Available online at www.sciencedirect.com
SCIENCE DIRECT
Thermochimica Acta 447 (2006) 30–35
thermochimica acta
www.elsevier.com/locate/tca

Thermal analysis on parchments I: DSC and TGA combined approach for heat damage assessment

D. Fessas [a,*], M. Signorelli [a], A. Schiraldi [a], C.J. Kennedy [b], T.J. Wess [b], B. Hassel [c], K. Nielsen [d]

[a] *Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università di Milano, via Celoria, 2, 201333 Milano, Italy*
[b] *Biophysics Division, School of Optometry and Vision Science, Cardiff University ,Cardiff CF10 3NB WALES, UK*
[c] *The Royal Danish Academy of Fine Arts, School of Conservation, Esplanaden 34, DK-1263 Copenhagen K, Denmark*
[d] *Department of Chemistry Kemitorvet Building 207, Technical University of Denmark, DK-2800 Kgs Lyngby, Denmark*

Received 24 January 2006; received in revised form 11 April 2006; accepted 20 April 2006
Available online 29 April 2006

Abstract

Ancient, new and artificially aged parchments were investigated with both differential scanning calorimetry (DSC) and thermogravimetry (TGA). Criteria to define a quantitative ranking of the damage experienced by the bulk collagen of historical parchments were assessed. A damage-related correlation was found between the collagen denaturation temperature and the moisture content of the parchment. Qualitative rules for the evaluation of the damage at the nano-and mesoscopic level were achieved on the basis of peculiarities of the shape and width of the DSC signals and confirmed by small angle X-ray scattering patterns.

Keywords: Parchments; DSC; TGA; Damage assessment; SAXS

1. Introduction

Due to the historical value of ancient parchments, damage assessment is very important in conservation practice. This article was developed in the frame of the "IDAP" (improved damage assessment of parchment) European project that aims at developing new methods for the assessment of damage in historical parchments at the macroscopic, microscopic, mesoscopic, nanoscopic and molecular level [1].

Ancient parchments are handcrafts of animal skin. The main component of the skin connective tissue matrix is collagen, a family of related proteins [2,3]. Different collagen types are present in skin, the most abundant ones being types I and III, which belong to the subfamily of fibril-forming collagens [4]. The structure formed has a characteristic coiled coil triple helical conformation that is stabilized by weak non covalent interchain bonds, mainly hydrogen bonds, and the result is a thin, long, rod-like molecule with a length of 300 nm and a diameter of about 1.1 nm. On a higher organization level, the collagen molecules are aligned side by side in a quarter staggered array with parallel main axis, forming fibrils. These supra-molecular structures at the fibril level are stabilized by further cross-links [5] and produce characteristic banding patterns viewed by electron microscopy upon staining [6]. To date electron microscopy, X-ray diffraction and to a lesser extent atomic force microscopy have been the major tools used to investigate the structural hierarchies of collagen based tissues.

Thermal analysis techniques (mainly calorimetric) are particularly suitable to investigate the stability of biological macromolecules (collagen in the case of parchment) as it allows detection of every state of modification within the system (e.g., protein denaturation and aggregation, gel-sol transitions, crystallization, fusion, glass transition, etc.) when it undergoes some temperature change.

A number of works have so far been devoted to the study of collagen and its aging [7], but only few of them deal with parchments [8,9]. Furthermore, most of the literature data about thermal analysis of parchments concern DSC investigations on aqueous suspensions of minutely cut material. The information drawn is mainly related to the triple-helix-to-random-coil

* Corresponding author. Tel.: +39 0250316637; fax: +39 0250316632.
E-mail address: Dimitrios.Fessas@unimi.it (D. Fessas).

0040-6031/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2006.04.007

conformational transition of collagen, which is, however, strongly affected by the environmental conditions, namely concentration, pH and ionic strength of the medium, as well as by the content of conformationally restricted amino-acids, such as proline and hydroxyproline [10].

Calorimetry on parchment samples in excess of water therefore allows the assessment of the damage at molecular level experienced by the collagen especially when the DSC data (transition enthalpy, transition temperature, shape of the signal) can be correlated with specific changes of other chemical and physical properties of the material (e.g., depletion of some amino acid, cross linking degree, etc.) determined with different techniques, such as HPLC and amino acid analysis [8]

Changes of the state of the parchment produced by a damaging process probably alter every level of the structural hierarchy. The changes at the mesoscopic and macroscopic level are modified or lost when the sample is dispersed in water. Changes at these levels are however worth assessing since the macroscopic integrity of parchment is dependent on the integrity at all the other structural levels and a more complete picture of damage can lead to better preservation techniques. The study of parchment samples in the intact state (i.e. without any previous treatment related to the experimental technique used), that can modify their structure, is therefore desirable.

