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THERMAL PROPERTIES OF ELASTIN–FATTY ACID COMPLEXES

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Differential scanning calorimetry has been applied to study the interaction of fibrous or of soluble elastin with fatty acids and their trialanine derivatives. The DSC curves of elastin–fatty acid preparations exhibited an endothermic transition in the temperature range -- 10° to + 50°. The peak temperatures and the enthalpy changes were independent of the chain length and of the saturation of the fatty acid. The interaction with the trialanine derivatives was similar to that with the fatty acids.

Such interactions between elastin and lipids might take place in vivo as well, resutling in the loss of elasticity of elastin fibres and consequently in the development of arteriosclerotic lesions.

The elastic fibres are important constituents of vertebrate arteries. They make up 10–40% of the total proteins in the different human arteries. Alterations of elastic fibers have been recognized as early signs of the aging of the arterial wall and of the development of arteriosclerotic lesions. One of these pathological alterations is the interaction between elastin and lipids [1–5]; such interactions are probably involved in the loss of elasticity of the arteries.

In the present investigation elastin–lipid interactions have been studied on model systems consisting of fibrous elastin or soluble elastin and various fatty acids and their trialanine derivatives by means of differential scanning calorimetry (DSC).

Experimental

Material and methods

Insoluble fibrous elastin was prepared from bovine ligamentum nuchae by the Lansing procedure [6].

Soluble (K) elastin was obtained from this substance by hydrolysis in 80% aqueous ethanol containing 1 *N* potassium hydroxide for 48 h at 37°, as described earlier [7]. The low molecular weight (12–16.000 dalton) peptide fraction of the alkaline hydrolysate was separated on a Sephadex G 100 column.

Selection of fatty acids

From among the biologically important fatty acids, four were selected for the experiments: caproic acid, lauric acid and stearic acid represented saturated fatty acids with increasing chain length, while oleic acid represented the unsaturated compounds. Fatty acids were purchased from Sigma, St. Louis.

Preparation of elastin–fatty acid derivatives

Fatty acids were dissolved in 0.05 *M* ammonium hydroxyde solution in 90% ethanol (50 mg/ml). Aliquots of this solution were added to fibrous or soluble elastin to obtain 2:1 mixtures of elastin and fatty acids. The solvent was evaporated off in vacuo at room temperature.

Preparation of fatty acid trialanine derivatives

The derivatives were prepared by acylation of trialanine with fatty acid chlorides in the presence of triethylamine.

0.5 mmol of trialanine (Sigma) was suspended in 2.5 ml of 80% ethanol, and the peptide was dissolved by addition of 0.78 mmol of triethylamine. 0.75 mmol of fatty acid chloride was added slowly in 10 min under agitation. The agitation was continued for 1 h at room temperature. Precipitation of the acyl derivative was completed by addition of 3 ml of water and 0.2 ml of glacial acetic acid. The crystals were filtered by suction, washed with water (5 X 10 ml) and then with ether (3 X 6 ml), and recrystallized from aqueous ethanol. The caproic acid derivatives, slightly soluble in water, were maintained for 1 h at 4° before filtering. The crystals were washed with 3 X 3 ml of ice-cold water. Results of chemical analysis of the preparations are summarized in Table 1.

Table 1 Analytical data of fatty acid–trialanine derivatives

Preparation	Chemical formula	Yield, %	Melting point C°	Calculated composition,			Found composition,		
				C	H	N	C	H	N
Caproyl-trialanine	$C_{15}H_{28}O_5N_3$	40.0	226	54.53	8.54	12.72	54.66	8.45	12.60
Lauroyl-trialanine	$C_{21}H_{40}O_5N_3$	68.0	219	68.84	9.73	10.14	60.59	9.71	10.10
Stearoyl-trialanine	$C_{27}H_{52}O_5N_3$	70.0	212	65.02	10.51	8.43	65.31	10.50	8.29
Oleyl-trialanine	$C_{27}H_{50}O_5N_3$	65.0	200 (decomp.)	65.29	10.18	8.46	65.03	10.04	8.47

Preparation of elastin–trialanine–fatty acid derivatives

Trialanine–fatty acid derivatives (50 mg) were dissolved in 2 ml (3 ml in the case of the stearyl derivative) 80% ethanol containing 3% ammonium hydroxide, and aliquots of this solution were added to the fibrous elastin, or to a solution of K-elastin (5% in 50% ethanol) to obtain a 1:1 mixture of elastin alkoyl-trialanine. The solvent was evaporated off in vacuo.

