PANEL DISCUSSION ON SOME APPLICATIONS OF COLLOIDAL CHEMISTRY IN MEATS RESEARCH

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MR. J. KASTELIC: Thank you, Dr. Pearson. Members of the Conference, and Friends:

I was not certain how I ought to approach the subject of some "Applications of Colloidal Chemistry in Meats Research," for I could only guess what my colleagues would say about this matter. There is no want of material to discuss. The fact that so-called protein solutions can be regarded as possessing certain features of behavior of "colloidal sols" makes it possible for us to use many different physical methods to study the properties of proteins. Light scattering ultracentrifugation, viscosity changes, electrophoresis are but a few well known examples which can be applied in studies of proteins.

Let us consider how some of these methods might be useful in meats research. Some years ago, Mihályi (1) Mihályi and Szent-Györgyi (2,3) published an interesting report on trypsin digestion of muscle proteins. Their primary objective was to determine whether the digestion of proteins by proteolytic enzymes followed one of the following courses:

(1) Gradual degrading with continuous splitting of low molecular weight products from the parent molecule;

(2) Explosion-like disintegration of the protein molecule into small fragments;

(3) Formation of well defined heavy intermediates which were further degraded into low molecular weight substances.

Their most interesting observation was that myosin incubated with trypsin shows a high rate of viscosity drop. This clearly suggested that the myosin molecule had undergone considerable hydrolysis in a matter of about 10 minutes. When undigested myosin and trypsin-treated myosin were subjected to ultracentrifugation, however, it was obvious that whereas nature myosin sediments with a sharp boundary, its single peak diminishes as digestion proceeds. After about 10 minutes two new peaks appear, one of which sediments faster and another more slowly than the original myosin. The sedimentation patterns would indicate that two homogenious components are produced.

These observations indicate that hydrolysis of myosin by trypsin is a well ordered process occurring in an all or none fashion. The viscosity and sedimentation measurements provided a great deal of information about the action of trypsin on myosin. It was subsequently shown that if trypsin digestion of myosin is permitted to continue, a secondary hydrolysis takes place, which unlike the initial digestion, is accompanied by a progressive appearance of non-protein nitrogen.

We have often discussed in this conference the problem of aging in beef. We have been interested in what happens to muscle proteins post-mortem. What do we mean when we say autolysis explains tenderness of aged beef? It occurs to me that if techniques such as those discussed today were used more frequently, perhaps we would develop a much clearer understanding about the processes involved. Perhaps we could describe the kinetics of proteolytic changes in aging beef. It is certain that the classic methods used to follow hydrolytic changes in intact animal muscle have failed to provide the desired information. Do you have questions?

Bibliography

1. Mihályi, E., J. Biol. Chem. 201, 197, 1953.

2. Mihályi, E. and Szent-Györgyi, A. G., J. Biol. Chem. 201, 189, 1953.

3. Mihályi, E. and Szent-Györgyi, A. G., J. Biol. Chem. 201, 211, 1953.

MR. PEARSON: I am going to call on Dr. Lillevik next. Hans.

MR. HANS LILLEVIK: Thank you very much, Dr. Pearson. Well, I must say, right at the outset, I am strictly among strangers in the meat protein business. I have been doing nothing but learning ever since the word "Mutton". All the discussions that I have been listening to have been most extremely interesting. They have raised a number of questions and I might throw another source of meat protein into the pot here which we have been dabbling around with. It is namely the proteins of fish muscle, actually a fish is quite an animal, a hunk of muscle, and together with Dr. Slammer, in the Department of Marine Biology, we have undertaken to make some examinations elector foredically (?) of muscle extraction of the caudal tail portion of the various fresh water species of fish here in Michigan. Prior work of this nature has been reported in England, regarding the salt water fish and muscle proteins, and they pointed out with the various species in that case the electric foretic patterns were quite distinct and characteristic for different species, and generic families of the

salt water fish. We finally tried it out here in the preliminary way, in connection with the fresh water fish and we found similarly very characteristic patterns. We took the species, various species of the family related to the sun fish, and it was possible by examining how we did it, we would take the muscle strip, grind them up in a wire blender, and extract them at pH 7, and run tests and electrofuse the pH 7. These patterns are most interesting, of course, show principles in the myosins, and myogens, and the proportions and general cations are very characteristic of different species, and furthermore, on hybrids, where you have crossed species of the same family, you can not hardly tell the difference externally and you look at the patterns, and you can see more where the dominant recessive contributions have been with regard to the muscle proteins.

