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Analysis of the molecular mobility of collagen and elastin in safe, atheromatous and aneurysmal aortas

Analyse de la mobilité moléculaire du collagène et de l'élastine dans les aortes saines, athéromateuses et anévrismales

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A B S T R A C T

Aim of the study. – In this study, we propose to use a thermal technique, Differential Scanning Calorimetry (DSC) to follow the evolution of elastin and collagen in safe and pathological cardiovascular tissues.
Patients and methods. – The first part of this study deals with the analysis of the elastin network and associated proteins during ageing (from children to old persons) in aortic walls. The second part is devoted to the characterization of the collagenic phase in aneurysms. In both cases, physical data are correlated with biochemical analyses.
Results and conclusion. – For old persons aortas with atheromatous stades, elastin and associated proteins are found to interpenetrate to form a homogenous phase. Abdominal aortic aneurysms (AAA) are characterized by structural alterations of the aortic wall resulting from the degradation of elastic fibers and an increase of collagen/elastin ratio. Notable modifications are evidenced between collagen from control tissue and collagen from AAA, particularly concerning the thermal denaturation. Biochemical and thermal results are compatible with the increase of new collagen deposition and/or impairment of the collagen phase stability in the extracellular matrix of AAAs.

Mots clés:
Transition vitreuse de l'élastine
Dénaturation du collagène
Analyse thermique
Anévrisme
Vieillissement
Paroi artérielle

R É S U M É

But de l'étude. – Nous nous proposons dans cette étude d'utiliser une technique d'analyse thermique, l'analyse diatherme à balayage (ADB) pour suivre l'évolution de l'élastine et du collagène dans des tissus cardiovasculaires sains et pathologiques.
Patients et méthodes. – La première partie de l'étude concerne le réseau élastique et les protéines associées dans la paroi aortique durant le vieillissement (depuis les jeunes enfants jusqu'aux personnes âgées). La seconde partie est consacrée à la caractérisation de la phase collagénique dans les parois d'anévrisme. Dans les deux cas, les données physiques sont corrélées aux analyses biochimiques.
Résultats et conclusion. – Pour les aortes des personnes âgées avec stade athéromateux, l'élastine et les protéines associées forment un réseau interpénétré assimilé à une phase homogène. Les anévrismes aortiques abdominaux (AAA) sont caractérisés par des variations structurales de la paroi aortique résultant de la dégradation des fibres élastiques et d'une augmentation du ratio collagène/élastine. Des modifications importantes sont mises en évidence entre le collagène des tissus témoins et le collagène issu d'anévrisme, en particulier pour le phénomène de dénaturation thermique. Les résultats biochimiques et thermiques sont compatibles avec l'augmentation du dépôt de collagène néosynthétisé et/ou l'altération de la stabilité thermique de la phase collagénique dans les AAA.

1. Introduction

Arteriosclerosis accompanies the normal or early ageing of arterial wall; the most visible manifestations are a decrease of cross-linked elastin biosynthesis, a fragmentation of elastic fibres and a thickening of the intima [1]. Accumulation of lipids such as cholesterol and cholesterol esters, which possess a strong affinity for elastin, constitutes one of the main causes of arteriosclerosis [2,3]. Lipid absorption leads to a loss of elasticity and enhances elastase activity [4]. Moreover, calcium fixation on peculiar sequences of elastin, well known as a promoter for elastolysis [5], is favoured by lipids accumulation and vice versa [6]. Early elastin expression and organization could also modify arterial ageing through their impact on both vascular cell physiology and structure and mechanics of blood vessels [7]. All these factors are important for arterial ageing. In general, arteriosclerosis precedes atherosclerosis. Extensive research has been done to determine the complex pathophysiology of atherosclerosis, although mechanisms for various aspects are still being elucidated. The advanced atherosclerotic lesion is characterized by accumulation of extracellular lipids, development of a lipid-rich necrotic core, formation of a fibrous cap and calcification. Atheroma plaques can lead to aneurysms, exposing the patient to a double risk of rupture and embolic migration [8]. Nevertheless, it must be pointed out that atherosclerosis and abdominal aortic aneurysms (AAA) are two distinct pathologic entities with different risk profiles [9,10]. Most patients with cardiovascular diseases do not develop AAA, and many AAA patients do not have peripheral carotid or coronary diseases [11].

Aortic aneurysm, responsible for 2% of human deaths in industrialized nations, is preferentially located on the infrarenal aorta-iliac artery. This peculiar localisation could be due to the decrease of the elastin/collagen composition from the suprarenal aorta to the infrarenal aorta, associated with a thickening of intima and adventitia [12,13]. The pathogenesis of AAA remains uncertain, and the lack of definitive insight suggests a complex, multifactorial process [14].

