Studies on Muscle Proteins

I. Behaviours of Muscle Proteins before and after the Heat-Coagulation in the 7M Urea-Containing Buffer Solution

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Introduction

A great deal of biochemical researches on the muscle proteins in raw or native state have been carried out. However, no investigation on the chemical changes of muscle protein occurring under the manufacturing process has been made, because a method to analyze the coagulated proteins subsequent to the heating process (cooking) has not been established, and many a technological problem on meat product manufacturing has been left to be solved.

Neelin and Rose[1] and Maire and Fischer[2] reported the post-mortem changes of raw meat but did not refer to the heat-coagulated muscle protein.

It is of much interest that, according to Melachouris and Tuckey[3], proteins in cheese made from milk treated under several conditions of heating are dissolved in the urea containing buffer solution and analyzed by means of polyacrylamide gel electrophoresis.

In view of meat product manufacturing, it is desirable to find out and establish a method to analyze muscle proteins and their components throughout the entire manufacturing processes.

From some preliminary experiments, it was ascertained that the heat-coagulated meat as well as the raw one could be solubilized by a buffer solution containing high concentration of urea. As high concentration of urea causes the cleavage of intra- or inter-molecular hydrogen bonds of protein and denatures the protein, excepting particular cases, it has not been used so widely in the range of dynamic and biochemical research about the native protein. However, since the heat-denaturation process of protein is unavoidable during meat product manufacturing, a buffer solution containing high concentration of urea is to be made use of rather positively to dissolve the coagulated protein.

In this report is given an account of the most suitable method to solubilize the heat-coagulated meat sample for the actual analyses by means of recent physicochemical technics : NaCl-gradient DEAE cellulose column chromatography, urea-containing starch gel electrophoresis, Sephadex column chromatography and ultracentrifugation, etc.

Experimental procedures

Materials

Species, age, sex of the individuals from which the samples were obtained and the

muscular names employed in this experiment are shown in Table 1.

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

Species	Age	Sex	Muscular names employed
Beef (Japanese brown cattle)	8 years	♀	M. longissimus dorsi
Pork (Landrace ♂X Birkshire ♀)	9 months	♂	M. longissimus dorsi
Chicken (Plymouthrock)	80 days	♂	M. pectoralis major
Mutton (unknown)	(unknown)	(unknown)	M. longissimus dorsi

These meat samples were frozen in the cold stocker (−25°*C*) within 5 hours after slaughtering except mutton, which was imported from New Zealand in a frozen state.

Prior to the employment of these samples to experiment, they were thawed through standing in the cold room (+4°*C*) overnight. And, the frozen sample meats were employed within two weeks after slaughtering.

Preparation of meat-urea solution

Of the sample meats thawed were removed the connective tissues and fats, and the meats were minced through a chopper plate with 3 *mm* holes, and then 10 *gm* of the minced meat was homogenized with high speed motor driven blade at 10,000 rpm, together with 90*ml* of 7.7*M* urea-containing 0.055 *M* Tris-HCl buffer (*pH* 8.6) for 5 min, ice cold. As a few foams were generated at this time, the homogenate was removed to a glass beaker and was made to stand for one hour in the cold room (+4°*C*), until these foams nearly disappeared. Next, the homogenate was centrifuged at 5,000 rpm for 5 min, and through the process, a little precipitates were sedimented at the bottom of the tube, and semi-transparent, brownish red coloured solution was obtained in the supernatant. As some floating substances apparently rich in fat were present on the surface, the supernatant was to be filtered through a double folded gauze to eliminate them.

For the preparation of heat-coagulated meat-urea solution, 10 *gm* of thawed and minced meat was weighed at first into the test tube (1.8×20 *cm*) and the tube was strongly shaken by hand to settle the meat down to the bottom of it and then it was sealed with a thick sheet of vinyl and gummy bands as tightly as possible. Heating of the test tube was performed in the water bath regulated at 75±1°*C* for 1 hour, cooling with tap water (ca 20°*C*) for 1 hour ensuing. All of the contents of the the tube, the coagulated meat and a little amount of juice yielded by heating, were homogenized and the following procedure was carried out same way as in the raw meat. The solution obtained by this, presented brown colour indicating the occurrence of Met-hemichromogen. The precipitants sedimented seemed to be of some scleroproteins present in the connective tissue of muscle.

DEAE cellulose column chromatography

In this experiment, DEAE cellulose which is 0.63 meq per *gm*, 70~100 mesh, from Brown Chem. Co. Ltd., was used. After activation with 1 *N* NaOH, it was stuffed into the glass column with a dimension of 5×15 *cm*, together with 1 *N* NaOH under the pressure of N_2 gas at 0.5 *kg* per cm^2, washed with distilled and deionized water, up to the

disappearance of phenolphthalein reaction, and finally bufferized with 1 litter of 7*M* urea containing 0.05*M* Tris-HCl buffer solution.

Ten ml of sample meat-urea solution was applied at the top of the column, and elution was started. The elution was performed by the NaCl and NaOH gradient, according to the buffer system shown in Table 2, in one mixing chamber, flow-rate being kept constantly 5.5-6.5 *ml* per min with the help of a press pump from Taiyo kagaku Kogyo Co. Ltd.

Table 2. The buffer system used for DEAE cellulose column chromatography of sample meat urea solution

Buffer	No.	NaCl	Volume
7*M* urea containing 0.05*M* Tris-HCl, *pH* 8.6	①	0 (*M*)	300 (*ml*)
	②	0.25	300
	③	0.50	300
	④	1.00	300
1*N* NaOH	⑤	—	1,000

The elution curves were automatically recorded with the transmittance value at 280 $m\mu$ by Uvicon 540 Optical Unit from Toyo kagaku Sangyo Co. Ltd.

