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THE INTERACTION BETWEEN ALKYL DERIVATIVES AND ELASTIN

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Elastin from bovine ligamentum nuchae is exposed to aqueous solutions of different alkyl sulfates and carboxylates (fatty acids). The substrates of alkyl chain lengths varying between C_8 and C_{17} bind to the elastin, the more so the longer the alkyl chain. However, the presence of two (or more) double bonds in the chain obstructs the penetration into the elastin network. As a result of absorption the elastin swells. The rate of binding is determined from the swelling of an elastin strip, that is monitored using a cathetometer. The diffusion of the substrate in the elastin is slower the longer the alkyl chain. The binding is reversible so that the Gibbs energy involved can be derived from the absorption isotherm. The values for the Gibbs energy of binding may amount to some tens of kJ per mol of substrate, with an increment of -4 kJ mol^{-1} per CH_2 group. From the influence of temperature it is concluded that the binding is entropically driven. This, as well as the observation that the glass transition temperature of elastin is not affected by the presence of the alkyl derivatives, suggests that the substrates are bound to the amino acid residues of the elastin, rather than to the polypeptide backbone. Stress-strain experiments reveal that the elasticity decreases markedly on swelling of the sample, irrespective of the type of substrate that is absorbed. The phenomena described in this paper may be similar to those that occur between fatty acids in blood and arterial elastin, which could be at the origin of the development of atherosclerosis.

1. Introduction

Elastin, one of nature's own rubbers, occurs abundantly in connective tissue and in blood vessel walls. In spite of its widespread occurrence, elastin has been subjected to only a relatively small number of studies. The three-dimensional structure of elastin is still a matter of controversy. There seems to be a consensus on a network structure of cross-linked polypeptide chains. However, some authors (e.g., refs. 1–3) propose that the polypeptide chains between the cross-links are in a random conformation, whereas others (e.g., refs. 4 and 5) claim evidence for favored conformations in the polypeptide chains. Although both models may explain elasticity, the glass transition, generally observed in elastin [6–8], can only be accounted for by the random chain model.

Whatever the exact structure is, elastin shows a strong tendency to associate with various natural and synthetic amphiphilic substances (e.g., refs. 9–16). The binding of fatty acids, bile acids, cholesterol and other lipoitic material from the blood plasma to the elastin in the arterial intima probably plays a significant role in the initial stages of the development of atherosclerotic lesions. For instance, Claire et al. [13] found a large accumulation of free, i.e., nonesterified, fatty acids in atherosclerotic aorta tissue. Kagan and coworkers [9–12] performed in vitro experiments showing that the binding of fatty acids, bile acids, etc., greatly facilitates the enzymatic proteolysis of elastin. The reason for the enhanced degradation is ascribed to the introduction of negative charge in the elastin network favoring the subsequent binding of the positively charged elastase mole-

cule. This could as well apply in vivo, since Robert et al. [17] observed an increased elastase activity in atherosclerotic vessels.

Thus, the binding of lipoitic substances, in particular free fatty acids, to elastin seems to be of great biomedical importance. However, only very little attention has been paid to the nature of the interaction [11,14,18]. In this article we describe the interaction between alkyl sulfates and carboxylates (fatty acids) with elastin. It is an attempt to characterize the interaction mechanism and to explain the effect of the binding on the elastic properties of the network.

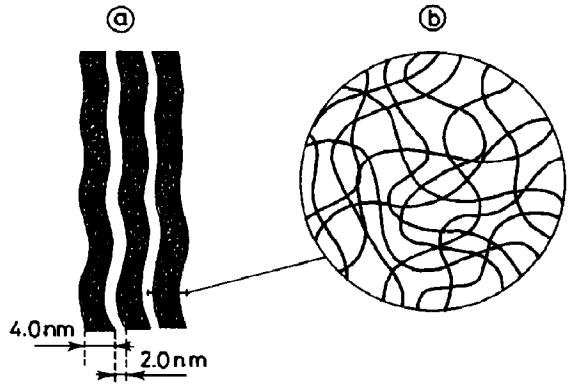

Fig. 1. Schematic illustration of the constitution of an elastin strip. The strip is made up by a bundle of filaments, shown in a. In the relaxed state the filament diameter is approx. 4 nm and the interfilamentary separation approx. 2 nm. (b) Cross-section through one filament, showing a network of polypeptide chains.

2. Materials and methods

2.1. Materials

2.1.1. Elastin

Elastin was purified from bovine ligamentum nuchae obtained at a local slaughterhouse. The samples were scraped free from extraneous tissue, cut into thin strips and extensively rinsed with distilled water. The strips were autoclaved twice for 8 h at 120°C and thoroughly washed again. Then they were dried at 80°C to constant weight and stored over P_2O_5 in a desiccator. The samples thus obtained were considered to be completely dry, since they did not lose further weight on drying at 100°C under reduced pressure. The amino acid composition, determined with a Beckman Multichrom Liquid Chromatograph 4255, is given in table 1. For comparison the values that, according to Mukherjee et al. [19], are valid for elastin of acceptable purity are included in table 1. It is noted that elastin contains only a small number of ionizable amino acid residues. Furthermore, a large number of hydrophobic amino acid residues renders the elastin relatively hydrophobic. Based on the scale of Eisenberg [20] the hydrophobicity is 9.8 J g^{-1}. It follows that water is a poor diluent for elastin. In an aqueous medium the saturation with water amounts to 0.5 g per g dry elastin. Therefore, the elastin network that is exposed to an aqueous environment may be considered as relatively compact.