Electron microscopy and NMR studies [15,16] confirmed that elastic fibre is the primary site of degeneration within the arterial wall, but the role of the different constituents of the extracellular matrix in aneurysm is not totally understood. AAA are characterized by structural alterations of the aortic wall resulting from the degradation of collagen and elastin, and several studies suggest that aneurysm formation in patients with atherosclerosis is related to the degree of expression of matrix metalloproteinases (MMPs), particularly MMP-2 [17] and MMP-9 [18–20], which show strong elastinolytic activity. A high concentration of MMP-8 could represent a potent pathway for collagen degradation in aneurysm [21]. A mixture of MMPs and cysteine collagenases could lead to the degradation of the fibrillar collagens of adventitia and media in AAA, which are highly resistant to proteolysis [22].

First studies reported a significant reduction in the elastin and collagen contents of aneurysms when compared with normal and atherosclerotic aortas [23], while more recent works bring to the light an important decrease of the relative quantity of elastin [24]. This decrease could be explained by a dilution of the elastin fraction (E fraction) with other components, the absolute amount of elastin remaining the same [24], or slightly greater [25]. Most of studies do not evidence a loss of cross-links, which would favour a degradation of mature elastin in AAA [25,26], but indicate an increase of collagen [14,24,25], which is considered as the major component of atheroma plaque. However, the evolution of the ratio between type I collagen and type III collagen is variable according to different reports [27,28]. Alteration of mechanical properties of aneurysmal artery has been associated with modification in collagen organization and deposition [29]. Structural alterations of the adventitial collagen organization have been evidenced in aneurysmal wall [14]. Investigation of vascular changes and protein composition by spectrometry techniques have been initiated and led to substantial information on extracellular matrix proteins alterations in aneurysm [15,16,28].

In this work, we have adapted a classical technique of characterization of synthetic polymers to pathological and unaffected cardiovascular tissues, in order to bring supplementary information on the possible evolution of collagen and elastin physical properties during ageing and in atheromatous arteries.

2. Patients and methods

2.1. Preparation of tissues for analysis

2.1.1. Thoracic aortas

Human aortas were collected within 4 hours of death from 11 patients of both sexes and separated in three groups:

- children (three samples) aged from 3 to 9 months;
- adults (four samples) aged from 24 to 32 years;
- old persons (four samples) aged from 66 to 86 years.

The atheromatous state was evaluated according to the classification of the World Health Organization (stages 0, I, II, III, IV). Elastin was extracted from the media of thoracic aorta in each sample according to the sequential enzymatic method of Ross and Bornstein [30] slightly modified by Spina [31], in a zone without atheromatous lesions. Biopsies were first decalcified with EDTA (EDTA 0.2 M, Tris-HCl 0.1 M) by two 24 h suspensions at 4 °C, pH 7.4. Then, EDTA was replaced by Guanidine HCL 6 M, EDTA 0.4% for a two 24 h suspension at 4 °C, pH 7.4 in order to remove glycoproteins. Structural glycoproteins were removed adding DTE 0.05 M in two 24 h extractions at 37 °C under N_2 at pH = 7.6. Collagen was removed incubating samples with collagenase (1/1000) with $CaCl_2$ 0.1 M in Tris-HCl 0.1 M during 2 × 24 h at 37 °C, pH 7.5. Then, samples were freeze-dried, resulting in a dry elastin-proteins fraction (EP fraction). Another set of samples were suspended in trypsin (1/10), NH_4CO_3 0.1 M for 2 × 6 h at 37 °C, pH 8 to extract the protein fraction. These samples were then freeze-dried resulting in E fraction. A detailed description of sampling is reported in Table 1.

2.1.2. Aneurysmal aortas

Specimens were collected from the anterior aspect of the infrarenal abdominal aorta during operation for non-specific AAA in 17 patients of both sexes, from 53 to 77 years of age following selection criteria and sampling procedure previously described [24]. Specimens from ten patients were used for quantification and amino acid analysis of elastin and collagen while other three were assayed for time-course release of hydroxyproline by pepsin digestion. The remaining four cases were dedicated to the characterization by thermal and dielectric techniques and comprised one specimen without evident lesions and three samples exhibiting atheroma plaque lesions. A detailed description of sampling from such specimens is reported in Table 2. An aortic valve leaflet from an aortic root (devoid of atherosclerotic lesions) obtained at autopsy (66 years, male) was chosen as control sample in order to compare AAA with a tissue not affected by evident disorders and having a comparable content of elastin and collagen. Quantification and amino acid composition of elastin and time-course release of hydroxyproline by pepsin digestion was also carried out on four age-matched control specimens of infrarenal abdominal aorta obtained at autopsy.

Table 1
Description of the elastin (E) and elastin-proteins (EP) fractions extracted from the media of thoracic aorta of human at different ages.

Group	Age	Atheromateous stage	Label°
Children	3 months		3M-E/3M-EP
	5 months		5M-E/5M-EP
	9 months		9M-E/9M-EP
Adults	24 years	I	24Y-E/24Y-EP
	25 years	0-I	25Y-E/25Y-EP
	26 years	I	26Y-E/26Y-EP
	32 years	0	32Y-E/32Y-EP
Old persons	66 years	III	66Y-E/66Y-EP
	72 years	III	72Y-E/72Y-EP
	75 years	II	75Y-E/75Y-EP
	86 years	III	86Y-E/86Y-EP

Table 2
Description of the pathologic tissues sampled from non-specific abdominal aortic aneurysms.