Condensation of each fraction

The eluates obtained through DEAE cellulose column chromatography of each sample meat solution were separated in three fractions as indicated in Figure 1. Each fraction was condensed to about 15*ml* by a batch method with Sephadex G-25, coarse. In spite of the presence of high concentration of urea, this condensation process was performed with facility.

Sephadex column chromatography

All of Sephadex employed in this study were from Pharmacia, Uppsala, Sweden. For the column chromatography of these condensates, Sephadex G-100 was employed. Buffer used in this experiment was only 7*M* urea containing 0.05*M* Tris-HCl buffer, and elution was carried out at the flow rate of 4 *ml* per minute, kept constantly under hydrostatic pressure at about 25 *cm*, without any gradient.

Other procedures used were managed in the same way as in the DEAE cellulose column chromatography.

Starch gel electrophoresis : (SGE)

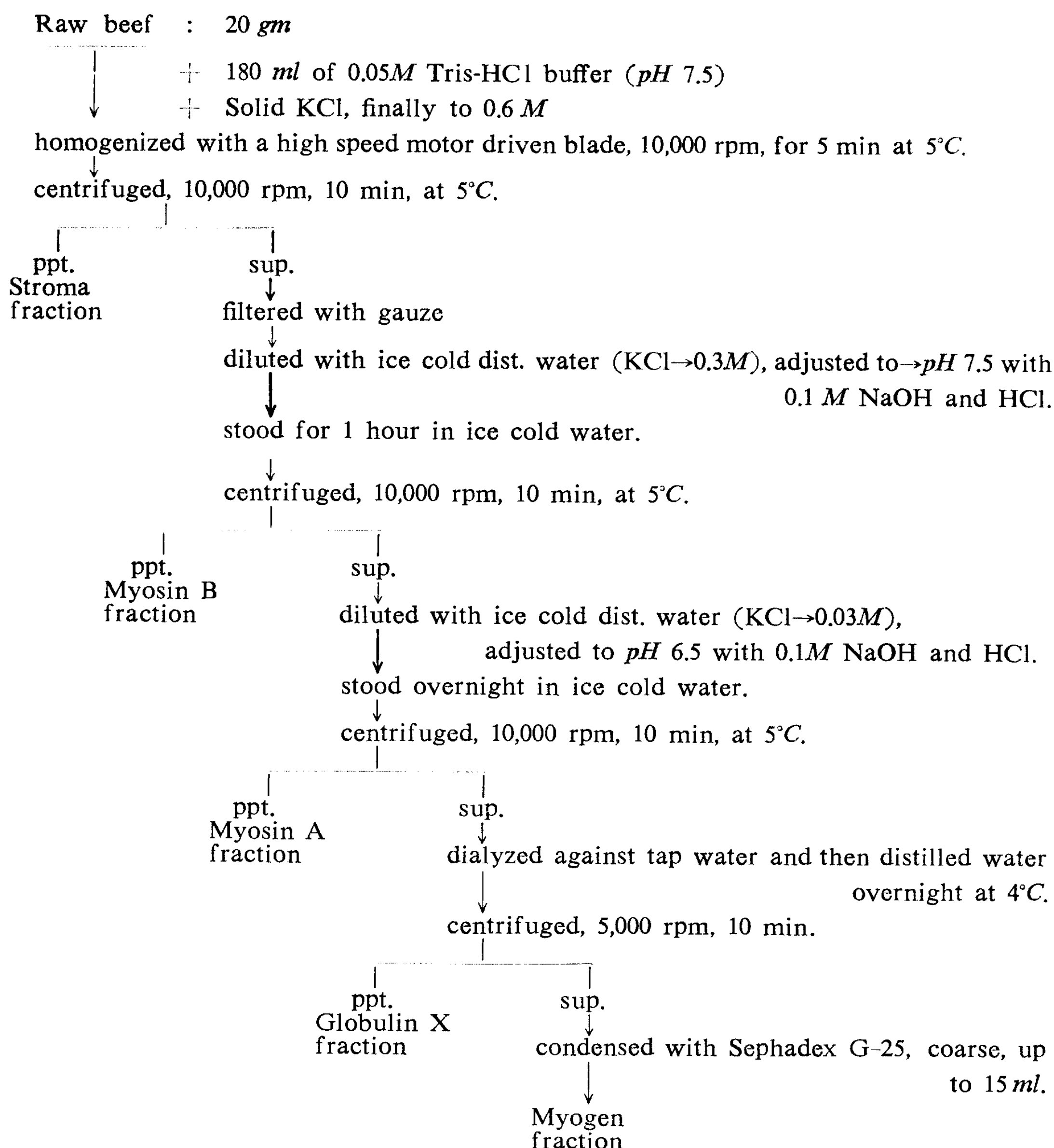
In this study, starch gel electrophoresis was carried out with our modified method[4], a method obtained through the improvement of Wake and Baldwin's method employing 7 *M* urea containing gel for milk casein analysis[5], performed to make it fit for the analysis of the meat sample solution. As the starch for preparing the gel, investigators generally employed partially hydrolyzed starch from Connanght Medical Research Laboratory, Toronto, Canada. Using potato starch, produced in Japan, we undertook, however, to find out the proper condition to prepare the suitable hydrolyzed starch for our analysis.