Fig. 1 gives a schematic illustration of the architecture of an elastin strip. The strip is made up by filaments that are arranged in a more or less parallel orientation. According to Gotte et al. [21] the diameter of the relaxed filaments is approx. 3–4 nm and the average separation between the

Table 1

Amino acid analysis of elastin in residues per 1000 residues

	Reference	
	This work	[19]
Cysteic acid	5.8	7.1
Hydroxyproline	–	–
Aspartic acid	6.4	7.0
Threonine	9.2	10.1
Serine	9.1	9.9
Glutamic acid	16.9	17.4
Proline	121.3	125.4
Glycine	330.6	316.2
Alanine	223.4	213.3
Valine	132.3	134.0
Isoleucine	25.2	26.6
Leucine	65.2	64.7
Tyrosine	7.2	6.1
Phenylalanine	31.8	33.6
Lysine	4.2	3.6
Histidine	0.5	0.5
Arginine	6.3	6.6
Isodesmosine	1.2	1.1
Desmosine	1.6	1.7

centers of two adjacent filaments is about 5 nm. These values were judged from high-magnification electron microscopy and may contain large uncertainties. The filaments themselves are composed of the three-dimensional network. The covalent cross-links consist of desmosin and isodesmosin units. The density of dry elastin is 1.33 g cm^{-3} [22].

2.1.2. Alkyl sulfates and alkyl carboxylates

One of the most important variables in this study is the number of carbon atoms in the alkyl chain. For chain lengths greater than C_{11} the sodium salts of the naturally occurring alkyl carboxylic acids (fatty acids) were used and, since such carboxylates were not available, the shorter chains were represented by the sodium sulfates. The alkyl carboxylates (Sigma) and the alkyl sulfates (Merck) are of 99–100% purity. Their gross formulas and some other relevant properties are summarized in table 2. The alkyl sulfates and carboxylates belong to the association colloids. This implies that beyond a given, characteristic concentration, the so-called critical micelle concentration (CMC), the monomers associate to form micelles. At total concentrations beyond the CMC the concentration of free, nonassociated molecules almost equals the CMC. Values for the CMC are included in table 2.

2.1.3. Other chemicals

All other chemicals were of analytical grade and the water was purified using a Milli-RO plus a Millipore Super Q purification system.

2.2. Methods

To achieve good solubility of all alkyl derivatives the experiments were performed under alkaline conditions, i.e., in borate buffer of pH 10 (0.05 M H_3BO_3 and 0.05 M NaOH), in which the ionic strength is adjusted to 0.15 M, using NaCl. To avoid oxidation of the unsaturated bonds in the alkyl chain, the experiments with oleate and linoleate were carried out in the dark.

2.2.1. Absorption kinetics

Dried elastin strips of 7 cm length, approx. 0.025 cm^2 cross-section and of known weight were immersed in the buffer solution, thermostatted at 70°C. After reaching equilibrium the strips were, in the same thermostat, vertically suspended in 100 cm^3 solution of the substrate (8 g dm^{-3}) in the same buffer. Due to the uptake of the alkyl derivative in the network the elastin sample swells. This swelling, together with the swelling of the elastin sample due to water uptake, would cause a decrease of the interfilamentary space. Hence, the elastin strip becomes more isotropic as swelling

Table 2

Relevant properties of alkyl derivatives used in this study

CMC values refer to 70°C and high pH. They are obtained from the tables published by Mukerjee and Mysels [22]. In some cases the values were derived by extrapolation from data given at other temperatures.

Substance	Gross formula	Molecular weight (g mol^{-1})	Density (g cm^{-3})	CMC (M)
Sulfates				
Octyl sulfate	$C_8H_{17}OSO_3^-\ Na^+$	232		1.6×10^{-1}
Decyl sulfate	$C_{10}H_{21}OSO_3^-\ Na^+$	260		4.0×10^{-2}
Dodecyl sulfate	$C_{12}H_{25}OSO_3^-\ Na^+$	288		1.1×10^{-2}
Carboxylates				
Lauric acid	$C_{11}H_{23}COO^-\ Na^+$	222	0.8679	2.0×10^{-2}
Myristic acid	$C_{13}H_{27}COO^-\ Na^+$	250	0.8439	2.9×10^{-3}
Palmitic acid	$C_{15}H_{31}COO^-\ Na^+$	278	0.8527	$3.9\text{–}6.5\times10^{-4}$
Oleic acid	$C_{17}H_{33}COO^-\ Na^+$	304	0.8935	1.4×10^{-3}
Linoleic acid	$C_{17}H_{31}COO^-\ Na^+$	302	0.9022	$>2\ \times10^{-3}$

proceeds. The swelling is recorded by measuring the length of the elastin strip as a function of time, using a cathetometer. At various time intervals the strip was removed from the solution and dried over P_2O_5 until constant weight, so that the amount of absorbed material could be determined from the material balance.

