Theoretical study of the thermoelastic properties of elastin model chains

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The origin of the elastic behaviour of the bioelastomer elastin has been a matter of some controversy in recent years, as has been the degree of stable secondary structure present in the native state. Available data on the primary sequence of the protein chains constituting the elastin network has indicated a significant incidence of three repeat peptides of length four, five and six, with all containing a Pro–Gly pair within them. The present investigation involved the calculation of the temperature coefficient of the mean-square end-to-end distance $\langle r^2 \rangle_0$ of random-coil model chains composed exclusively of one of the three repeat peptides. This configuration-dependent property is directly related to the experimentally obtained energetic component of the elastic force. The values of $d\ln\langle r^2 \rangle_0/dT$ for each model chain were evaluated through the use of semi-empirical energy calculations on terminally-blocked residues present in the aforementioned repeat peptides, and subsequent use of established matrix techniques for calculating $\langle r^2 \rangle_0$ for polypeptides within a scheme involving independently rotating virtual bonds. Three levels of hydrogen bonding inclusion were investigated to help establish the importance of this contribution. The small positive value of the energetic component of the elastic force for elastin in the literature was found to be adequately reproduced in these calculations on the random-coil model chains.

(Keywords: thermoelasticity; elastin; chain model; protein chains)

INTRODUCTION

Elastin is a biological elastomer located in specific tissues of various mammalian species where its fully recoverable elastic character is properly utilized, such as in ligaments, skin, the lungs, and in the arteries leading from the heart[1]. The mechanism of the elastic response along with the magnitude and sign of the energetic component to the elastic force have been a matter of some controversy over the years[2–9], especially as to whether it is necessary to invoke ordered morphologies on the molecular level *versus* a treatment based on a simpler model of a crosslinked network of random-coil polypeptide chains. This latter model mirrors that for most synthetic elastomers under most physical conditions. It is the purpose of this theoretical study to examine the energetic component of the elastic force for elastin model chains, by use of the random-coil rotational isomeric state model and to determine whether it is sufficient to gain agreement with the experimental value for the quantity.

In the native state, elastin is a crosslinked network of identical protein chains, in which lysine residues present in the primary sequence are the loci for the formation of unique, enzymatically produced crosslinks. The native fibres are amorphous and contain fat, collagen, and soluble protein material, which can be extracted *in vitro* to yield nearly pure, insoluble elastin. The crosslinks are formed by the catalysed condensation of the termini of four lysine side chains into desmosine and isodesmosine structures[10,11].

Until relatively recently, the analysis of the actual primary sequence of elastin was all but impossible, since any attempt at obtaining isolated protein chains by severing the crosslink structure to a sufficient degree led to significant degradation and modification of the primary structure. Some information could be obtained on the residues in the vicinity of the crosslinks, especially C-terminal to them; however, such information is both fragmentary and of limited utility. This problem was partially circumvented by the discovery and use of a precursor polypeptide, tropoelastin, extracted from the aortas of copper deficient swine by Foster, Sandberg and coworkers[12]. Partial sequence analysis of this molecule has led to the observation that, as in the case of many fibrous proteins such as collagen and silk fibroin, certain repetitive amino acid sequences exist in the primary structure. Recent identification, isolation and nucleic acid sequence analysis of the elastin gene by Sandberg and coworkers[13] has for the most part completed the determination of the primary sequence and supported the above observations. In particular, the following three peptide sequences are found to contain approximately 30% of the total number of amino acid residues:

Tetrapeptide	–Val–Pro–Gly–Gly–
Pentapeptide	–Val–Pro–Gly–Val–Gly–
Hexapeptide	–Val–Ala–Pro–Gly–Val–Gly–

The pentapeptide for example is found to exist in a sequential run of eleven repeat units in bovine tropoelastin, while in chick tropoelastin, the length of the run is thirteen repeat units.

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The presence of a common Pro–Gly dipeptide centred in each of the three repeat sequences, as shown, is of particular interest. Specifically, this amino acid pair has been implicated in the past as having a high incidence of 1–4 β-bend structures in proteins in the absence of other driving forces. Work of Urry and coworkers on the above and similar peptide sequences by n.m.r., c.d. and other means strongly reinforces this observation[9,14–20]. The unique rigidity of the proline residue coupled with the inherent flexibility of the succeeding residue of glycine allows for the proper stabilizing hydrogen bonds to be formed in higher incidence than with most amino acid pairs. The residues in the aforementioned repeat peptides are quite hydrophobic, and perusal of the other residues in elastin show that very few hydrophilic residues are present, except for the lysines which are necessary for crosslink formation. The high incidence of hydrophobic residues has been used by Weis-Fogh[7,8] as the basis for a model of elastin in which the hydrophobic residues are viewed as phase separated globules whose surface areas are increased upon extension, exposing the residues to polar solvent. This mechanism is in stark contrast to the random-coil model where the reactive force is intramolecular in origin and incorporates the details of the primary structure.