No report has ever been found as to the preparing method of starch for zone electrophoresis except Smithies'[6] in which hydrolysis was carried out at 38.5°*C*, for about 40 min with concentrated HCl in acetone.

In order to clarify the relationship between the hydrolyzing condition of starch and the quality of the gel prepared from the starch hydrolyzed by that condition, some experiments were operated and reported in the previous paper[4]. As the result, judging from 7*M* urea containing gel electrophoresis by Smithies method, the best condition of hydrolysis was ascertained to be 45–50 min at 38.5°*C*, the Alkali lability number[7] of the starch being 10 to 11.

Scheme

Preparation method of Myosin A, B, Globulin X, Myogen and Stroma fraction.



Electrophoresis was carried out horizontally, 2mA per cm^2 for 7 to 10 hours at 4°*C*, and Amido Black 10B was used for staining.

Ultracentrifugal sedimentation

An attempt of ultracentrifugal sedimentation of beef-urea solution in 7*M* urea containing 0.05*M* Tris-HCl buffer (*pH* 8.6) was undertaken, too. Runs were carried out in the Spinco Model E, 56,100 rpm, in the cell with the depth of 12 *mm*, at 20°*C*, and the protein concentration used was about 1 %.

Preparation of Myosin A, B, Globulin X, Myogen and Stroma fraction

To identify the components in the respective patterns obtained from DEAE cellulose and Sephadex column chromatography and SGE, several protein fractions were separated from beef muscle by the method of Portzehl et al.[8] as shown in the scheme. These protein fractions were solubilized in the urea containing buffer same way as in the preparation of the sample meat-urea solution.

Protein concentration of the sample meat solution was controlled by diluting with buffer based on 1/10 of the value which was gained by multiplying 6.25 to total nitrogen content determined by micro Kjeldahl method before being solubilized in the urea containing buffer. All the operations were carried out at the room temperature unless otherwise stated.

Results and discussion

Selection of urea concentration

For the purpose of giving rise to denaturation of protein as a result of hydrogen bond cleavage, 8*M* of urea has been employed hitherto. But, 8*M* of urea is near to 50 % and because of its high viscosity and easily crystallizing character at low temperature, is too high in concentration to perform the various operations. And so far as the samples are to be dissolved completely, low concentration of urea may rather be desirable.

As the SGE must be carried out in the gel containing 7*M* of urea, it was surveyed whether the heat-coagulated meat samples could be dissolved in 7*M* urea containing buffer, and if dissolved, at any *pH* value.

Consequently, it was found that even the heat-coagulated muscle proteins could be dissolved entirely in the buffer at *pH* 8.5 or at the value higher than this. (Table 3).

Tris-aminomethane-HCl buffer is one of the most effective buffering solutions for biochemical analysis, owing to its difficulty in crystallizing at low temperature (near to

Tabe 3. Solubility of the heat-coagulated beef meat in 7*M* urea containing 0.05*M* Tris-HCl buffer ; relationship between solubility of the meat and the *pH* of buffer used.

	pH of buffer				
	6.5	7.0	7.5	8.0	8.5
Precipitates	⧻	++	+	±	—

To try to solubilize 10*gm* of sample meat, 90 *ml* of buffer was used.