2.2.2. Absorption isotherms

Elastin strips were chopped into small pieces. About 150 mg of dry elastin were immersed in 50-cm^3 solutions of the substrates of varying concentration, at constant temperature. After reaching equilibrium (the equilibrium time may be judged from the kinetic experiments) the elastin pieces were taken out of the solution, dried over P_2O_5 and the absorbed amount was gravimetrically determined.

2.2.3. Stress-strain measurements

Dried elastin strips of approx. 6 cm length, and approx. 0.025 cm^2 cross-section and of known weight were incubated in 8 g dm^{-3} solutions of the substrates at 70°C. After reaching absorption saturation the strips were removed from the solution and dried to constant weight. The strips were subsequently desiccated at room temperature in various humidities which leads to different extents of water content in the strip. After weighing, the strips were immediately immersed in paraffin oil to keep the amounts of alkyl derivative and water in the strip constant. Then, stress-strain properties were determined using an Overload Dynamics tensile tester (model S 100).

3. Results and discussion

3.1. Rate of absorption

Since the elastin network is isotropic, absorption of the alkyl derivatives causes a swelling that

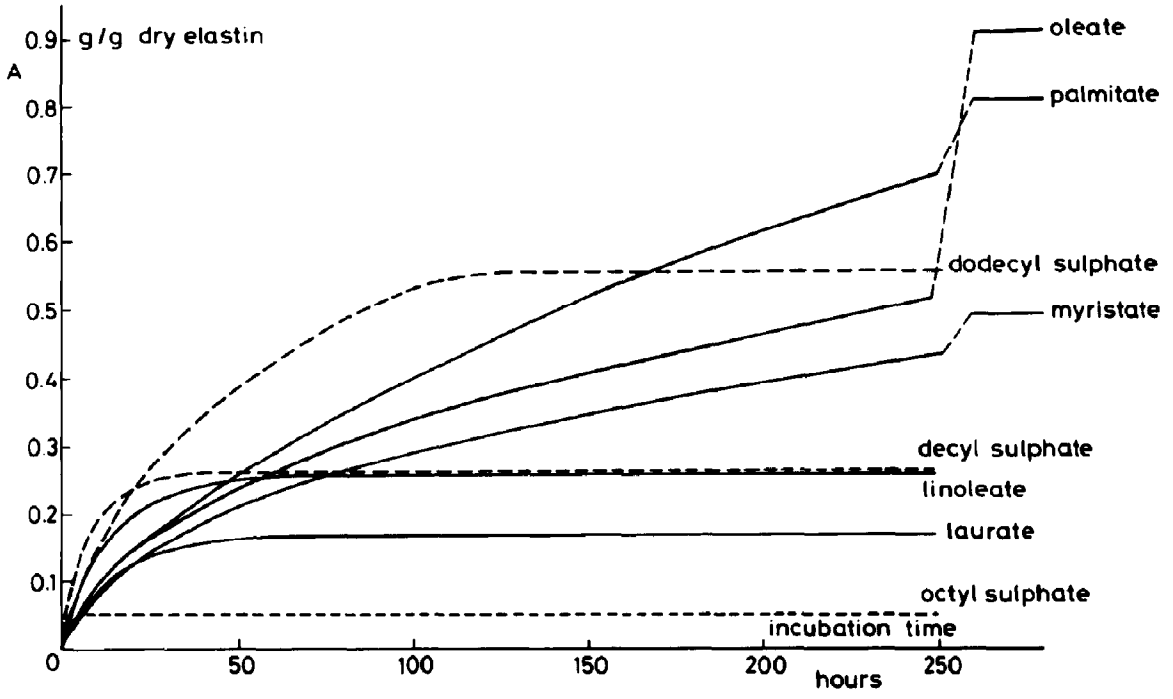

Fig. 2. Uptake of various alkyl derivatives by elastin. $T = 70°C$; ionic strength 0.15 M; pH = 10.

is the same in each direction. Consequently,

$$\left(\frac{L_0 + \Delta L}{L_0}\right)^3 = \frac{V_0 + \Delta V}{V_0} \quad (1)$$

where L_0 and V_0 are the length and volume of the strip, respectively, prior to addition of the alkyl derivative to the solution and ΔL and ΔV the changes in length and volume, due to the uptake of the substrate. For each substrate a linear relation has been found between the absorbed amount A and ΔV, as calculated from eq. 1. Fig. 2 shows A as a function of the incubation time for the various substrates. These data were obtained at 70°C. Preliminary experiments revealed that the absorption rates slow down considerably on decreasing the temperature. A direct correlation appears to exist between the alkyl chain length and A. This is most clearly observed with the alkyl sulfates. Increasing the number of carbon atoms from 8 (octyl sulfate) to 12 (dodecyl sulfate) causes more than a 10-fold increase of A. The alkyl carboxylates show the same trend. A minimum length of the alkyl chain seems to be required to realize binding to the elastin. However, the differences between the sulfates and the carboxylates indicate that the nature of the anionic head group plays an important role in the interaction as well. The initial absorption rates of the sulfates are higher than those of the carboxylates having a comparable number of CH_2 groups in the alkyl chain. Furthermore, the sulfates attain higher absorption levels than the corresponding carboxylates. For the longer carboxylates the saturation values are indicated in the far-right part of fig. 2.

