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Dynamic mechanical relaxations in swollen elastin networks

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Summary

Samples of elastin obtained from ox *ligamentum nuchae* were swelled with one of several glycols and then studied with regard to their dynamic mechanical properties. The glass transition temperatures of these swollen networks were substantially lower than that of dry elastin. Two low-temperature transitions were observed when longer-chain glycols were used as the diluent. The relaxation processes in elastin/triethylene glycol combinations has a higher activation energy than that reported for elastin/ethylene glycol systems. This is thought to be due to the bound diluent increasing the mass and bulk of the relaxing main-chain segments. The data are consistent with elastin being an amorphous, randomly-coiled, somewhat hydrophilic, polymeric material interacting with diluents in the same way as do non-biological polymers.

Introduction

The main component of biological tissue is the amorphous protein elastin. Its importance in conferring elasticity to vital body tissues such as ligaments, arteries, and skin, and its unique macromolecular structure with several repeating polypeptide sequences has lead to numerous studies on the mechanism of its elasticity.

Early microscopic and gel filtration data lead Partridge[1] to propose a regular assembly of globular uinits as the main structural feature of elastin. This seemed to be supported by calorimetric studies and a two-phase model of elastin, often referred to as the "liquid-drop" model, was therefore developed.[2] According to it, the elastic response in elastin is primarily energetic in origin. Fluorescence probe analyses[3] and nuclear spin relaxation data[4] on elastin have also been thought to be consistent with such a model.

However, essentially all other rubbery materials are known to be one-phase networks of macromolecules having random-coil configurations. Thermoelastic studies by Hoeve and Flory,[5] Volpin and Ciferri,[6] and Andrady and Mark,[7] suggest elastin also to have such a structure, and that the origin of its elasticity is primarily entropic. This classical, random-network model is also supported by NMR data on elastin.[8]

Dynamic mechanical analysis should be very useful for addressing such issues, since it is a convenient method for investigating the structural features of polymeric materials in general. In fact, Pezzin and Scandola[9] did study the low-temperature relaxations of dry elastin using this technique. They established the existence of a transition below the glass transition temperature T_g, which was attributed to main-chain relaxation. In a second study,[10] elastin swollen with either water or ethylene glycol was studied using the same technique. Interestingly, the two diluents were found to have significantly different effects on the dynamic mechanical properties of elastin.

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The present, preliminary study was undertaken to determine the dynamic mechanical behavior of elastin/glycol systems, including those with longer-chain glycols as diluents. The data are expected to provide information on the interaction between solvents and the polypeptide backbone, and therefore also to clarify some of the structural features of this important protein.

Experimental

Ox *ligamentum nuchae* from freshly slaughtered animals was cleaned of adhering surface fat and tissue with a scalpel. Strips of tissue were extracted with a chloroform: methanol (3:1) mixture, at 4 °C for 24 hrs. The tissue was then extracted with 0.2 M aqueous sodium chloride for 48 hrs. at 4 °C. The elastin was then washed with distilled water, and extracted with water in an autoclave at 121 °C for 1 hr. The autoclaving was repeated 8 times, finally giving an aqueous extractant which was negative to the biuret test. The extracted tissue was partially dried in vacuo for 24 hrs., and the semi-dry elastin was sliced to give thin sheets about 0.5 mm thick, using a microtome. The slices were further dried under vacuo and stored in a desicator at ambient temperature.

Three of the the five diluents, namely water, ethylene glycol, and diethylene glycol, were introduced into the elastin by allowing strips of it to swell in them, at ambient temperature. The amount of diluent was determined gravimetrically, and adjusted to give a volume fraction v_2 of polymer equal to 0.49 ± 0.02, by controlling the duration of contact with the diluent.

In the case of triethylene glycol and tetraethylene glycol, the swelling was too slow at ambient temperature, and the swelling was therefore carried out at 100 °C for about twenty minutes. The volume fraction of the diluent was adjusted to the same volume fraction as that in the others by evaporating the diluents under vacuum at room temperature, or by placing the swollen elastin networks in acetone at room temperature for short (pre-determined) intervals of time to extract some of the excess diluent.