Aneurysm	Tissue	Age	Label°
Non-specific	Aortic wall	53	A1
Non-specific, familial tendency	Aortic wall + atheroma plaque	72	A2
Non-specific	Superior part of atheroma plaque	77	A3a
	Fragments of atheroma plaque		A3b
	Aortic wall supporting atheroma plaque		A3c
Non-specific, polyaneurysm condition	Aortic wall + atheroma plaque	73	A4a
	Aortic wall + atheroma plaque		A4b
	Fragments of atheroma plaque		A4c

Biopsies and control samples washed with ice-cold PBS in presence of protease inhibitors and stored at –80 °C [24]. To remove possibly interfering residual blood, readily soluble fractions, minerals, lipids and cell components the samples investigated by thermal techniques were first suspended in a hypotonic, 10 times diluted, PBS pH 7.4 containing protease inhibitors and 10% DMSO (dimethylsulfoxide) and gently extracted at 4 °C for 3 hours before decalcification with EDTA (EDTA 5 M, PBS buffer) and defatting by successive 2-hour extractions at 4 °C in chloroform:methanol (2:1, vol/vol), chloroform:methanol (3:1, vol/vol), ethanol:-distilled water (1:1, vol/vol) and distilled water. Cell remnant removal was completed by replacing DMSO with 1% (w/w) Triton X-100 in PBS buffer and extraction for 2 × 10 h periods at 4 °C. In turn, Triton X-100 was replaced by 10 mM sodium cholate for 2 × 10 h treatments at room temperature. Samples were successively washed with 10% isopropanol in saline and deionized water before freeze-drying and final weighing after storage under reduced pressure over P_2O_5 (dddW, dry defatted decalcified weight).

2.2. Biochemical analyses on aneurismal aortas

Isolation of elastin was carried out by a multi-step extraction based on chaotropic agent followed by treatment with purified collagenase and by trypsin to remove age-related polar proteins deposited on elastin [24]. The content and composition of collagen and elastin in the resulting insoluble matrix was determined by weight of insoluble elastin and amino acid analysis of the collagenase solubilized fraction [31]. Hydroxyproline was also determined separately according to Woessner [32]. Sample hydrolysis and amino acid analysis was performed as previously described [31].

Solubilization of collagen by pepsin was evaluated on the basis of time-course release of hydroxyproline as determined on aliquots of the supernatants and related to total hydroxyproline comprising the final insoluble residual pellet. Freeze-dried AAA and control samples of infrarenal aorta obtained at autopsy were minced with scissors, suspended in 0.5 M acetic acid for 48 h at 4 °C and then treated by addition of pepsin at 35 mg/g dddW under constant stirring for 24 h at 4 °C [32]. Aliquot of supernatant was taken after 6, 16 and 24 h, respectively, and separated from solids by high-speed centrifugation. The insoluble material separated by centrifugation was re-suspended in 0.5 M acetic acid and treated with fresh pepsin in the same conditions for additional 24 hours prior to further supernatant separation. Such procedure was successively repeated for two 48 h and one final 120 h period for a total of 264 hours.

2.3. Differential Scanning Calorimetry (DSC)

The DSC thermograms were recorded with a Perkin Elmer DSC7 Differential Scanning Calorimeter. The calorimeter was calibrated using indium and tin as standard. Samples (5–10 mg) were sealed into aluminum pans, and empty pans were used as references. Investigations were performed between 30 and 250 °C with 20 °C/min heating rates. This heating rate was chosen to obtain well-defined glass transitions and to explore the 20–250 °C range in a fast experiment reducing the degradation phenomena.

For each set of samples, DSC experiments were performed in triplicate and Origin Software was used for statistics.

3. Results and discussion

3.1. Thoracic aortas of different ages

Fig. 1 presents the DSC thermograms of elastin (E) and elastin-proteins (EP) fractions from thoracic aortas of children. On the first scan (not show here), a broad endothermic peak is observed at around 100 °C. This peak, which vanishes on the second scan, is attributed to the evaporation and vaporization of bound water adsorbed by the sample stored under a 50-80% relative humidity [33–35]. Therefore, this thermal transition can be considered as an extrinsic transition. A second thermal event, noted as a jump in the specific heat, appears between 190 and 220 °C on the second scans and third scans for all the samples: this reversible transition is associated with the glass transition of elastin in the freeze-dried state [33–35] namely the transition from a vitreous to a rubbery state. By analogy with synthetic polymers, this intrinsic transition is characterized by its temperature (Tg), corresponding to the middle point of the specific heat jump of the step. The value of Tg is an important parameter of the chain dynamics of elastin, and gives specific information on the mobility and stiffness of the protein in the artery. The values of Tg for all the samples corresponding to children aortas were reported in Table 3. For the E fraction, an endothermic peak is superimposed on the glass transition phenomenon on the second scan, vanishing on the third scan. This over-shoot was often reported in literature on a large class of polymers and attributed to the breaking of physical bonds (in the case of proteins, generally hydrogen bonds) at the glass transition, when the system has a sufficient mobility [36]. In children group, the cross-linking of elastin is not totally achieved and the lateral chain groups that are not implied in cross-links are able to interact via hydrogen bonds, giving rise to the observed over-shoot at the first cross of Tg. This over-shoot is only observed on pure elastin, the presence of associated proteins avoiding the formation of such hydrogen bonds within the elastin network. Moreover, the glass transition temperatures of the EP fractions are lower than values corresponding to elastin fractions: as already observed in elastic