With most substrates the uptake in the elastin strip proceeds for several days. Assuming that the elastin strip is structured as illustrated in fig. 1 and considering the dimensions of the alkyl derivatives it is likely that they enter the interfilamentary space without much restraint. Binding at the periphery of the filaments will be a relatively fast process. However, penetration into the elastin network within a filament will be much more difficult, the more so the larger the molecules are. Moreover, unsaturated bonds in the alkyl chain decrease its flexibility, which, in turn, may obstruct its diffusion into the network. The low saturation value for linoleate could well be caused for that reason.

Under the experimental conditions (high pH, 0.15 M ionic strength, 70°C) the hydrodynamic radius of the micelles of the substrates used is in the range of a few nanometers [23]. It is likely that, especially in the initial stages of the absorption process, the micelles are able to penetrate the interfilamentary regions. Obviously, micelles cannot enter the network.

Assuming the uptake of the alkyl derivatives to be diffusion-controlled, the $A(t)$ curves allow for the calculation of the diffusion coefficient. To that end, the elastin strip is considered to be an isotropically structured cylinder. A theoretical description of diffusion in such a geometry is given by Crank [24]. In the case of radial diffusion, i.e., neglecting the contributions at both ends of the cylinder, the diffusion process is described by

$$\frac{\delta c}{\delta t} = \frac{1}{r} \cdot \frac{\delta}{\delta r}\left(rD\frac{\delta c}{\delta r}\right) \quad (2)$$

where c is the concentration at a distance r from the cylinder center, t the time and D the diffusion coefficient. In the derivation of eq. 2 it is assumed that D is independent of r, which, in view of the network model, seems reasonable.

Eq. 2 can be solved observing the following boundary conditions: (i) at $t = 0$ c (the concentration in the cylinder) $= 0$, (ii) the concentration c_0 of the diffusing molecules outside the cylinder remains constant during the diffusion process and (iii) the radius a of the cylinder is constant. A solution of eq. 2 is

$$c = c_0 - \frac{2}{a}c_0 \sum_{n=1}^{\infty} \frac{J_0(r\alpha_n)e^{-D\alpha_n^2 t}}{\alpha_n J_1(a\alpha_n)} \quad (3)$$

where $J_0(x)$ and $J_1(x)$ are Bessel functions of the first kind of zeroth and first order, respectively, and where α_n terms are the positive roots of $J_0(a\alpha_n) = 0$.

The first condition is obviously fulfilled. The second condition is approximately met, since during the diffusion process the total concentration of the substrates is beyond the CMC. The micelles are too large to penetrate into the elastin network. Hence, the effective concentration is the monomer

concentration, which is almost constant throughout the diffusion process. The third condition, unchanged dimensions of the cylinder, is not fulfilled. As a result of the absorbate uptake the network swells and a increases proportionally. It is, however, mathematically very difficult to account for the increase in a during the diffusion experiment.

Crank has modified eq. 3 as follows:

$$\frac{A_t}{A_\infty} = 1 - \frac{4}{a^2} \sum_{n=1}^{\infty} \frac{e^{-D\alpha_n^2 t}}{\alpha_n^2} \tag{4}$$

where A_t and A_∞ are the masses of the diffused material inside the cylinder at $t = t$ and $t = \infty$, respectively. Substituting the roots of the Bessel function [25] and introducing $t_{1/2}$ for t at which $A_t = \frac{1}{2}A_\infty$, it can be derived from eq. 4 that

$$D = 0.06524 \frac{a^2}{t_{1/2}} \tag{5}$$

If the diffusion process is accompanied by a concomitant binding the effective concentration, with respect to diffusion, is reduced and, hence, application of eq. 5 gives an underestimation of the diffusion coefficient. If S represents the concentration of bound molecules, eq. 2 must be extended with an additional term

$$\frac{\delta c}{\delta t} = \frac{1}{r} \cdot \frac{\delta}{\delta r}\left(rD\frac{\delta c}{\delta r}\right) - \frac{\delta S}{\delta t} \tag{2a}$$

If S is proportional to the concentration c of free molecules, i.e., $S = Rc$

$$\frac{\delta c}{\delta t} = \frac{1}{r} \cdot \frac{\delta}{\delta r}\left[r\left(\frac{D}{R+1}\right)\frac{\delta c}{\delta r}\right] \tag{2b}$$

Analogous to the foregoing reasoning (eqs. 2–5) it is derived that

$$\frac{D}{R+1} = 0.06524 \frac{a^2}{t_{1/2}} \tag{5a}$$

Values for $t_{1/2}$ can be taken from fig. 2 and those for a^2 from the degree of swelling:

$$a^2_{t_{1/2}} = \frac{L_{t_{1/2}}}{L_{\text{dry elastin}}} \cdot a^2_{\text{dry elastin}}$$

R may be obtained from the initial linear parts of the binding isotherms (see section 3.2). The results are collected in table 3. Absorption isotherms for octyl and decyl sulfate have not been determined so that for these substances R values are not available.

