Postmortem Tenderization of Chicken Muscle: Stability of Phenylalanine as the C-Terminal Amino Acid of Actin

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The possible involvement of the myofibrillar protein actin in the tenderization that occurs during postmortem aging of chicken breast and leg muscles has been investigated using the enzyme carboxypeptidase A as an analytical tool. The amount of actin that could be isolated from the muscles did not change during the aging period. Phenylalanine was the only amino acid that was subsequently released from actin by the enzyme in major amount. The amount of phenylalanine that was released, corresponding to a molecular weight of actin of 49,000, was the same whether actin was isolated from tough muscle (3 hr postmortem) or from tender muscle (24 hr postmortem). Smaller amounts of cysteic acid and lysine were detected in the enzymatic digests, suggesting that the C-terminal tripeptide of actin is Lys–Cys–Phe. The results indicate that postmortem tenderization of chicken muscle does not involve the removal from actin of its C-terminal amino acid phenylalanine.

The tenderness of meat, whether the source is avian, mammalian, or piscine, is a quality factor of major importance to the prospective consumer. Although it is generally recognized that meat becomes more tender during postmortem aging, the exact mechanism by which this occurs has not been elucidated. Penny (1968) and Davey and Gilbert (1968a,b) have recently reported studies with myofibrils from rabbit and beef muscle which indicate that the extractability of actin, a major myofibrillar protein, increases during postmortem aging. It is not clear whether the extractability of actin increases because of a weakening of the bonds which hold the actin filaments to the Z-line, or whether it increases because of a degradation of the Z-line material itself.

In an attempt to shed some light on this problem, the present study reports the results of some experiments which were designed to determine whether there was any change in the C-terminal amino acid of actin during postmortem aging. The results indicate that the C-terminal amino acid of actin remains unchanged during postmortem tenderization of chicken muscle.

EXPERIMENTAL

Slaughter and Chilling. Commercially obtained meat-type chickens, ranging in live weight from 1.7–2.6 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, killed by slashing the throat, and skinned. 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 portions of the carcass were sealed in polyethylene bags, placed immediately in a cold room at 2°, and held here for 3 hr. The inner and outer breast muscles (or leg and thigh muscles) from one side of the carcass were then excised, and the remainder of the carcass was sealed in a polyethylene bag and replaced in the cold room for an additional 21 hr. The muscles from the other side of the carcass portion were then excised similarly to the 3-hr sampling. The carcasses were sampled at 3 and 24 hr because at these times the muscles are uniformly tough or tender, respectively (de Fremery and Streeter, 1969).

Purification of Actin. The first step in the purification of actin is the preparation of an acetone powder from the ground muscle. This was done following Scheme II of Seraydarian *et al.* (1967), except that the muscle suspension was centrifuged at 27,000 × *g* for 20 min after having been stirred with dilute $CaCl_2$. Purified G-actin was prepared from the acetone powder by the procedure of Rees and Young (1967), except that the final column fractionation was performed with Sephadex G-100 (Pharmacia). Protein concentration was determined by the biuret procedure of Gornall *et al.* (1949). The biuret color yield was standardized against solutions of purified G-actin whose dry weight had been determined by air-drying for 16 hr at 105° C.

Determination of C-Terminal Amino Acid. Carboxypeptidase A (CPA), which hydrolyzes C-terminal aromatic amino acids rapidly, was used in these experiments because the C-terminal amino acid of actin has been established as phenylalanine (Laki and Standaert, 1960; Locker, 1954). The sample of CPA that was used was a commercially obtained preparation that had been treated with diisopropylphosphofluoridate to eliminate tryptic and chymotryptic activity. Actin preparations were treated with CPA by a modification of the procedure described by Ambler (1967). Washed crystals of CPA were dissolved to a final concentration of 3.5 mg/ml in 10% LiCl containing 0.04 *M* barbital, pH 7.9. Samples of actin (70–90 mg) were incubated with CPA (0.03 mg CPA/mg actin) at 25° C for varying periods of time. The reaction was carried out in 2.5% LiCl containing 0.01 *M* barbital, pH 7.9; actin was present at a concentration of 1.2–2.2 mg/ml. The reaction was terminated by the addition of $HClO_4$ to a final concentration of 0.5 *M*, and the precipitated protein was removed by centrifugation. The supernatant solution was neutralized to pH 2–3 with KOH, and the precipitated $KClO_4$ was removed by filtration. Free amino acids were adsorbed on and eluted from a cation exchange resin as described by Stark (1962). The NH_4OH was removed by evaporation, and the amino acids in the dried residue were determined quantitatively on an amino acid analyzer using the accelerated system of Spackman (1963).

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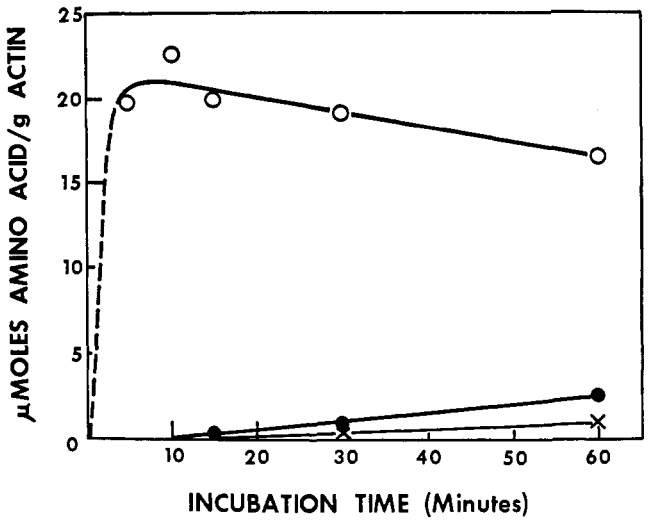

Figure 1. Rate of release of amino acids from actin by carboxypeptidase A. ○, phenylalanine; ●, cysteic acid; ×, lysine

RESULTS AND DISCUSSION

The amount of acetone powder that could be obtained from fresh muscle was constant throughout the aging period (Table I). However, the yield was dependent on the source of muscle tissue. The higher yield from mixed leg muscles is probably caused by the higher content of connective tissue in leg muscles (de Fremery and Streeter, 1969).