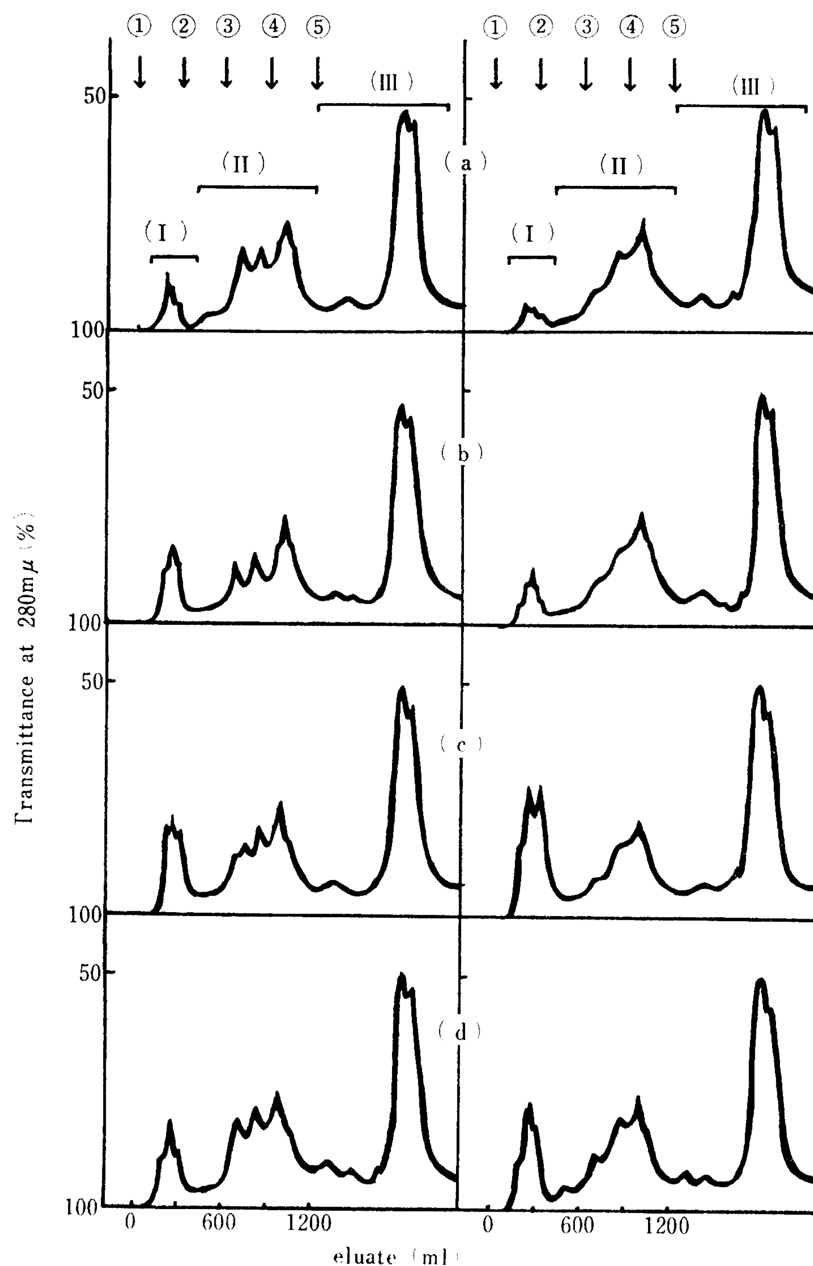


Fig. 1. DEAE cellulose column chromatograms with NaCl and NaOH gradient elution of several sample meat urea solution before and after heating at 75°*C* for 1 hour, in 7*M* urea containing 0.05*M* Tris-HCl buffer, *pH* 8.6. Ten *ml* of the solution was chromatographed in each case.

(a) Beef, (b) Pork, (c) Mutton, (d) Chicken.

Left; before heating, Right; after heating.

Numbers in circle indicate the changing points of each buffer described in Table 2.

(I), (II) and (III) show the fraction number to be collected for the following operations.

0°*C*), to its being intense in buffering capacity at alkaline side in even 0.05 *M*, and not binding to the proteins as phosphate.

For the reasons mentioned above, 7*M* urea containing 0.05 *M* Tris-HCl buffer was selected as the most suitable solvent to solubilize the meat samples before and after the heat-coagulation.

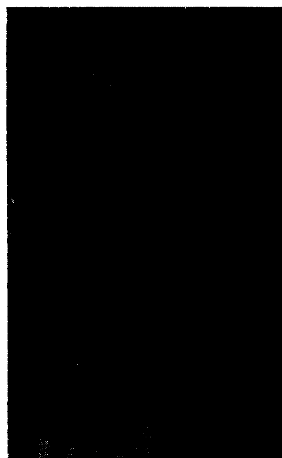
DEAE cellulose column chromatography and starch gel electrophoresis

Chromatograms of various meat samples before and after heating at 75°*C* for 1 hour, are shown in Figure 1. Arrows in the raw beef pattern are the symbols of the changing position of each buffer in the system shown in Table 2. Observation of these patterns, in the first fraction (I), eluted with buffer ①, shows that there are components exchangeable a little, that is, components which are very weakly charged in this experimental condition. In the second fraction (II), eluted with buffer ②-④ of NaCl gradient, the main components including myofibrillar proteins seem to be contained. And in the finally eluted fraction (III) with NaOH gradient is considered, in general, to be containing the column denatured proteins.

Among these fractions, (II) is the most characteristic as it is subject to the changes induced by heat, (I) is the next and (III) is scarcely affected by heating as far as the observation in these patterns goes. Perhaps, however, contents in each fraction may change considerably before and after the heating. (I) does not show so characteristic pattern as (II) does, but owing to the presence of only one peak indicating decrease in quantity after heating, and judging from the complexity of the shape of curves, presence of many kinds of component in this fraction may be presumable. (II) shows very characteristic and complex shape of the peak shared by shoulders in all raw meat samples,

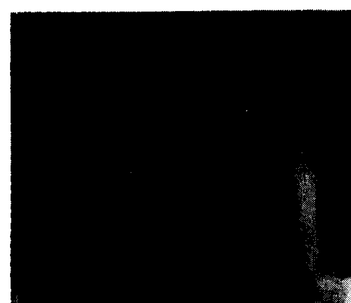
(b) (a) (d) (c)

Fig. 2. Starch gel electrophoretic patterns of beef and pork meat urea solutions, before and after heat coagulation through 7*M* urea containing gel.
(a) Raw pork,
(c) Raw beef,
(b) Heated pork,
(d) Heated deef.



(a) (c) (d) (b)

Fig. 3. Starch gel electrophoretic patterns of beef and pork meat urea solutions, before and after heat coagulation through 7*M* urea containing gel.
(a) Raw chicken,
(c) Raw mutton,
(b) Heated chicken,
(d) Heated mutton.



(a) (b) (c) (d) (e) (f)

Fig. 4. Starch gel electrophoretic patterns of protein fractions separated from beef muscle.
(a) Raw beef, (b) Stroma fraction, (c) Myosin B fraction, (d) Myosin A fraction, (e) Globulin X fraction, (f) Myogen fraction.

but in the heat coagulated sample meat, these shoulders disappeared partially. Out of these peaks, it is noticeable that a main peak which is located near the last of NaCl gradient, that is, at 1,100 *ml* of eluate from the starting point of elution, remains to be almost intact even after heating. This is very noteworthy, and is prone to indicate the main component constituting muscle, myosin B or its fragment.

At the same time, these sample meat-urea solutions were analyzed with SGE, and the results are shown in Figure 2 and 3. From these patterns, though there might be some differences in details, the behaviour of the components given as bands and zones, is similar approximately in mobility through the gel, notwithstanding the mutual differences in species.