Due to the invalidity of one of the model assumptions and the approximations in the mathematical treatment the values calculated for D may contain a large systematic error. However, the data obtained may be used for comparison between the various alkyl derivatives. Thus, the diffusion coefficients of the fatty acids decrease with increasing chain length, as expected. Linoleate is an exception, however. It shows a remarkably high diffusion constant. This, in combination with the exceptional low saturation absorption suggests that linoleate diffuses only in the interfilamentary space, but not into the individual filaments. Apparently, the two double bonds in the alkyl chain reduce the flexibility so much that penetration into the network is hampered.

Another remarkable feature is the relatively high value for D of sodium dodecyl sulfate. On the

Table 3

Diffusion of alkyl derivatives into elastin network (T = 70°C)

Component	$t_{1/2}$ (s)	$a^2_{t_{1/2}}$ (m^2)	R	D (m^2 s^{-1})
Octyl sulfate	7200			
Decyl sulfate	14400			
Dodecyl sulfate	97200	1.5×10^{-6}	620	5.4×10^{-10}
Laurate	28800	1.4×10^{-6}	120	3.8×10^{-10}
Myristate	198000	1.5×10^{-6}	680	3.3×10^{-10}
Palmitate	367200	1.7×10^{-6}	840	2.5×10^{-10}
Oleate	712800	1.8×10^{-6}	900	1.5×10^{-10}
Linoleate	28800	1.3×10^{-6}	320	9.5×10^{-10}

basis of molecular dimensions the value would be expected to be between those of laurate and myristate. An explanation is not given here, but, in this context the large saturation absorption of the sulfates, relative to the carboxylates, is mentioned.

3.2. Absorption isotherms

The isotherms at 70°C for the various carboxylates and for dodecyl sulfate are given in fig. 3, where the amount A of absorbed material is plotted against the equilibrium concentrations c_{eq} in solution. In all cases the absorption is completely reversible, i.e., the isotherms obtained by absorption and desorption (on diluting the system) coincide.

For all substrates A increases gradually with increasing c_{eq} leading to a definite plateau value, A_s. This value is larger for the longer alkyl chains, unless uptake is blocked by steric restrictions (e.g., linoleate). It is noted that for the long fatty acids A continues to increase even at $c_{eq} >$ CMC, the latter quantity being indicated by an arrow in fig. 3. For laurate and dodecyl sulfate that have almost reached plateau absorption at $c_{eq} \leqslant$ CMC the corresponding initial concentration is in most cases also beyond the CMC. Hence, with all substrates,

Table 4

Standard Gibbs energies for the binding of alkyl derivatives to elastin

Substrate	ΔG° (kJ mol^{-1})
Dodecyl sulfate	−15.8
Laurate	−13.1
Myristate	−16.5
Palmitate	−21.1
Oleate	−18.7
Linoleate	−16.5

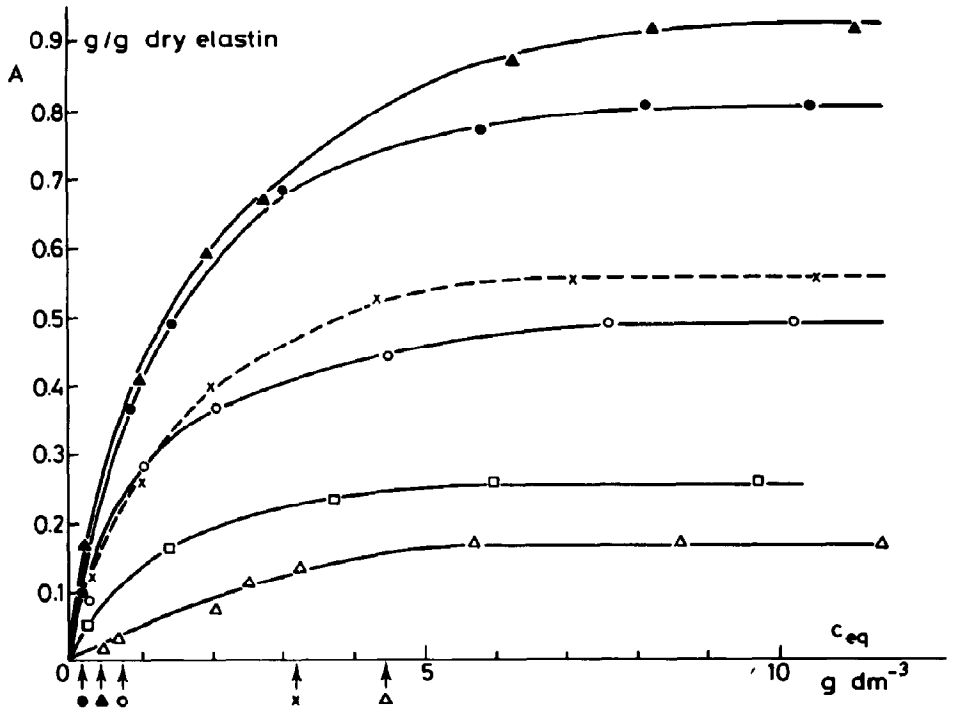

Fig. 3. Absorption isotherms for various alkyl derivatives in elastin. Conditions as in fig. 2. (×) Dodecyl sulfate, (△) laurate, (○) myristate, (●) palmitate; (▲) oleate, (□) linoleate.

