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DIFFERENTIAL CALORIMETRIC MEASUREMENTS OF CHEMICALLY TREATED MOHAIR IN CONJUNCTION WITH SMALL-ANGLE X-RAY DATA

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ABSTRACT

The structural features and decomposition reactions of mohair and chemically treated samples can be described in detail as a result of differential scanning calorimetry (DSC) measurements made in conjunction with small-angle X-ray data. α-Helix peaks can be observed in samples which have been treated with tributyl phosphine and pepsin; however, this endotherm is observed at lower temperatures compared with untreated mohair, depending on the extent of degradation due to the reduction of stabilizing cystine links. In contrast, samples which have only been nitrated show an order in X-ray measurements which cannot be observed in the DSC curve. Parallel measurements under atmospheric oxygen proved that the disulfide dissociation and subsequent oxidation are consecutive reactions which follow the fusion process of the α-keratin.

INTRODUCTION

Although research into the structural features of wool and other fibre keratins has been underway for some time, our knowledge about the submicroscopic structure of fibre keratin is still fragmentary. One way of obtaining further insight into the structure of fibre proteins is small-angle X-ray (SAX) investigation of chemically treated keratin. This leads to a defined modification of different structural areas which can be analyzed very well by means of SAX (as has been done by Spei et al. [1—3]). On the basis of these SAX investigations, observations by differential scanning calorimetry (DSC) in conjunction with SAX have been carried out in the present work. Thermoanalytical observations can assist in the acquisition of additional information about keratin properties. Earlier work (inter alia that of Crighton et al. [4—6]) describes physical and chemical transitions in keratin and modified keratin. This paper analyzes treated mohair samples which, based on the results of Spei et al. [1—3], provides special details of the structural properties of fibre keratin. In addition, enzymatically digested samples were investigated. Enzyme treatment of fibre protein represents a specific method of hydrolysis enabling a complete analysis of the amino acids as well as offering the possibility of tracing self-crosslinking reactions (isopeptide crosslinks) in heat-treated fibre proteins (as postulated elsewhere [7—10]). The results

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obtained from the thermoanalytical studies are correlated with the results from SAX experiments, the object being to determine the extent of correlation between these two methods and to procure additional information about the keratin structure and properties.

EXPERIMENTAL

Differential scanning calorimetry

A Perkin-Elmer DSC-1B calorimeter was used. The rate of heating was 16°C min^{-1} at a sensitivity of 5 mcal s^{-1}. The tests were conducted at ambient pressures and under a nitrogen flow of 30 cm^3 min^{-1}, which was dried over silica gel. The fibres were cut into lengths of 0.2 mm by means of a microtome in order to ensure optimum heat transfer between sample and sample pan. An empty aluminium pan served as a control reference. All pans were sealed with a lid. The sample weight was 2—5 mg.

X-ray tests

The tests were carried out in a Kiessig camera with a nickel-filtered copper $K\alpha$ beam (λ = 1.54 Å). MoS_2 was used as an inner standard [1].

Treatment of samples

South African mohair CSFH (diameter 37.5 μm) was extracted with methylene chloride followed by isoionic washing and drying at room temperature for 6 h in a vacuum oven (12 mm Hg).

Nitration

One g of dry mohair fibres was treated with 100 ml of a 1.0 N nitric acid solution at 60°C for 20 h. Two-hundred mg of these samples were treated (a) with 100 ml 0.001 N Na decysulfate solution at pH 4.0 (4 h at room temperature, 20°C), or (b) with 100 ml 0.01 N dodecylamine solution of pH 4.0 (22 h at room temperature).

Reduction and carboxymethylation

This treatment of the mohair samples followed that described by MacLaren et al. [11,12] with tributyl phosphine (TBP) and iodic acetic acid in buffered water—*n*—propanol solution. The desired degree of reduction was achieved by using different amounts of TBP. The determination of the degree of reduction (in this case 86 and 69%) was accomplished following the polarographic method of Leach [13].

Enzymic treatment

The enzymic treatment was carried out in a thermostatically controlled glass tube whilst being stirred gently (about 100 rotations min^{-1}) at 39°C.