Although the three repeat peptides constitute only about 30% of the primary structure, it is the major thrust of this investigation to study polypeptide chains constructed from the repeat peptides, assuming that to the first approximation they mirror the elastic and thermoelastic behaviour of elastin. This is on the surface a somewhat tenuous assumption; however, one justification is the observation by Urry that crosslinked networks prepared from polypeptide chains composed solely of the pentapeptide repeat unit have elastic character much like elastin[9,20].

THEORY

General approach

The computational procedure is as follows[21,22]. The initial stage is to perform semi-empirical molecular mechanics energy calculations on the amino acid residues present in the three repeat sequences of elastin. The process generates a (ϕ,ψ) Ramachandran energy map for each type of residue, where ϕ and ψ are the rotational angles about the bonds spanning the central α-carbon of the residue, and includes the interactions between atoms/groups whose distances depend solely on this pair of angles. The next step involves the Boltzmann averaging of an appropriate transformation matrix over each of the generated energy maps at two chosen temperatures. The final step of the calculation introduces the averaged transformation matrix into a 5×5 generator matrix for each residue at each temperature. These in turn are sequentially multiplied in the order of the residues in the primary sequence structure of the model polypeptide to yield the unperturbed mean-square end-to-end distance of the chain, $\langle r^2 \rangle_0$. Using the results obtained for the quantity at the two temperatures, its temperature coefficient (TC) expressed as $\mathrm{d}\ln\langle r^2 \rangle_0/\mathrm{d}T$ can be immediately evaluated. It is this quantity which is related to the energetic component of the elastic force, where the total force f is decomposed into the sum of the entropic component f_s and the energetic component f_e. The energetic component is functionally related to the temperature coefficient of $\langle r^2 \rangle_0$ as follows:

$$\mathrm{d}\ln\langle r^2 \rangle_0/\mathrm{d}T = f_e/fT \qquad (1)$$

The value of the quantity on the right has been obtained experimentally by stress–temperature measurements on a swollen network in a judiciously chosen solvent, and the theoretically obtained value can be directly compared with it.

Geometric and energy parameters

In order to perform semi-empirical energy calculations on the appropriate amino acid residues, it is necessary to decide on a suitable set of geometric parameters for the residues, torsional potentials and barriers (where applicable), charge distributions, and parameters and functions for non-bonded and hydrogen-bonded interactions. *Figure 1* depicts an important aspect of the theoretical treatment used in this investigation, i.e., the use of a virtual bond scheme[21]. Due to the planar-*trans* nature assumed to predominate for the peptide linkage, which follows from the partial double bond character of the C′–N backbone bond, the distance between adjacent C^α centres is constant for a given geometry of the unit which they span. This rather extended chemical structure allows for the treatment of the conformational energy surface generated by rotations about ϕ_i and ψ_i as independent of rotations about adjacent (ϕ, ψ) pairs. This aspect significantly simplifies the theoretical treatment of such chains, since it allows subsequent Boltzmann averaging of the transformation matrix representing a pair of consecutive virtual bonds over the energy map for the residue which they span. The angles η, ξ, θ, ϕ_i and ψ_i all enter the transformation matrix, with the first three fixed for a given geometry as shown in *Figure 1*.

The accepted bond angles and bond lengths for the relevant amino acid residues are given in *Figure 2*, and the partial charge distributions in *Figure 3*. These values are the best currently available as derived from extensive work on peptides and proteins by Scheraga and coworkers, with the partial charges derived from CNDO/2 calculations[23]. The geometric parameters also agree quite well with recent X-ray diffraction results on several elastin model peptides[24–26].

The total conformational energy of a particular residue as shown in *Figures 2* and *3* is partitioned into four contributions. The torsional energy about single bonds is ignored under the Scheraga treatment, except for amino acid side chains. For the residues under consideration

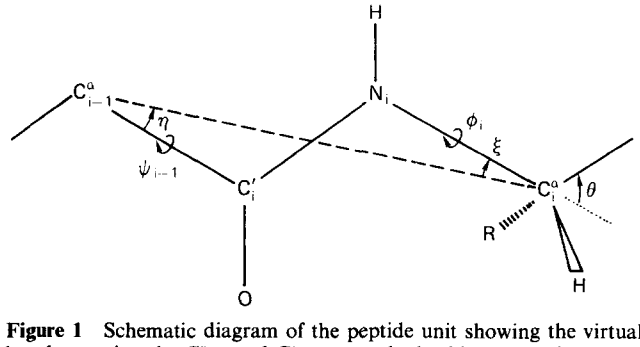

Figure 1 Schematic diagram of the peptide unit showing the virtual bond spanning the C^α_{i-1} and C^α_i centres, the backbone rotations ψ_{i-1} and ϕ_i, and the angles η, ξ and θ needed to transform between adjacent virtual bonds