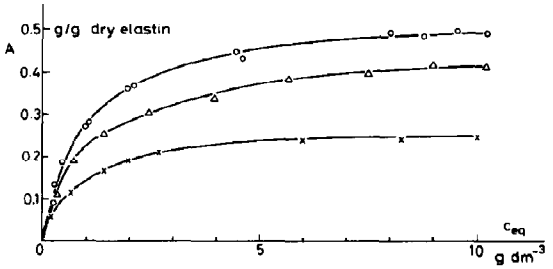

Fig. 4. Absorption isotherms for myristate in elastin. Ionic strength 0.15 M; pH = 10. (○) 70°C, (△) 55°C, (×) 40°C.

the uptake implies to a large extent the conversion of micellar material into elastin-bound material.

The absorption isotherms may be analyzed according to Langmuir [26], reversibility being the most relevant prerequisite. Applying the equation

$$\frac{A}{A_s - A} = Kc_{eq} \tag{6}$$

the binding constant K can be derived. The standard (per unit molarity) Gibbs energy ΔG° due to absorption then follows from

$$\Delta G^\circ = -RT \ln K \tag{7}$$

The values for ΔG° thus obtained for the three substrates are summarized in table 4. They are comparable to the Gibbs energies of surfactant binding to globular proteins in the cooperative hydrophobic binding region [27]. The values become more negative with increasing length of the alkyl chain. In the homologous series laurate-myristate-palmitate the increment per CH_2 groups is approx. -4 kJ mol^{-1}, which is comparable to that for alcohols being transferred from water into their own bulk liquid [28]. It further appears that, for a given chain length, the absorption affinity decreases with increasing number of unsaturated bonds, i.e., with decreasing flexibility of the molecule.

Table 5

Standard thermodynamic functions of state for the binding of sodium myristate to elastin

Temperature (°C)	ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	ΔS° (J mol^{-1} K^{-1})
40	−13.7	+11.3	80
55	−14.9	+17.8	100
70	−16.5		

From the variation of the isotherm with the temperature the contribution from the enthalpy ΔH° and the entropy ΔS° to ΔG° can be derived

$$\left(\frac{\delta \Delta G^\circ}{\delta T}\right)_P = -\Delta S^\circ \tag{8}$$

and

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{9}$$

The temperature effect has been studied for myristate only. Fig. 4 contains the isotherms at 40, 55 and 70°C. The values for ΔG°, ΔH° and ΔS° are given in table 5. The binding of myristate and, probably, of the other substrates as well is driven by an increase of entropy that more than compensates for the unfavorable enthalpy change. It is likely that the large entropy gain stems from dehydration of the hydrophobic alkyl chains and, possibly, from the hydrophobic amino acid residues of the elastin that are involved in the binding.

3.3. Stress-strain measurements

At constant volume V the change in free energy F of any elastic material that is deformed by applying a force is given by

$$(\mathrm{d}F)_V = -S\mathrm{d}T + \tau \mathrm{d}L \tag{10}$$

where τ is the force per unit area (the stress), S the entropy and L the length in the direction of the force. Then, if the temperature is also kept constant

$$\tau = \left(\frac{\delta F}{\delta L}\right)_{T,V} = \left(\frac{\delta U}{\delta L}\right)_{T,V} - T\left(\frac{\delta S}{\delta L}\right)_{T,V} \tag{11}$$

where U is the internal energy. Written in a different notation

$$\tau = \tau_u + \tau_s \tag{11a}$$

In section 1 it has already been mentioned that elastin behaves like a rubber. Rubber elasticity is characterized by $\tau_u = 0$ and, hence, $\tau = \tau_s$ [29]. The polypeptide chains between two cross-links in the

Table 6

Elasticity moduli, E (N cm^{-2}), of elastin and elastin-alkyl derivative complexes at various water contents ($T = 37°C$)
A, g alkyl derivative per 100 g dry elastin; y, g water per 100 g dry material (elastin + alkyl derivative).