Ten mg were suspended in 2 ml diluted HCl, pH 1.9 and, in order to avoid bacterial growth, small crystals of thymol were added. After addition of 2% pepsin (Fa. Boehringer/Mannheim), the digestion took 18 h [10].

RESULTS AND DISCUSSION

All observations were compared with untreated CSFH mohair as reference (see thermogram in Fig. 1). As shown by the works of other authors [14—16], the three processes, viz. dehydration, disordering of the α-helix and chemical changes, can be recorded from this sample. A broad and distinct endothermic profile, viz. loss of moisture from the fibre proteins, occurs in the initial heating range and runs on to approximately 180°C. By heating with continuous evaporation, a much sharper endotherm with a peak now observed at a considerably lower temperature, i.e. 50—80°C, has been reported by Crighton and Happey [17]. Similar endothermic peaks have been observed on heating inert gas flows, other proteins, peptides and polyamides. The shift of the major dehydration endotherm to lower temperatures can uncover a small endotherm at 140—150°C which has been linked to the release of those relatively small quantities of water strongly bound to hydrophilic sites [14,15]. In the present study, the major endotherm occurs at such a temperature under the nitrogen flow and 16 K min^{-1} heating, and is of such a size that the presence of this latter contribution to the net thermal effect cannot be identified.

Analogous tests on model peptides, in the case of hydrophilic side groups,

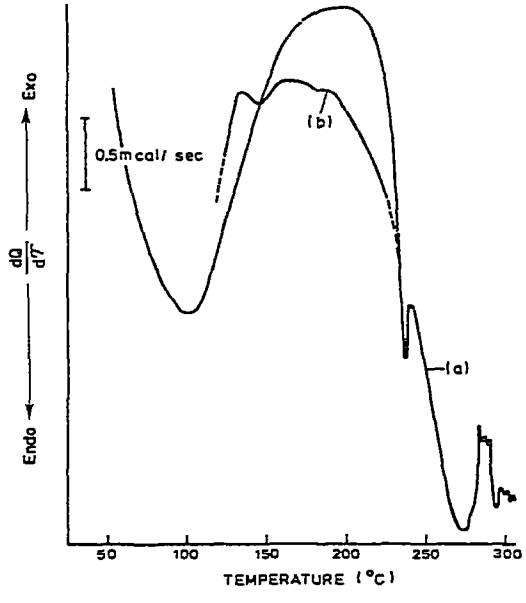


Fig. 1. (a) DSC curve of an untreated CSFH mohair sample, heating rate 16°C min^{-1}. (b) Schematic representation of the results obtained by Felix et al. [14] and Menefee and Yee [15].

manifest a continuous rather than a stepwise dehydration process [18]. Consequently, interpreting the dehydration process at 140—150° C in terms of different strong water bonds seems too simple an explanation. Also the relative low enthalpy of this process in view of the numerous hydrophilic side groups in fibre proteins should favour an extended interpretation.

One can argue that defined hydrophilic structural areas in the fibre protein embed water molecules, which are in a certain "trapped" stage. Increasing the temperature now leads to structural relaxation processes and conformational changes which then release the water molecules.

Consequently, this dehydration behaviour of the keratin is independent of the different strong physical forces between hydrophilic protein segments and water molecules, but dependent upon special structural features which enable the formation of stabilized water clusters. DSC studies in connection with wide-angle X-ray investigations on radiation grafted wool tend to support this view, showing that there are defined areas in wool which are penetrable only by very hydrophilic molecules [19].

The second-order transition found by Crighton and Happey [17] in the 165—180° C range, which associates with the glass or rubber-like transition in synthetic polymers, could only be detected in the nitrated sample [Fig. 3(a)]. In recent DSC [18] studies on model peptides similar transitions could be detected in the 50—80° C range. These transitions should be similar to those occurring in the case of the keratin, which are attributed to specific molecular conformation or rotation transitions of the side groups. The temperature difference is caused by the higher structure stability among fibre proteins due to crosslinking and fixation of intercrystalline peptide chains by crystalline segments. According to Crighton et al. [9], combined gas chromatography and mass spectrometry revealed the formation of water and ammonia in this temperature range. It can now be argued that due to the removal of structural barriers, an enhanced molecular mobility could lead to these glass-temperature-like transitions. A relationship could possibly exist between this physical phenomenon and the dehydration process discussed.

