. The pH of each solution was checked again and if necessary readjusted before the system gelled. In the second experiment gels were prepared, each containing one of the following reagents: 0.2 M 2-mercaptoethanol, 10% v/v propylene glycol, 5 M urea, 0.5 M NaCl, and 0.5 M NaSCN (Aldrich Chemical Co. Inc., Milwaukee, WI). These concentrations were selected as determined from preliminary experiments. Control gels had no added reagent. All gels in this second experiment were set at pH 5.5. In both experiments, a duplicate set of gels was prepared, and gels were set at 25 °C for 1 h.

Gels for both experiments were covered with aluminum foil and allowed to firm at 4 °C overnight in beakers, after which one of the set of gels was heated in a 92 °C water bath for 40 min. The gels were kept covered during heating. All gels were then held at room temperature and allowed to equilibrate to room temperature prior to measurement of gel strength.

Determination of Gel Strength. Gels were retained in beakers when analyzed. Strength of all gels was measured at room temperature using a Model TM Instron universal testing machine (Instron Corp., Canton, MA), equipped with a machined aluminum probe consisting of a 10-cm rod with a 3.5-cm-diameter disk attached to the end of the rod. The probe was lowered into the gel at 2 cm/min and allowed to penetrate 4 cm below the gel surface. Peak penetration force (force required to rupture the gels) was recorded with a strip chart recorder and used as a measure of gel strength (Xiong and Brekke, 1989). All gels ruptured before the end of the test.

Statistical Analysis. The first experiment was a completely randomized block 2 × 2 × 5 factorial design with two protein levels (0, 2.5%), two heat treatments (25, 92 °C), and five different pH values (5.2, 5.5, 5.8, 6.0, 6.2). The second experiment (effect of different reagents) was designed to allow data using each of the five additives/reagents to be analyzed as a separate experiment. Therefore, no comparisons were made between reagents. The second experiment was a completely randomized 2 × 2 × 2 factorial design with two levels of reagent (without or with 5 M urea, 0.5 M NaCl, 0.5 M NaSCN, 10% propylene glycol, or 2-mercaptoethanol), two levels of protein concentration (0, 2.5%), and two temperature treatments (25, 92 °C).

Each treatment was replicated four times in all experiments. Data were analyzed by the general linear model (GLM) using a Statistical Analysis System (SAS, 1985) statistical package. Appropriate orthogonal contrasts were used to delineate treatment differences.

RESULTS

Effect of pH. The relationship between gel strength and pH is shown in Figure 1. Calcium alginate (CA; 1% w/v) alone formed gels at room temperature (25 °C) within the pH range tested (pH 5.2–6.2), but the gel strength decreased with an increase in pH. The effect of 2.5% (w/v) myofibrillar protein (MP) on the CA gels was pH dependent, showing small reinforcement at pH 5.2–5.5 (although this was not statistically significant, it was consistent throughout all replications) but an antagonism above pH 5.5 (Figure 1). Heating significantly ($P < 0.01$) increased CA gel strength, whether MP was present or not. The pH dependence profile of the nonheated gels, however, was unchanged. Post-mortem pork used for the manufacture of restructured steaks has a typical pH around 5.5 (Lawrie, 1974), at which CA or CA/MP mixture formed relatively strong gels (Figure 1). Thus, in this research, the effect of various reagents on CA/MP gelation was investigated at pH 5.5.

