Actin Extractability during Postmortem Tenderization of Chicken Muscle

Donald de Fremery

Total protein extractability is relatively unaffected by aging for 0.5 hr (prerigor), 3 hr (rigor), or 24 hr (postrigor), based on tests with acetone-dried powders of minced chicken muscle. However, actin extractability is increased at the expense of the nonpolymerizable fraction, with most of the increase occurring during the onset of *rigor mortis*. The nonpolymerizable fraction has a larger molecular weight than actin, indicating that this fraction may be a partial polymer of actin. In further studies there was no significant correlation between tenderness of the cooked breast meat from chickens that had aged 5 hr and extractability of actin from acetone-dried powders of breast muscle. Since most of the changes in the extractability of actin occur during the first 3 hr postmortem, it is concluded that these changes bear no relation to postmortem tenderization but are concerned only with the onset of *rigor mortis*.

The chemical mechanism of the tenderization of meat that occurs during postmortem aging is still not clear, even though scientists have been studying it for many years. Recent studies with native myofibrils from rabbit and beef muscle by Penny (1968) and Davey and Gilbert (1968a,b) indicate that the extractability of actin, a major myofibrillar protein, increases during postmortem aging. Their data do not allow one to decide whether this apparent increase is caused by a weakening of the bonds which hold the actin filaments to the Z-line, or whether the increase is a result of the degradation of the Z-line material itself. More recently, de Fremery (1971) has shown that there is no marked change in extractability of actin during postrigor aging when studies were made on acetone-dried powders prepared from minced chicken muscle. The chemical changes that occur in the proteins of muscle during postmortem aging have recently been reviewed extensively by Goll *et al.* (1970).

The present paper reports studies undertaken to determine whether or not tenderness is related to the extractability of actin from acetone-dried powders of minced chicken muscle sampled in the prerigor, rigor, and postrigor states. By "actin" is meant water-soluble muscle protein which can be polymerized in the presence of 0.1 *M* KCl and sedimented in an ultracentrifugal field. These experiments were carried out on breast and leg muscles of chickens in the prerigor state (0.5 hr postmortem), rigor state (3 hr postmortem), and postrigor state (24 hr postmortem). Other experiments compared the actin extractability in one breast muscle of a bird with shear resistance in cooked meat from the other breast muscle for 16 birds that had been aged for 5 hr postmortem, a time when shear resistance is the most variable. The results show that actin extractability increases slightly in chicken leg and breast muscles during postmortem aging, but actin extractability does not correlate significantly with the variable shear resistance (1.4–8.3 kg) that occurs in muscles held for 5 hr.

EXPERIMENTAL SECTION

Slaughter and Chilling. Commercially obtained meat-type fryer chickens, ranging in live weight from 1.8 to 3.5 kg, were used in these experiments. The birds were allowed free access to food and water prior to slaughter. They were stunned electrically for 5 sec and killed by severing the veins and arteries in the neck. Birds that were to be tested only for actin extractability were processed as follows. They were first skinned and eviscerated. When breast muscles were to be used, the thoracic region was removed from the carcass in such a way that the attachments of the pectoralis muscles to the bone structure were not broken. When leg muscles were to be used, the posterior portion of the carcass was treated similarly, *i.e.*, the muscle–bone attachments were retained. The excised portion of the carcass was then sealed in a polyethylene bag, placed immediately in a cold room at 2°, and held there until the initial muscle sample was to be taken. Three sampling-time comparisons were made as follows: 0.5 hr *vs.* 3 hr; 0.5 hr *vs.* 24 hr; and 3 hr *vs.* 24 hr. In each case, muscles from one side of a carcass at one aging time were compared directly with corresponding muscles from the other side of the carcass at a second aging time. The inner and outer breast muscles (or leg and thigh muscles) from one side of the carcass were excised, and the remainder of the carcass was sealed in a polyethylene bag and replaced in the cold room until the second sample was taken in a similar manner.

A different processing technique was used in experiments comparing actin extractability with shear resistance. Following exsanguination, the birds were scalded at 54° for 60 sec, plucked by hand, eviscerated, and placed in ice slush within 15 min postmortem. After aging for 5 hr, a time at which variation in cooked shear resistance is maximal (de Fremery and Streeter, 1969), both breast muscles were excised from the carcass. One muscle (alternately right and left) was cooked and tested for shear resistance, and its pair was tested for actin extractability.

