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DETECTION OF NONCOVALENT INTERACTIONS IN THE HEAT-INDUCED AGGREGATION OF SKELETAL MUSCLE MYOSIN

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Background:

The binding of chunks or ground pieces of meat in cooking is a heat-induced phenomenon which involves protein-protein interactions, since raw meat pieces do not cohere to any significant extent. In this respect, a number of intresting findings have been reported by some researchers, including identification of the proteins which provides a binding force in cured processed meat , and the mechanism of binding in meat (Fukazawa et al., 1961,Zieglar and Acton, 1984,Asghar et al.,1985). According to those studies, the heat-induced gelation process of skeletal myosin is essential to products with desired properties. Thermal gelation of myosin can be studied in two phases: myosin denaturation/unfolding and myosin aggregation. Because myosin is a multi-domain protein, the unfolding and interactions of discrete myosin domains might be important to gelation ability (Samejima et al., 1981, Ishioroshi et al.,1982). Therefore, the mechanism of myosin gelation has been investigated extensively during the last three decades.

Objectives:

From the results of gelation and denaturation studies of myosin, Yasui and Samejima (1990) suggested that the gelation of myosin consists of two satges, i.e., aggregation of myosin molecules through their heads at low temperature (~43℃) and another cross-linking reaction due to helix-coil transition of the tail portion of the molecules at higher temperature (~55℃) . They concluded that the hydrophobic residues exposed by unfolding of rod portion produce intermolecular cross-linkages among molecules and their network throughout the sysetem. Generally, it is thought that the gelation of thermally unfolded proteins is caused by the results of inter-molecular covalent and noncovalent bonds. However, it has been hard to detect the participation of noncovalent interations in the heat-induced gelation of myosin. The objective of this study is to find a role of the noncovalent interactions in the gelation mechanism of myosin by using of chemical cross-linker, EDC [1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide].

Methods:

Myosin was prepared according to the method described previously (Ishioroshi et al.,1982) from chicken breast muscle and myosin subfragments were prepared according to the methods of Weeds and Pope (1977). Chymotryptic S-1 and rod was made from insoluble myosin filaments (in 0.12M KCl) in the presence of 1mM EDTA and 1mM DTT.

Myosin and subfragment (0.5mg/ml) solution in 0.2~0.5M NaCl and 20mM PIPES (pH6.0) was heated at 30~80℃ for 20min, and then EDC was added to make a final concentration of 0~2.0mM and the solution was incubated for 30min at 25℃. After incubation, the cross-linking reaction was terminated by the addition of 2-fold excess concenteration of dithiothreitol.

Sodium dodecyl salfate polyacrylamide gel electrophoresis (SDS-PAGE) was carrid out by the procedure of Laemmli (1970). The contents of myosin and subfragments were determinded with Imaging Densitometer (*BIO-RAD,GS-700*).

Results and discussions:

Two kinds of bonds are usually present in the heat-induced gelation of proteins. There are covalent and noncovalent bonds. Chemical changes of the amino acid side chains of a protein usually affect the force of protein-protein interaction. The nature of the noncovalent bonds was studied by introducing a zero-length cross-linker. EDC is cross-linking adjacent region of amino and carboxyl groups and occuring in hydrophilic interaction. The feature of cross-linking by EDC, which cross-links the aggregation sites of protein with electrostatic interaction, was different among myosin and its subfragments.

Fig.1 shows the changes in the amounts of cross-linked myosin, S-1 and rod with EDC before heating in 0.5M NaCl at pH6.0. The cross-linked myosin and rod were increased with increasing of EDC concentration, but S-1 was not cross-linked. In 0.2M NaCl,

however, myosin and rod were cross-linked greater extent than in 0.5M NaCl by EDC. This difference may be due to whether filament or monomer status of myosin molecule.