The various diluents used were themselves subjected to dynamic mechanical analysis by retaining them on strips of Whatman GF/C glass-fiber paper. In a similar manner, the elastin-relevant poly(α-amino acids) polyglycine, polyalanine, polyvaline, and polyleucine were dissolved in trifluoroacetic acid at room temperature and then absorbed onto other glass-fiber paper strips. The solvent was then removed under vacuo for several days at room temperature.

The dynamic mechanical measurements were made on a Rheovibron DDV IIC instrument. The test pieces were placed in the grips of the viscoelastometer and were rapidly cooled to about -120 °C, altering the tension to prevent rupture of the sample due to contraction. The temperature was then allowed to rise at the rate of about 1 °C/min. Repeated measurements on a single test piece showed good reproducibility. The water, ethylene glycol, and diethylene glycol tended to evaporate at temperatures above ~ -30°C, due to the use of a stream of precooled nitrogen. This source of error was minimized by closing off the nitrogen and placing a drying agent on the bottom of the sample chamber. Comparison of weights of test pieces measured under such conditions did not show any significant changes in weight during the measurements.

Results and Discussion

Dynamic Mechanical Relaxation in the Elastin/Tri-Ethylene Glycol System

Over the temperature range -110 to about +20 °C, the elastin sample containing triethylene glycol exhibits three relaxation peaks. Previous workers[9,10] have used lower-boiling diluents, consequently limiting the temperature range over which measurements could be made. The use of triethylene glycol as the diluent in the present study permitted observations to be made at temperatures as high as 30 °C without any significant loss of diluent.

As seen in Figure 1, the variation of tan δ with temperature shows a high-temperature peak, which occurs at 16 °C at 3.2 Hz (and $v_2 \approx 0.49 \pm 0.02$). This peak. which occurs at approximately the same temperature at 11.0 Hz, corresponds to the glass transition of the system. The glass transition temperature of pure (unswollen) elastin has been reported[9,11] as being around 200 °C. However, Hoeve and Hoeve[11] have shown the T_g of elastin to fall dramatically in

the presence of ethylene glycol. The present value of T_g is similar to that observed in the case of elastin samples containing approximately the same volume fraction of ethylene glycol.

As shown in Figure 1, the system exhibits two lower-temperature relaxation peaks.

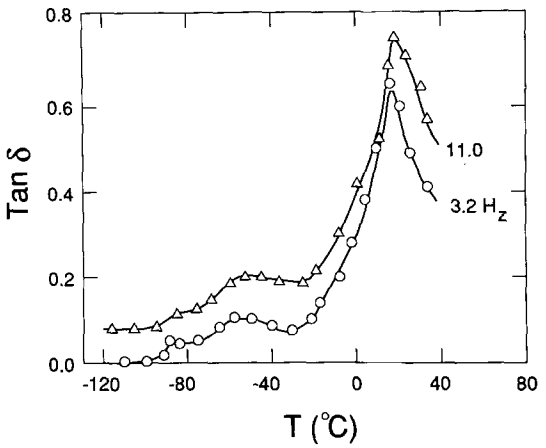


Fig. 1 Temperature dependence of the dynamic mechanical loss tangent for the elastin/triethylene glycol system ($v_2 = 0.49 \pm 0.02$) for the two specified values of the frequency. The upper curve has been shifted upward by 0.08 unit, for purposes of clarity.

These are termed the β and γ peaks, the latter being the one observed at the lower temperature. (Earlier workers[1,2] refer to a β peak in elastin/ethylene glycol and elastin/water systems. In the present study, it was found that three peaks were reproducibly observed with some diluents, and thus the β peak referred to by earlier workers possibly corresponds to the γ peak in the present study).

The origin of the β peak in Figure 1 is not clear. Some recent evidence suggests this peak in polymer systems to be complex, and made up of several overlapping peaks. The broadness and diffuse nature of the β peak observed for elastin/glycol is consistent with this view. Ishida et al.[12] tabulated values of T_β and T_α [the temperatures (°K) at which the respective peaks occur (at 100 Hz)] and found the ratio T_α/T_β to be 0.75. His result was based on polymers for which the β peak was believed to be caused by main-chain movements. Boyer[13] has made a similar study on polymers having long side chains, or having heterogroups in the polymer main chain. In this case, the ratio was significantly lower than 0.75. However, polypeptides were not one of the groups studied by these workers.[12,13] The present results indicate the ratio is indeed close to 0.75, as seen from the data in Table I. The T_α/T_γ ratio, however, is 0.64.