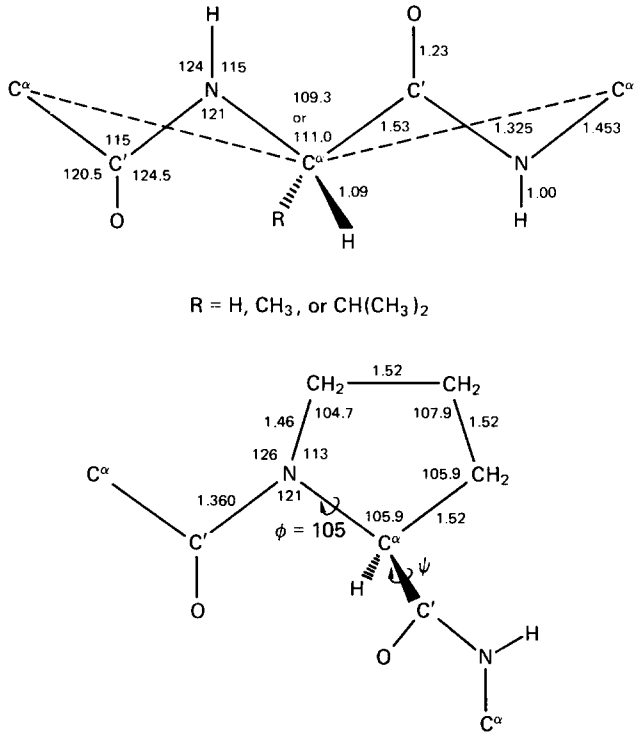

Figure 2 Bond length and bond angle geometric parameters of the amino (glycine, alanine and valine) and imino (proline) acid residues present in the repeat peptide sequences in elastin

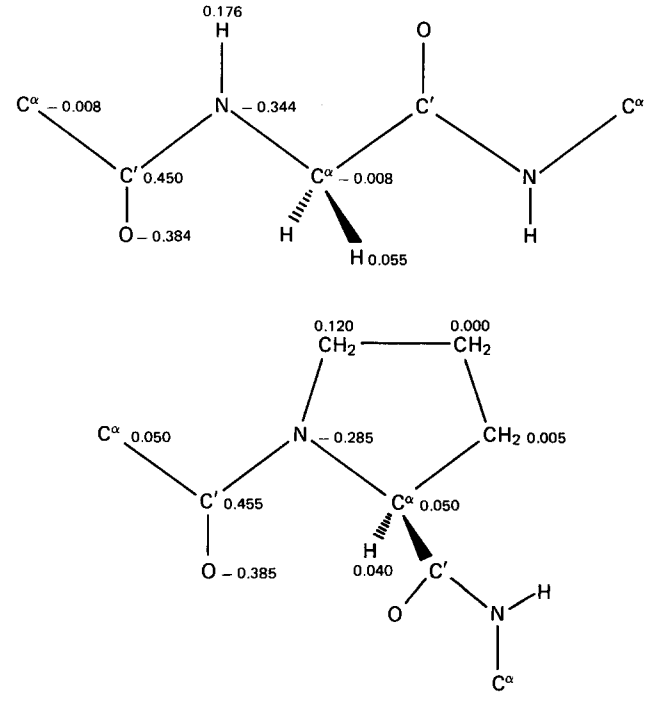

Figure 3 Partial charges placed on the constituent atoms of the amino and imino acid residues and used to calculate the coulombic interactions in the dipeptide units

here, only the rotation of the valine isopropyl side chain is included. It is assumed to have the functional form of a three-fold cosine expression as per usual, with a 3.0 kcal mol^{-1} energy barrier. All other torsional potentials are viewed as being incorporated within the non-bonded energy terms. The coulombic interactions are treated using the partial charges shown in *Figure 3* along with a dielectric constant of 2.0. This value is chosen so that the lower calculated dipole moments corresponding to the theoretical charge distributions are compensated for artificially. The non-bonded terms are treated by a 6–12 Lennard-Jones potential function, in which the attractive inverse sixth-power parameters are calculated for each type of pair interaction between atoms/groups using the Slater–Kirkwood equation. The repulsive inverse twelfth-power constants are evaluated as those values which yield a minimum of the 6–12 energy function at a distance of the sum of the van der Waals radii of the interacting pair. In addition, the repulsive terms for pair interactions separated by three bonds are scaled by a factor of 0.5, which was found to reproduce crystal lattice energies of small molecules and to allow for the virtual elimination of torsional potentials, except as discussed above. It should be stated that the CH_2 and CH_3 groups are treated each as single interacting species, rather than as individual atoms of carbon and hydrogen. Finally, the hydrogen bonding present between the carbonyl oxygen and amide hydrogen atoms is treated by a 10–12 general hydrogen bonding function. For the H···O interaction, this 10–12 function replaces the 6–12 non-bonded term in the total conformational energy sum. The A'_{ij} and C'_{ij} constants are taken from the literature and are characteristic of this type of hydrogen bond[23]. The total conformational energy of a given conformation is the sum over all relevant pair interactions and necessary side-chain rotational angles of the residue spanning two virtual bonds.