Analytical method

Calorimetric experiments were performed in a Du Pont 910 DSC cell. Experiments were performed in 10 μl sealed Al pans at a heating/cooling rate of 5 deg/min.

Results and discussion

Fibrous elastin is highly cross-linked and is insoluble even in dissociative solvent systems. Interaction with other molecules takes place only on the surface of the fibres. The reactive elastin peptides responsible for several structural features at the molecular level of the fibrous elastin [7–9] can be extracted following partial degradation by acid [8] or by alkaline hydrolysis [10]. In our first series of investigations the interaction between fibrous elastin and fatty acids was studied. Further in order to approach the problem at the molecular level, a well-defined fraction of the alkaline hydrolysis product of fibrous elastin (κ-elastin, K-elastin) was extracted, and the properties of its fatty acid derivatives were also examined.

In addition to the interaction of elastin with fatty acids, the reaction between elastin and the trialanine derivatives of the fatty acids also seemed to be of interest. It has been demonstrated that oligopeptides with repeating alanine residues are predominant in the partial hydrolysate of elastin [11], and that they interact with the β-sheet configuration regions of the polypeptide chain of fibrous elastin [9]. Thus, trialanine residues present on the surface of fibrous elastin might contribute to the interaction of the fatty acids with elastin.

Within the temperature interval $-20°$ to $+50°$, trialanine, K-elastin and fibrous elastin did not show any reaction accompanied by an enthalpy change.

As concerns the investigated fatty acids, caproic acid, oleic acid and lauric acid melt at $-1.5°$, $+14°$ and $+48°$, respectively. Stearic acid melts at higher temperature (70°). The corresponding endothermic peaks could be identified in the DSC curves. Binding to trialanine did not alter the thermal behaviour of the fatty acids.

In the DSC curves of the K-elastin–fatty acid preparations (Fig. 1), and also in those of the K-elastin–trialanine–fatty acid derivatives, an endothermic transition could be established which could not be observed for the individual constituents. The peak temperatures and the enthalpy changes for the oleic acid, lauric acid and stearic acid derivatives were similar, while that for caproic acid–K-elastin was slightly lower.

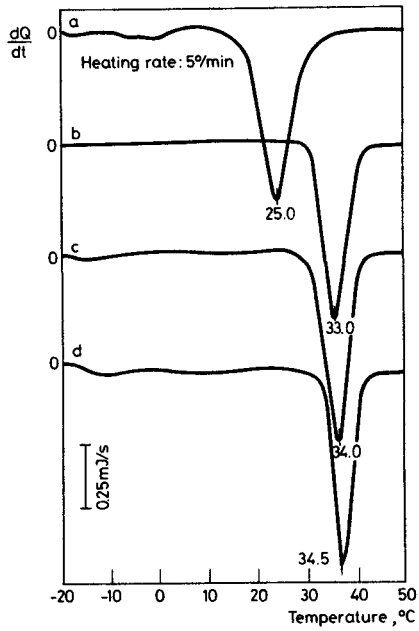

Fig. 1 Differential scanning calorimetry (DSC) curves and transition enthalpy (ΔH) values of soluble-K-elastin–fatty acid complexes. Heating rate: 5 deg/min. a: caproic acid complex sample weight: 13.3 mg, $\Delta H = 9$ mJ/mg, b: oleic acid complex sample weight: 12.5 mg, $\Delta H = 10$ mJ/mg, c: lauric acid complex sample weight: 11.5 mg, $\Delta H = 12$ mJ/mg, d: stearic acid complex sample weight: 13.6 mg, $\Delta H = 9$ mJ/mg

In the samples prepared from fibrous elastin, analogous processes took place; the corresponding DSC peak temperatures were found to be somewhat lower (Fig. 2).