Table I gives the T_α, T_β and T_γ values for the elastin/triethylene glycol system and their frequency dependence. By considering the frequency dependence of the peak maxima the energy of activation of the relaxation processes might be obtained as

Table I. The Occurence of Tan δ Peaks in the Elastin/Triethylene Glycol System ($v_2 = 0.49 \pm 0.02$)

	Frequency, Hz					10^{-4} d (ln υ_{max})		
Temperature, °C	110	32	11	3.2	1.0	d(1/T_{max})	ΔH, kcal mol^{-1}	r*
T_α	24	–	18	16	–	-3.69	73.5	0.995
T_β	-42	-50	-52	-55	-57	-1.32	26.0	0.993
T_γ	-80	-83	-85	-88	-88	-1.59	31.5	0.999

* Correlation coefficient for data used to obtain ΔH.

$$\Delta H = \frac{R \, d \ln \upsilon_{max}}{d\,(1/T_{max})} \qquad (1)$$

where υ_{max} is the frequency and ΔH the energy of activation. Table I also gives values of ΔH and the correlation coefficient r for the data. Comparison of the ΔH value for the γ relaxation from Table I with that published by Pezzin et al.[9] for elastin/ethylene glycol systems show the former to be much higher (by about 17 - 20 kcal mol^{-1}). The γ relaxation process thus seems to require a higher activation energy in the triethylene glycol system relative to the elastin/ethylene glycol system.

It is interesting to compare the relaxation spectrum in Figure 1 with that for pure triethylene glycol supported on glass fiber paper. The latter shows a sharp dissipation peak at -65 °C which is not seen in any elastin samples which contain triethylene glycol. This seems to suggest that no "free" triethylene glycol domains exist in the swollen test pieces. In the case of ethylene glycol, however, the tan δ peak (of the diluent) is in the same temperature range as the γ peak for the elastin/ethylene glycol system. This makes the interpretation of data less ambiguous in the case of the elastin/triethylene glycol system.

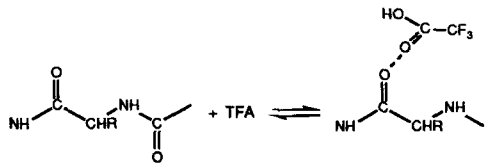
Molecular Interpretation of the Loss Peaks

The molecular significance of the β and γ peaks in swollen elastin is difficult to determine due to the limited data obtained here and available in the literature. Direct extension of results obtained from studies on different synthetic macromolecules to interpret the results of elastin is not satisfactory due to extreme differences in structure of polypeptides relative to that of most synthetic polymers. Such structural features are an important consideration as they dictate the degree of chain flexibility in amorphous domains.

Work done on poly α-amino acid systems might be reasonably extended to interpret the results obtained for elastin. However, in the case of these compounds as well, the assignment of the loss peaks is not clear. Some workers[14] believe the β and γ peaks to be due to side-chain movements, but it has also been suggested that the β loss peak arises from main-chain motions. The nature of these processes are, however, unknown.

The dynamic mechanical relaxation results for elastin/triethylene glycol were compared with those obtained for the same system with a small amount of trifluoroacetic acid in it. This

acid[15] has been shown to hydrogen bond with the carbonyl group of the peptide unit as shown in the reaction



Such an interaction would affect the main chain mobility while not affecting the mobility of non-polar side chains, which make up 90 % of the amino acid residues in elastin. The comparison of data showed the γ peak position to be virtually undisturbed (at 11Hz) by the addition of the acid, while the β peak shifted significantly (by about 8 °C) to a higher temperature. While this result is consistent with the assignment of the γ peak to side-chain movements, an exact assignment must await further study.