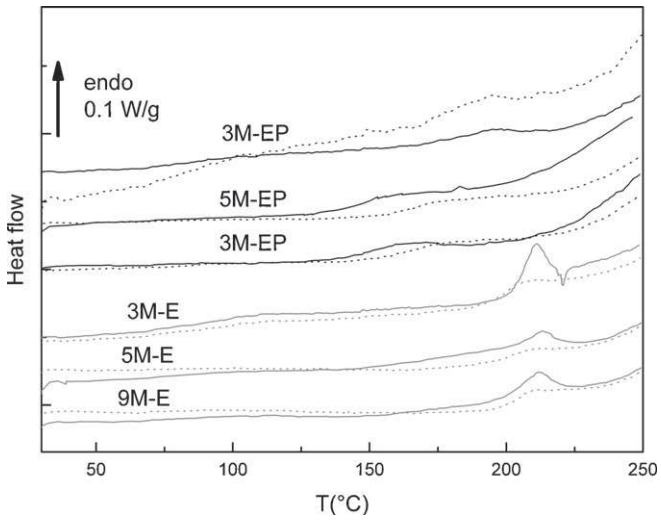

Fig. 1. Differential Scanning Calorimetry thermograms (2nd scans in solid line and 3rd scans in dot line) of elastin (E) and elastin-proteins (EP) fractions from children thoracic aortas.

Table 3
Glass transition temperature of elastin (E) and elastin-proteins fraction (EP) extracted from human thoracic aortas of children, adults and old persons.

	Tg 2nd scan (°C)		Tg 3rd scan (°C)	
	E	EP	E	EP
3 months	206.9 (over-shoot)	180.9	197.7	180.4
5 months	208.2 (over-shoot)	145.6	202.3	166.4
9 months	207 (over-shoot)	153.1	202.4	163.5
24 years	204.4	204.7	203.1	204.3
25 years	207.1	203.7	205.3	202.5
26 years	203.7	206.8	202.4	202.2
32 years	178.3	205.9	203.5	197.3
66 years	207.3	204.8	206.2	202.8
72 years	203.8	200.3	202.6	197.3
75 years	205.3	Not measurable	203.2	202.0
86 years	207.0	Not measurable	205.8	203.7

tissues [35], the associated proteins have a plasticizing effect on elastin, certainly by a swelling effect on the elastin network.

The thermograms corresponding to the 2nd and 3rd scans of elastin and EP fractions of thoracic aortas from adults are reported on Fig. 2. The major thermal event is the step of the specific heat associated with the glass transition of elastin. All the values of Tg are reported in Table 3. In this case, no over-shoot could be detected on the 2nd scan of pure elastin: cross-links are achieved in elastin of adults aortas, avoiding the formation of physical bonds on normal conservation. In contrast with children's group, there was no significant difference between the values of the glass transition of elastin and elastin-protein fractions; as confirmed by biochemical analysis which does not evidence differences in amino acids composition [37], we can affirm in this case that the E fraction is comparable to the EP fraction. The proteins associated with elastin are mainly removed before the trypsin extraction. A special feature is reported for EP fraction of the 24 years patient; in this case an endothermic peak is detectable at 220 °C, a zone corresponding to the denaturation of β-sheets and helical structured proteins; This endothermic event is not observed on the E fraction of the same patient, and must be associated with the presence of structured proteins in the E fraction, removed by the trypsin attack.

The thermograms corresponding to the 2nd and 3rd scans of elastin and EP fractions of thoracic aortas from old persons are

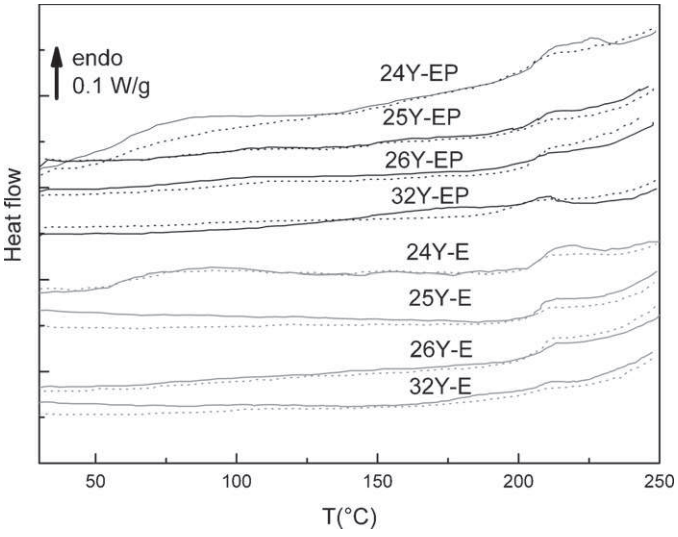

Fig. 2. Differential Scanning Calorimetry thermograms (2nd scans in solid line and 3rd scans in dot line) of elastin (E) and elastin-proteins (EP) fractions from adults thoracic aortas.