Results from analyses with SGE of the separated protein fractions from beef muscle are shown in Figure 4. Since each protein fraction is crude, many bands and zones appear in their patterns, but on staining, those of the main component aimed to separate must appear intensely of all.

Indeed, though myosin A fraction has many bands and zones, the most intensely stained part is arranged from near the slot to the half way up to the moving front. On myosin B fraction, resembling to the myosin A, the most intensely stained zone is found at the section nearest to the slot.

Stroma fraction has all of the bands and zones included in the whole meat-urea solution itself. Both globulin X and myogn fraction have a slowly moving zone, the half moving zones and the fast moving bands situated near to the front. Slowly moving zone in globulin X is clear but a little slower than that of the myosin A and that of myogen is far slower, though faster than that of myosin B. The most characteristic feature in these two fractions is found in the bands lying near to the front in the pattern of myogen fraction, which is stained as intensely as the bands of the whole meat-urea solution are, assuming a yellowish orange hue in the gel before staining, after electrophoresis. As myogen fraction is by nature slightly yellow coloured, hemeprotein, myoglobin or heme itself separated from it, may reasonably be supposed to be included in this fraction, and it may be represented by the bands mentioned above.

The results from analysis by SGE with an aliquot of condensates which is derived from fraction (II) by DEAE cellulose column chromatography are shown in Figure 5. As a rule, these patterns are approximately similar each other notwithstanding the differences in species, and they represent the occurrence of fast bands, namely a zone moving half

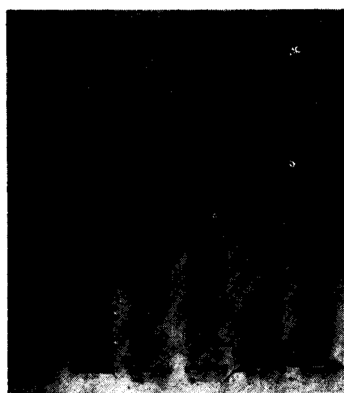


Fig. 5. Starch gel electrophoretic patterns of the condensates of fraction (II) resulting from DEAE cellulose column chromatography of pork, beef and chicken meat-urea solution, before and after heat coagulation.
(a) Raw pork, (b) Raw beef, (c) Raw chicken.
Marked alphabets show those sample solutions after heat coagulation.

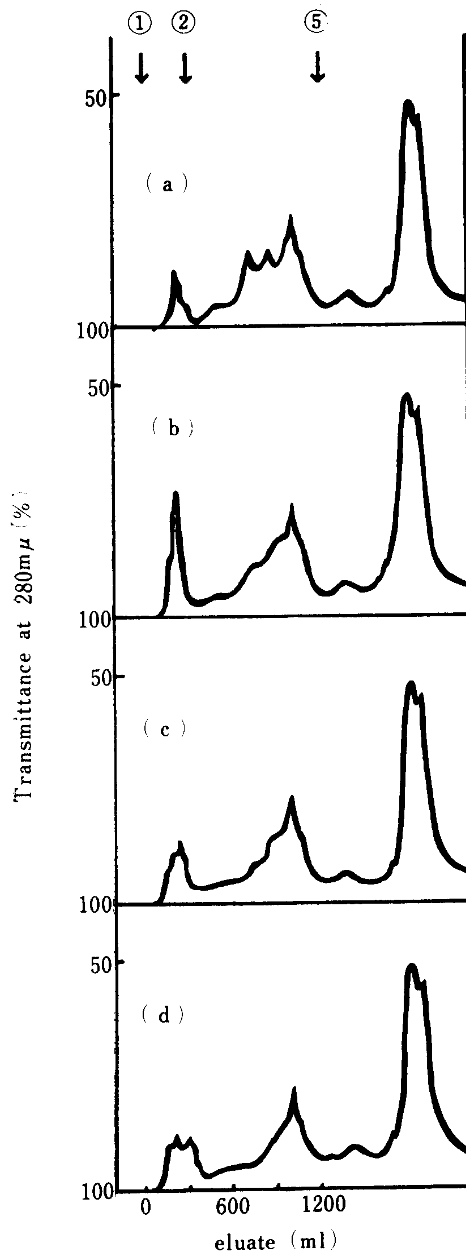


Fig. 6. Changes of DEAE cellulose column chromatograms of the pork meat urea solution at several steps of heating temperature.

(a) Raw, (b) heated at 50°*C*, (c) heated at 75°*C*, (d) heated at 90°*C*.

All the heating processes were carried out for 1 hour and after heating, the samples were cooled with tap water before solubilizing for 1 hour.

way up to the front and intensely stained, and some zones spread near to the slot corresponding to myosin A and B. Expectedly, judging from these patterns, the fraction (II) seems to contain not only mainly myosin A and B, but, though in a small quantity, sarcoplasmic proteins, that is, myoglobin, globulin X and myogen.

Observation over the patterns of sample solution before and after heating shows that the fast moving bands corresponding to sarcoplasmic protein and zones, and to myosins of the sample after heating, are to be stained weakly, in comparison with those bands before heating. This seems to imply that this is due to the denaturation of the proteins, which is especially strong in sarcoplasmic proteins and myosin A, which have been known to be subject to denature even in the muscle post mortem.

