DSC STUDIES OF MUSCLE TISSUE PROTEIN DENATURATION

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A preliminary study of the potential uses of Differential Scanning Calorimetry (DSC) for describing the thermal denaturation of muscle proteins is reported.

The general thermogram pattern appeared to vary little between different types of muscle tissues. Four distinct endothermal denaturation peaks were found in the characteristic thermograms, - with maxima at 58, 65, 68 and 80.5°C, respectively. One of the peaks was associated with the denaturation of connective tissue.

The addition of NaCl changed the meat thermogram drastically.

The drip liquid from thawing calf muscle showed three incompletely separated denaturation peaks with maxima at 62 - 66, 70 - 72 and 75°C, respectively.

Pre rigor muscle from calf and cow showed a strongly exothermal peak between 50°C and 60°C, whereafter the thermogram was similar to that of post rigor tissue from the same muscle.

DSC-ÉTUDES DE LA DÉNATURATION DE PROTÉINE MUSCULAIRE

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Une étude préliminaire d'application potentielle de "Differential Scanning Calorimetry" (DCS) pour décrire la dénaturation thermale de protéine musculaire a été rapporté.

Les variations entre les types différents de tissu musculaire dans le modèle générale de thermogramme étaient, paraît-il, petites. Quatre sommets distincts endothermaux de dénaturation ont été trouvés dans les thermogrammes caractéristiques avec des maxima à 58, 65, 68 et 80,5°C respectivement. En ce qui concerne un des sommets il a été lié à la denaturation de tissu conjonctif.

L'addition de NaCl a rigoureusement changé la thermogramme de viande.

Le suc au jus de viande en goutte venant de muscle de veau degelent a montré trois sommets de dénaturation incomplètement séparés, avec des maxima à 62-66, 70-72 et 75°C respectivement.

Des muscles pré-rigides de veau et vache ont montré un sommet fort exothermal entre 50°C et 60°C, et après ça le thermogramme était le même que celui de tissu après-rigide venant du même muscle.

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DSC-STUDIEN DER DENATURIERUNG DER MUSKELPROTEINE

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Eine vorläufige Studie über die mögliche Verwendung der differentialen scanning Kalorimetrie (DSC), um die hitzebedingte Denaturierung der Muskelproteine zu beschreiben, ist gegeben.

Das gewöhnliche Muster des Thermodiagramms erwies eine geringe Variation zwischen verschiedenen Typen von Muskelgeweben. Man fand vier deutliche endothermale Denaturierungspiks in dem typischen Thermodiagramm, mit Maksimalwerten bei den Temperaturen 58, 65, 68 bzw. 80,5°C. Einer der Piks gehörte der Denaturierung des Bindegewebes an.

Bei der Zufuhr von NaCl wurde das Thermodiagramm stark verändert.

Der abgegebene Saft von aufgetautem Kalbfleisch zeigte drei unvollständig getrennte Denaturierungspiks mit Maksimalwerten bei den Temperaturen 62 - 66, 70 - 72 bzw. 75°C.

Prärigor Muskulatur von Kalb oder Grossvieh gab einen grossen, exothermalen Pik zwischen 50°C und 60°C, aber die Fortsetzung des Thermodiagramms war ähnlich mit dem von postrigor Gewebe derselben Muskulatur.

ИССЛЕДОВАНИЕ ДЕНАТУРАЦИИ МЫШЕЧНОГО БЕЛКА ДИФФЕРЕНЦИАЛЬНОЙ СКАНИРУЮЩЕЙ КАЛОРИМЕТРИЕЙ (ДСК)

ХАРАЛЬД МАРТЕНС и ЭЛЛЕФ ВОЛД

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Изучались перспективы применения ДСК для описания термической денатурации мышечных белков.

Мышечные ткани разного рода дали термограммы мало различающиеся между собой. В типичных термограммах наблюдалось четыре четких эндотермических пика денатурации, максимумы которых соответственно 58, 65, 68 и 80,5°C. Один пик связан с денатурацией соединительной ткани.

Добавление NaCl приводило к острым изменениям в термограммах мяса.