Generation of (ϕ, ψ) energy surfaces

Conformational energy surfaces for a glycine residue are shown in *Figures 4* and *5*. The former Figure excludes hydrogen bonding of any kind, or more specifically retains the simple 6–12 non-bonded term for the H···O interaction. The results in the latter figure include the hydrogen bonding as discussed in the previous section, which will be referred to henceforth as 'local' hydrogen bonding. Both surfaces display a centre of inversion about

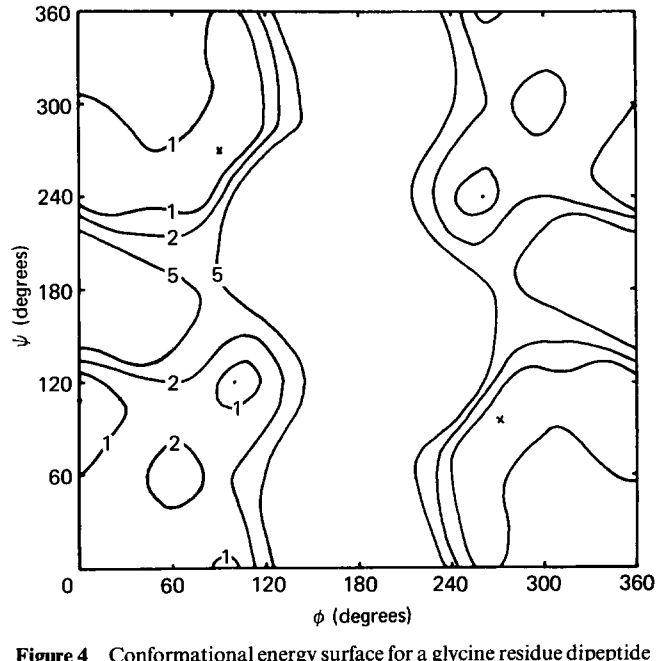

Figure 4 Conformational energy surface for a glycine residue dipeptide unit, when no hydrogen bonding interactions are included

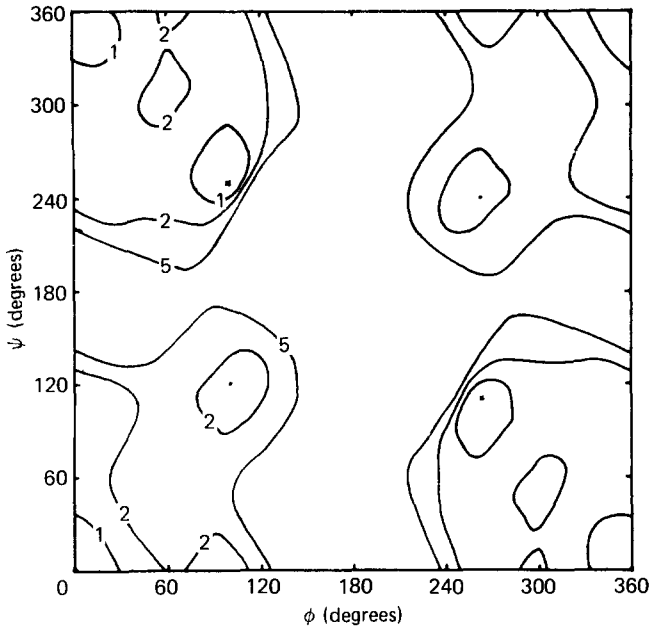

Figure 5 Conformational energy surface for a glycine residue dipeptide unit, where a normal (local) hydrogen bonding function has been used in its generation