The effect of heating temperature on the amount of cross-linked myosin, S-1 and rod is shown in Fig.2. Cross-linking of S-1 occured at relatively low temperature (40~50℃), but decreased at higher temperature than that in the low temperature. On the other hand, myosin and rod was cross-linked at higher temperature (50~70℃).

Heat-induced gelation process of myosin is contributed by two reaction. One is the aggregation of heads of myosin molecules, which occurs at relatively low temperature such as 40℃. Second is helix-coil transition of tail portion followed by the entanglement each other, which occurs at higher temperature, and plays an essential role in the gelation. It has been pointed out that hydrophobic interaction contributed to latter reaction. Our results suggest that additional interaction i.e.electrostatic interaction takes part in the entanglement among the tail portions of myosin molecules but not in the aggregation of head portions of myosin molecule.

Conclusion:

In 0.56M NaCl the cross-linked myosin and rod were increased with increasing of EDC without heating, but S-1 was not cross-linked. In 0.2M NaCl myosin and rod were cross-linked greater extent than in 0.5M NaCl. Cross-linking of S-1 treared with EDC occured at relatively low temperature (40~50℃), but myosin and rod was cross-linked at higher temperature (50~70℃). These results indicate that the application of the chemical crosslinker might be useful method to get new information on the heat-induced gelation mechanism of proteins.

Pertinent literature:

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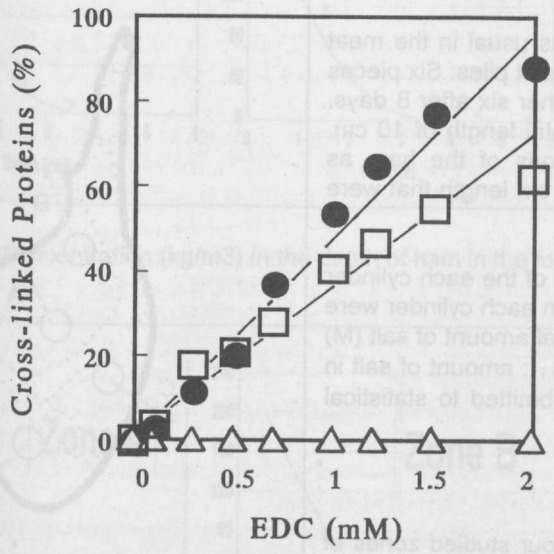

Fig.1 Effect of the cocentration of EDC on the amount of cross-linked myosin and subfragments. The cross-linked proteins were expressed as a percentage to the untreated preparation. myosin(●), S-1(△), and rod(□)

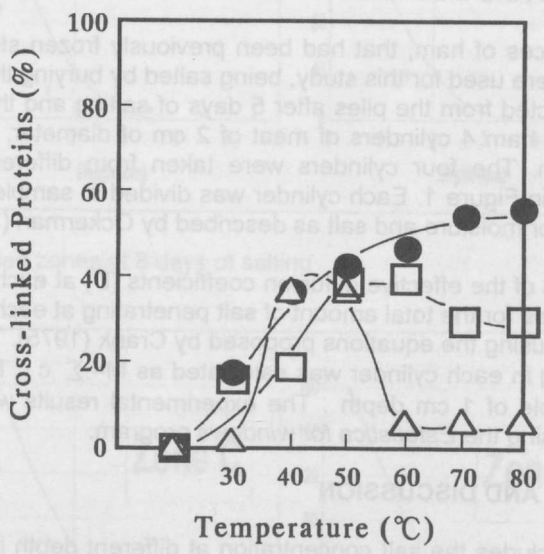

Fig.2 Effect of the heating temperature on the amount of cross-linked myosin and subfragments.Protein(0.5mg/ml) solutions in 0.5M NaCl and 20mM PIPES(pH6.0)were heated at various temperature for 20min and cooled at room temperature. Sample solutions were treated with 0.3 mM EDC and then reaction was stopped by the addition of 0.6mM DTT. Symbols are the same as in Fig.1.