At 240° C we observe the melting of the α-helix occurring in all fibre proteins. Another endothermic shoulder which was reported to exist at 234° C and is associated with different thermal stabilities of the α-helices stemming from the different environments of the α-helices (i.e. the fibrillar region and the high-sulphur-content matrix) could not be detected. This interpretation can be substantiated by the fact that mohair is essentially composed of only one type of cortical cell. The fusion of the helical areas is accompanied by scission of the disulfide links under elimination of H_2S, followed by the commencement of splitting reactions in the side chains [20].

Investigations of low-sulfur molecular peptides confirm these results [18], i.e. the collapse of the physical structure, rapidly followed by decomposition of the sulfur bond. This process can be observed in the case of peptides and proteins as a single endothermic reaction, whereas in the case of fibre proteins with their heterogeneous structure, numerous different processes take place simultaneously, so that detection of a definite single process is not possible.

Thermogravimetic studies on mohair done by Hack [20] point to a

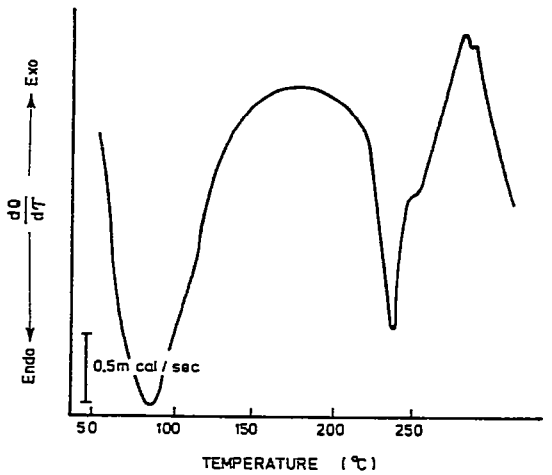


Fig. 2. DSC curve of untreated CSFH mohair in air.

defined weight decrease beginning at 220° C. Curie-point gas chromatography investigations by the same author [20] show unambiguously that H_2S can be traced at 250° C. One can now argue that the disruption of the helical structures, the cleavage of the sulfur bridge and the elimination of volatile sulfur products are almost simultaneous reactions. This conclusion can also be drawn in view of the fact that, contrary to the sample tested under nitrogen, a drastic exothermic shift takes place in the DSC curve after the fusion peak (Fig. 2). This is caused by the immediate oxidation of the sulfur fragments in the presence of air oxygen. The shoulder just after the fusion peak indicates that at this stage the endothermic dissociation of the sulfur bridges and the exothermic oxidation of these fragments contribute equally to the overall energy flow. Increasing the temperature, however, leads to a strong exothermic shift, showing that the oxidation reaction becomes the energetically prevailing reaction.

Considerable differences are observed when the treated samples are compared with the untreated material. In the case of the nitrated mohair samples (Fig. 3), the α-helix peak has either disappeared completely or is only vaguely discernible. It would be incorrect to assume that the ordered structure ranges are destroyed, as in the case of the crystalline to amorphous transition in synthetic polymers. X-Ray tests by Spei [1] on similar nitric acid treated mohair samples show however, that the meridional reflections of 66 Å (third order), 39 Å (fifth order) and 28 Å (seventh order) do not disappear completely, as would be expected if a defined structural conformation were completely removed. This observation can be explained by the fact that fragmentation of the cystine and oxidation yields SO_3 groups [21]. The newly formed cysteic acid residues can form strong salt bridges to the basic lysine and arginine residues [22]. By means of these new bridges the helical structure has been partly restabilized so that although the X-ray pictures measured at room temperature show a disturbed pattern, nevertheless a distinct picture is obtained [1]. These salt bridges, which exert their maximum stabilizing functions at room temperature, are destroyed by heating the