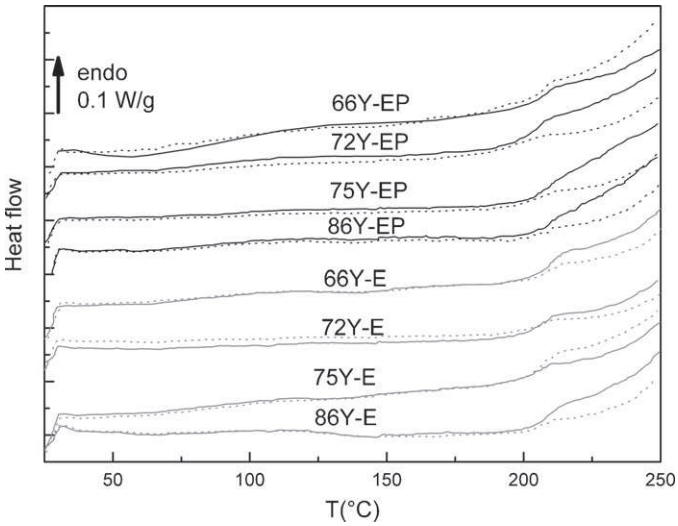

Fig. 3. Differential Scanning Calorimetry thermograms (2nd scans in solid line and 3rd scans in dot line) of elastin (E) and elastin-proteins (EP) fractions from olds persons thoracic aortas.

reported on Fig. 3. The main event is the glass transition of elastin. The corresponding values are displayed on Table 3. No significant differences were found between the glass transition temperature of elastin in adults and old persons, suggesting that the mechanical properties of elastin are conserved during ageing. As shown by mechanical analysis, the contribution of ageing to vascular stiffness is minor [38]. It was shown that an increase of stiffness was significant only with advanced stages of plaque formation, certainly due to an increase of collagen content and calcification. It is noteworthy that for EP fractions, an increase of the specific heat is observed between 210 and 250 °C, and associated with a degradation phenomenon. This degradation phenomenon is superimposed to the glass transition, which cannot be measured for the two oldest patients (75 and 86 years) at the second scan. On the 3rd scan, this degradation phenomenon is not yet detectable. This could be attributed to the denaturation of elastin-associated proteins. As a matter of fact, in this temperature zone are often observed the denaturation phenomena of β sheet or helical structured proteins. This assumption is corroborated by biochemical analyses which showed an important increase of polar amino acids in the EP fractions of old persons: aspartic acid represents 21% of the total amino acids in the elastin-protein fraction of the aortas of old persons (5.3% in adults) and the proportion of arginine and lysine is doubled; this increase in polar amino acids is concomitant to a decrease in apolar amino acids [37]. There is also an increase in hydroxylated amino acids, like serine and threonine. This protein fraction was also shown to increase in this case, reaching 18% in weight of the total EP fraction. Previous works associated this protein fraction to microfibrils and type IV collagen, which would form in the case of old persons an homogenous phase with elastin by ionic bonds [37].

3.2. Abdominal aortic aneurysms

3.2.1. Biochemical analysis

3.2.1.1. Elastin and collagen content. Concentration of elastin and insoluble collagen content in non-specific AAA ($n = 10$) accounted for 3.2 ± 1.3 and 41.5 ± 9.8, respectively, while in control infrarenal aorta ($n = 4$) it accounted for 18.6 ± 1.9 and 40.4 ± 9.2, respectively, (% dry defatted weight). Elastin and collagen contents of the tissues sampled from the 4 AAA specimens selected for thermal and dielectric

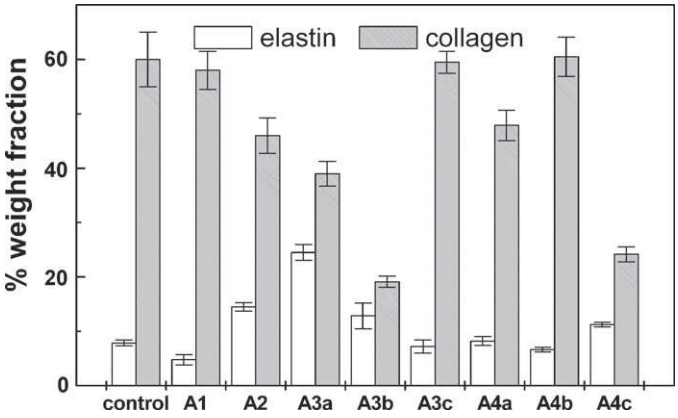

Fig. 4. Elastin and collagen contents of tissues sampled from non-specific aneurysms (Table 2) and of control sample (aortic leaflet).

