Hydrophobic Hydration Is an Important Source of Elasticity in Elastin-Based Biopolymers

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Abstract: Molecular dynamics simulations with explicit waters have been employed to investigate the dominant source of elastin's elasticity. An elastin-like peptide, $(VPGVG)_{18}$, was pulled and released in molecular dynamics simulations, at 10 and 42 °C, lasting several nanoseconds, which is consistent with the experimentally determined dielectric and NMR relaxation time scales. At elastin's physiological temperature and degree of extension, the simulations indicate that the orientational entropy of waters hydrating hydrophobic groups decreases during pulling of the molecule, but it increases upon release. In contrast, the main-chain fluctuations and other measures of mobility suggest that elastin's backbone is more dynamic in the extended than released state. These results and the agreement between the simulations with various experimental observations suggest that hydrophobic hydration is an important source of the entropy-based elasticity of elastin. Moreover, elastin tends to reorder itself to form a hydrophobic globule when it was held in its extended state, indicating that the hydrophobic effect also contributes in the holding process. On the whole, our simulations support the hydrophobic mechanism of elasticity and provide a framework for description of the molecular basis of this phenomenon.

Introduction

Elastin is responsible for elasticity and resilience in many tissues, and it is well-known for its extreme durability and ability to deform reversibly.[1] Recently, elastin-derived biopolymers, represented by $(VPGVG)_n$, have attracted much attention due to their potential uses as biomaterials[2] and for other industrial[3] applications. For example, one can modulate the transition temperature of a given elastin polymer by many physicochemical changes, such as pH,[4] pressure,[5] salt,[6] organic solutes,[4] and oxidation state of a side chain or functional group.[2] These properties could be exploited to provide materials with particular sensing capabilities or molecular machines for interconversion of energies.[7] Elastin has also been used as a model system to study hydrophobic interactions of proteins and to determine hydrophobicity scales for different amino acids.[8]

Due to the extreme mobility of the elastin backbone,[1] high-resolution NMR and X-ray structures of elastin remain elusive. This lack of detailed structural information has prevented the elucidation of the link between structure and function. Currently, there are three mechanisms to explain the entropic origins of elastin elasticity. Gosline's group suggested that polymer−water interactions are the source of elasticity, such that the hydrophobic effect drives the process.[9] Urry and co-workers built a model structure of an elastin-like peptide, $(VPGVG)_n$, as a continuous coil of β-turns interconnected by glycine residues.[10] They suggested that the elastomeric restoring force originates from the reduction of librational entropy in the peptide segments linking the β-turns as the elastin coil is stretched, which they coined the "librational elasticity mechanism".[11] In addition, Hoeve and co-workers suggested that elastin follows the classical theory of rubber elasticity: the collection of chains have a random distribution of end-to-end chain lengths and the displacement from this position of highest entropy provides the source of the elastic restoring force.[12] On the whole, each elasticity mechanism is consistent with, or even based on, some of the experimental results, but the lack of detailed structural information makes it very difficult to compare these mechanisms and arrive at the molecular basis for elasticity.

Previous NMR and CD studies[13] suggest that the entropy change of each single $(VPGVG)_n$ chain, rather than the effects of cross-linking, provides the dominant source of elastomeric force. In addition, gene analysis shows that tropoelastin is divided into several hydrophobic and cross-linking sections,[14] and $(VPGVG)_{18}$ appears to nicely represent one of these hydrophobic sections. These results opened the door for investigating elasticity using this relatively small peptide instead of a large cross-linked polymer. For example, Urry's group simulated elasticity in vacuo[15] while Wasserman and co-workers included water in their simulations.[16] Both studies began with