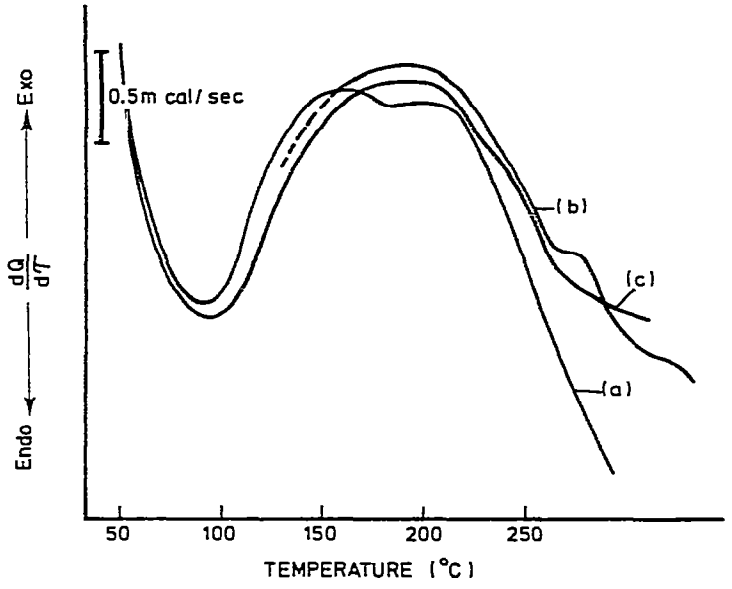


Fig. 3. DSC curves of treated wool samples. (a) 20 h with 1 N nitric acid at 60°C; (b) treatment (a) + 24 h with 0.001 N sodium decylsulfate solution at pH 4.0 and room temperature; (c) treatment (a) + 22 h at room temperature with 0.01 N dodecylamine solution.

samples in the DSC cell. This process cannot be detected, since the amount of heat generated in the reaction is too small to register on the apparatus. However, nitrated and detergent treated samples [Fig. 3(b) and (c)] still show two characteristic endothermic shoulders in the range 200 (sample c)—250° C (sample b).

The insertion of the detergent molecules (which for the first time led to the detection of the fundamental 198 Å periodicity on the SAX diffraction pattern) happens at a vertical orientation to the fibre axis [1,22]. The reaction of the nitrated mohair with the two detergents leads to a cleavage of the salt bonds; however, the relative stability, as observed by the DSC tests, points to a structure stabilization due to hydrophobic interactions of the isolated fibrils with the aliphatic residues of the detergent. The temperature difference of the two endothermic shoulders [Fig. 3(b) and (c)] can be interpreted in terms of the levels of interaction of the detergent (under the applied treatment conditions), and the heterogeneous structure of the fibre substrate with consequent effects on the thermal stability of the helix. The corresponding SAX studies reveal a meridional diffraction pattern with an enhanced 39 Å reflection. The 28 Å reflection is already weaker (Fig. 4). This substantiates the view that in fibre keratin there are predominant ionogenic amino acid residues followed by areas with hydrophobic ones. Thus an optimal stabilization of the fibre structure, as a consequence of salt bonds and hydrophobic interactions, is possible. Treating the fibre with an ionic detergent now leads to a certain restabilization of the partly disintegrated structure due to the re-established secondary interaction forces.

The treatment with tributyl phosphine (TBP) represents a specific scission

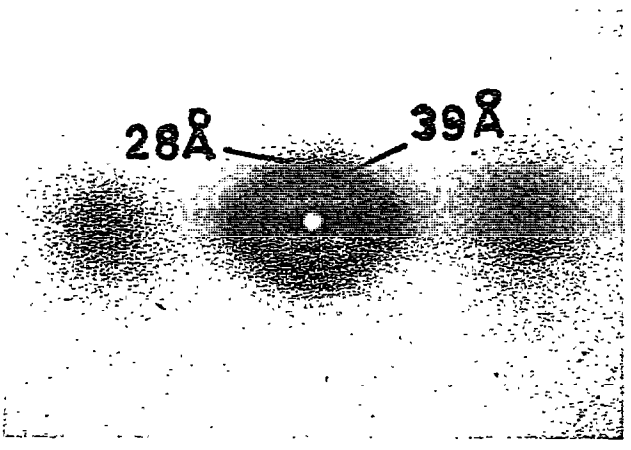
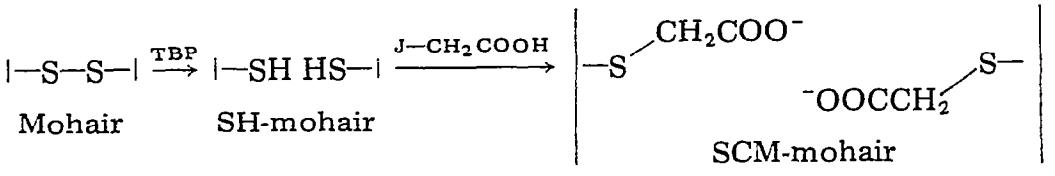