Elastin											
A	–	–	–	–	–	–	–				
y	17	23	25	39	48	53	55				
E	225	192	194	179	157	148	159				
Elastin-laurate											
A	11	15	15	15	22	22	22				
y	99	42	71	115	18	31	44				
E	89	129	108	66	155	140	124				
Elastin-myristate											
A	27	39	39	39	40	40	40	49	52		
y	112	20	37	44	36	86	113	118	127		
E	70	123	100	107	92	56	44	34	34		
Elastin-palmitate											
A	13	42	42	52	52	52	52	68	69	69	71	71
y	76	74	110	17	27	41	66	29	49	75	56	118
E	90	43	38	118	104	76	73	96	53	38	45	19
Elastin-oleate											
A	61	71	71	74	90						
y	54	107	166	122	57						
E	45	29	27	31	40						
Elastin-linoleate											
A	14	14	14	16	16	16	16	25	25		
y	22	38	55	36	75	75	80	49	92		
E	164	137	124	137	106	93	96	108	73		
Elastin-dodecyl sulphate											
A	50	50	50								
y	23	38	85								
E	110	84	60								

relaxed elastin network are in rapid Brownian motion. This rapid motion is only possible if a large number of conformations are available to the chains. In rubber-like materials, like elastin, these conformations all have the same energy. On stretching the network the polypeptide chains are elongated. This does not affect the internal energy. However, the decrease of the number of the conformational degrees of freedom reduces the entropy of the network.

The elasticity of the material may be expressed through its elasticity modulus E, defined by

$$\tau = E\frac{\Delta L}{L} \tag{12}$$

where $\Delta L/L$ is the relative length increase (the strain). In the case of an ideal rubber, for relatively small elongations, E may be approximated by

$$E = 3nkT \tag{13}$$

where n is the number of chains between two adjacent cross-links per unit volume. Absorption of water and of the alkyl derivatives causes swelling of the elastin and, hence, a reduction of n. As a result E decreases.

Rubber elasticity is observed only over a limited temperature range. Below a certain temperature, the glass transition temperature T_g, the material becomes glassy, implying an increase of E by

several orders of magnitude. More specifically, the glass transition takes place over a small temperature interval of, say, 5–10 degrees, after which rotational transitions of the polypeptide chains become rare during the time of the measurement. The temperature at the midpoint of the rubber-glass transition is usually denoted as T_g. From the foregoing it may be clear that T_g is not a uniquely defined temperature. Its value depends on the detection method, notably the time scale of the measurement.

Table 6 summarizes the elasticity moduli for elastin and the elastin-alkyl derivative complexes (A g absorbate per 100 g dry elastin) containing varying amounts of water (y g water per 100 g dry material). The moduli were calculated from stress-strain measurements performed at a frequency of 1.5 Hz and at 37°C, mimicking the conditions of the arterial wall. The maximum elongation of the elastin strip was less than 10%. The data reveal that E decreases systematically with increasing amount of absorbate, irrespective of whether this is alkyl derivative or water, in the sample. Since uptake of the substrates causes a swelling of the elastin network n decreases with the amounts absorbed. If, upon absorption, no cross-links are formed (e.g., by bridging between two polypeptide chains via the alkyl derivative molecule) or broken, n is inversely proportional to the volume of the sample containing a given amount of pure elastin: $dn = \alpha dV^{-1}$, in which α is a constant. Substituting this condition in the differential form of eq. 13 yields

$$dE = 3kT\alpha dV^{-1} \tag{14}$$

In fig. 5 E is plotted against V^{-1}. The values of V refer to samples containing 100 g dry elastin. They were calculated according to

$$V = \frac{m}{\rho} = m\left(\frac{X_e}{\rho_e} + \frac{X_w}{\rho_w} + \frac{X_a}{\rho_a}\right) \tag{15}$$

where m is the weight of the sample (containing 100 g elastin), ρ the density and X the weight fraction of the indicated component in the sample (e, elastin; w, water; a, alkyl derivative). Fig. 5 shows, for the various samples, a linear relation between E and V^{-1}. Hence, n varies proportionally to V^{-1}, which confirms that no cross-links are formed or broken due to the uptake of the substrates. Furthermore, for all samples $E(V^{-1})$ can be represented by one single master curve. The conclusion is that the variation in E is exclusively determined by the degree of swelling and is not affected by any specific interaction between the elastin and the absorbed components. The reduction in E is, therefore, the larger the higher the fatty acid content which, in turn, results in a larger water uptake. As a result the elasticity modulus decreases by more than a factor of 5, except for the short fatty acids and the multi-unsaturated ones.

Finally, the elasticity of several samples has been determined as a function of temperature, in order to establish the glass transition temperature, T_g. The results are presented in fig. 6. The data obtained for the elastin-water systems compare