In order to make the fact more evident, a series of analyses were performed, employing only pork meat, by both DEAE cellulose column chromatography and SGE, heated at the steps of 50°, 60°, 75°, 80°, 90° and 100° (98°) *C*, for 1 hour in the test tube. All these samples were easily solubilized with 7.7 *M* urea containing buffer solution, but exceptionally, in the sample heated at 100°*C* (actually 98°*C*, in the boiling water bath) a considerable amount of gelous precipitates was found to be existing, when centrifuged at 5,000 rpm for 5 min, and this was assumed to have resulted from the formation of gelatine by gelation of collagen. DEAE cellulose column chromatograms of meat solution heated at 50°, 75°, and 90°*C*, in contrast to the raw meat, are shown in Figure 6. Some distinguishable changes are to be found also in the fraction (I) and (II). (I) shows remarkable rise and fall in quantity and irregular change in the shape of curves,

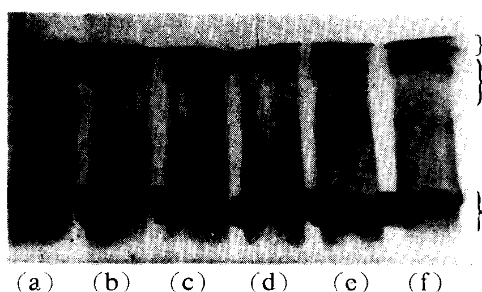


Fig. 7. Starch gel electrophoretic patterns of pork meat urea solution, at several steps of heating temperature for 1 hour.

(a) Raw pork, (b) Heated at 50°*C*, (c) Heated at 60°*C*, (d) Heated at 75°*C*, (e) Heated at 80°*C*, (f) Heated at 90°*C*.

Electrophoretic run was made for 7 hours at 4°*C*.

Three parts which are shown by braces, are particularly heat labile.

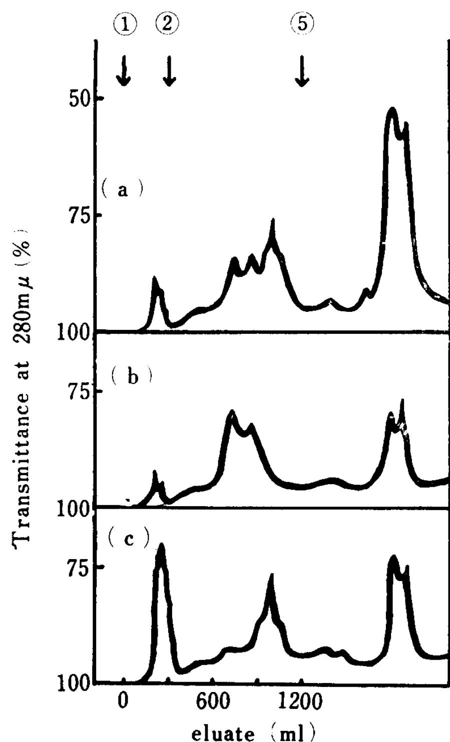


Fig. 8. An attempt to identify the main components, myosin A and myosin B, found in NaCl gradient of DEAE cellulose column chromatography with the beef meat-urea solution.

(a) Raw beef, (b) Myosin A fraction, (c) Myosin B fraction.

Protein concentration of the myosin A and B were 0.5%.

illustrating the variation of contents, and (II) shows a constant change indicating the decrease of the shoulders in accordance with the elevation of the heating temperature. It is noteworthy, however, that the main peak maintains its shape and quantity after the heating even at 90°*C* for 1 hour. Resulting patterns from SGE are shown in Figure 7, which indicate the disappearance of zones and bands at the three parts suggested in the figure, in accordance with the elevation of heating temperature and may be applied for the confirmation of the changes of the various meat samples described above.

An attempt for the identification of the main peak on DEAE cellulose column chromatogram

It is needful to clarify the main peaks unchangeable after heating in Figure 1, which one corresponds to a specific component in the muscle.

So an attempt was made to identify it with the myosin A or B fraction separated from beef muscle, relying on the presumption that either is corresponding to it, comparing with the raw beef meat-urea solution, by means of DEAE cellulose column chromatography. The results obtained are shown in Figure 8. As it is evident from these patterns, the main peak in the NaCl gradient corresponds to that of myosin B, and one of its shoulders, to myosin A. Perhaps, the rest of the shoulders may correspond to a part of sarcoplasmic proteins including the heme protein.

Sephadex column chromatography

Fraction(I), (II) and (III) separated by DEAE cellulose column chromatography are condensed and desalted with Sephadex G-25, coarse, by batch method up to 15*ml*, and analyzed in part, with