Сок, выделившийся при оттаивании телятины, показал три неполно разделенных пика денатурации, максимумы которых соответственно 62-66, 70-72 и 75°C.

До наступления посмертного трупного окоченения мышцы телятины и говядины показали остро экзотермический пик в области 50-60°C, после чего термограмма равна термограмме той же мышцы после посмертного трупного окоченения.

DSC STUDIES OF MUSCLE PROTEIN DENATURATION

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Introduction

It is generally accepted (Privalov, 1974) that heat-induced unimolecular protein unfolding denaturation is an endothermal process readily studied by Differential Scanning Calorimetry (DSC), while multimolecular protein aggregation is often exothermal and less well understood.
In a DSC run, a sample and a reference are both heated at a constant heating rate, e.g. 10°C/min., from e.g. 20 to 100°C. If the reference contains pure water and the sample contains a solution of a protein which unfolds at a certain temperature, the unfolding will be registered by the DSC by the additional energy required by the protein sample in order to keep up with the linear temperature increase of the reference.
Conventionally, DSC has primarily been used for the study of the unfolding of pure proteins in dilute water solutions, but the method may also be used for intact meat tissue, as well as isolated meat protein fractions. Subsequent aggregation phenomena may complicate the DSC measurements, but Karmas and DiMarco (1970) have shown that the method yields interesting results from beef muscle. The authors used a Perkin-Elmer DSC 1B. They found a complex endothermal peak starting at 50°C, with a maximum at 66°C. A second, more well defined peak started at 73°C, with a maximum at 82°C, tailing off at about 90°C. The first peak was assumed to reflect the denaturation of proteins, while the second peak was discussed in terms of collapse of water structure. By cooling the samples and heating them a second time in the calorimeter, they found the transitions to be irreversible. A salt-soluble extract did not yield any peaks in their experiment.

Materials and methods

Muscle tissue from m. sterneomandibularis was obtained from newly slaughtered calves. Several other types of mammalean muscle tissues were also investigated, as well as cod and chicken muscle tissue.

When whole muscle was to be examined, a 10-15 mg wet sample was dissected with a razor blade from a freshly cut surface of the meat, taking care to avoid visible fat and connective tissue.
The meat sample was placed in a 20 microliter Perkin-Elmer standard "volative sample holder", which was sealed to prevent the evaporation of water. The samples were prepared at room temperature.
The samples were scanned in a Perkin-Elmer DSC 2 calorimeter with an empty sample holder with an extra lid as a reference. The scan rate was 10°C/min., sensitivity was 0.5 mcal/sec. full scale, and paper speed 2 cm/min.
Starting temperature was 1°C (using Perkin Elmer Intracooler II). Upper temperature limit was 100-105°C.
After scanning, the samples were cooled to room temperature, punctured in the lid by a needle and dried at 105°C over night. The holders were weighed empty, with wet meat and after drying, and the wet and dry weight of the meat sample were thus found.
The temperature scale of the DSC was calibrated by the melting of benzil (m.p. 95°C) and azobenzene (m.p. 68°C). The ordinate scale was calibrated by the melting of indium. The temperature scale is accurate to about ±1°C. At least three replicate DSC runs were performed for each meat sample, and typical curves are reported here.

Results and discussion

A DSC curve is called a "thermogram", and is a plot of the differential heat input vs. time. Since the heating rate is constant, the time abcissa is also a temperature abcissa and the differential heat input ordinate is equal to the appearent differential heat capacity.
The thermogram of an equilibrated mixture of 70% whale myoglobin and 30% water (Hägerdal and Martens, 1976) is shown in figure 1 in order to illustrate the general phenomenon of thermal unfolding of protein in DSC, and for comparison to the typical meat thermogram given in the lower half of the figure.
For myoglobin the differential heat input (dQ/dt) increases linearly (a) with temperature as the sample is heated from the starting temperature towards the unfolding temperature. A peak (b) is then obtained as the temperature passes through the region in which the protein unfolds, above which the curve continues to rise more or less linearly (c). The area under the peak (b) is equal to the total entalpy change caused by the unfolding. Privalov (1974) has treated these phenomena in detail.