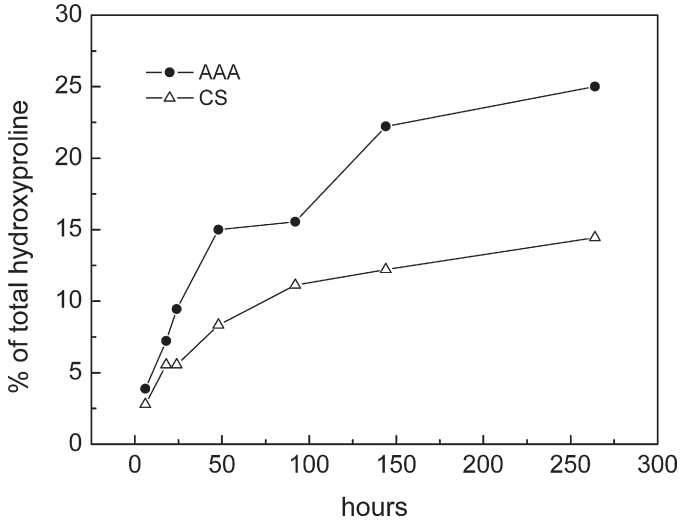

Fig. 5. Pepsin solubilization of insoluble collagen: hydroxyproline release. Each value is the mean of the determinations carried out on three control samples (CS) and three non-specific aneurysms (AAA), respectively.

investigation are given on Fig. 4 (dddW). Although there is a great variability of elastin and collagen concentrations in the different samples (the plaque fragments contained the lowest amount of elastin-collagen tissues), the proportion of collagen was always largely in excess with respect to elastin, as reported in the literature [25]. This is the reason why age-matched human valvular leaflet, composed by 50 to 60% collagen and 12% elastin [39], was chosen as reference tissue to follow the evolution of the structural and dynamical parameters of extracellular matrix biopolymers in aneurysms.

3.2.1.2. Composition of the elastin fraction in control tissue and aneurysms. As reported in Table 4, the amino acids composition of control and AAA elastin shows some significant differences. AAA elastin exhibits an increase of polar residues with a concomitant decrease of the hydrophobic residues, typical of human elastin from non-aneurysmal aortic wall. In fact elastin from AAA is contaminated by an additional insoluble polar protein fraction. Considering that in human aorta the ageing process is related to the progressive deposition of polar glycoproteins (removed by trypsin treatment), the presence of a protein resistant to trypsin is indicative of a component likely different from the fraction deposited during ageing in non-aneurysmal aortic tissues [24].

Table 4
Amino acid composition (mean ± SD) of elastin isolated from non-specific aneurysms (AAA) and control samples (CS); data are expressed as res/1000 total res.

	AAA ($n=10$)	CS ($n=4$)
Lys	15 ± 6	8 ± 2
Hys	7 ± 3	4 ± 1
Arg	18 ± 5	13 ± 2
Cys	9 ± 3	5 ± 1
Hyp	16 ± 3	15 ± 3
Asx	29 ± 11	14 ± 5
Thr	26 ± 6	19 ± 2
Ser	26 ± 8	15 ± 3
Glx	46 ± 9	31 ± 6
Pro	105 ± 10	119 ± 6
Gly	238 ± 26	261 ± 14
Ala	181 ± 17	211 ± 10
Val	105 ± 6	121 ± 5
Met	8 ± 1	6 ± 2
Ile	29 ± 2	27 ± 1
Leu	71 ± 7	64 ± 2
Tyr	28 ± 1	26 ± 2
Phe	31 ± 4	26 ± 2
Ide + Des	10 ± 3	13 ± 3

3.2.1.3. Time course solubilization of insoluble collagen by pepsin. Fig. 5 shows time-course solubilization of insoluble collagen by pepsin; a larger fraction of collagen is solubilized by pepsin from AAA with respect to age-matched non-aneurysmal abdominal aorta (CS). It is well known that the triple helical domain of collagen is resistant to pepsin attack in contrast to non-ordered telopeptide regions; therefore, the difference of solubilization could be seen as expression of a lower order in collagen from aneurysms. Two hypotheses can be driven from this observation. On one hand, the large release of AAA collagen by pepsin is compatible with the presence of less cross-linked neosynthesized collagen, owing to its recent deposition, or to some impairment of its stable incorporation into the extracellular matrix. In fact, collagen has been reported to become more resistant to pepsin with ageing in non-aneurysmal aortic tissues [40]. On the other hand, weakening of the AAA insoluble extracellular matrix cannot be excluded as a result of previous nicking by MMPs enzymatic attack.

3.2.2. Thermal transitions

The thermograms of aortic valvular leaflet corresponding to two successive scans between 30 and 150 °C and 250 °C are reported on Fig. 6.