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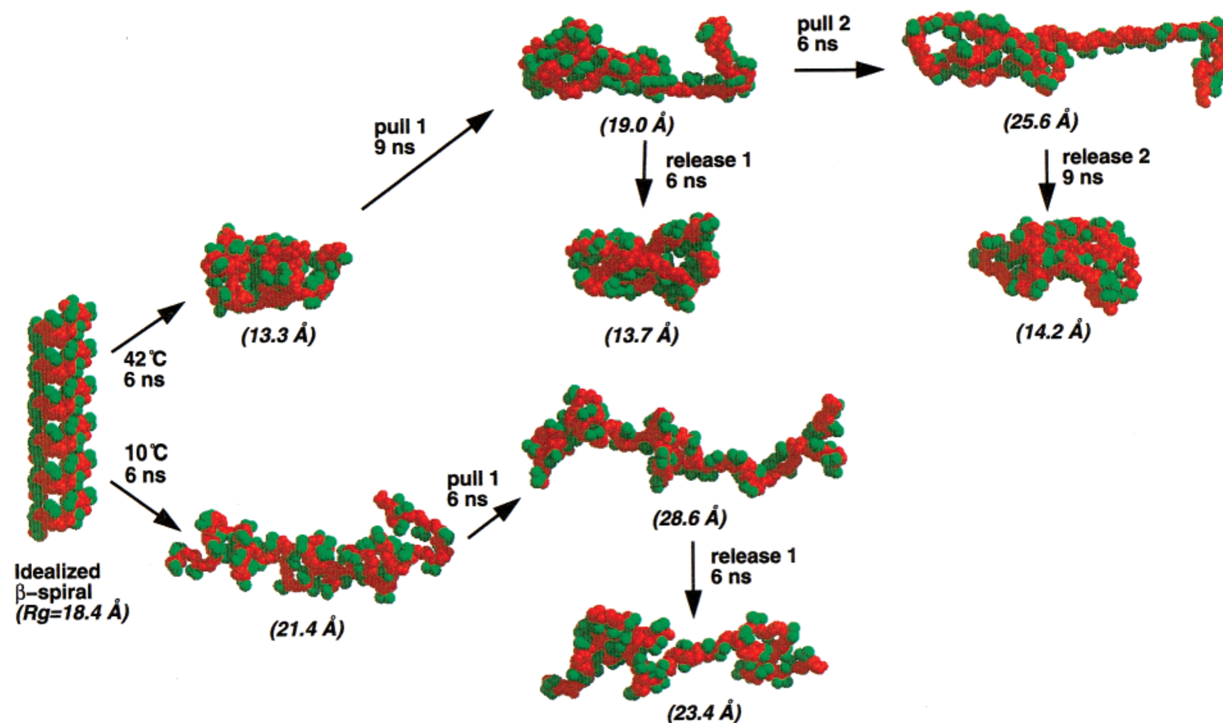

Figure 1. Conformational behavior of elastin as probed by molecular dynamics simulations in water at 10 and 42 °C. The simulations began from the idealized β-spiral structure, which was allowed to adapt to its environment for 6 ns of MD. Then, various cycles of pulling, holding, and releasing the molecule were performed. The C_α-based radius of gyration is given below each structure. The final structure from each simulation is shown with the main chain in red and side-chain atoms in green.

Urry's idealized β-spiral model as the starting structure. In both cases, larger main-chain (ϕ, ψ) fluctuations of the segments linking the β-turns were observed in the relaxed state than in the extended state, thereby supporting the librational elasticity mechanism.[15,16] However, they did not discuss the role of the hydration waters, even though only water-swollen elastin shows elasticity.[17]