Tenderness Evaluation. Tenderness of the cooked meat was determined with a Warner–Bratzler shear-force apparatus. Breast muscles were cooked in boiling water following the technique of de Fremery and Pool (1960). The excised muscle was clamped between metal plates that were maintained 0.95 cm apart, immersed in a vigorously boiling water bath for 10 min, and then cooled in running tap water. Strips (1.9 cm wide) were cut parallel to the fibers for determination of shear resistance.

Actin Extractability. The extractability of actin was determined from acetone-dried powders of minced muscle. The powders were prepared following Scheme II of Seraydarian *et al.* (1967), except that the muscle suspension was centrifuged at 27,000 × *g* for 20 min after stirring with dilute $CaCl_2$. The dried material was powdered further in a Wiley mill and passed through a 20-mesh screen.

Western Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Berkeley, California 94710.

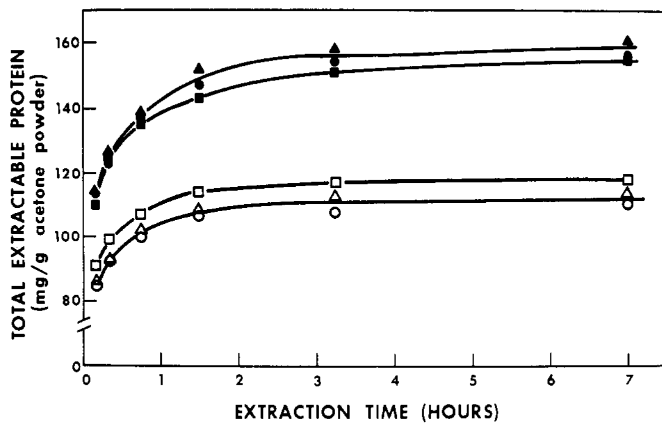

Figure 1. Extractability of total protein from acetone-dried powder. Solid symbols, breast muscles; open symbols, leg muscles. ○, 0.5 hr; △, 3 hr; □, 24 hr

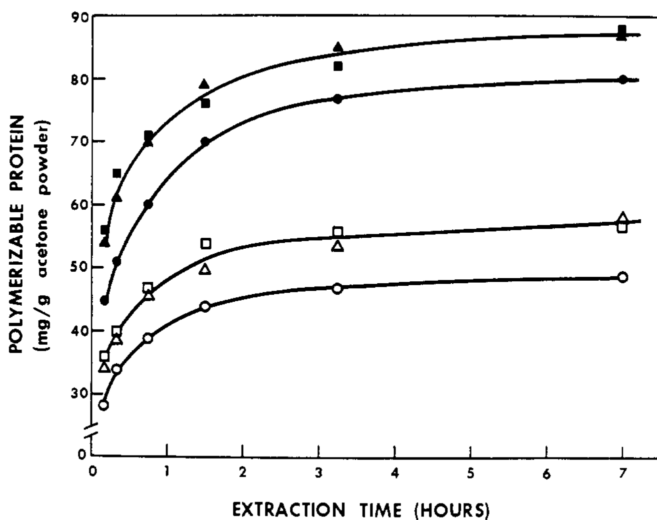

Figure 2. Extractability of polymerizable protein from acetone-dried powder. Solid symbols, breast muscles; open symbols, leg muscles. ○, 0.5 hr; △, 3 hr; □, 24 hr

Actin was extracted from the acetone-dried powders using the extraction solvent described by Rees and Young (1967). This solvent is composed of 0.5 m*M* of adenosine triphosphate, 0.5 m*M* of 2-mercaptoethanol, and 0.2 m*M* of $CaCl_2$, adjusted to pH 7.5 with dilute NaOH. Breast muscle powders were stirred with 35 ml of solvent per g of acetone powder at 0° for periods of time ranging from 10 min to 7 hr. Leg muscle powders were treated identically, except that 30 ml of solvent was used per g of acetone powder. In all cases, the moisture contents of the powders were determined on separate samples by air-drying for 16 hr at 105°. The muscle residue was removed by centrifugation at 48,000 × *g* for 15 min, and the supernatant solution was filtered to remove a small amount of suspended material. The protein concentration of the clarified extract was determined by the biuret procedure of Gornall *et al.* (1949). The biuret color yield was standardized against solutions of chromatographically purified G-actin (Rees and Young, 1967) whose dry weight had been determined by air-drying for 16 hr at 105°.