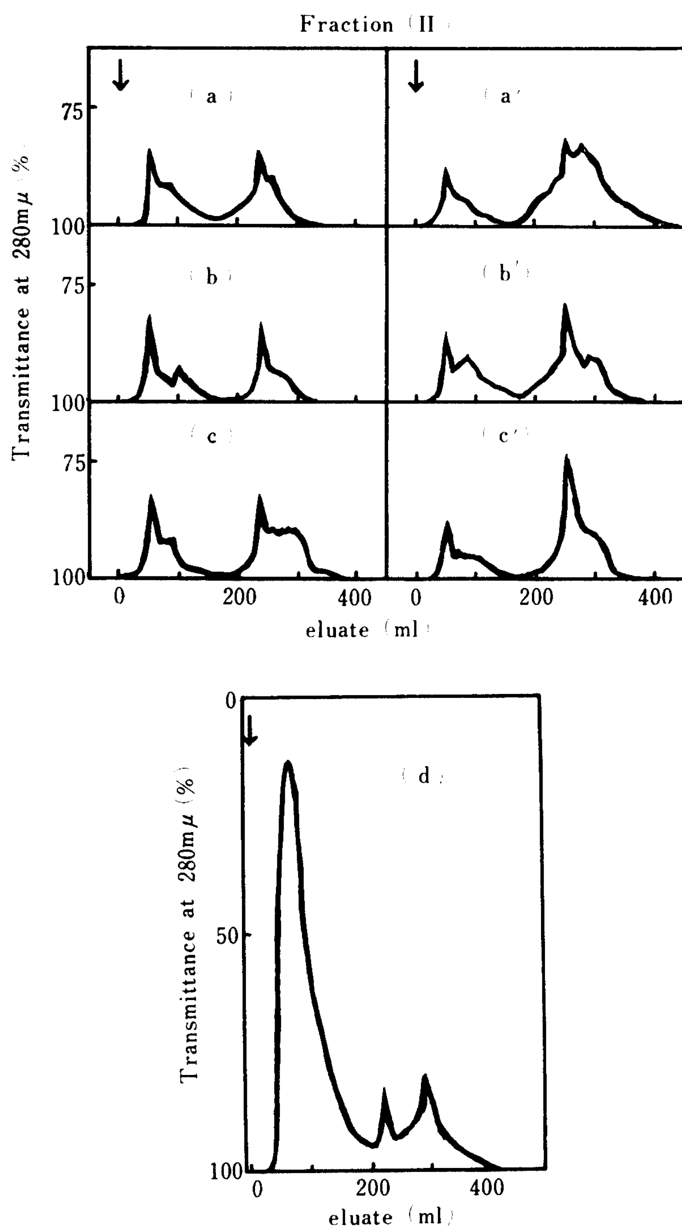


Fig. 9. Sephadex G-100 column chromatograms of condensates of fraction (II) resulting from DEAE cellulose chromatography of beef, pork and chicken meat urea solution, before and after heat coagulation, in contrast to myosin A fraction of beef.

Arrows show the starting points of elution.
(a) Raw beef, (b) Raw pork, (c) Raw chicken, (d) Myosin A fraction of beef.

Marked alphabets show those sample solutions after heat coagulation. Protein concentration of myosin A was about 5%.

Sephadex G-100 column, though smaller components absorbed in Sephadex G-25 were neglected.

Results obtained from fraction (II) are shown in Figure 9. At first, as a measure of content in pattern to be obtained, myosin A fraction prepared above was analyzed, and three peaks appeared in the chromatogram, among which, judging from molecular sieving quality of Sephadex G-100, the peak eluted fastest, being maximum in quantity and with a little tailing, was supposed to be a substance with fairly large molecular weight, though the environment of the column employed in this experiment was ascertained to be far more abnormal when compared with that in routine technics. Under the normal condition which is equilibrated with the buffer containing no urea, substance up to about 100,000 of molecular weight was reported to be capable of entering into the dextran matrix of Sephadex G-100, delaying its eluting time. In this study, however, as 7*M* urea containing buffer is used, analysis will not be performed the same way as in the above, that is, in such an environment, myofibrillar proteins are partially divided into smaller units in consequence of the cleavage of hydrogen bond, whereas Sephadex particles may not be made to be swollen to the extent as in the buffer containing no urea, so that the effect of the molec-

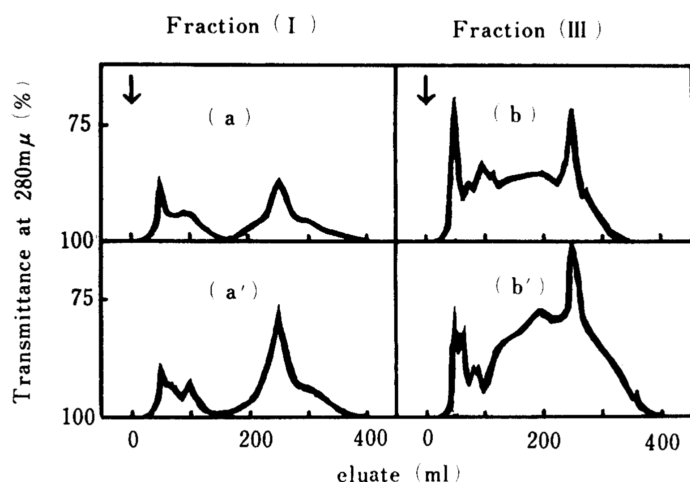


Fig. 10. Sephadex G-100 column chromatography of the condensates of fraction (I) and (III) resulting from DEAE cellulose column chromatography of beef meat urea solution before and after heat coagulation.
(a) Fraction (I), (b) Fraction (III).
Marked alphabets show those sample solutions after heat coagulation.

ular sieving may not be expected as in the above. The constituent of the maximum peak obtained from this analysis of myosin A fraction is supposed to be mainly consisting of the very myosin A accompanied with some contamination of myosin B and with polymerized actins. At the same time, it is suggested that myosin A may hold fairly large molecular shape even in such a high concentration of urea as 7*M* containing buffer, though partial degradation of helical construction or depolymerization is inevitable. In the figures, consulting the results from SGE, smaller peaks following to the peak of myosin A are supposed to be actin and minor groups of sarcoplasmic proteins. The fast eluted peak of myosin A fraction has a tailing, and at that position in the patterns of fraction (II) of each sample, a small shoulder appears, suggesting the occurrence of subunits of myosin A and B as the contaminant, and the slowly eluted two peaks are not so clearly separated into two as in case of myosin A fraction, but form rather one peak with one shoulder, which corresponds to the last peak found in myosin A fraction. Results from the heat coagulated meat sample shown to the right side, indicate that they are not so different from those from the raw meat sample, but the slowly eluted peak tends to increase in quantity, its shape being more complex. Thus, small molecular substances seem to increase considerably as the result of heat denaturation of protein.