To this aim, ancient, new and artificially aged parchments were investigated, without further preparation, with both differential scanning calorimetry (DSC) and thermogravimetry (TGA). Collected data encompassed a very wide range of moisture content up to conditions not far from those of the aqueous suspensions considered in previous studies. A bridge between the results obtained from “dry” and “aqueous” parchment samples could therefore be envisaged.

The scope of the study was the assessment of criteria on intact parchment that allow definition of a quantitative damage degree in order to rank the damage experienced by the bulk collagen of historical parchments. The nano/meso structure of the parchment was characterised with small angle X-ray scattering (SAXS), in order to correlate changes of structural features at this level with alterations in the thermochemical signals.

2. Materials and methods

2.1. Materials

Ancient (16th century), new and artificially aged calf parchment were supplied by the Royal Danish Academy of Fine Arts, School of Conservation. The artificial accelerated aging was aimed at mimicking potential heat damages that ancient parchments can experience in their long shelf life. For the present work the heat treatment of freshly prepared parchments was produced in drying oven for 48 h at 60, 80, 100, 120, 140, 150, 160, 165 and 170 °C. Details of these procedures are reported elsewhere [8].

2.2. DSC investigations

The instrument used was a DSC 6 (Perkin–Elmer, USA) operating with sealed cells (an empty cell was used as the reference). The typical sample was a small (about 5 mg mass) piece of a new or artificially aged parchment sheet. Although small, such a sample had both the grain and the flesh side of the parchment. Heating runs in the temperature range 20–150 °C at 1 and/or 5 °C min^{-1} scanning rate were performed with at least two replicas for each run. The raw data were analysed with the software IFESTOS [11] in order to obtain the excess (with respect to the native state) heat capacity of the sample, Cp^{exc} (T), J K^{-1} g^{-1} (per gram of sample).

2.3. TGA investigations

TGA was used to assess the sample moisture and water release during the temperature scan. The instrument used was a TG-DSC 111 (SETARAM, France). Measurements were performed on new and artificially aged parchments at 2 °C min^{-1} heating rate in the 20–200 °C temperature range. A typical DTG trace (i.e. the time derivative of the mass loss), of a parchment sample shows a broad peak with an underlying area that corresponds to the overall water content of the sample (the heat flow signal is used to check that the mass loss is related only to water release; decomposition phenomena start at higher temperatures). The position of the DTG peak maximum and the shape of the peak are related to the state of water within the sample [12] and therefore are a reliable parameter when comparing different samples. To simplify this evaluation, the TGA data collected in the present work were normalized with respect to the overall moisture of the sample to 100 mg of water. Accordingly the DTG traces were expressed as milligrams of lost water per degree K (with reference to the scanning rate used). Each run was repeated at least twice.

2.4. SAXS investigations

For small angle X-ray scattering (SAXS) measurements, the samples were loaded into the sample chamber of the NanoSTAR (Bruker AXS, Karlsruhe) X-ray facility at the University of Cardiff. The data collection procedure was the same as that described in detail by Wess et al. [13] Scattering profiles were taken over 6 h exposures using a sample-to-detector distance of 1.25 m. Collected data were corrected for camera distortions, a background image was subtracted, and images were analyzed with an in-house software. The two-dimensional detector output was converted into one-dimensional profile.

3. Results

Fig. 1 reports the DSC traces of samples from two intact historical parchments and represents a typical example of the data observed with this technique. Curve a shows only one sharp endothermic peak at a rather high temperature (above 100 °C), whereas curve b shows three phenomena, a high-T peak with a shoulder in the rising branch, and a complex signal, at low temperature (around 55 °C), with endothermic and exothermic components. Similar data have been reported in the literature [14].