The G-actin in the clarified extract was transformed to F-actin by the addition of 0.1 vol of 1.1 *M* KCl and 11 m*M* of $MgCl_2$. The solutions were held for 30 min at room temperature and then stored overnight at 2°. The polymerized actin was then sedimented by ultracentrifugation for 2 hr at 105,000 × *g*. The protein concentration of the supernatant solution was determined by the biuret procedure, using the same colorimetric factor as before. Protein concentrations are expressed as mg of protein per g of acetone powder (dry weight basis). Total protein is the soluble protein in the original extract; nonpolymerizable protein is the protein in the supernatant solution following ultracentrifugation; and polymerizable protein, assumed to be actin, is the difference between the two.

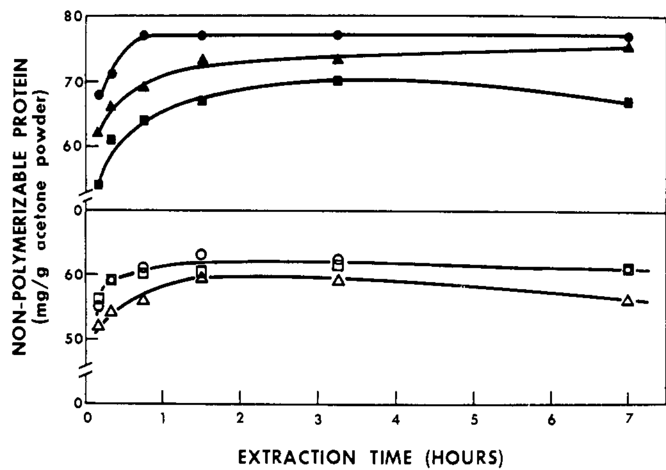

Figure 3. Extractability of nonpolymerizable protein from acetone-dried powder. Solid symbols, breast muscles; open symbols, leg muscles. ○, 0.5 hr; △, 3 hr; □, 24 hr

In the experiments in which actin extractability was compared with shear resistance, the extractability of actin was determined exactly as described above, except that the acetone powder was extracted with solvent for one time period only (1.5 hr).

RESULTS AND DISCUSSION

The total protein extractable from acetone-dried powders of muscle during extraction periods of 10 min to 7 hr increases for about 2 hr and then remains constant (Figure 1). It is interesting to observe that with breast muscle the samples aged for 24 hr have the *lowest* extractability (4% less than the 3-hr samples), whereas, with leg muscle, the samples aged for 24 hr have the highest extractability (5 to 7% greater than either the 0.5- or 3-hr samples). This differential response, as well as the small size of the differences, makes the significance of this observation difficult to assess.

The extractability of polymerizable protein (actin), shown in Figure 2, tends to become constant after extracting for about 3 hr. The samples aged for only 0.5 hr have a consistently lower extractability than either the rigor or postrigor samples. This lower extractability (about 15% less) is statistically significant and indicates that actin becomes more extractable during the onset of *rigor mortis.* As aging proceeds beyond that point, however, extractability does not increase. This confirms the previous findings of de Fremery (1971), who compared actin extractability from rigor and postrigor muscle only. The observation that actin extractability is less from prerigor muscle than from either rigor or postrigor muscle is a significant extension of these findings. Although the studies of Penny (1968) and Davey and Gilbert (1968a,b) showed that actin extractability continued to increase well into the postrigor period, their experiments are not strictly comparable with the ones reported in this paper.

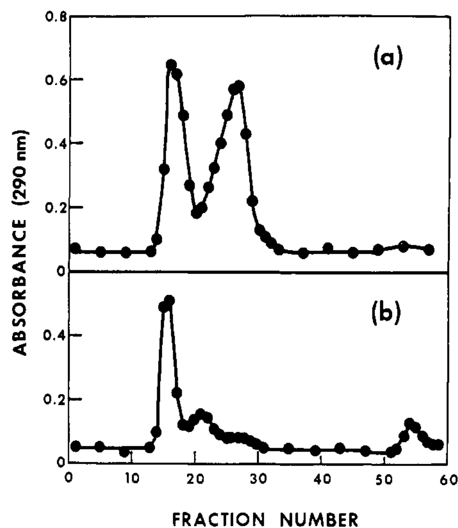

Figure 4. Elution diagrams of 1.5-hr extract of acetone-dried powder from breast muscle aged for 24 hr; (a) before polymerization, (b) after polymerization and ultracentrifugation. Column packing, Sephadex G-100; column dimensions, 2.5 × 40 cm; sample size, 5 ml; fraction size, 4 ml. Columns were operated at 2°