Fig. 4. SAX diagram of a nitrated and sodium-decylsulfate treated mohair sample.

method which allows a stepwise cleavage of the disulfide bonds. The thiol groups so formed (SH-mohair) can be alkylated with iodoacetic acid. The cystindisulfide bridges can be replaced by two S-carboxy groups (SCM-mohair).



The sample which was reduced by 69% has a "normal" DSC curve [Fig. 5(a)] with a shift of the fusion peak to 225°C. Although we have to consider certain cystine re-oxidation effects during the heating phase [6], the fusion temperature which is lower by 20°C than that of the untreated fibre protein, reveals the fundamental destabilizing effect that the reduced cystine has on the thermal stability of the helical areas. Analogous X-ray experiments (Fig. 6) show an unchanged small-angle diagram (as also found elswhere [2, 11,12]). From the comparison of these results with the nitrated sample, we can conclude that it is not only the cleavage of the disulfide bridges which causes the thermal instability, but also the electrostatic repulsion built up by the strong acidic sulfonic groups. This hypothesis can be substantiated by means of the reduced and carboxylated sample [Fig. 5(a)]. By forming additional carboxy groups we build up a similar electrostatic repulsion force to that formed by the sulfonic groups, although this effect is not all that strongly developed here. The introduction of carboxy groups should also exert a certain steric force on the conformational structure of the peptide chains. This leads to a decreased thermal stability with the disordering of the α-helix being observed at 20°C lower than normal. Corresponding SAX experiments show that the diffraction diagram is weak and diffused (Fig. 7). This was also observed in the case of 75% reduction grade [2]. The samples treated with pepsin have a relative high enthalpy value [Fig. 8(a)] in comparison with the sample which had been nitrated first [Fig. 8(b)]. The amino acid specificity

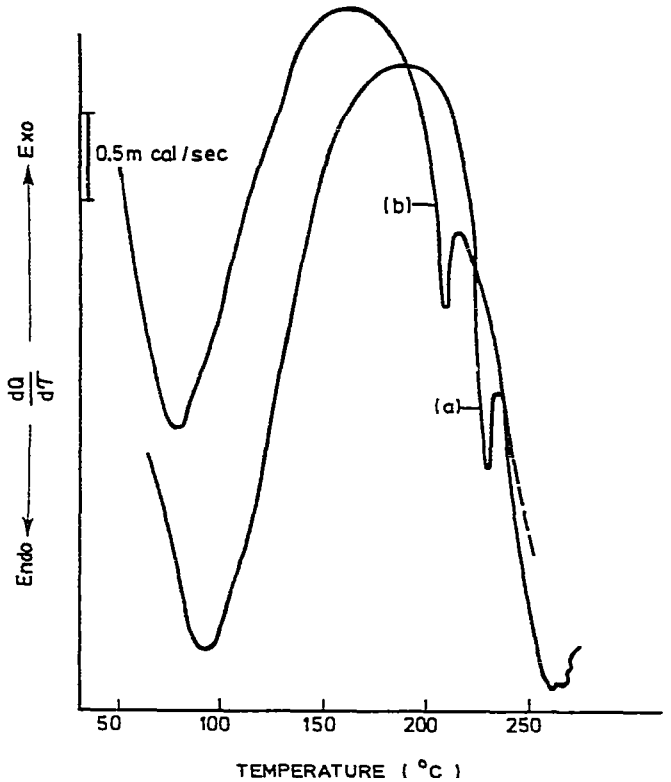


Fig. 5. DSC curves of CSFH mohair treated with tributylphosphine. (a) 69% reduced with tributyl phosphine; (b) reduced with tributyl phosphine and carboxylated with iodic-acetic acid.

of pepsin is not yet fully understood. The enzyme hydrolyses peptide bonds but not amides and esters. This specific hydrolysis method enables one to trace, in particular, isopeptide crosslinks which, according to some authors [7—9], are formed by heating the keratin. However, investigations by