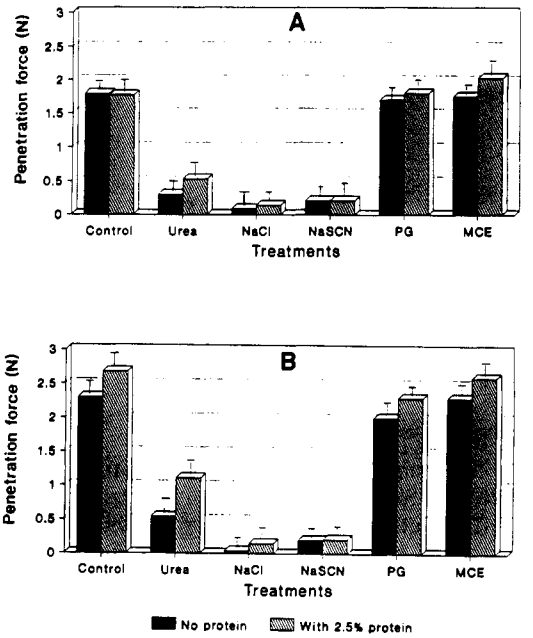

Figure 2. Strength of 1% calcium alginate (CA) gels at pH 5.5 with or without 2.5% myofibrillar protein (MP) and the following reagents: 5 M urea, 0.5 M NaCl, 0.5 M NaSCN, 10% propylene glycol (PG), and 0.2 M 2-mercaptoethanol (MCE) Gels were prepared at 25 °C (A) and at 92 °C (B).

Effect of Various Reagents. The five additives/reagents used in this study showed quite different effects on CA gelation. Overall, the addition of 5 M urea, 0.5 M NaCl, or 0.5 M NaSCN markedly weakened CA gels, while propylene glycol (PG) and 2-mercaptoethanol (MCE) exhibited a smaller effect (Figure 2A). The most detrimental effect on CA gel formation was observed with the addition of 0.5 M NaCl and 0.5 M NaSCN. More specifically, CA formed gels in the presence of 5 M urea, 10% PG, and 0.2 M MCE, but completely failed to gel in 0.5 M NaCl and 0.5 M NaSCN. The addition of MP appeared to reinforce CA gels. For instance, in 0.5 M urea, CA gel strength decreased approximately 5 times (from 1.8 to 0.3 N), whereas gel strength of CA/MP decreased only 2.5 times (from 1.8 to 0.5 N). Furthermore, MCE showed an effect on alginate gels only in the presence of MP.

Although CA or CA/MP gels did not require heat to set, heating at 92 °C did significantly ($P < 0.01$) increase the rigidity of the gels which were previously formed at 25 °C (Figure 2A,B). For CA/MP composite gels, the result of heating was more pronounced than for CA gels. However, in the presence of NaCl or NaSCN, the CA system, with or without MP, remained liquid or semiliquid after it was heated to 92 °C (Figure 2B).

DISCUSSION

Mechanisms of macromolecular gelation have been the subject of numerous investigations (Imeson et al., 1977;

Ziegler and Acton, 1984; Utsumi and Kinsella, 1985; Xiong and Kinsella, 1990). When gelation conditions are varied and various reagents are used to perturb intra- and intermolecular bondings, the forces involved in protein and polysaccharide gel networks can be identified. In our study, CA gel strength was relatively pH independent within pH 5.2–6.0, but in the presence of MP, gel strength decreased markedly ($P < 0.01$) above pH 5.5 (Figure 1). This result suggests that electrostatic interactions were involved and a balanced charge interaction is important for stability of protein/polysaccharide composite gels. Imeson et al. (1977) also observed a strong, pH-dependent interaction between myoglobin and alginate, which was ascribed to electrostatic forces. The weakening effect of increasing pH on CA/MP gels likely resulted from repulsion between carboxyl groups in alginate and those in the myofibrillar protein. Above the isoelectric point (around pH 5.2), myofibrillar protein carries a net negative charge which generally increases electronegativity with an increase in pH.

The increased gel strength of CA and CA/MP composites caused by heating reflected a possible rearrangement of intra- and intermolecular bonding resulting from molecular unfolding (Kilara and Sharkasi, 1986). In our preliminary study we observed no gel formation for MP heated to 92 °C, whether the additives were present or not. Rather, MP would only form a coagulum with excessive syneresis, probably because the heating temperature was too high or the ionic environment was unfavorable for MP gelation. However, heating appeared to facilitate polysaccharide–protein interaction, since heat-induced augment (extent) in gel strength of CA/MP composite was different from that of CA. Alginate possesses certain secondary structure (Morris, 1980), which may be unfolded upon heating. This would result in an increased exposure of reactive groups and enhance molecular flexibility and molecular entanglement. Therefore, improved CA and CA/MP gel strength, caused by alginate conformational changes, can be expected.

