Studies on Muscle Proteins

III. Behaviours of Actins in 7M Urea-Containing Buffer Solution

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Introduction

In the previous investigations,[1,2] among the muscle proteins was found a component unchangeable both before and after heating, or during the period when the whole meat-product-manufacturing processes were carried out, where each meat sample was analyzed, after being dissolved in 7*M* urea-containing Tris-HCl buffer solution. This component was assumed to be F-actin through preliminary experiments,[2] though its confirmation was left to further research. The confirmation of this point was attempted in this paper. At first, Straub's[3] acetone dry powders were prepared from beef, pork and chicken muscle, and then G-actins were extracted with ATP-ascorbic acid solution, respectively. Next, G-actins extracted above were concentrated with Sephadex and were transformed to F-actins. According to the methods approximately similar to those described in the previous papers,[1,2] analyses were carried out concerning these concentrated F-actin solutions.

Experimental procedures

Materials

Species, sex and age of the individuals from which the samples were obtained and the muscular names employed in this experiment are shown in Table 1. After the preliminary removal of fats and connective tissues, these muscles were removed quickly from the carcasses after slaughtering, each muscle being minced through a chopper plate with holes, 3 *mm* in diameter.

Table 1. Species, sex and age of the individuals from which the samples were obtained and the muscular names employed in this experiment.

Species	Age	Sex	Muscular names employed
Beef (Japanese Black Cattle)	6 years	♂♀	M. longissimus dorsi
Pork (Landrace ♀ × Berkshire ♂)	9 months	♂♀	M. longissimus dorsi
Chicken (Plymouth Rock)	70 days	♂	M. pectoralis superfacialis and profundus

Preparation of F-actin solutions

The preparations of the muscle-acetone-dry-powder were essentially those of Straub's.[3] All the preparations were heat-sealed in the polyethylene coated cellophane bag in vacuo and stored in the cold stocker (−25°C) up to use. When needed, at first, G-actin was extracted according to Krans et al.[4] employing 0.02 *mM* ascorbic acid-0.2 *mM* ATP aquous solutions. The G-actin solutions obtained were condensed with Sephadex G-25, coarse, by the batch method to give them 4 % of protein concentration and then were transformed to F-actin according to Krans et al.[5]

To attain completion of this G-F transformation, the solutions were left standing overnight at 4°*C* and diluted with an equal volume of 7 *M* urea containing 0.05 *M* Tris-HCl buffer (*pH* 8.6), and dialyzed against the same buffer for 24 hours to give it ca 2 % of protein concentration, and then immediately analyzed.

Analytical methods

DEAE cellulose column chromatography and starch gel electrophoresis were adopted according to the same procedure as described in the previous paper,[1,2] excepting that UV absorption analyses were carried out at 280 *mμ* or 260 *mμ* in case of need, changing the metal filter attached.

Results and discussion

DEAE cellulose column chromatography

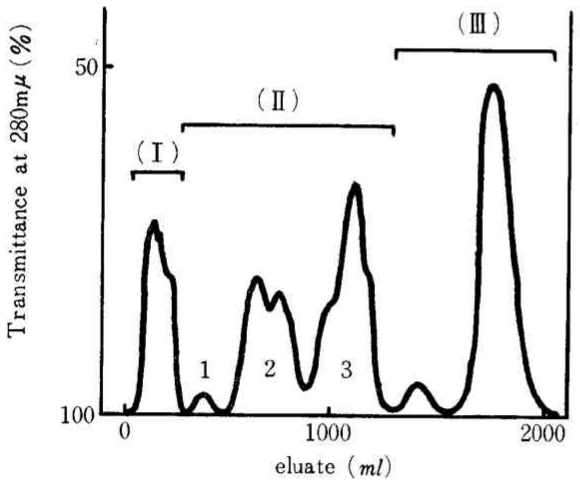

Fig. 1. The genuine DEAE cellulose column chromatogram with NaCl-and NaOH-gradient elution of the raw beef solution obtained by the same procedures as those shown in the previous papers.[1,2]
(I), (II) and (III) show the fraction number divided by the property of the eluting buffer, and the numbers indicate the order of the peaks appeared during the NaCl-gradient.

Fig. 1 shows a *DEAE* cellulose column chromatogram of raw beef's whole muscle-7*M* urea containing 0.05 *M* Tris-HCl buffer solution (*pH* 8.6), reported in the previous paper.[2] As described before, this chromatogram was considered to be capable of being divided into three fractions (I, II, III). And besides, it was presumed that the so called myofibrillar proteins were included in the fraction (II) in which several peaks were contained and at the same time, that the largest and highest peak (3) among them might be corresponding to fragments of F-actin.