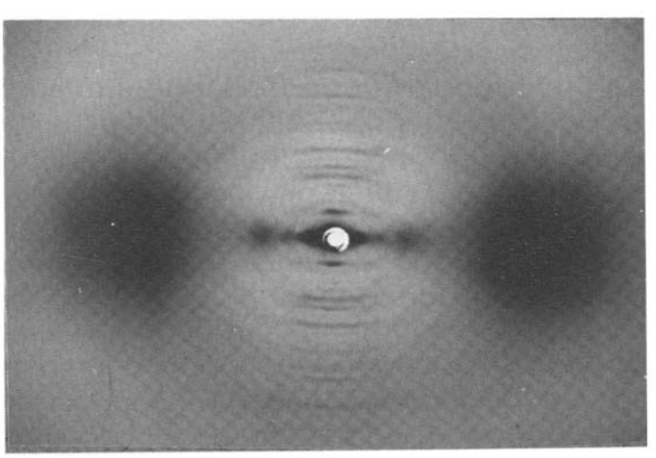


Fig. 6. SAX diagram of a tributyl phosphine reduced sample (reduction grade 69%).

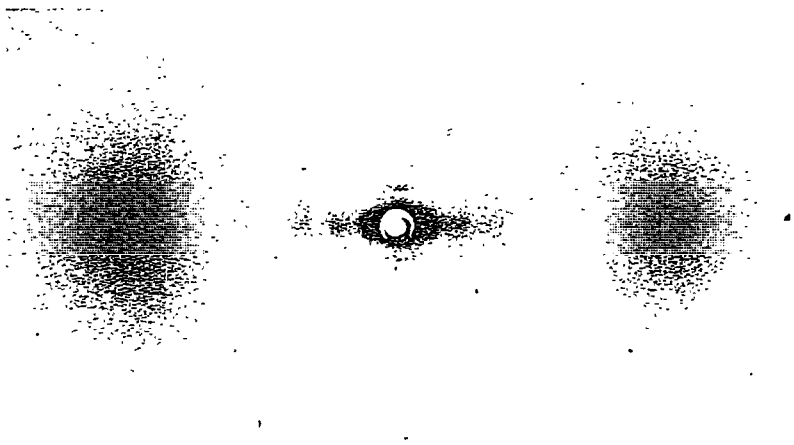


Fig. 7. SAX diagram of a mohair sample treated with tributyl phosphine (86% reduction grade) and carboxymethylated.

Schmitz [10] revealed, by means of this digestion technique, that self-cross-linking reactions and isopeptide formation (as postulated by a few authors) do not occur at all or, alternatively, occur to a much lower extent than stated. Starting with the principle that disulphide bridges are not hydrolysed by pepsin, the enthalpy differences of these two samples indicate the extent

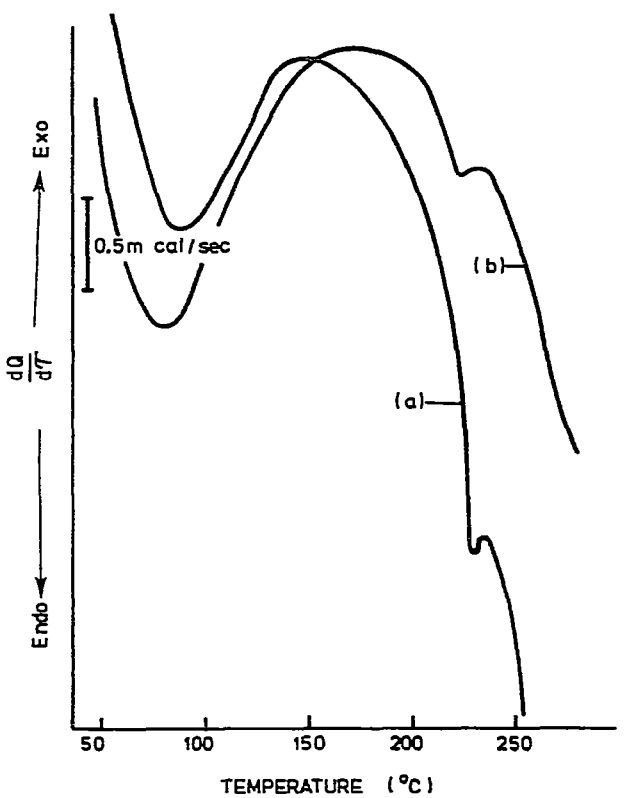


Fig. 8. DSC curves of CSFH mohair digested with pepsin (both under nitrogen). (a) Digested with 2% pepsin after 7.5 h at 37°C; (b) nitrated at 60°C with 1 N nitric acid for 20 h and then digested with 2% pepsin.