The CA/MP gels did not show visible syneresis. This would suggest possible compatibility between CA and MP. Tolstoguzov and Braudo (1983) proposed that in a multicomponent gelling system three types of macromolecular interactions were possible, i.e., to form filled, complex, or mixed gels. Whether the added MP acted as a reactive filler or copolymer in CA gel matrices requires further investigation. The microstructure of the CA/MP gels was not examined in this study, but because gel strength can be affected by the geometry of the gel network, the actual structure of the gels could help further explain the improved gel strength of CA by the added MP. Alginate-restructured pork steaks may be cooked on a grill, causing some parts of the steak to be heated above 92 °C. This would allow binding of meat via alginate–protein interaction. The effect of heating below 92 °C should be further investigated to establish conditions for optimum CA/MP binding reactions.

The failure for mixed CA/MP to form a gel in 0.5 M NaCl or NaSCN was apparently an ionic effect. Ensor et al. (1991) observed a considerable decrease in MP thermal stability when alginate was added to the protein system. The destabilizing effect by concentrated NaCl and NaSCN was reported also in other gelling systems, such as soy protein gels (Utsumi and Kinsella, 1985). Bernal et al. (1987) also observed a disruptive effect of NaCl and NaSCN on CA or CA/MP gels even at a rather low salt concentration (0.05 M). However, this effect was much smaller than what we observed in the present study with a high concentration of salt (0.5 M). Because of their ionic nature, both NaCl and NaSCN were capable of modifying the electrostatic interactions between alginate and myofibrillar protein.

Urea at high concentrations is known for its ability to diminish hydrogen bonds and weaken hydrophobic interactions by altering water structure and increasing solubility of hydrophobic amino acid side chains (Lapanje, 1978). The marked reduction in CA and CA/MP gel strength suggests the involvement of hydrogen bonds and/or hydrophobic interactions in gel networks. The weakened gel was reinforced upon heating, counteracting the added urea, which may be due to an increased exposure of hydrophobic and hydroxyl groups in protein. Hence, protein–protein and protein–polysaccharide interactions could be facilitated. The protein–temperature interaction was significant ($P < 0.01$), suggesting that in the CA/MP gelling systems the stabilizing effects of MP and heat were synergistic. Our results differed from those previously reported which showed no effect of urea on CA/MP gels (Bernal et al., 1987), probably because of the low concentration of urea (0.05 M) used by the previous researchers.

The addition of 10% PG caused little ($P > 0.01$) change in CA gel strength with or without MP, but when heated, gels were slightly ($P < 0.01$) weakened (Figure 2A,B). This indicates that protein denaturation, or unfolding of the reactive sites, was a prerequisite for PG–protein or PG–alginate interaction to occur. PG may disrupt hydrophobic forces and enhance hydrogen bonds and electrostatic interactions by lowering the dielectric constant (Utsumi and Kinsella, 1985). Our results would suggest that hydrogen and electrostatic bonding were more important than hydrophobic forces in the alginate/protein systems. This can be substantiated because when both hydrogen bonds and hydrophobic interactions were disrupted, as in the urea-added CA/MP systems, the gelling ability of CA/MP composite was largely suppressed (Figure 2A,B).

As expected, MCE had no effect on CA gelation unless MP was also present. The added MCE acted as a reducing agent to cleave inter- and intramolecular disulfide bonds to facilitate protein unfolding and increase the exposure of reactive groups that may be involved in hydrogen bonding and ionic and hydrophobic interactions. It is possible that the increased gel strength for the unheated CA/MP mixture may involve sulfhydryl–disulfide interchanges. Sulfhydryl–disulfide interchange reactions are important and are common in the protein matrix (Schmidt, 1981; Xiong and Kinsella, 1990). Furthermore, molecular entanglement between alginate and protein due to increased flexibility of peptide chains may also contribute to the increased CA/MP gel strength.

Our results suggest that electrostatic interactions and hydrogen bonds were the major forces cooperatively maintaining alginate or mixed alginate/myofibrillar protein gel networks. Hydrophobic and disulfide bonds appeared to be secondary in contributing to CA/MP gelation. Heating caused a great exposure of reactive sites in alginate and protein, thereby facilitating intermolecular cross-linkages. These reactions were pH dependent. Therefore, in the manufacture of restructured pork products using calcium alginate binding mechanisms, processing conditions, including temperature, pH, and ionic environment (salt type and concentration), need to be carefully controlled.

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