Elastin is composed of glycine, alanine, and valine as the principal amino acid residues. The number of residues of each of these, and of leucine, per 1000 residues is listed in Table II. Study of these poly α-amino acid/triethylene glycol mixtures supported on fiber glass paper

Table II. Transition Temperatures for Some Poly α-amino Acids Relevant to Elastin

Amino acid (in polypeptide)	No of residues/ 1000 in elastin	Transition temperatures of poly α-amino acid/triethylene glycol systems, °C*		
		T_γ	T_β	T_α
Glycine	327	-76	-40	-2
Alanine	220	-82	-42	-18
Valine	154	-75	41	–
Leucine	65	-88	-67	–
Elastin**	–	-85	-52	18

* All data for 11.0 Hz, obtained using polymer and diluent supported on glass-fiber paper.
** Included for purposes of comparison.

revealed their dynamic mechanical loss tangent peaks to be similar in form to those obtained using elastin. Their T_α and T_β values are indicated in Table II. It is interesting that the peak positions approximately correspond to those for elastin measured under similar conditions. If a plot of temperature vs. tan δ for elastin/triethylene glycol (at 11.0 Hz) is superposed over each of the poly α-amino acid/triethylene glycol plots, it is seen that the width of each poly α-amino acid peak (γ and β) is less intense than (and therefore falls within) the relevant elastin peak. In the case of the β peak, this is not particularly surprising since in elastin it is rather broad and not well defined. However, it suggests the possibility of side-chain movements being the origin of the γ loss peak, or strongly influencing it. The activation energy ΔH was calculated in the case of the alanine polypeptide and was found to be 10 kcal mol^{-1}, which is lower than that for elastin. This is to be expected in view of the simplicity of the side chain involved.

Effect of Diluent on the Dynamic Mechanical Relaxation in Elastin

Five diluents of increasing molecular weight (from water to tetraethylene glycol) were used in this study. In each case, the volume fraction of elastin was adjusted to be approximately 0.49 ± 0.02. Figure 2 shows the β and γ regions of the tan δ vs. temperature curves obtained in each case at 11.0 Hz.

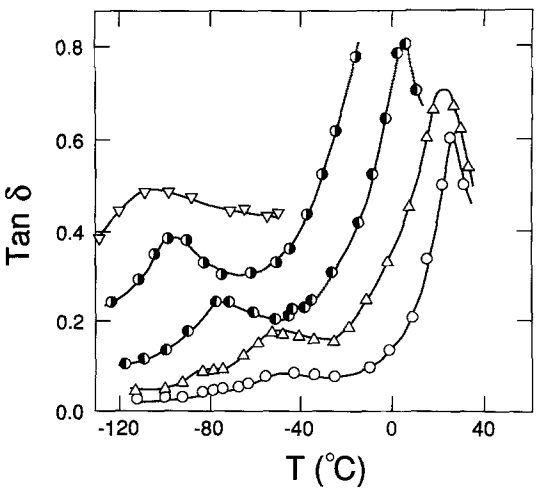


Fig. 2 Dynamic mechanical loss behavior of elastin/diluent systems for several glycol diluents. The diluents were water (▽), ethylene glycol (◑), diethylene glycol (◐), triethylene glycol (△), and tetraethylene glycol (○). The four upper curves were shifted upwards by 0.05, 0.15, 0.20, and 0.40 unit, respectively, with the curve for water also scaled upward by a factor of ten.

At one extreme, water shows only a predominant γ peak while at the other extreme, tetraethylene glycol shows predominantly the β peak. Scandola et al.[10] refer to the single peak obtained in the elastin/water system as a β peak, and it was found to vary considerably with v_2. They found a single low-temperature relaxation peak corresponding to the γ transition listed in Table II, for the elastin/ethylene glycol system. Again, the loss tangent was found to depend on v_2. This suggests gradual "binding" of diluent at various sites on the elastin network. Due to the predominantly non-polar nature of the amino acid side chains present in elastin, such interactions might be expected mainly to involve main-chain locations. The increased free volume available for side-chain movement in swollen elastin might then have contributed to the decrease in the γ transition temperature. Increasing the molecular weight of diluent (from ethylene glycol to triethylene glycol) increases the β relaxation while suppressing the α peak. The latter effect is probably a result of the decrease in available free volume in the glassy state for side-chain relaxation. The appearance of the β peak might be due to possible stronger interaction of diluent with the main-chain of the polymer. Unlike the case of water and ethylene glycol, the di- and triethylene glycols are bulkier and change the effective dimensions and the relaxation characteristics of the main-chain segments involved. In the case of tetraethylene glycol, the γ peak is virtually absent and the β peak is predominant. Table III summarizes the results obtained in this series of experiments. The values of T_γ, T_β, tan $\delta_{max}(\gamma)$, tan $\delta_{max}(\beta)$, tan $\delta_{max}(\alpha)$ and the ratio of tan $\delta_{max}(\beta)$/tan $\delta_{max}(\gamma)$ are given. The last quantity compares the relative