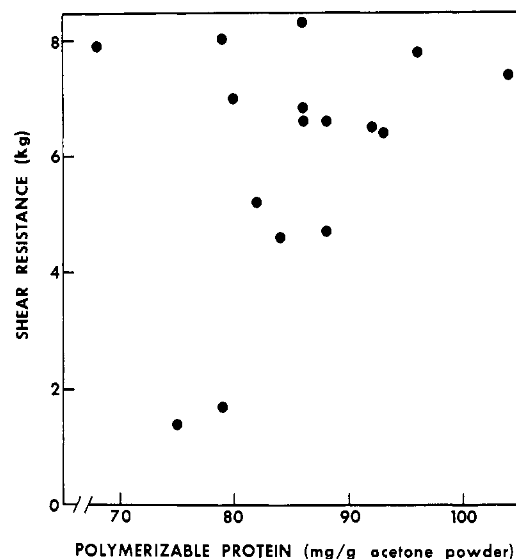

Figure 5. Relation between shear resistance of cooked meat and extractability of polymerizable protein from acetone-dried powders of minced raw muscle. Paired breast muscles from 16 chickens aged for 5 hr postmortem before cooking or extraction

Not only were they studying mammalian muscle rather than avian muscle, but, more importantly, their extractability experiments were carried out on native myofibrils rather than acetone-dried muscle. In their samples, the presence of native myosin, which binds strongly to actin, should exert a marked influence on the extractability of actin.

The extractability of nonpolymerizable protein is shown in Figure 3. In breast muscle there is a progressive inhibition of extraction (about 15%) as the muscle passes from the prerigor state to the postrigor state. In leg muscle, however, the only significant difference is the 6% greater extractability of postrigor muscle compared to rigor muscle. Although this result is contrary to the data from breast muscle, it is consistent with the observation that the extractability of *total* protein from leg muscle is greatest in the 24-hr sample.

It is difficult to include all of the data into a consistent hypothesis of actin extractability during postmortem aging. The observation that total protein extractability is relatively unaffected by aging, whereas actin extractability increases at the expense of the nonpolymerizable fraction, leads to the speculation that the nonpolymerizable fraction is actually a shielded, or modified, form of actin. This implies that the extractability of actin is actually unchanged during a 24-hr aging period, but its ability to polymerize in 0.1 *M* KCl and to be sedimented in an ultracentrifugal field is enhanced as aging proceeds.

The composition of the original clarified extract of acetone-dried powder has been investigated, and the results are shown in Figure 4. Figure 4a shows an elution diagram from Sephadex G-100 of the original extract of an acetone powder prepared from breast muscle that had been aged for 24 hr. Figure 4b shows a similar diagram of the same extract following polymerization in 0.1 *M* KCl and ultracentrifugation to remove the F-actin. The two major peaks in Figure 4a, in order of their emergence, correspond to the nonactin fraction and actin, respectively, since the slower fraction has disappeared from the ultracentrifugal supernatant in Figure 4b. (The small peak observed at fractions 53–57 in Figure 4b did not contain protein and was not investigated further.) If the hypothesis stated above is correct, then the modified actin must be a partial polymer of actin whose molecular size is still small enough to avoid sedimentation at 105,000 × *g*, since larger molecules migrate faster in gel filtration. There is no evidence to indicate the nature of the change in the actin molecule which increases its susceptibility to polymerization. The elution diagrams shown in Figure 4 are typical of many such diagrams from both breast and leg muscles and from the prerigor, rigor, and postrigor states. Although the protein concentrations varied between samples, the general shape of the elution diagrams was similar.

The experiments described above have shown that the extractability of actin increases moderately during postmortem aging, particularly so during the early stages. Since meat becomes tender also during postmortem aging, the correlation between polymerizable protein (actin) and shear resistance was measured in muscles that had been aged for 5 hr postmortem, the time of maximal variation in shear resistance. The results of an experiment with 16 chickens are shown in Figure 5. Statistically, the correlation coefficient is +0.30, nonsignificant for 16 pairs. Since data on total protein extractability and nonpolymerizable protein extractability were obtained in this same experiment, the correlation coefficients between these quantities and shear resistance were calculated also. The correlation coefficient between shear resistance and total protein extractability is +0.36; that between shear resistance and nonpolymerizable protein extrability is +0.38. These values also are nonsignificant for 16 pairs.

The lack of a significant correlation between shear resistance and extractability of actin from acetone-dried muscle is evidence against the involvement of actin in the molecular changes accompanying tenderization. Since most of the changes in actin extractability occur during the first 3 hr postmortem, and since there is no tenderization in breast muscle during this period (de Fremery and Streeter, 1969), the results presented in this paper can best be interpreted as reflecting changes accompanying the formation of the rigor state rather than changes accompanying tenderization.