Well, this is still in the preliminary stages, and we are drawing up a preliminary report, and possibly we will get that published. Now, listening to some of the remarks made previously here this afternoon, a few questions come to my mind. I certainly felt my reaction very similar to Dr. Kastelic, from Illinois, here just a moment ago, about the application of physical chemical methods towards studying these changes that come about, that occur in them and effect hydration capacity. What happens to these proteins with respect to the muscle proteins, the fraction of these extractions, the myosins and myogens, if one checked those individually this, of course, is a big job, and the process of isolation and studying the protein components of muscle oxygen. I was wondering in connection with the various effects of treatment with the muscle proteins whether or not there might be a like heat treatment, and that type of operation, whether or not that might destroy certain amino acid residues in the proteins, and consequently, some of the more heat liable residues may be effected by heat treatments, cooking, and so forth. Whether that does not alter the hydration capacities, and so forth.

Also, in the various changes of hydration capacity, is there not possibly some incipient proteolysis perhaps non catilyzed type of break down that has altered the hydration capacity of the meat proteins. This may be that people have looked into these questions. As I say, I am not overwhelmingly familiar with the proteins of meat, despite all the work that we have done. There is another point raised by Dr. Briskey, in discussing the changes setting in of rigor mortis, particularly where cathepsin may be taking over. I was wondering has any investigation been made as to which of the proteins substrates is most greatly affected by the catheptic enzyme, and what is the nature of the change there. What extent do they reach? These are just some questions that may be highly naive questions. Perhaps I might suggest these.

(Applause)

MR. PEARSON: Thank you. I am now going to call on Dr. Doty. He is substituting on our panel for Dr. Schweigert. He was suggested as an able substitute for him. Dr. Doty.

DR. DOTY: Al, Gentlemen, Lady: When Dr. Schweigert said he couldn't attend this conference and asked me to attend in his place, I suggested to him that he might tell me what he expected to talk about on this subject. He simply said he did not know, and I certainly didn't know, and I still don't know entirely, so here I am.

It has been very gratifying this afternoon to hear a great many of the speakers talk about the protein complexion of meat, and its collodial properties, and what influence it may have on some of our more practical aspects of meat chemistry, because this recalls to my mind a statement that was made by a very emminent protein physical biochemist a great many years ago. I refer to Dr. Gortner of Minnesota, who stated that in nature proteins were not separated that we had a protein complex and you will recall all of you that we went through a series of, or went through a period of a great many years when we forgot this very profound statement made by Dr. Gortner, and worked pretty much with isolated protein systems. These have a place in any research program involving colloids or proteins, but certainly the reactivity and the nature of the response which we may get in isolated protein components may not be the same that we get when we have the entire protein complexion present as we have in the meat, and I don't believe that it makes much difference how we look at it in terms of response, whether we look at it as a mixture of proteins, as Dr. Deatherage did in stating perhaps the difference in the response of meat to heating was due to a difference in response of the different proteins present, or whether we say that heating causes a different effect upon the complete and organized protein complex of meat. Actually, it may be easier to interpret if one looks at it as a single colloidal complexion, if you please, rather than a separate group of chemical entities. There are three or four things that I think that we need to consider in thinking about the aspects of polychemistry, which relates to meat research. First of all, colloids, as we learned in the first discussion, are large molecules and as such have to be treated in a way which is entirely different than the inorganic small molecules. We have certain diffusion techniques which we are all familiar with. Some of you, I am sure have made separations of meat fractions, and we sometimes forget that the separations of these fractions are dependent upon molecular size and molecular change, if you please. I suspect if one were to ask each of you individually what size molecule do you think will defuse from cellophane membrane, how many membranes will go through the cellophane membrane? You would get all the way from two to several hundred. Actually, we know in terms of amino acid residues do fuse through normal membranes, we have a range of sizes from one or two up to as high as a hundred. Amino acid residue peptides that will diffuse through a membrane so that when we start talking of meat fractions, or what is solubility, and insolubility, and using fusability as a means of separation, we must remember that this is dependent upon a lot of properties other than molecular size, but that molecular size is important. We have heard this afternoon some reference to enzymes, and their relationship, perhaps to aging changes that take place. There are places, I think, where poly-chemistry techniques, or techniques of poly-chemistry may enter into the investigation.