As previously observed in porcine aortic valves and leaflets [35], the first scan is characterized by a large endothermic peak with maximum at 97.7 °C associated with the loss of residual bound water. The second scan presents the denaturation endotherm of collagen (i.e. the transition from an ordered state – triple helix arrangement – to a random state–gelatin) at 231.6 °C, with an enthalpy of 13.2 ± 1 $J.g^{-1}$ (normalized to the weight of collagen in the tissue). The third scan evidences the glass transition of gelatin at 207.6 °C, which can be assimilated to an amorphous polymer. It is noteworthy that the thermal signature of elastin, namely a reversible glass transition that would occur at around 200 °C on the second scan for freeze-dried tissues [35] is not detectable, due to the low content of elastin in aortic leaflet.

For this study, we will mainly focus on the denaturation parameters of collagen, which constitute a good evaluation of triple helix stability. DSC thermograms of aneurysms were recorded according to the same protocol as control tissue; in all the cases, second scans are characterized by the collagen denaturation peak; we have plotted on Fig. 7 this peculiar zone

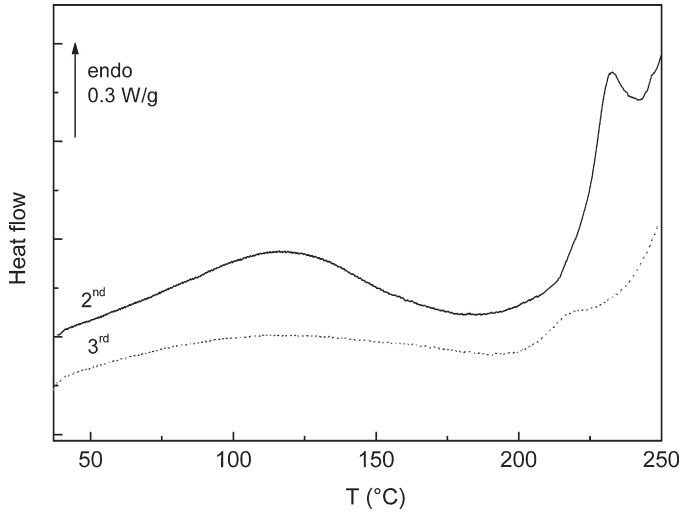

Fig. 6. Differential Scanning Calorimetry thermograms of control sample (human aortic valvular leaflet).

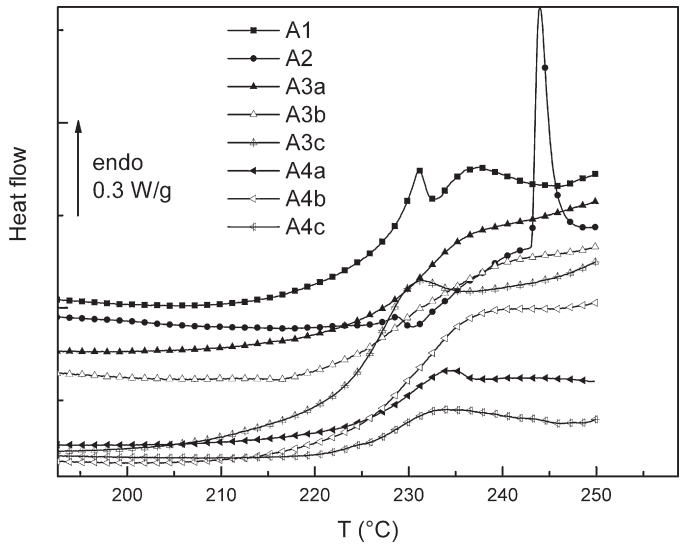

Fig. 7. Differential Scanning Calorimetry thermograms of aneurysms (2nd scans, denaturation zone).

for all the tissues. Although the thermograms of pathologic tissues have a similar aspect than the thermogram of normal tissues, some of them can be distinguished by the broadness, the multiplicity and the intensity of endothermic events observed above 200 °C and the occurrence of a shoulder at around 170/180 °C in two sets of samples (not shown here).

The thermal parameters of collagen denaturation are reported in Table 5. Other thermal events are also noticed and reported in Table 5. Various modifications are observed on DSC thermograms; as a matter of fact, the collagen denaturation signal is multiple in some pathologic tissues; this feature is never observed in normal aortic tissues. In literature data, the multiplicity of collagen denaturation peak has been reported for different kinds of tissues in solution. Most of the authors attribute this sequential melting to a distribution of collagen molecules with a distinct degree of thermal stability [41]. In some cases, this multiplicity would arise from the heterogeneity of samples [42]; it was also interpreted as the melting, in a same triple helix, of domains of distinct cooperativity [42]. For Flandin et al. [43], who studied the evolution of collagen denaturation peaks with ageing, there is a correlation between the multiplicity of this denaturation and the presence of different cross-links (labile cross-links and thermally stable cross-links) in the protein. Finally, complementary studies on lyophilized collagen from immature rat skin have allowed us to confirm the existence of such a doublet of denaturation [44].