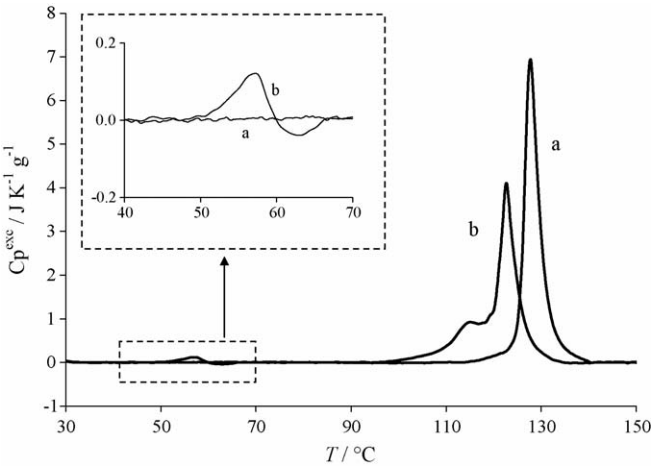

Fig. 1. DSC traces of samples from two "as is" historical parchments (scanning rate 5 °C/min).

Fig. 2 shows DSC traces obtained from samples of new parchments (either of the same or of different animal origin), which apparently are characterized by different position and intensity of the main endotherm.

To define the information related to the collagen thermal stability, new parchment samples with different moisture contents were compared (Fig. 3). Curves a–c concern excess, 64% and native moisture content, respectively. The first curve is typical of collagen aqueous suspensions, the endotherm concealing a number of molecular details relevant to the unfolding and denaturation of the collagen molecule. Detailed investigation on these processes is beyond the scope of the present work. Here it is sufficient to say that the position of the overall peak is shifted toward higher temperatures on decreasing the moisture content. The shape of the peak becomes without shoulders as in a cooperative process. The enthalpy remains substantially unmodified ($43 \pm 2\,J\,g^{-1}$) although a slight increase (about $2–3\,J\,g^{-1}$) is found in parchment samples with their original moisture content (curve c in Fig. 3) These stabilizing effects can come from the close environment of the collagen, namely the local concentration of macromolecules and the molecular architecture at mesoscopic level (see below).

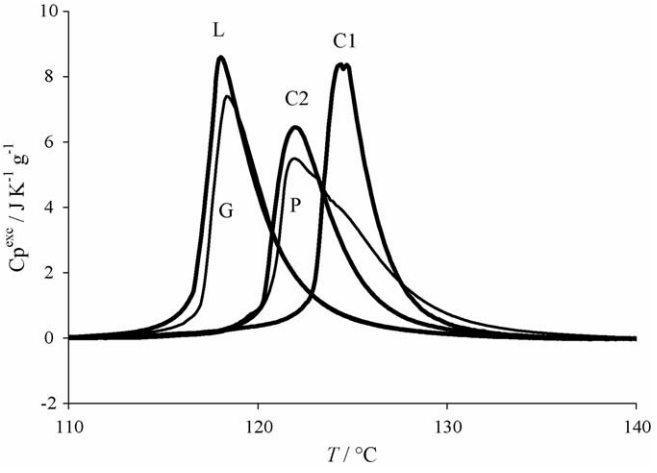

Fig. 2. DSC traces of samples of new parchment. Calf (C1 prepared in 1985, C2 prepared in 2000); goat (G); pig (P), lamb (L) (scanning rate 5 °C/min).

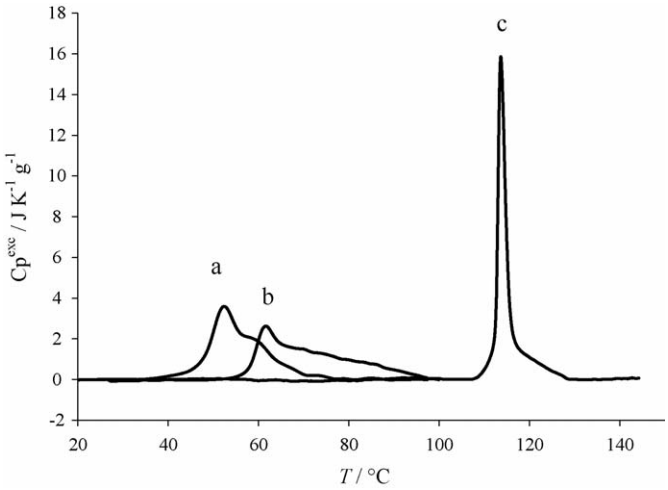

Fig. 3. DSC traces of new parchments samples with different moisture contents. Curves a–c concern excess, 64% and native moisture content, respectively (scanning rate 1 °C/min).