ACKNOWLEDGMENT

The author thanks B. E. Mackey for statistical analysis of

the data and acknowledges the expert technical assistance of I. V. Streeter, M. E. May, and N. B. Nutt.

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Received for review April 24, 1972. Accepted July 3, 1972.

Effect of Inorganic Salts on the Strength of Muscle Fibers

Ryo Nakamura

To study the effect of inorganic salts on the strength of muscle fibers, tensile strength was measured about the muscle fibers prepared from the chicken pectoralis major muscles after incubation with various kinds of salt solutions. The tensile strength of muscle fibers changed little between pH 5.0 and 8.0. Although monovalent cations and most of anions had a relatively small effect, divalent cations and phosphates decreased greatly the tensile strength of muscle fibers. The decreasing action of divalent cations and phosphates on the shear force value of muscles was also noted in the experiment of the rehydration of the lyophilized muscles in these salt solutions.

In previous work it was shown that the tensile strength of muscle fibers decreased greatly during postmortem aging and also in the presence of small amounts of Ca ions (Nakamura, 1972). From these results it was suggested that the cause of the changes in the tensile strength of muscle fibers might be the released Ca ions from muscles during postmortem aging. The action of Ca ions on the muscle fibers, however, has not been clarified yet.

In this work, in order to obtain more information about the action of Ca ions on muscle fibers, the effect of various inorganic salts on the tensile strength of muscle fibers was studied. The effect of the inorganic salts on the tenderness of aged meat was also studied.

EXPERIMENTAL SECTION

Pectoralis major muscles were obtained from 12- to 14-month-old chickens (White Leghorn, female). All birds were killed by cutting the jugular vein and carotid arteries, were skinned without scalding, and were eviscerated. The carcass was placed in a plastic bag and aged in drained crushed ice. Muscle fiber bundles were prepared at 1–2 hr after slaughter and kept in 50% glycerol as described previously (Nakamura, 1972). They were dipped in 50% glycerol to which various amounts of inorganic salts were added, were kept overnight at 0°, and their tensile strength was measured. Each muscle bundle was 2–3 cm in length and contained about 25 single fibers.

Tensile strength measurements were made on about 8–10 muscle fiber bundles by the use of a strain gauge attached to an automatic recorder (Nakamura, 1972).

Shear force value was measured about the muscles aged for 24 hr at 0°. To study the effect of inorganic salts, muscles (approximately 5–7 cm wide, 15–18 cm long, and 1–1.5 cm thick) were lyophilized and rehydrated in 5 m*M* solution of various inorganic salts for 2 days at 0°. A small amount of thymol was added to each solution to suppress the bacterial growth during rehydration. Shear force value was measured by a Warner–Bratzler type apparatus after cooking; muscle samples were clamped in a special mold designed according to de Fremery and Pool (1960) and cooked to an internal temperature of 82–85° (about 1 hr in boiling water). Strips 0.25 cm^2 in cross-section were cut and 8–12 determinations were made on each sample. To obviate the effect of bird-to-bird variation, comparisons were made between left and right halves of one bird; one half was dipped in 5 m*M* KCl solution as control sample and the other half was dipped in various kinds of salt solutions with the same concentration.

RESULTS AND DISCUSSIONS

The tensile strength of muscle fibers did not change with the addition of KCl or NaCl until the concentration of 20 m*M* was reached (Table I). As this concentration is much higher than that of the effective concentration of $CaCl_2$ described in the previous work (Nakamura, 1972), 3 m*M*, the effect of $CaCl_2$ on the muscle fibers seems to be rather specific.

To compare the effect of $CaCl_2$ with that of other inorganic salts, experiments were made about various kinds of inorganic salts. In this case, there is a possibility that the effect of inorganic salts is that of the change in pH caused by their presence. As shown in Figure 1, the tensile strength of muscle fibers was affected greatly by the pH of the solution; it decreased greatly below pH 5.2 and above pH 8.1. Most pH of the glycerol solution with various amounts of inorganic salts tested, however, was between 5 and 8. When the pH of the glycerol solution tested was above 8 or below 5 (in the case of either 10 m*M* K_2HPO_4 or 10 m*M* KH_2PO_4 in Table II), comparisons were not made with other experimental results. This means that the change in pH caused by the addition of

Laboratory of Food Science and Technology (Animal Products), Faculty of Agriculture, Nagoya University, Nagoya, Japan.