And on this peak, only a little change was observed even after heating at 75°*C* for 1 hour or during the meat-product-manufacturing processes.

On the other hand, the two conspicuous bands left unchanged before and after heating or during the meat-product-manufacturing processes were found in the

starch gel electrophoresis, and these bands were also presumed to correspond to F-actin.

However, clear evidence showing that these peak or bands surely indicated F-actin or its fragments was not provided yet.

To make this more confirmable, actins of three kinds of muscle were prepared and at first, behaviours of G-actins were compared with those of F-actins in the 7*M* urea-containing Tris-HCl buffer solution.

Fig. 2 shows the DEAE cellulose column chromatograms of the beef, pork and chicken G-actin respectively. In these chromatograms, no peak was found, corresponding to the one characteristically observable during NaCl gradient elution, excepting only a few small

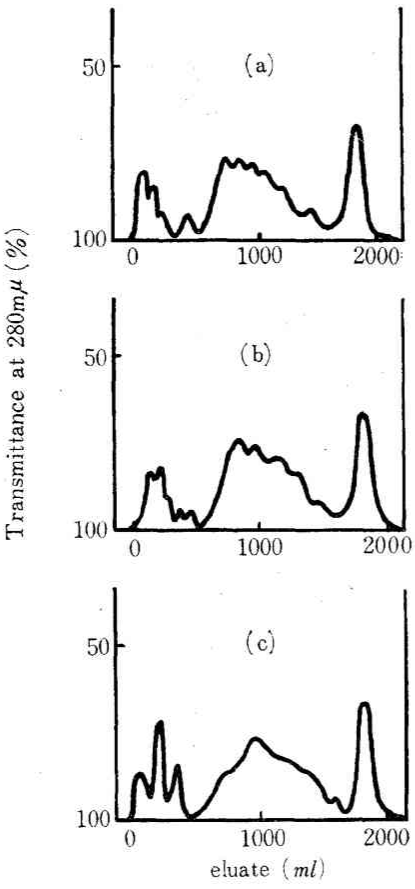

Fig. 2. The genuine DEAE cellulose column chromatograms with NaCl- and NaOH-gradient elution of the urea solutions of three kinds of G-actin, obtained by the same procedures as those shown in the previous papers.[1,2]
(a) Beef G-actin, (b) Pork G-actin, (c) chicken G-actin. Protein concentration was about 2% in all.

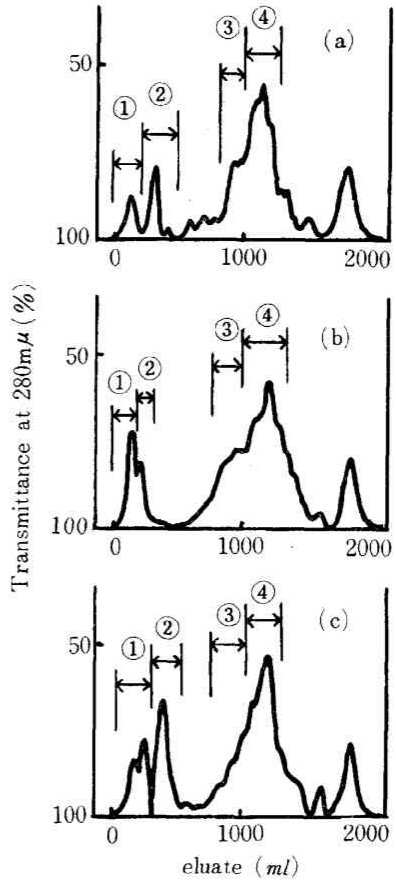

Fig. 3. The genuine DEAE cellulose column chromatograms with NaCl- and NaOH-gradient elution of the urea solutions of three kinds of F-actin, obtained by the same procedures as those shown in the previous papers.[1,2]
(a) Beef F-actin, (b) Pork F-actin, (c) chicken F-actin. Protein concentration was about 2% in all.
Each section indicated with arrows and numbered were fractionated, condensed and analyzed further by SGE.

peaks observable there, though a small but distinguishable peak was observed at the position in the previous experiment[2] carried out without ATP.