In order to single out these effects from those related to possible damages experienced by the material (e.g., for historical parchments), the behaviour of new undamaged parchments was assessed. To this end, TGA and DSC investigations were performed on parchment samples with different moisture contents. High moisture levels were obtained by adding distilled water to the sample in the DSC pan, while lower moisture levels corresponded to the endogenous water content of the parchments (which was assessed by TGA).

Fig. 4 shows the correlation between the temperature of the peak maximum, T_d, for a given heating rate, and the moisture content. The trend follows a decreasing straight line for moisture contents below 40%. For larger moisture content the decrease

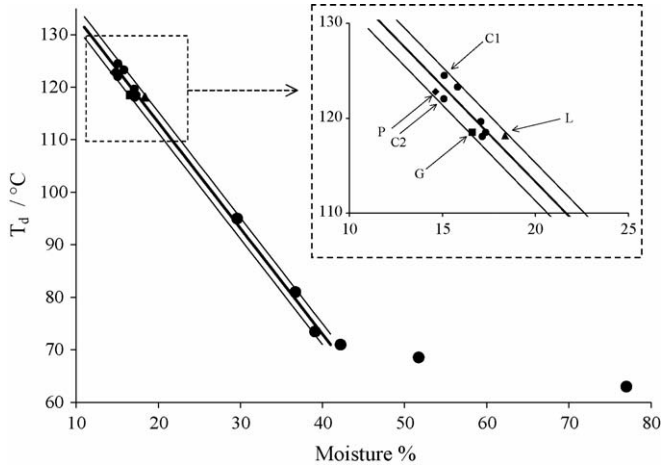

Fig. 4. Correlation between T_d, (from DSC, 5 °C/min heating rate) and moisture content. Full circles are calf parchments equilibrated at different moisture contents. Data within the insert are from "as is" samples. Lettering is the same as in Fig. 3, i.e. calf prepared in 1985 (C1); calf prepared in 2000 (C2); goat (G); pig (P), lamb (L). The straight line correlation for data below 40% moisture is described by the equation T_d (°C) = −2.0089 × (%moisture) + 153.37, with $R^2 = 0.9965$.

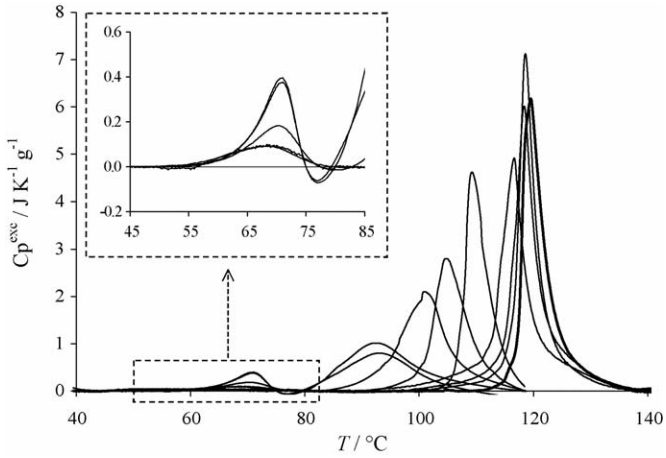

Fig. 5. DSC traces from new parchments that had experienced heat damage of various extent. The heavy line corresponds to the untreated reference parchment. From right to left, increasing treatment temperature. The insert shows the increasing intensity of the low T signal that starts to appear for treatment temperatures above 120 °C. The traces reported in the insert correspond (from bottom to top) to treatments at 120, 140, 150, 160, 165 °C.

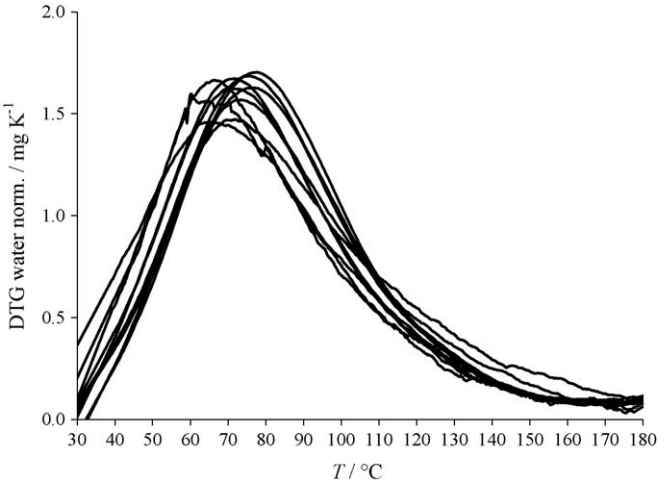

Fig. 6. DTG traces from the heat treated parchments. The moisture content of these samples is assessed after several months storage after the heat treatment. DSC analysis of the samples was performed at the same time.