From these phenomena it might be concluded that the binding of fatty acids to elastin alters the thermal properties of the individual components. The transition takes place both with soluble K-elastin and with insoluble fibrous elastin, and seems to be independent of the chain length or of the saturation of the fatty acid (between C_{12} and C_{18}).

Interaction with the trialanine derivatives of the fatty acids was similar to that observed with the fatty acids themselves. This finding suggests that different regions of elastin are involved in the interaction with fatty acids and with hydrophobic oligopeptides. The latter probably interact via H-bonds with the peptide linkages of the β-sheets, while the fatty acids are bound by hydrophobic linkages to other regions of the elastin.

The results of our investigations on model systems might be of physiological importance. Pathological alterations within the vessel wall might lead to a similar interaction between elastin and lipids, and the resulting changes in the stability of the complex molecule might give an explanation of the in vivo irreversibility and of the high in vitro resistance of the elastin–lipid complexes.

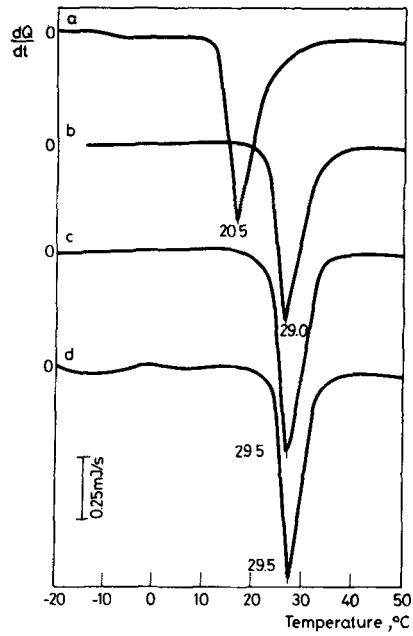

Fig. 2 Differential scanning calorimetry (DSC) curves and transition enthalpy (ΔH) values of insoluble fibrous elastin–fatty acid complexes. Heating rate: 5 deg/min. a: caproic acid complex sample weight: 9.2 mg, $\Delta H = 12$ mJ/mg, b: oleic acid complex sample weight: 11.5 mg, $\Delta H = 11$ mJ/mg, c: lauric acid complex sample weight: 12.0 mg, $\Delta H = 10$ mJ/mg, d: stearic acid complex sample weight: 12.5 mg, $\Delta H = 10$ mJ/mg

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Zusammenfassung – Zur Untersuchung der Wechselwirkung zwischen dem fibrösen oder lösbaren Elastin, und den Fettsäuren und ihren Trialanin-Derivaten wurde die DSC-Methode angewandt. Die DSC-Kurven der Elastin–Fettsäure-Präparate zeigten in dem Temperaturbereich von – 10° bis + 50° einen endothermischen Übergang. Die Peak-Temperaturen und die Änderungen der Enthalpie waren von der Kettenlänge und der Sättigung der Fettsäure unabhängig. Die Wechselwirkung mit den Trialanin-Derivaten war der mit den Fettsäuren ähnlich.

Solche Wechselwirkungen zwischen Elastin und den Lipiden können auch in vivo stattfinden, und die Abnahme der Elastizität der Elastin-Fibern, und folglich die Entfaltung der arteriosklerotischen Schädigungen resultieren.

Резюме – Дифференциальная сканирующая калориметрия была использована для изучения взаимодействия волокнистого или растворимого эластина с жирными кислотами и их триаланинпроизводными. ДСК-кривые препаратов эластин – жирная кислота показали наличие эндотермического перехода в области температур – 10° – + 50°. Температурные пики и изменения энтальпии не зависят ни от длины цени, ни от степени насыщенности жирных кислот. Взаимодействие с триаланинпроизводными было подобно тому, как и с жирными кислотами. Такие взаимодействия между эластином и липидами могут происходить и в естественных условиях, вызывая потерю эластичности эластиновых волокон, что и приводит к развитию артериосклеротических нарушений.