Our thermal results on aneurysms are consistent with biochemical and literature data; different factors can explain the multiplicity of collagen denaturation peaks in some AAAs: accumulation of neosynthesized collagen evidenced by Gargiulo et al.[24] and sharp increase of C-telopeptide fragments in AAA wall [10] associated with collagen degradation by cysteine proteases. The different pepsin solubilization and thermal stability of collagen in the aneurysms are likely to reflect an abnormality in regulation of collagen turnover, as suggested by different studies [25,27]. In the present study, difference of denaturation temperature cannot be correlated with difference of proline hydroxylation as generally observed. In its greatest part, the AAA collagen phase is not subject to type I-type III deregulation but rather to other chemical and/or physical changes (increased proportion of neosynthesized collagen, collagen fragmented by enzymatic attacks).

Moreover, an endothermic peak was evidenced between 170 and 180 °C in some thermograms. Even if the identification of this peak is not straightforward, this thermal event shows the existence of structures of weak stability in AAA walls. These results must be correlated with thermogravimetric studies performed on unaffected and pathologic arterial tissues, which reveal an alteration of the thermal stability of elastin and collagen in the atheroma plaque [45] and could be related to the degree of MMPs expressions [17,21].

A further comparative study of denaturation parameters can be performed by normalizing the denaturation enthalpy to the weight of collagen in the dry and delipided tissue. These results are reported in Table 5. The denaturation enthalpies are comprised between 9.5 and 20 $J.g^{-1}$. The values that strongly deviate from the reference value (13.2 ± 1 $J.g^{-1}$) correspond to fragments of atheroma plaque, in which collagen alterations are certainly the most pronounced.

Table 5
Characteristic thermal transition parameters of tissues sampled from abdominal aortic aneurysms (AAA).

Label	Nature	Tpeak (°C)	ΔHpeak ($J.g^{-1}$)	ΔHd (J/g of collagen)	Other transitions
A1	Doublet	231.1 ± 0.6; 236 ± 0.4	8.1	14.0 ± 0.5	Shoulder at 170 °C
A2	Triplet	229 ± 0.5; 239 ± 0.3; 244 ± 0.5	7.6	16.5 ± 1.2	Shoulder at 180 °C
A3a	Singlet	$236{,}9 \pm 0.4$	3.7	9.5 ± 0.6	
A3b	Singlet	239.2 ± 0.4	3.8	19.9 ± 1.0	
A3c	Singlet	230.4 ± 0.5	8.6	14.5 ± 0.5	
A4a	Singlet	234 ± 0.3	8.2	17 ± 1	
A4b	Singlet	236.5 ± 0.5	7.2	11.9 ± 0.7	
A4c	Singlet	232.8 ± 0.5	4.3	17.8 ± 1	

The global evolution of the characteristic parameters confirms an increase of the molecular mobility of collagen in aneurysms, at different scales of mobility.

Finally, it is noteworthy that the sample which exhibits distinct thermal characteristics resulting from multiple steps of collagen denaturation, in the unaffected tissue, derives from the patient affected by polyaneurysm (genetic basis or general predisposition). These changes illustrate a profound alteration of the collagenic phase, in spite of its content similar to that of other aneurysmal tissues.

4. Conclusions

The thermal characterization of the elastin and E fraction from the media of thoracic aortas suggested to follow the evolution of the mechanical properties during ageing: it was shown that in the thoracic aortas from children, proteins induced a more flexible structure of the elastic component in contrast with that observed in the thoracic aortas from the adult group, due to the complete cross-linking of elastin. In this case, there is a segregation between proteins and elastin. During ageing, there is an interpenetration between elastin and other proteins, giving rise to a more homogenous phase. This assumption is confirmed by biochemical analysis, with an increase of polar amino acids in the elastin-protein fraction in the thoracic aortas from group of old persons. Elastin is contaminated by a protein fraction that can reach 18% of the total elastin-protein fraction, with an increase of polar amino acids [37].

As for the aneurysms, the first concern is the great variability of pathologic tissues in their biological composition (fragments of atheromatous plaque exhibit a lower elastin-collagen ratio as compared to samples from plaques and aneurysms) as well as in their thermal and dielectric properties. This general trend underlies the heterogeneous character of AAA wall when compared with controls. However, we cannot exclude that heterogeneity at the structural level could result at least in part from the coexistence of earlier with the more recently deposited extracellular matrix. Nevertheless, some common features of the whole set of aneurysmal samples were demonstrated by thermal and dielectric techniques, as far as the peculiar alterations of the collagen phase are concerned. This study shows the adequacy of thermal techniques, usually reserved for the study of synthetic polymers, to get an insight into the chain dynamics of biopolymers in pathological cardiovascular tissues. Our results can be compared to the recent thermal-dielectric study [46] evidencing the alteration of the elastin network in arteries cultured in presence of homocysteine, shown to induce histological anomalies in human arteries.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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