of T_d becomes much weaker. The slope of the correlation line depends on the DSC scanning rate. In view of a future application to a large number of historical parchments, we chose 5 °C/min as a compromise between resolution and measurement time.

Fig. 5 shows DSC thermograms obtained from new parchments that had experienced heat damage of various extent (see Section 2). On increasing the heat damage, the main endothermic signal moves toward lower temperature and its shape broadens. Up to a heat treatment temperature of 100 °C an almost constant value of the enthalpies ($41 \pm 2\,J\,g^{-1}$, gram of dry matter) was observed. For higher temperature treatments a decreasing trend was observed (the lowest enthalpy value, $12 \pm 2\,J\,g^{-1}$, was observed for the sample treated at the highest temperature, 165 °C) which means that, beyond the 100 °C threshold, the artificially heat-treated samples contain smaller amounts of residual native collagen. Severely damaged samples showed an extra signal around 70 °C (see insert) the intensity of which increases with the damage extent.

The shift of the DSC peak maximum was correlated with the moisture content of the samples as assessed by TGA. Fig. 6 shows the relevant DTG traces. The close similarity among these traces suggests that different heat damage did not cause differences in the mechanism of dehydration and that water distribution was homogeneous through the sample (Fig. 7.).

The T_d versus moisture plot showed substantial deviations from the straight-line trend observed for the undamaged materials (see Figs. 4 and 7). The difference, ΔT, between the observed T_d and that expected according to the straight-line correlation is shown in Fig. 8 and may be used as a damage index. However, for treatment temperatures below 100 °C, ΔT became insignificant. This fact is in line with the above discussion on the enthalpies data. Similar threshold effects are reported in the literature [15]. This threshold temperature is just below the onset temperature of the collagen denaturation observed by DSC in the reference intact parchment (see Fig. 5).

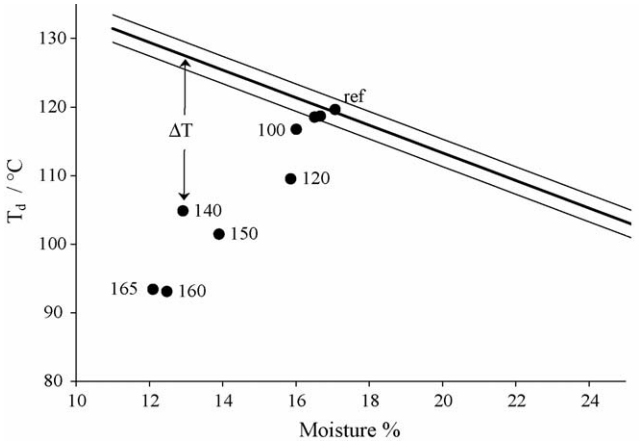

Fig. 7. T_d vs. moisture plot. Substantial deviations from the straight-line trend (see Fig. 4) were observed for the heat-treated parchments (lettering indicates the treatment temperature in °C). ΔT is the difference between the observed T_d and that expected according to the straight-line correlation.

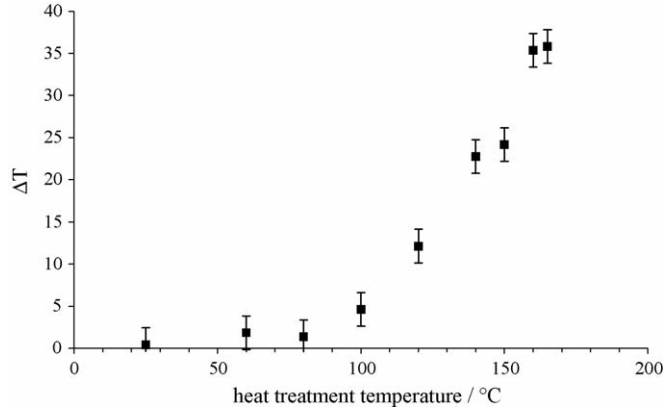

Fig. 8. ΔT vs. the heat treatment temperature. The trend may be used as a damage index.