This seems to be caused by the following; as the ATP-ascorbic acid solution was used in this experiment, the actins extracted were almost likely to be a G-form which was considerably depolymerized. On the contrary, in the previous experiment[2] ATP was not employed on the extraction of actin. So it was assumed that not only G-form but also F-form of actin had been extraced.

Fig. 3 shows the chromatograms of F-actins from three kinds of muscle respectively. In those, obviously large peaks appeared in the range of NaCl gradient elution respectively and they were situated at the situations of which were confirmed to be identical with those of the peaks in question.

From these results, it was suggested that the characteristic peak was formed by F-actin and not by G-actin. In the muscles post mortem, myosin A and F-actin (not G-actin) bind together, constructing actomyosin (myosin B), as known well. The results from this experiment seemed to indicate an actual proof of the above mentioned.

In this experiment, however, F-actin treated by heating process was left uninvestigated. It is of interest to ascertain the behaviour of the heat-treated F-actin in the urea containing buffer, which was left to the future solution.

Starch gel electrophoresis (SGE)

In the previous experiment,[2] the author found the two bands characterized in their being unchangeable before and after the heating in the starch gel electrophoretic patterns, which were presumed to be some fragments of F-actin. To confirm these findings further, G- and F-actins from the three kinds of muscle were compared each other, with the results obtained shown in Fig. 4. In the patterns, two bands which were situated in the middle of each run and a zone which was situated near the slot, narrow in F-actin and broad in G-actin, were found throughout all kinds of muscle. The bands and zone of F-actin were generally a little smaller in movability compared with those of G-actin. This phenomenon seemed to be caused by the molecular sieving effect of starch gel in the course of electrophoretical run of both the actins, namely, when G-actin was transformed to F-actin, G-actin was polymerized and its molecular size grew so much bigger that it was slightly difficult to pass through the gaps of starch gel.

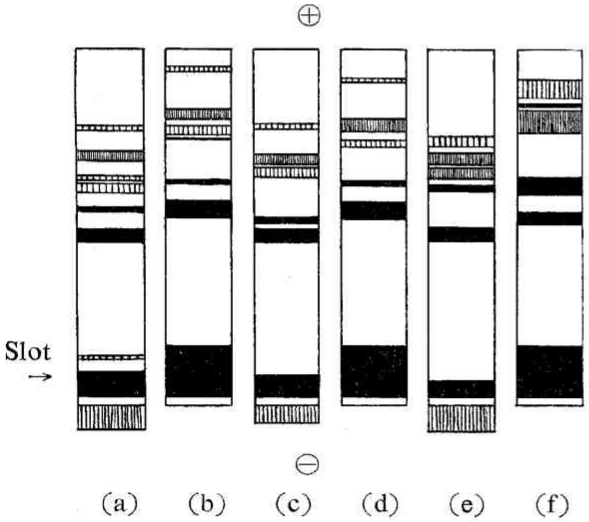

Fig. 4. Starch gel electrophoretic patterns of the urea solutions of G- and F-actin from beef, pork and chicken, at *pH* 8. 6, Tris-HCl buffer, 7*M* urea.
(a) F-actin of beef, (b) G-actin of beef, (c) F-actin of pork, (d) G-actin of pork, (e) F-actin of chicken, (f) G-actin of chicken.

Judging from the results obtained by the experiment above, it was clear that

the actins extracted from the muscles of three kinds of animal, behaved in an analog on G-F transformation, and the positions at which the two bands existed in the patterns of F-actins corresponded to those of the meat-urea solution before and after heating described in the previous papers.[1,2] So it may reasonably be asserted that the two bands indicated the fragments of F-actin.

Thus the component contained in the highest and largest peak on the DEAE cellulose column chromatogram was assumed to be F-actin, whereas the components in the two bands found in the SGE patterns were also presumed to be the fragments of F-actin. Then, it was required to confirm whether the both components were of the same quality or not.

So, the fractions shown with arrows on the DEAE cellulose column chromatograms of the respective F-actin (Fig. 3) were removed and condensed with Sephadex G-25, coarse, and analyzed further by SGE technique. Results obtained were shown in Fig. 5, 6 and 7. As evident from the figures, the characteristic two bands were contained in the fraction 4 which corresponded to the highest peak on the chromatograms. Thus the components contained in the highest and largest peak on the DEAE cellulose column chromatogram coincided with the component in the specific two bands of the SGE pattern, and these were proved to be a part of F-actin.