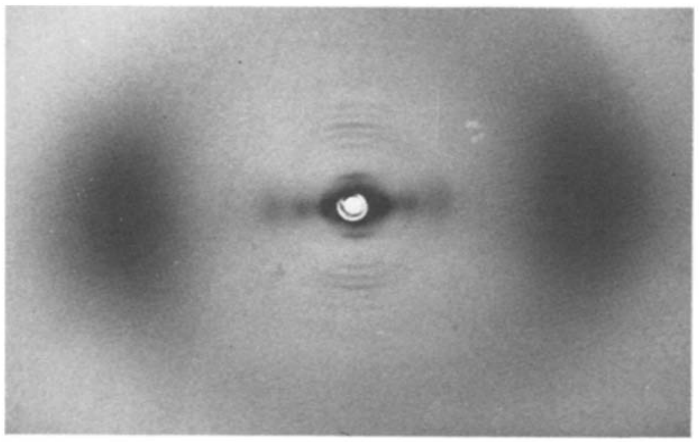


Fig. 9. SAX diagram of a nitrated and pepsin treated mohair.

to which disulfide bonds contribute to the α-helix stability. In the case of the prenitrated sample, disulfide bonds have been partly decomposed so that the subsequent digestion with pepsin can also occur in helical areas, which are normally impenetrable for pepsin. The X-ray scatter pattern of the nitrated and digested sample becomes distinctly weaker (Fig. 9) as compared with the virtually normal SAX diagram of only pepsin treated mohair (Fig. 10). This shows that, due to the nitration, the original helical areas are split into smaller parts which are partly disorientated.

CONCLUSIONS

Chemically and enzymatically treated mohair samples, which on the basis of SAX contribute immensely to a deeper understanding of keratin structures, can also reveal some interesting aspects by means of thermoanalytical studies. The degree of decomposition being caused by the described treat-

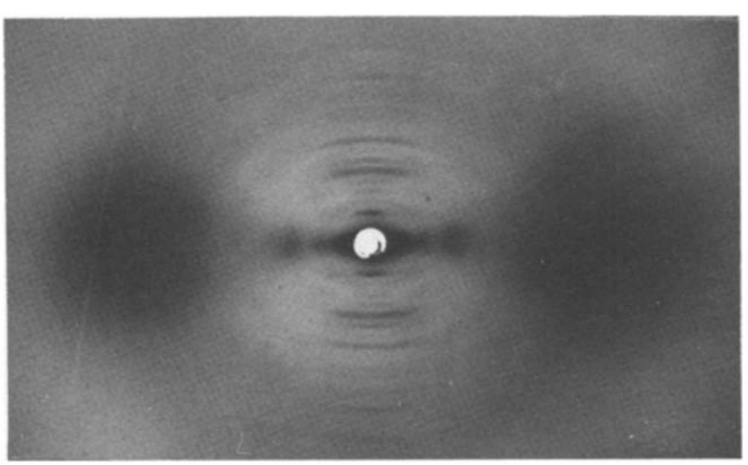


Fig. 10. SAX diagram of a pepsin digested mohair sample.

ments can be detected accurately. The results obtained from the X-ray experiments give a view of the structural state at ambient temperatures. It could be shown that these results can be correlated well with the results obtained from the corresponding DSC observations, which indicates the thermal stability of α-keratin. Thus we obtain further information about the kind of decomposition resulting from the different treatments and the structural features of the fibre protein. Although a complete correlation of the DSC data with all the available SAX data is impossible (since some of the interpretations in both fields are still in the realm of hypothesis), the combination of these two methods should, however, enable us to obtain further information on the keratin structure and a better basis for interpretation.

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