Table III. Dynamic Mechanical Data for Elastin/Glycol Systems at 11.0 Hz ($v_2 = 0.49 \pm 0.02$)

Diluent	Molecular weight, g mol-1	toC			tan γ_{max}			tan δ_{max} (β)/ tan δ_{max}(γ)
		+α	-β	-γ	α	β	γ	
Water	18	-	-	105	-	-	0.09	-
Ethylene glycol	62	-	-	98	-	-	0.18	-
Diethylene glycol	106	5	40	78	0.65	0.06	0.07	0.86
Triethylene glycol	151	18	52	85	0.65	0.13	0.05	2.6
Tetraethylene glycol	914	35	45	75	0.59	0.08	0.03	7.4

strengths of β and γ relaxations. Direct comparison of a tan δ_{max} value for different diluents is not satisfactory as different test pieces were used with each diluent. (Test-piece to test-piece variance might be large in case of elastin). The observed trend in the variation of T_γ is shown in Figure 3, where the molecular weight of diluent is correlated with these values for results at 11.0 Hz and $v_2 = 0.49 \pm 0.02$. In the case of T_γ, the values for the poly α-amino acids (containing approximately 16 % by wt of triethylene glycol) at the same frequency are included. Also included for comparison are the T_{max} values for the relaxation of the pure diluents (ethylene glycol and triethylene glycol). The T_γ for elastin is seen to increase approximately linearly with the molecular mass of the diluent.

The dependence of both T_γ and tan $\delta_{max}(\gamma)$ on the glycol weight fraction has been reported by Pezzin[10] for elastin/ethylene glycol systems. His results were confirmed in the present study in that lowering of v_2 from 0.58 ± 0.02 to 0.49 ± 0.02 was accompanied by a decrease in the T_γ values by two degrees and a decrease in the tan $\delta_{max}(\gamma)$ values for both ethylene glycol and triethylene glycol. In the case of the β peak for triethylene glycol/elastin, T_β increased by two degrees while tan $\delta_{max}(\beta)$ also increased significantly when the value of v_2 was similarly lowered. While the differences in T_β and T_γ values at the respective v_2 values might not be significant (being within experimental error) the difference in tan δ_{max}seems to be significant.

It is difficult to interpret these observations with any degree of certainty, however, due to the preliminary nature of the results. Nonetheless, it is likely that in the triethylene glycol/elastin system, the interaction of the polypeptide with the bulky triethylene glycol molecules limit the mobility of both the main-chain segments and the side-chain segments without altering the nature of the motion itself. Progressively longer-chain glycols attached at one end[16] to the elastin chain would increase the size of relaxing main-chain segments, thereby increasing the activation energy for relaxation. In general, glycols affect the relaxation behavior of elastin to an extent determined by the volume fraction of glycol in the system, and chain length of the glycol. The dynamic mechanical properties observed are consistent with that of an amorphous, random-coil polymer interacting with diluents in the usual manner for non-biological polymers. There is thus no evidence whatever for formation of a two-phase arrangement such as that assumed in the "liquid-drop" model.[2]

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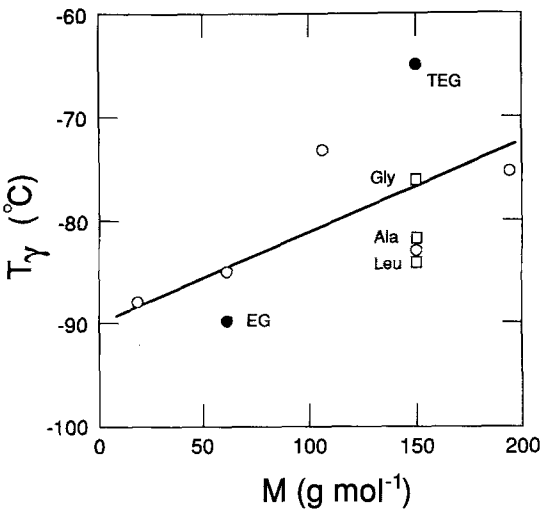

Fig. 3 Dependence of the transitions temperatures T_γ (°C) of elastin/diluent systems on the molecular mass of the diluent.

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