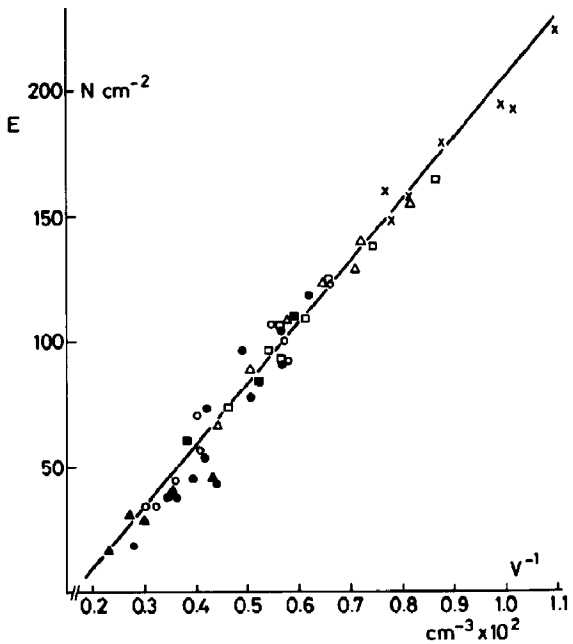

Fig. 6. The glass transition temperature, T_g, of elastin and elastin-alkyl derivative complexes (A g alkyl derivative per 100 g dry elastin) as a function of their water content, y (in g per 100 g dry material). (×) Elastin, (△) elastin-laurate ($A = 10$), (○) elastin-myristate ($A = 44$), (●) elastin-palmitate ($A = 70$), (▲) elastin-oleate ($A = 76$), (□) elastin-linoleate ($A = 16$), (■) elastin-dodecyl sulfate ($A = 46$).

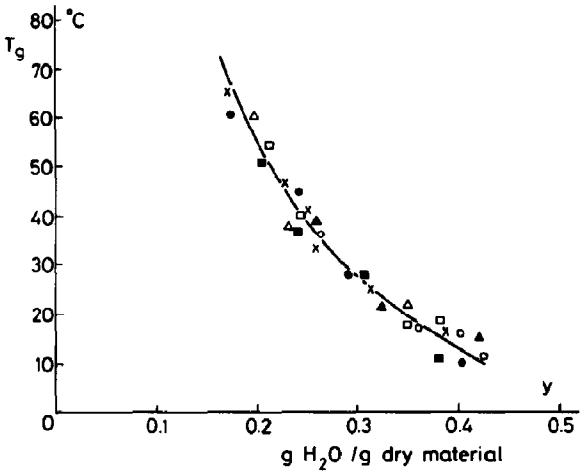

Fig. 5. The elasticity modulus, E, of elastin-alkyl derivative-water complexes as a function of the reciprocal volume of the sample. This plot is a graphical representation of the data given in table 6. $T = 37°C$. (×) Elastin, (△) elastin-laurate, (○) elastin-myristate, (●) elastin-palmitate, (▲) elastin-oleate, (□) elastin-linolcate, (■) elastin-dodecyl sulfate.

well with those presented in the literature [7]. It appears that, within the range studied, at a given water content T_g is not significantly affected by the presence of the alkyl derivative in the elastin. It means that the alkyl derivatives do not affect the rotational freedom along the polypeptide chains between the cross-links, suggesting that these substances do not bind to the peptide units in the main chain but rather to the residual amino acid side groups. This is in agreement with the suggestion of dehydration of hydrophobic amino acid residues which contributes to the entropy increase upon binding.

As a consequence of the presence of the alkyl derivatives the samples contain an increased water content. Therefore, absorption of fatty acids indirectly lowers T_g. This, in itself, is not physiologically relevant, since the effects on T_g occur well below the physiological temperature of 37°C.

4. Conclusion

Alkyl sulfates and alkyl carboxylates (the naturally occurring fatty acids) show a strong tendency of binding to elastin. This tendency depends on the structure of the molecule. It decreases with decreasing length of the alkyl chain and it is reduced to almost zero for chain lengths less than C_{10}.

The absorption rate of the substrates in the elastin network also depends on the chain length. At 70°C it takes some tens of hours to saturate the elastin with laurate (C_{11} chain), but some hundreds of hours in the case of palmitate and oleate (C_{15} and C_{17} chain, respectively). Furthermore, if the alkyl chain contains two (linoleate) or more double bonds the molecule is too stiff to penetrate the elastin network. Elastin contains a relatively large amount of hydrophobic amino acids and different experiments suggest that the alkyl derivatives bind to the hydrophobic side chains rather than to the polypeptide backbone of elastin. The uptake of the anionic material introduces negative charges in the network which leads to electrostatic repulsion. As a result, the network swells, reaching equilibrium when the electrostatic repulsion is balanced by the swelling pressure. Obviously, the swollen network contains an increased amount of diluent, i.e., water.

Although in the experiments described here, elastin from bovine ligamentum nuchae is exposed to solutions of only one type of alkyl derivative, the events that occur on contacting arterial elastin to a mixture of fatty acids probably are comparable. The composition and properties of arterial elastin are very similar to that of elastin from ligamentum nuchae [30]. If in the artery the endothelial lining is damaged, the elastin of the intima is exposed to the blood that contains free fatty acids of various kinds. It is then expected that the longer fatty acids, except the multi-unsaturated ones, are taken up. Because of the temperature difference, the rate of absorption will be considerably slower than that in the present study. The fatty acid absorption may lead to a local reduction of the elasticity of the artery by, say, a factor of 5 (cf. fig. 5). The mechanical response becomes more sluggish so that various components from the blood may be deposited in that region. Plaque formation has started, which may finally result in a total occlusion of the artery. Indeed, as mentioned in section 1, large amounts of free fatty acids have been found in atherosclerotic tissue. Thus, the

absorption of fatty acids from the blood in the elastin of arterial intima could well be at the origin of atherosclerosis. Further research, preferably similar experiments in situ, will reveal whether the mechanism described in this paper is indeed of pathological significance.

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