Bound adenosinediphosphate (ADP)

It is well known that adenosinediphosphate (ADP) binds to F-actin in the proportion of 1 mol per 60,000 *gm*[3,6] and nucleotide containing adenine indicates the maximal absorbance at 260 *mμ* which is assumed to be caused by the construction of adenine. So, to give one more certainty to the conclusion above, the following experiment was carried

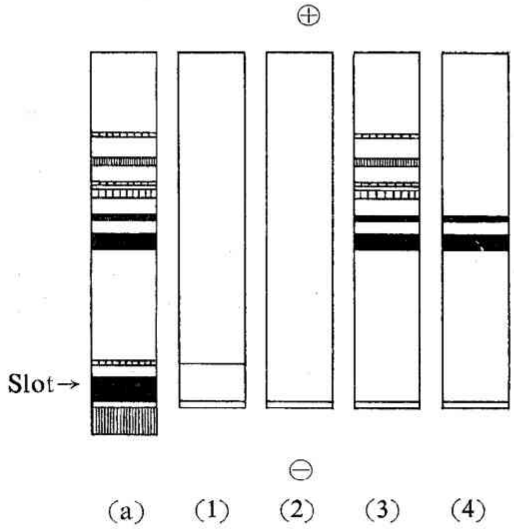

Fig. 5. Starch gel electrophoretic patterns of the respective fractions obtained from chromatography of beef F-actin, at *pH* 8.6, Tris-HCl buffer, 7*M* urea.
(a) Whole F-actin of beef. Numbers in the brackets show the respective fraction shown in Fig. 3.

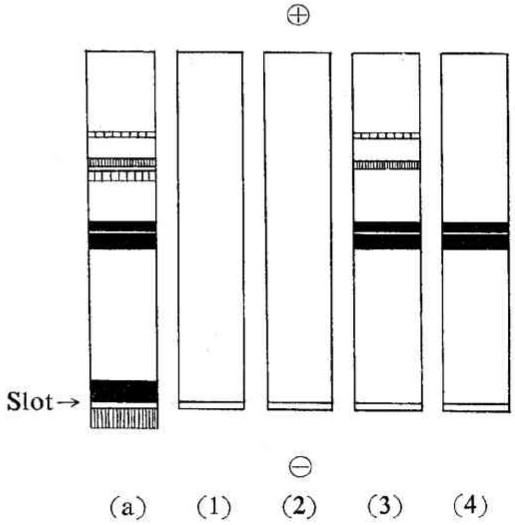

Fig. 6. Starch gel electrophoretic patterns of the respecti ve fractions obtained from chromatography of pork F-actin, at *pH* 8.6, Tris-HCl buffer, 7*M* urea.
(a) Whole F-actin of pork. Other remarks are the same as those in Fig. 5.

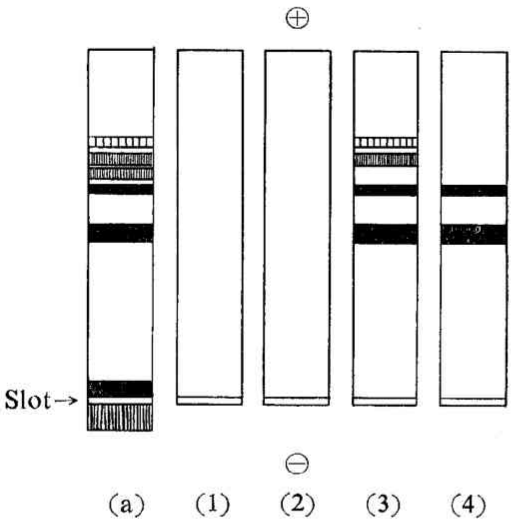

Fig. 7. Starch gel electrophoretic patterns of the respective fractions obtained from chromatography of chicken F-actin, at *pH*8.6, Tris-HCl buffer, 7*M* urea.
(a) Whole F-actin of chicken. Other remarks are the same as those in Fig. 5.