First, enzymes, themselves, are proteins, and have many characteristics of the other colloids. We can use the principles of colloid chemistry in separating enzymes and determining some of their characteristics. Secondly, as has been suggested, we can also use the techniques of colloid chemistry and techniques of large molecule chemistry, if you please, for separating the components that are formed, the end products of action upon meat proteins themselves. Such studies are under way in our laboratory at the present time. I just told Dr. Kastelic maybe "X" years from now when we have the answers, whether the enzymes which are present in meat do have the effect of tenderizing meats themselves. In this same connection, it might be mentioned while the classical approaches for determining whether or not one has had proteolysis or not, have not been very productive. One has only to look at the relatively few members of the amino carboxic (?) groups that would have to be liberated from the chain to make a drastic increase in drastic size, if you please. To know the amino carboxic group would not be productive, whether tenderness was due to proteolysis or not. One can determine and it has been done a number of times in a number of laboratories, that aminos are liberated in protest of that. Liberation of these groups is not quantitatively related to the amount of tenderness that occurs. If we remember we are dealing with large molecules and only a few breaks are necessary to give you degrees in molecular size, it is not surprising one can make these determinations and find a change that would not be able to quantitate these changes to the tenderness change. Surprisingly enough, not many people have mentioned the color of meat this afternoon in connection with the colloidal characteristics of meat, and how they are related. Actually, many of the problems of meat color will depend for their ultimate solution upon a recognition that meat pigments are after all also proteins and they are colloidal in nature. Some of the aspects that we have been talking about in connection with tenderness and other characteristics also applied to studies and relate to meat pigments. We should not forget also that meat, even though it is shown by the criteria that was suggested by someone else is still a highly organized system, and there are still certain membranes and certain potentials present which do influence its characteristics very greatly. This was brought forceably to our attention some years ago when we started studying meat hydration, and if you dehydrate meat from the unfrozen condition, you immediately get a shift as has been suggested, of inorganic ion. But, they do not diffuse very readily throughout the meat, only to the periphery of the muscle fiber. I am speaking of potassium ions. The sarcolema of the fiber is not permanent to the potassium ions even in meat. Not only in the animal itself, but in meat after the death of the animal. We are dealing with membrane, potential, despite the fact the structure has been changed to some extent.

Actually, I could go on and on, and discuss many characteristics of the meat, and how they may be perhaps identified more clearly with careful consideration of the principle of colloid chemistry, but I'll leave the rest of this to Dr. Deatherage, I think. Thank you.

(Applause)

DR. DEATHERAGE: I have said quite enough this afternoon. We have been sitting long enough. I said I appreciate the kind words said about me this afternoon, but I would like to say, Joe, in a bit of defense, the reason I got interested in meat is high temperature aging, twenty years ago. When the question was asked me what were my ideas concerning the fact if you chill a piece of meat out and put it in a room of 68 degrees, why it didn't get more tender if you held the meat three days for 48 degrees than it did if you held it somewhat less than two days. I couldn't answer the question, and I said I don't believe it is true, but it is true. That is what I spent time on, a lot of it has not been in the literature, except in certain patterns, nevertheless, it did get me involved in a mixture of things that sometimes you don't get too far.

I might say I think it might be of interest that in our lab, we don't know, but we never were successful in finding any non-protein fragments, Dr. Doty, that form hydrolysis. I don't believe we ever made any attempt to fracture the protein any further. I think it is a better start that I read the last week or so, I got the annual report of New Zealand Meat Investigation, a long title, NS something or another. Anyway, Dr. Notting down there made the study of the end group in proteins of the skeletal muscle, and reports the maximum increase in number of end groups on the number of proteins in meat at point of the pH fraction of increased 6%. Only 6% of the protein molecules had a bone cleaved, and that is not certainly an awful lot of protein to be chopped apart, but if they happen to be chopped in the right spot, they would call it a reasonable change.

I said it is time to, and I am going to sit down now. If anybody wants to ask any questions, then it is up to them. Thank you very much.

DR. PEARSON: Are there any questions of any of the speakers or panel this afternoon? If anyone has any questions, we will entertain them at this time. I think you are about right. I am inclined to dismiss. We will turn this thing back to our Chairman.

MR. ADAMS: Thank you, Al. We all appreciate very much the work this group has gone into on this committee this afternoon, in preparing the information that they have presented to us. I think that it will give us good deal of thought.

Are there any announcements to be brought before the conference this afternoon? If not, tomorrow morning at eight o'clock, we will meet in this room, begin with the Pork Carcass Evaluation Committee. Woody, I would appreciate it if you will have your members ready for this part of the program.

We are adjourned until eight o'clock tomorrow morning.

(Meeting adjourned.)

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