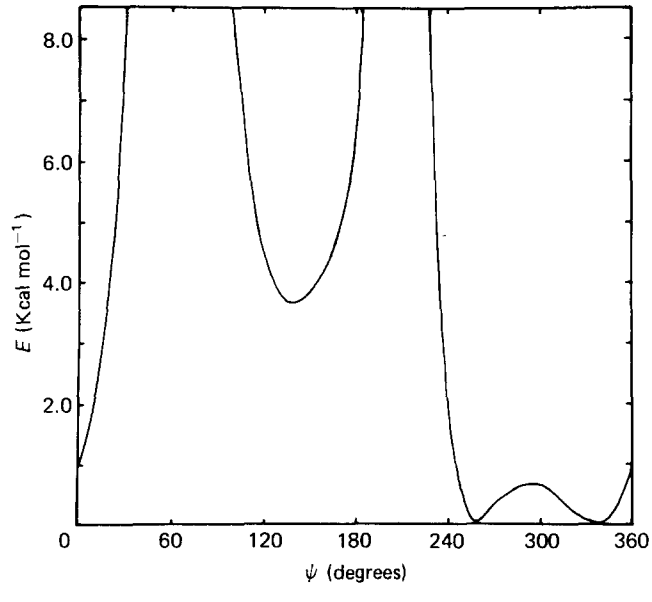

Figure 6 Effect of the rotation angle ψ on the conformational energy of a proline residue dipeptide unit (ϕ fixed at 105°)

$(\phi, \psi) = (180°, 180°)$ as expected from the symmetry of the α-carbon and the *trans*-planarity of the peptide linkage. Contours for all maps discussed are drawn at 1.0, 2.0 and 5.0 kcal mol^{-1} above the absolute minimum of each map which is denoted by an 'x', while local minima are specified by dots. The conformational energy surfaces for glycine show large areas which are accessible. The importance of this fact and the similarities of the minima in the four quadrants will be discussed in due course with regards to their effects on $\langle r^2 \rangle_0$ and $d\ln\langle r^2 \rangle_0 / dT$.

The energy surfaces for proline, valine, valine (followed by proline) and alanine (followed by proline) respectively are shown in *Figures 6–9*. The relative area of the surface accessible to valine is much less than that for glycine, and the relative areas and energies are significantly different. Each of these facets is brought about by the replacement of a hydrogen by an isopropyl group at the central α-carbon, introducing both asymmetry and greater steric interactions. For the proline residue the 'map' is one-dimensional in that the angle ϕ is fixed at 105.0° by the geometry of the pyrrolidine ring. It is assumed in these calculations that the ring is in the puckered-down conformation, which has been found to be the conformation of minimum energy. The valine (proline) and alanine (proline) residue maps differ significantly from those corresponding to valine and alanine without the succeeding proline residue. The pure alanine map is not shown since an isolated alanine residue is not present

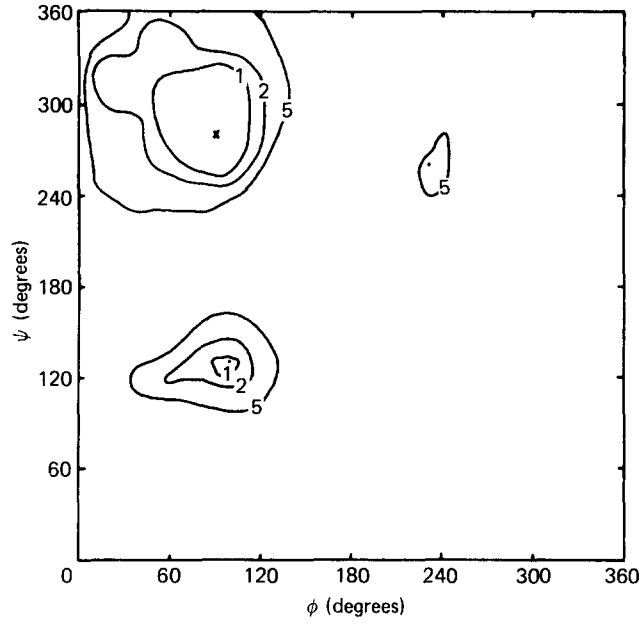

Figure 7 Conformational energy surface of a valine residue dipeptide unit with the isopropyl side chain energy minimized at each calculated point on the (ϕ, ψ) map

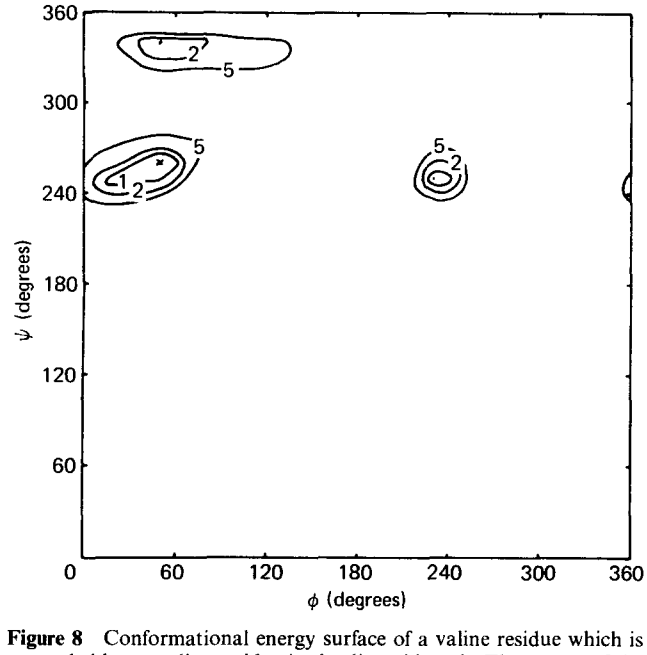

Figure 8 Conformational energy surface of a valine residue which is succeeded by a proline residue in the dipeptide unit. The energy of the isopropyl side chain is once again minimized for each calculated point on the map