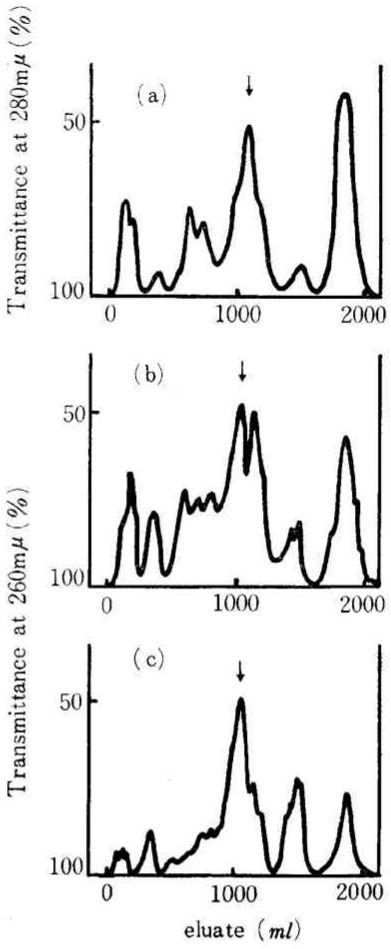

Fig. 8. The genuine DEAE cellulose column chromatograms with NaCl- and NaOH-gradient elution of the urea solutions of raw pork and its F-actin, obtained by the same procedures as those shown in the previous papers.[1,2]
(a) Raw pork's chromatogram obtained at 280 *mμ*, (b) That obtained at 260*mμ*, (c) F-actin's chromatogram obtained at 260 *mμ*. Arrow in each figure indicates the characteristic peakin question.

out. As examples, pork muscle ane pork F-actin were dissolved in the urea-containing buffer solution and analyzed by DEAE cellulose column chromatography, employing the absorbance at 260 *mμ*.

Results obtained were shown in Fig. 8. As evident from the figures, the maximal peak on the chromatogram at 260 *mμ* was observed at the same position as that on the chromatogram at 280 *mμ* appeared.

Accordingly, it was doubtless that F-actin consisted on this position of the chromatogram.

Judging from the results in the previous report, this fact seems to be common not only concerning muscle or F-actin from other kind of animal and but also in the samples after heating or meat product manufacturing processes.

Hereafter, it will be further investigated whether the component found in the characteristic peak on the DEAE cellulose column chromatography and in the bands on the SGE pattern of the whole muscle-urea-solution, corresponds to F-actin, sharing the equal chemical composition, or not. And moreover, studies will be carried out about the differences, if any, observable among the chemical compositions of the components obtained out of the muscles of various domestic animals.

Summary

The preparations of G- and F-actin from beef, pork and chicken were performed by the methods of Straub and Krans et al. and they were analyzed through DEAE cellulose column chromatography and starch gel electrophoresis in 7*M* urea containing buffer solution (*pH* 8.6) by the method almost the same as those described in the previous paper.

From the results obtained out of chromatography, it was evident that the principal peak of F-actin (not G-actin) corresponded to that of the maximal peak of the chromatogram of the meat-urea solution in all kinds of the animal tested, as was noted in the previous study.

And from the results of starch gel electrophoresis, the conspicuous two bands, situated in the middle of each run, which had been found in the patterns of raw and cooked meat-urea solutions, were found in F-actin, at the position identical with the one mentioned above.

Besides, the fraction corresponded to the principal peak on the DEAE cellulose column chromatogram was collected and condensed, and the component in it was analyzed by SGE technique. The result showed the perfect coincidence of the both components, that is, one was the component that appeared on the chromatogram as a principal peak, the other was the component which was revealed on the SGE pattern as two conspicuous bands.

Finally, to give one more certainty to this conclusion above, the presence of the bound ADP in the component which is specific to F-actin, was investigated by measuring the absorbance at 260 $m\mu$ through DEAE cellulose column chromatography. The result proved the obvious presence of the bound ADP.

Acknowledgement

The author expresses his thanks to Prof. N. Andō, Kyushu University, Prof. T. Tsugo, the University of Tokyo, and Prof. M. Kojima, Kagoshima University for their kindly guidance and suggestions, and to Mr. K. Arita and Mr. T. Kamei for their technical assistances.

References

1. KAKŌ, Y.: *Memoirs Fac. Agr. Kagoshima Univ.* **6**, 161 (1968).
2. KAKŌ, Y.: ibid.: **6**, 175 (1968).
3. STRAUB, F. B. and FEUER, G.: *Biochim, Biophys. Acta*: **4**, 445 (1950).
4. KRANS, H. M. J., VAN EIJK, H. G. and WESTENBRINK, H. G. K.: ibid.: **100**, 193 (1965).
5. KRANS, H. M. J., VAN EIJK, H. G. and WESTENBRINK, H. G. K.: ibid.: **65**, 166 (1962).
6. MOMMAERTS, W. F.: *J. Biol. Chem*, **198**, 468 (1952).