The thermogram of the calf m. sterneomandibularis (figure 1) shows a mixture of peaks, as one would expect, considering the many different protein species present in the muscle. But the general phenomena of rising heat capacity "baseline" below the peaks, as well as

the occurence of unfolding-like peaks are similar to the thermogram of pure proteins. It is difficult to determine the direction and level of the "base-line" below the complex meat thermogram peaks, since it is known that the heat capacity of proteins increase during unfolding (Privalov, 1974). Therefore, quantitative entalpy data will not be given in this preliminary communication.
The m. sterneomandibularis sample shows 3 distinct peaks on top of a large general "hill". The first peak (I) starts at 49-50°C, with a maximum at 57°C. The starting temperature of the second peak (II) is hidden by the first peak, but its maximum is at 65°C. The third peak (III) appears to start at about 75°C, but may possibly start earlier, and has a maximum near 80°C.

We have found (not shown here) that the addition of 6% NaCl in cow minced meat, changes the thermogram pattern drastically, by removing more or less completely the two first peaks, leaving only a broad "hill" with a single sharp peak at about 70°C that apparently corresponds to peak III, the peak at 80°C in normal meat thermograms.

The thermogram of different types of animals

Figure 2 shows typical thermograms from porc diaphragma, chicken pectoral muscle and cod filet muscle. The endothermal peaks occur at a lower temperature in the cold-blooded cod fish than in the warm-blooded chicken and swine, but the patterns are somewhat similar, especially regarding the peak around 80°C.

The rigor mortis

Figure 3 shows thermograms from m. sterneomandibularis from calf at 2 hrs (a), 8 hrs (b), and 27 hrs (c) after slaughtering. The muscle shown in the figure was kept at room temperature from 1 hr after slaugtering till the experiment was finished, but similar curves were obtained when the muscle was kept on ice instead. Up till at least 8 hrs after slaughtering a strong exothermal reaction in the 48-58°C region is observed. This peak is absent after 27 hours (at 20 as well as at 0°C). Also, the pattern of endothermal peaks changes with time after slaughter: The first observable peak (I) is shifted from 65 to 58°C and the second one (II) from 67 to 65°C. The relative sizes of the two peaks also change drastically. The major peak near 80°C (III) remains more or less constant in shape and position.

The factors responsible for the thermograms

The origin of the exothermal reaction in pre rigor meat is at present not clear. It is possibly caused by some super-activation of one or more metabolic enzymes, possibly breaking down ATP or some other high-energy compounds. The endothermal transition peaks could be due to the melting of lipids, nucleic acids, polysaccharides or proteins, or the breakdown of water structure (Karmas and DiMarco, 1970). Polysaccharides and nucleic acids probably do not occur in DSC-detectable amounts in muscle. Visible fat in cow shows a strong, complex melting peak between 29 and 45°C (not shown here), and it is improbably that significant amounts of higher-melting fats exists in the tissue, although this cannot be ruled out. The water structure hypothesis of Karmas and DiMarco (1970) for the major peak at 80°C appears unlikely, since we have found that the similar peak in eggwhite, on which the authors based their hypothesis, is in fact due to the unfolding of ovalbumin and other globular egg proteins.
Thus, we interpret the endothermal peaks in the meat thermograms as protein unfolding.

It is interesting to relate the thermogram shape to other known effects of heat on the physical and chemical properties of meat and isolated meat proteins. Many authors have reported drastic changes in the 30-50°C region for both whole meat and for isolated meat proteins. Hamm (1972) reports older data showing that the water binding capacity of whole meat starts falling already at 30°C and more drastically above 40°C, while the pH and the level of free divalent cations starts rising drastically at 40°C. Goodno and Swenson (1975 a, b) found by pH-studies a sharp transition in both isolated myosin and its subfragments, starting below 30°, with a maximum at about 40°, being finished at about 60°C. Burke *et al.* (1973) found by ORD studies on isolated myosin fragments two different transition regions, the lower of which started at about 30° and reached a maximum at 44°C. In our DSC thermograms of meat we find no major peak in the 30-50°C region when visible fat is avoided. The first peak observed in our meat thermograms starts at 49-50°C, rising rapidly into the first peak complex. This may correspond to the intermediate plateau between 50 and 55°C in the physical and chemical parameters of meat, reported by Hamm (1972), as well as to the second ORD transition region found by Burke *et al.* (1973) for isolated myosin subfragments, apparently starting a few degrees below 50°C, reaching a maximum at 55-52°C, and dropping sharply again just below 60°C.
Given our high scanning rate, it may also correspond to the plateau in the 45-50°C region in the decrease of easily available dye-binding groups in isolated actomyosin (Hamm, 1966). Thus, it appears that the unfolding of myosin contributes to the peak(s) in the 50-65°C range, possibly peak I.
Peak II resembles the single peak obtained from manually dissected intramuscular connective tissue from the same muscle, starting at about 61°C, reaching a maximum at about 66.5°C, tailing off at about 72°C (not shown here).
This also agrees well with other investigations of intact connective tissue (Mohr, 1971).