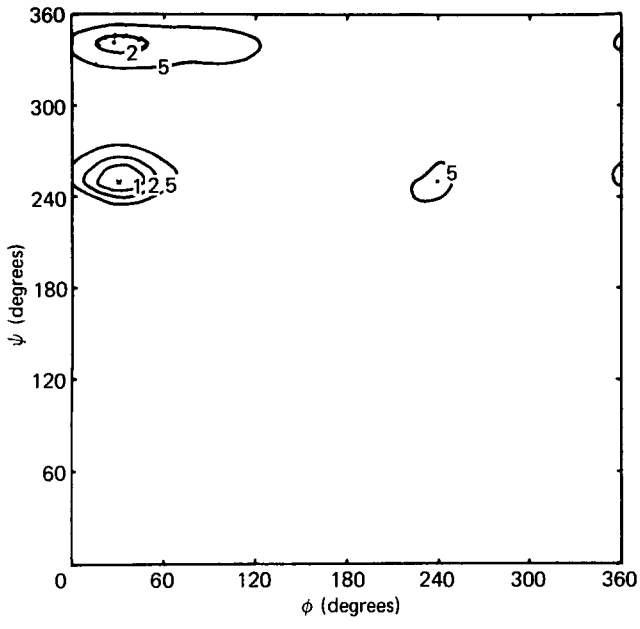

Figure 9 Conformational energy surface of an alanine residue which is succeeded in the dipeptide unit by a proline residue

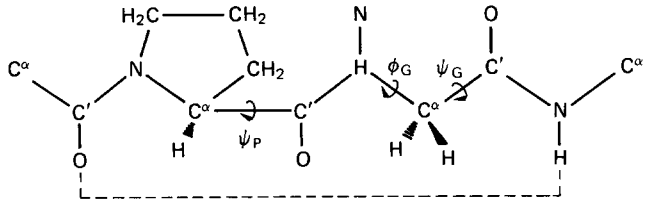

Figure 10 Schematic diagram of the proline–glycine–X tripeptide unit used to incorporate the longer-range hydrogen bond (depicted by the broken line) into the conformational energy surface of the glycine residue, through minimization of the energy as a function of ψ_P for each point on the (ϕ_G, ψ_G) map

in any of the three elastin repeat peptides. The severe restriction of available conformational energy surface occurs only when a proline succeeds such residues rather than preceeds them, and stems from the replacement of the succeeding amide hydrogen by the methylene group of the pyrrolidine ring. Such a situation exists in all three repeat peptides and is thus of importance. Each value of the total conformational energy used to generate the contours of the valine and valine (proline) maps are those calculated after minimization of the total energy with respect to rotation about the angle χ_i of the isopropyl side chain of valine.

As mentioned in the Introduction, work of Urry and coworkers has implicated the proline–glycine pair of sequential residues as the location of a rather stable type II β-bend. They have developed a model of elastin which accentuates this conformational aspect[18–20]. It uses a regular helical structure called a β-spiral and an argument employing librational entropy to explain the source of the elastic behaviour. Thus, it was felt necessary to attempt to include the hydrogen-bond interaction formed between the residues on either side of the Pro–Gly pair as shown in *Figure 10*. The (ϕ_G, ψ_G) map for the glycine residue was calculated for the structure shown incorporating all interactions present in the structure, with the energy being simultaneously minimized with respect to the single variable backbone rotation angle ψ_P of the preceeding proline residue. The glycine (ϕ, ψ) energy surface is shown in *Figure 11*. This energy surface is different from its two glycine predecessors discussed (*Figures 4* and *5*) in that it no longer possesses a centre of inversion due to the lack of symmetry of the structure involved. The longer-range hydrogen bonding interaction stabilizes the minimum at (260°, 100°) at the expense of the other low energy regions. Subsequent reference to this glycine energy surface will be denoted as incorporating interactions of longer-range (L.R.) as opposed to no hydrogen bonding or local hydrogen bonding. This longer range interaction is not incorporated into any other energy surfaces, since it is the peculiar character of the Pro–Gly pair which increases the likelihood of the β-bend structure and allows for its treatment as stated above. Once again, it is the rigidity of the proline residue and the limited configuration space which that implies coupled with the flexibility of the glycine residue and relative low energy already in the vicinity of (260°, 100°) which make its inclusion solely in the glycine (L.R.) map both possible and relatively exclusive relative to other residues.