Recently, we have successfully simulated the "inverse temperature transition"[18] of elastin using the ENCAD molecular dynamics program,[19] F3C water model,[20] and Urry's β-spiral model as the starting structure. Those simulations suggest that water is critical in determining elastin's conformational behavior and consequently its inverse temperature transition. Above its transition temperature, elastin is best described as a compact amorphous hydrophobic globule with distorted β-strands and fluctuating turns. Moreover, the hydrophobic domain of elastin formed in water is extremely dynamic with molecular expansion/contraction on the nanosecond time scale.[18] Therefore it is a "dynamic-compact amorphous globule", which is distinct from the traditional view of compact globular proteins. Below the transition temperature, the strands and turns are less prevalent and hydrophobic collapse does not occur, such that elastin is more extended.[18] Here, we extend our earlier work by performing elasticity simulations using conformations from our previous simulations as starting structures. Specifically, we have simulated pulling/releasing cycles on the nanosecond time scale at 10 and 42 °C, which are below and above, respectively, the transition temperature of elastin. The results presented here suggest that hydrophobic hydration of elastin is a very important, if not essential, source of elastin's elasticity.

Methods

NMR or X-ray structures of elastin are not available due to the extremely high mobility of the elastin backbone.[21,22] Instead, previous elasticity simulations[15,16] used an idealized β-spiral model[10] as the starting structure. However, our recent MD simulations (which also started with the β-spiral model structure)[18] and various experimental studies[23,24] suggest that elastin does not form a stable extended β-spiral structure and instead should be described as an amorphous hydrophobic globule. Therefore, we extracted the 6 ns structures from the previous simulations[18] at 10 and 42 °C, and used them as the starting structures for the elasticity simulations described here.

Previous elasticity simulations[15,16] assumed that elastin has a helical structure of 2.7 pentamer units per turn (β-spiral) in both the relaxed and extended states, therefore they simulated the stretching process of elastin by raising the axial distance per turn of the β-spiral. In the current research, we pulled a pair of elastin main-chain atoms, instead of many pairs of atoms,[15,16] to release the assumption of the helical structure in elastin's extended state. Since elastin forms a hydrophobic core at 42 °C,[18] pulling just the ends of the molecule can cause the termini to separate from the main body of the peptide. As a result, we selected another pair of atoms (C_α atoms of Pro 12 and Gly 78) for the pulling simulation at 42 °C to minimize end effects (Figures 1 and 2). We have also completed several other elasticity simulations by selecting other pairs of atoms. The overall results are similar though the detailed information may differ from one simulation to another (data not shown). In addition, in each pulling simulation, we continued the simulation

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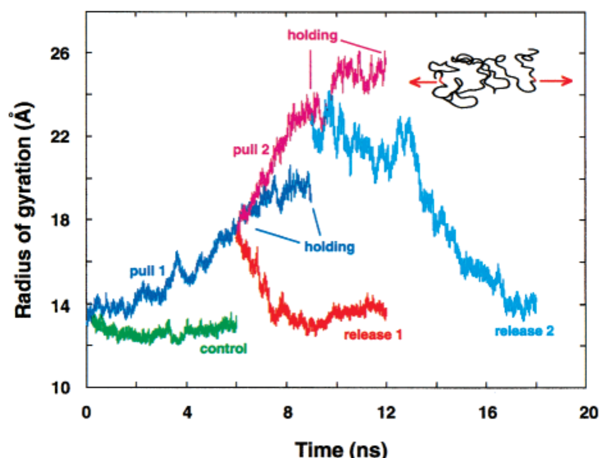

Figure 2. Elastin's response to force at 42 °C. The inserted structure shows the pair of atoms selected for pulling.

for 3 ns after the distance of the select pair of atoms reached the designated extension to collect conformations and statistics of elastin in its extended state (we call this the holding process, Figure 2). At 10 °C, the C_α atoms of Val 1 and Val 79 were selected for pulling (Figure 1).