The amount of purified actin that could be isolated from the acetone powder also was independent of aging time (Table I). The slight variation that did occur appeared to be random in nature. These results differ from those of Penny (1968) and Davey and Gilbert (1968a,b), who reported an increased extractability of actin during postmortem aging. The difference may be due to the fact that their studies were carried out on native myofibrils isolated from mammalian muscle, whereas the experiments reported in this paper were conducted on acetone powders prepared from minced chicken muscle. Since the object of the isolation procedure was highest purity of G-actin, no attempt was made to isolate the protein in quantitative yield. Nevertheless, the fact that the yields were essentially the same at the two aging periods indicates that actin had not been modified during postmortem aging in a way which altered its solubility properties to any major extent. Experiments on actin isolated from fully-aged muscle thus represent studies on total actin and not studies merely on an unmodified fraction of the total.

In preliminary experiments, the amino acids released from actin by CPA were determined as a function of incubation time. When the time was varied from 5 to 60 min, phenylalanine was the only amino acid that appeared to a major extent (Figure 1). Maximum release occurred in 10–15 min. An incubation time of 15 min was arbitrarily selected for further experiments.

The rapid appearance of phenylalanine was followed by a slow steady release of cysteic acid and lysine (Figure 1). The 60-min release of cysteic acid and lysine was 13% and 5%, respectively, of the 15-min phenylalanine release. Although Bailey (1962) has reported that C-terminal cysteine and lysine are resistant to the action of CPA, the data are consistent with a C-terminal tripeptide structure of Lys–Cys–Phe, the cysteic acid arising from oxidation of cysteine in the presence of 0.5 *M* $HClO_4$. This finding agrees with the results of Johnson and Perry (1968), who reported that the C-terminal structure of actin was Cys–Phe preceded by either lysine or arginine. Only two other amino acids (not shown in Figure 1) appeared in the chromatographic eluates. Valine occurred at a constant low level (<2% of the maximum phenylalanine level), and an unknown compound (approximately 5% of the maximum phenylalanine value) appeared in the eluates just before the emergence of glycine.

Table I. Yield of Muscle Fraction

Muscle	Acetone powder (g/g fresh muscle)	G-actin (g/g acetone powder)
Breast		
Aged 3 hr	0.11	0.039
Aged 24 hr	0.11	0.041
Leg		
Aged 3 hr	0.14	0.029
Aged 24 hr	0.14	0.027

Table II. Amount of Phenylalanine Released from Actin by Carboxypeptidase A

Muscle	Aging time: 3 hr μmol/g actin	Aging time: 24 hr μmol/g actin
Breast	20.5	20.1
Leg	20.1	20.8

The amount of phenylalanine which is released from actin by CPA during an incubation period of 15 min is shown in Table II. Regardless of the muscle from which actin has been purified and regardless of the length of time the muscle has aged on the carcass, the amount of phenylalanine that is released by CPA remains unchanged. If it is assumed that actin consists of a single polypeptide chain terminating in phenylalanine (Rees and Young, 1967), the molecular weight of actin, based on the data of Table II, is calculated to be 49,000. This value falls in the range of values reported by Johnson and Perry (1968) using varying concentrations of urea (43,000–60,000).

The results presented in this paper provide conclusive evidence that the C-terminal amino acid of actin is not removed or modified during postmortem aging.

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LITERATURE CITED

Ambler, R. P., in "Methods in Enzymology," C. H. W. Hirs, Ed., Academic Press, New York, N.Y., 1967, p 155.
Bailey, J. L., "Techniques in Protein Chemistry," Elsevier Publishing Company, Amsterdam, 1962, pp 202–5.
Davey, C. L., Gilbert, K. V., *J. Food Sci.* **33,** 2 (1968a).
Davey, C. L., Gilbert, K. V., *J. Food Sci.* **33,** 343 (1968b).
de Fremery, D., Streeter, I. V., *J. Food Sci.* **34,** 176 (1969).
Gornall, A. G., Bardawill, C. J., David, M. M., *J. Biol. Chem.* **177,** 751 (1949).
Johnson, P., Perry, S. V., *Biochem. J.* **110,** 207 (1968).
Laki, K., Standaert, J., *Arch. Biochem. Biophys.* **86,** 16 (1960).
Locker, R. H., *Biochim. Biophys. Acta* **14,** 533 (1954).
Penny, I. F., *J. Sci. Food Agr.* **19,** 518 (1968).
Rees, M. K., Young, M., *J. Biol. Chem.* **242,** 4449 (1967).
Seraydarian, K., Briskey, E. J., Mommaerts, W. F. H. M., *Biochim. Biophys. Acta* **133,** 399 (1967).
Spackman, D. H., *Federation Proc.* **22,** 244 (1963).
Stark, J. B., *Anal. Biochem.* **4,** 103 (1962).

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