Data on alteration in the collagen axial lattice came from SAXS experiments performed on heat-treated samples which showed modulations to the characteristic diffraction pattern of dry collagen [16,17]. The main features observed were the collagen meridional series, which give an indication of the periodicity and crystallinity within collagen fibril axial order. The axial spacing of dry collagen results in a 64 nm lattice with the exact value dependent on heating. Up to eleven orders of diffraction were observed with the sixth and ninth orders displaying the highest intensity. When associated in fibrils, the collagen molecules (approximately 300 nm in length) display a molecular alignment based on a relative stagger (*D*-spacing) of 65.5 nm in skin.

Measurements on the heat treated samples, in selected regions where some collagen is still bunched in fibrils, showed evidence of a reduced *D*-spacing after heat treatment, namely, 61.1, 60.3, 59.96, 60, 59.96, 57.78, 57.58, 57.11 and 55.79 nm for:- untreated and 60, 80, 100, 120, 150, 160, 165 and 170 °C heat treated samples, respectively. The most obvious modifications to the axial structure occurred at temperatures higher than 120 °C. The breadth of the diffraction peaks also gives important information about the extent of crystallinity or the variability of the lattice parameters. The paucity of the data observed here does not allow a conclusive decision to be made as to the physical basis for broadening. However variability of the collagen axial period is rarely observed and it is more likely that a significant reduction in the lattice coherence contributes to Bragg peak broadening. In the sample here, fitting of the peak as a Lorenzian function and applying the Sherrer equation showed that the axial coherence of the scattering unit decreased as a function of temperature. In the control sample, the extent of coherence is greater than the sensitivity of the technique, however heating regimes reduced the axial coherence to that of 350 nm at D60 and 250 nm at D170, the latter corresponding to about four unit cells.

4. Discussion

The data reported in the Figs. 1 and 2 show that, in spite of the common overall aspect of the DSC traces, some substantial differences relevant to the temperature of the peak maximum and its enthalpy can be often found when parchment is of different animal origin, or from different lots of the same animal source, or different regions of a given skin are considered, even in the case of freshly produced materials.

This apparent discrepancy clearly indicates that the DSC approach cannot be used blindly, but requires basic criteria that allow a quantitative interpretation of the real phenomena and the elimination of, or accounting for parasite effects. For example, enthalpy is an extensive quantity, i.e. it depends on the actual content of collagen in the skin samples considered.

Since the scope of the work is the definition of a damage index based on DSC data collected from intact samples for historical parchments which may have largely different collagen contents (with no reliable "standard" material), it seemed more reasonable to focus the attention principally on the denaturation temperature, which is an intensive parameter.

The data reported in Fig. 4 show a decreasing straight line correlation between the temperature of the peak maximum, T_d, and the moisture content (for moisture contents below 40%) in the case of new intact parchments. The slope of the correlation line depends on the DSC scanning rate since kinetic effects are present during the collagen denaturation in "as is" parchments (the transitions are irreversible) which makes the position of the signal dependent on the heating rate of the DSC run.

The data (T_d versus moisture) relevant to the thermograms reported in Fig. 2 are aligned along the straight-line region, irrespective of the animal origin of the parchments (see insert in Fig. 4). This finding suggests that such a correlation may be used as a rule to identify undamaged materials and attribute observed deviations to extra damage experienced by the parchment.

The application of this method (Figs. 5, 7 and 8) to samples of new parchments, that had experienced heat damage of various extent, allows definition of an onset temperature threshold and quantitative evaluation (although phenomenological) of the damage produced by the heat treatment (for a given treatment time).

This evidence may support the tentative interpretation that the denaturation of collagen observed with the DSC in the damaged parchment can take place within a changing environment: a lower T_d would reflect a decreased stability of the residual native (not denatured during the previous artificial damage) molecules, while the broad shape of the peak and/or the presence of shoulders (Fig. 5) would correspond to an increased dispersion in terms of stability of the still intact fibres.

As for the signal at lower temperature, which increases in more severely damaged parchments, one has to take into account that it is indeed the convolution of endo-and exo-thermic effects (see insert in Fig. 5). The same kind of signal was already observed [9] for the ancient parchments from the Libreria Nazionale (Turin, Italy) which partially escaped the disaster of an inferno that completely destroyed many valuable tomes. This signal was supposed to reflect modifications of the mesoscopic structure of the parchment, as confirmed by TEM investigations [9].