Evaluation of $\langle \mathbf{T} \rangle_i$ matrices and calculation of $\langle r^2 \rangle_0$ and its temperature coefficient

The matrix $\mathbf{T}_i$ transforms a vector based in the usual coordinate system of the virtual bond (i) into the corresponding coordinate system of virtual bond (i–1)[21]. This transformation is best visualized and the matrix $\mathbf{T}_i$ constructed from three sequential transformations; i.e. from the virtual bond (i) to the backbone C′–C$^\alpha$ bond, then to the preceding C$^\alpha$–N bond, and to the virtual bond (i–1). To evaluate the averaged matrix $\langle \mathbf{T} \rangle_i$, it is necessary to perform the Boltzmann average of the transformation matrix $\mathbf{T}_i$ over the energy surface of the residue in question as given in equation (1). For all residues except proline, this is a double sum

$$\langle \mathbf{T} \rangle_i = \left[\sum_{\phi_i} \sum_{\psi_i} \mathbf{T}_i \cdot \exp(-E(\phi_i,\psi_i)/RT) \right] \Big/ \sum_{\phi_i} \sum_{\psi_i} \exp(-E(\phi_i,\psi_i)/RT) \quad (2)$$

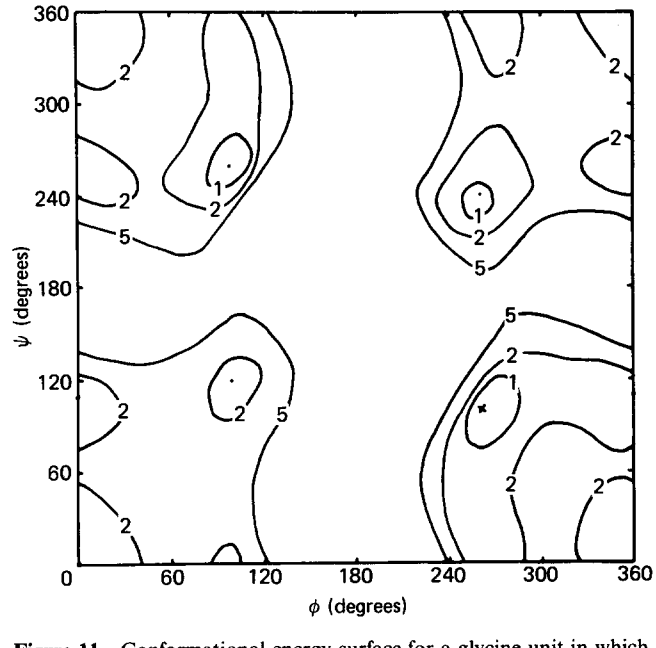

Figure 11 Conformational energy surface for a glycine unit in which long-range hydrogen bonding has been included, as described in *Figure 10*

ϕ_i and ψ_i at 30° intervals, while for proline it is taken as a single sum over the angle ψ_i at 20° intervals. The averaged matrices were evaluated at 300 K and 350 K.

The calculation of the mean-square end-to-end distance $\langle r^2\rangle_0$ for a given repeat sequence and at a given temperature is performed within the virtual bond scheme, assuming independent rotational potentials between polypeptide residues. The 5×5 generator matrix $\mathbf{G}_i$ shown in equation (3) is constructed using the averaged $\langle\mathbf{T}\rangle_i$ matrix for the residue and the virtual bond vector based in its own coordinate system, i.e. $\mathbf{l}_v = \mathrm{Col}(l_v,0,0)$.

$$\mathbf{G}_i = \begin{bmatrix} 1 & 2\mathbf{l}^T\langle\mathbf{T}\rangle_i & l_v^2 \\ 0 & \langle\mathbf{T}\rangle_i & \mathbf{l}_v \\ 0 & 0 & 1 \end{bmatrix} \tag{3}$$

The G_i matrices are then sequentially multiplied in the order of the primary sequence of the chain and to any degree of polymerization desired. The value of $\langle r^2\rangle_0$ is extracted from the final 5×5 matrix by pre- and post-multiplication by the vectors row (1,0,0,0,0) and Col (1,0,0,0,0), respectively. The value of $\langle r^2\rangle_0$ is usually expressed as a characteristic ratio, $C_n = \langle r^2\rangle_0/nl_v^2$, which scaled the mean-square end-to-end distance by the value it would have for a corresponding freely-jointed chain of n virtual bonds. The evaluation of $\mathrm{d}\ln\langle r^2\rangle_0/\mathrm{d}T$ for the chain of a given sequence is calculated from the results for $\langle r^2\rangle_0$ at the two aforementioned temperatures.