With the condensates from fraction (I) and (III), analysis was carried out only of beef meat sample before and after heating. Results obtained are shown in figure 10. In the patterns of sample meat before heating, those of fraction (I) represent a fast eluted

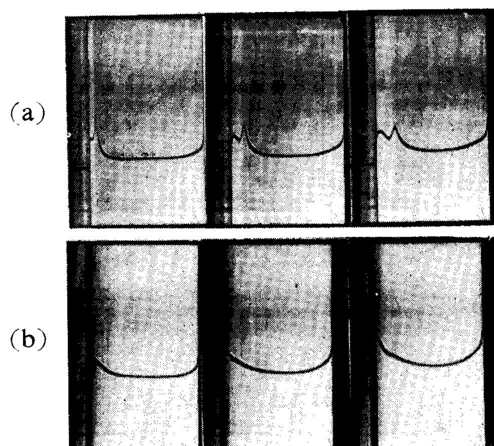


Fig. 11. Ultracentrifugal sedimentation patterns of beef urea solution before and after heat coagulation.

Protein concentration 1 %, 56, 100 rpm, 20°C, 7*M* urea containing 0.05*M* Tris-HCl buffer, *pH* 8.6.

At the intervals of every 16 minutes after constant speed, from left to right.
(a) Raw beef, (b) Heat coagulated beef.

peak with a shoulder and a slowly eluted simple peak, whereas those of fraction (III) also represent two peaks, fast and slow, but there is something between them, which makes the pattern particular. In those patterns after heating, shoulder of the fast peak in fraction (I) becomes more noticeable and the shape of the fast peak in fraction (III) becomes more complex.

Furthermore, it is interesting that, in fraction (I) and (III), the former is higher than the latter or equivalent before heating, but after heating, the latter becomes higher. This is an evidence showing that smaller molecular substances resulting from denaturation of proteins increase after heating. However, contents of these peaks are unexplainable within the limits of the present experiment.

Ultracentrifugal sedimentation

Ultracentrifugal sedimentation patterns of beef muscle before and after heating, are shown in Figure 11. One sharp peak is found in the pattern of the raw meat sample, which is sedimented gradually in 7*M* urea containing buffer and after 48 min rotation, comes to be separated into two peaks. In the heated meat sample, no peak can be found in this experimental condition. Perhaps, this means that proteins are degradated into an approximately similar dimenson by heating and by the high concentration of urea. Concerning this, therefore, further investigation will necessarily be required with higher concentration of protein.

From the above-mentioned facts it becomes evident that the various samples may be analyzed by several methods, provided that they are solubilized with 7.7*M* urea containing Tris-HCl buffer, though many problems which should be solved are included in the results obtained. Further investigation about them, therefore, will be done hereafter, and besides, changes at each step of meat and meat product manufacturing process shall be pursued in close touch with the technological practice.

Summary

Muscle proteins in the raw state have been investigated hitherto, but on denatured ones through the heat coagulating process which is quite unavoidable to the meat product manufacturing, no essential studies have been made yet. So, at first, it was undertaken to find out a method for solubilizing the heat-coagulated meat proteins to get the most suitable sample solution applicable to the several routine analytical methods.

Consequently, a procedure in which the sample meat was to be not only homogenized with 7.7*M* urea containing 0.055*M* Tris-HCl (*pH*8.6) buffer solution but solubilized, was found as the best method for the purpose of considering the various aspects of analyses to be followed.

The sample solutions from several kinds of meat which were utilized as common raw materials for the meat product manufacturing were tried to be analyzed actually by DEAE cellulose, Sephadex column chromatography, starch gel electrophoresis and ultracentrifugal sedimentation, in 7*M* urea containing buffer (pH 8.6).

From these results, it became evident that the sample solutions obtained by the method might be analyzed sufficiently by various analytical methods, and it was assumed that muscle proteins were distinguishable from each other in such a high concentration of

urea containing buffer solution as $7M$, and that after heat coagulation, substances of low molecular weight were prone to come out considerably in quantity.

About the contents of the results obtained in this experiment, further investigation was supposed to be indispensable before any explanation in details might be obtained, and besides, changes at each step of meat and meat product manufacturing process shall be pursued in close touch with the technological practice.

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References

1. NEELIN, J. M. and D. ROSE : *J. Food Sci.*, **29**, 544 (1964).
2. MAIRE, G. E. and R. L. FISCHER : *J. Food Sci.*, **31**, 482 (1966).
3. MELACHOURIS, N. P. and S. L. TUCKEY : *J. Dairy Sci.*, **49**, 800 (1966).
4. KAKŌ, Y. and N. ANDŌ : *Sci, Bull. Fac. Agr., Kyushu Univ.*, **22**, 301 (1966).
5. WAKE R. G. and *R. L.* BALDWIN : *Biochim. Biophys. Acta*, **47**, 225 (1961).
6. SMITHIES, *O.* : *Biochem. J.*, **61**, 629 (1955).
7. SCHOCH, T. J. and C. C. JENSEN : *Ind. Eng. Chem., Anal. Ed.*, **12**, 531 (1940).
8. PORTZEHL, H., G. SCHRAMM and H. H. WEBER : *Z. Naturforsch.*, **5b**, 61 (1950).