As already mentioned, denaturation enthalpy, determined from DSC investigations of "as is" samples, is not an appropriate heat damage index for historical parchments. Nonetheless denaturation enthalpy changes can be considered in different experimental conditions, e.g. microcalorimetry investigations on parchment samples in excess of water aimed at achieving an estimation of the damage. However, also in this case, well reliable criteria must be defined to use the experimental data. For this reason a separate work is in preparation and has been devoted to discuss these aspects on the basis of microcalorimetry data combined with those of amino acid analysis.

5. Conclusions

Combined DSC and TGA data allow assessment of the overall bulk damage experienced by parchments. The criteria to manage "as is" parchment samples have been defined. A correlation between the denaturation temperature, T_d, with the moisture content allows a quantitative ranking of the damage experienced.

Qualitative evaluation of the changes at the mesoscopic level can also be achieved with SAXS investigations.

Acknowledgement

This work was carried out within the framework of the IDAP (Improved Damage Assessment of Parchment) EU Project EVK4-2001-00061.

References

[1] R. Larsen, D.V. Poulsen, F. Juchauld, H. Jerosch, M. Odlyha, J. de Groot, Q. Wang, C. Theodorakopoulos, T. Wess, J. Hiller, C. Kennedy, G. Della Gatta, E. Badea, A. Masic, S. Boghosian, D. Fessas. Damage assessment of parchment: complexity and relations at different structural levels, in preprints ICOM-CC 14th Triennial Meeting, September 12-16 2005. The Hague, The Netherlands, pp 199-208.
[2] D.J. Prockop, K.I. Kivirikko, Annu. Rev. Biochem. 64 (1995) 403–434.
[3] D. Parry, Advances in Protein chemistry: Fibrous Proteins: Coiled-Coils, Collagen and Elastomers, vol. 70, Academic Press, 2005.
[4] K.A. Holbrook, L.T. Smith, in: P.M. Royce, B. Steinmann (Eds.), Connective Tissue and its Heritable Disorders. Molecular, Genetic and Medical Aspects, Wiley, New York, 1993, p. 51.
[5] D.R. Eyre, M.A. Paz, P.M. Gallop, Annu. Rev. Biochem. 53 (1984) 717–748.
[6] J.A. Chapman, D.J.S. Hulmes, in: A. Ruggeri, P.M. Motta (Eds.), Ultrastructure of The Connective Tissue Matrix, Boston, Nijhoff, 1984, p. 205.
[7] F. Flandin, C. Buffevant, D. Herbage, Biochim. Biophys. Acta 791 (1984) 205–211.
[8] B. Hassel. Examination of Heat Damaged Parchment', Master Thesis. School of Conservation The Royal Danisch Academy of Fine Arts, Copenhagen, 2001.
[9] D. Fessas, A. Schiraldi, R. Tenni, L. Vitellaro Zuccarello, A. Bairati, A. Facchini, Thermochim. Acta 348 (2000) 129–137.
[10] P.L. Privalov, Adv. Protein Chem. 35 (1982) 1–104.
[11] D. Fessas, A. Schiraldi, J. Therm. Anal. Calorim. 61 (2000) 411–423.
[12] D. Fessas, A. Schiraldi, Food Chem. 72 (2001) 237–244.
[13] T.J. Wess, M. Drakopoulos, A. Snigirev, J. Wouters, O. Paris, P. Fratzl, M. Collins, J. Hiller, K. Nielsen, Archaeomerty 43 (2001) 117–129.
[14] G. Della Gatta, E. Badea, R. Ceccarelli, T. Usacheva, A. Mašić and S. Coluccia. Assessment of damage in old parchments by DSC and SEM. J. Therm. Anal. Calorim. 82 (2005) 637–649.
[15] R. Ronald, Ancient Skins, Parchments and Leathers, Seminar Press, New York, 1972.
[16] A.J. Hodge, J.A. Petruska, Recent studies with the electron microscope on ordered aggregates of the tropocollagen molecule, in: G.N. Ramachandran (Ed.), Aspects of Protein Structure, Academic Press, New York, 1963, pp. 289–300.
[17] T.J. Wess, J.P. Orgel, Thermochim. Acta. 365 (2000) 119–128.