RESULTS

The calculated characteristic ratio and temperature coefficients of polypeptide chains for various homopolymers and copolymers of residues present in the repeat peptides of elastin are shown in *Table 1*. Three things should be noted with respect to these results. First, the characteristic ratios of the homopolymers are in general agreement with both previous calculated results and limited experimental data[27,28]. These facts give some assurance that the energy surfaces for these residues are reasonable. Second, the temperature coefficients of most of the structures shown are large in magnitude and negative in sign with the exception of glycine. The negative sign stems from the fact that these chains are either homopolymers or alternating binary copolymers. Specifically, the increased incidence of the more collapsed α-helical conformations at the expense of those in the β-region occurs as the temperature is increased. Finally, the inclusion or exclusion of the 10–12 hydrogen bonding term has in some cases a significant yet unpredictable effect, especially on the values of the temperature coefficients. The sensitivity of this quantity is due to the importance of the separated regions of the energy surface on the magnitude and sign of the temperature coefficient.

Table 2 shows the calculated results for the characteristic ratio and the temperature coefficient of the polypeptide chains composed exclusively of one of the three repeat peptides of elastin and for the three options

Table 1 Peptide characteristic ratios and temperature coefficients (homo- and copolymers)

Repeat sequence	Temperature (K)	No hydrogen bonding		Hydrogen bonding (local)	
		C_∞	$10^3\,TC$ (K^{-1})	C_∞	$10^3\,TC$ (K^{-1})
	300	2.49		2.82	
–Gly–			+0.57		+0.09
	350	2.56		2.83	
	300	12.60		10.68	
–Val–			−3.64		−2.72
	350	10.48		9.33	
	300	12.88		11.28	
–Ala–			−2.60		−2.40
	350	11.31		10.01	
	300	18.19		8.93	
$-Ala_p-Pro-$			−2.06		−3.22
	350	16.41		7.59	
	300	16.76		10.18	
$-Val_p-Pro-$			−2.80		−0.88
	350	14.56		9.74	

Table 2 Peptide characteristic ratios and temperature coefficients (model compounds)

Repeat sequence	Temperature (K)	No hydrogen bonding		Hydrogen bonding (local)		Hydrogen bonding (L.R.)	
		C_∞	$10^3\,TC$ (K^{-1})	C_∞	$10^3\,TC$ (K^{-1})	C_∞	$10^3\,TC$ (K^{-1})
$-Val_p-Pro-Gly-Gly-$	300	3.36		3.43		3.05	
(Model tetrapeptide)			+0.34		+0.08		+0.69
	350	3.42		3.44		3.15	
$-Val_p-Pro-Gly-Val-Gly-$	300	2.76		2.57		2.51	
(Model pentapeptide)			+0.14		+0.52		+0.80
	350	2.78		2.64		2.61	
$-Val-Ala_p-Pro-Gly-Val-Gly-$	300	3.29		2.61		2.92	
(Model hexapeptide)			−0.20		+0.69		+0.26
	350	3.26		2.71		2.96	

Experimental value (Andrady and Mark) (*Biopolymers* 1980, **19**, 849): $TC = +0.85(\pm0.29)\times10^{-3}\,K^{-1}$ (at 293 K) [i.e. $(f_e/f) = 0.26\pm0.09$]

regarding hydrogen bonding. It is the values of the temperature coefficients which are of primary interest here. Examination of the Table shows that all values of $d\ln\langle r^2\rangle_0/dT$ are positive with but one exception, and that the magnitudes are much smaller as well. The small characteristic ratios and relatively small positive temperature coefficients are a consequence of the dominance of the glycine residues in determining these two properties, acting much like flexible disordered joints between the more rigid other residues of the repeat units. Particularly rewarding is the rather good agreement between the calculated temperature coefficient for the model chains composed of the repeat tetra- or pentapeptides and the experimental value of 0.85 $(\pm 0.29)\times 10^{-3}\,K^{-1}$ obtained by Andrady and Mark[5] from stress–temperature measurements on bovine elastin swollen by triethylene glycol, at 293 K. The inclusion of some form of hydrogen bonding is necessary to achieve this agreement, with that including the interaction of longer range seeming to be the better of the two. One should be cautious and not overemphasize this agreement, however, since the elastin primary structure contains but 30% or so of the repeat peptides. The results are gratifying nonetheless. If the conserved nature of the repeat peptides present in various forms of elastin is indicative of the importance placed on them as the source of much of the elastic behaviour of elastin, then these results are of much more relevance than the 30% incidence would suggest.

One significant conclusion based on the calculations of the temperature coefficient of $\langle r^2\rangle_0$ is that no exotic topological structure or physical mechanism is necessary to explain the thermoelastic behaviour of elastin if one feels secure with the assumption of the model polypeptides mirroring the elastin structure. Simple random-coil polypeptide chains suitably crosslinked by lysine side-chain condensation seem to be a sufficient model for elastin and its elastomeric properties.

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