The soluble sarcoplasmatic proteins constitute a heterogenous mixture of heat labile proteins (Lee et al., 1974). Drip juice from thawing calf m. sterneomandibularis shows a complex thermogram (not shown here) starting at 55°C, with distinct peaks at 66 and 75°C, tailing off at 80°C.
Thus, soluble proteins may also contribute to the general "hill" in the meat thermogram. The peak near 80°C is quite constant and well defined in all meat samples investigated, and the peak appears to have significance for the sensory quality of the meat. According to data from Machlik and Draudt (1963), as summarized by Forrest et al. (1975), temperatures at or near the starting temperature of this peak causes a drastic hardening of the meat.
When the inner temperature of beef reaches about 60°C, the meat is "rear", when it reaches about 71-75°C it is "medium", and when it reaches 77-82°C it is "well done" (Forrest et al., 1975; Paul, 1975).
The identity of this peak is not clear. Possibly it represents the unfolding of actin, the only major muscle protein component not yet accounted for in the thermogram. According to Hamm (1972), the I-band (actin) is destroyed by heating meat to 70°C, as seen in electron microscope. The starting point of the peak in the DSC thermogram could possibly be as early as 70°C, but obscurred by the earlier peaks.

Differential Scanning Calorimetry (DSC) may increase our understanding of the molecular processes which take place in meat during heating, and may make it possible to distinguish between unimolecular protein unfolding transitions and multimolecular aggregation phenomena.

Litterature

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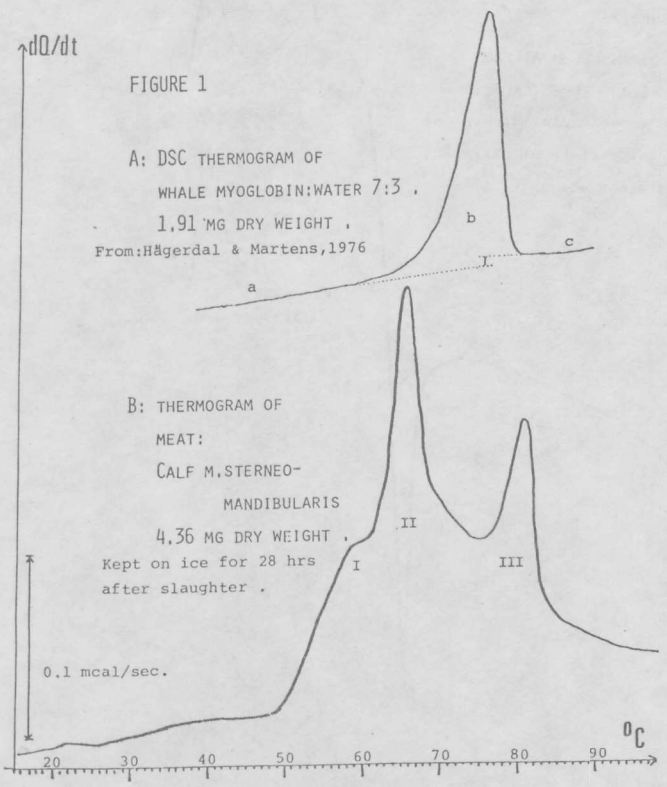

FIGURE 1