Molecular dynamics simulations were performed by using an in-house version of the program ENCAD[19] and a previously described force field.[20,25] Each molecular system simulated was described by using an all-atom representation for both the protein and the solvent. Each of the starting structures was minimized for 500 steps. The protein was then solvated by a water box extending at least 10 Å in all directions for the pulling simulations and 8 Å in the control and releasing simulations. The water density was set to the experimental value for the temperature of interest (0.9997 and 0.9915 g/mL for 10 and 42 °C, respectively).[26] The systems were then equilibrated by minimizing the water for 2000 steps, performing MD of the water for 2000 steps, minimizing the water for 2000 steps, minimizing the protein for 2000 steps, and minimizing the protein and water system for 2000 steps. After these preparatory steps, the systems were heated to the designated temperatures. Initial atomic velocities were chosen from a Maxwellian distribution and they were periodically scaled until the target temperature was reached. Production MD simulations were then run using a 2-fs time step. The nonbonded list was updated every 5 steps, and the nonbonded cutoff was 8 Å. Periodic boundary conditions and the minimal image convention were employed to reduce edge effects. In each step of the pulling simulation, a 1 Å increment per step on the selected atom pair was achieved by using a 420 $kJ\cdot Mol^{-1}\cdot Å^{-2}$ force constant, followed by 200 ps of molecular dynamics simulation to equilibrate at the new distance. There were 30 steps in the *pull* 1 simulation at 42 °C and 15 steps for the other pulling simulations. The 1 Å increment per step caused only a slight increase in the energy of the system such that the total energy was well conserved throughout the trajectories. Structures were saved every 0.2 ps for analysis, yielding 30 000 structures per 6 ns simulation and 45 000 structures per 9 ns simulation.

Results

Global Description of the Elasticity Simulations. We used structures from our previous simulations at 10 and 42 °C as the starting points for the elasticity simulations (Figure 1). At both temperatures, the radius of gyration became larger after pulling the molecule, and it relaxed back toward the starting value when released (Figure 1). In addition, like our previous results,[18] the 42 °C simulations formed a hydrophobic globule, while none of the 10 °C simulations did (Figure 1).

The average values of various properties are shown in Table 1. Elastin has a large solvent accessible surface area and a low number of hydrophobic contacts after pulling, while the solvent accessible surface area decreased and the intrachain contacts increased when released. Also, the number of hydration waters decreased upon release when elastin underwent hydrophobically driven collapse (Table 1). The properties of the simulations after elastin was released were quite similar to those of the corresponding reference simulations at both temperatures. These results, together with the data presented in Figures 1 and 2, suggest that our simulations provide a reasonable representation of the pulling/releasing (elasticity) processes of elastin.

Hydration Changes during Pulling/Releasing Cycles of Elastin. The orientations of waters around hydrophobic groups of elastin are almost identical for extended and relaxed states, and they are similar at 10 and 42 °C (Figure 3). These data are in good agreement with our previous simulation results of hydrophobic hydration.[29,30] Regarding temperature-dependent differences, Figure 3 shows an additional peak with a distance of 3.5−4.0 Å and an angle of 100−110° in the lower temperature simulations that is not present at the higher temperature. These peaks and the distributions indicate that ∼50% of the water molecules in elastin's closest hydration layer at low temperature are nearly parallel to the protein surface, indicating that the hydration layer is more structured at lower temperature.

The average number of hydrogen bonds per water molecule remains constant during each pulling/releasing cycle (Figure 4A). However, the number is higher at 10 than 42 °C, suggesting that the water solvating elastin is more structured at lower temperatures (Figure 4A). Overall, both the hydrogen bonding and water orientation data suggest that the properties of elastin's hydration waters remained almost the same through pulling/releasing cycles at a particular temperature. However, the number of hydration waters increased and decreased in response to pulling and releasing the chain (Table 1).

Figure 4B shows the water orientational entropy around both the main-chain and side-chain groups of elastin. Upon releasing the molecule, the entropy of the elastin side-chain associated hydration shell increased, while the entropy of main-chain hydration waters decreased. The entropy gain from the side-chain hydration as the chain collapsed dominated the entropy loss of the main-chain hydration waters (Figure 4B) and elastin returned to its relaxed states (Figure 1). Therefore, it appears that hydrophobic hydration, but not hydrophilic hydration, provides the entropic source of elasticity.

Changes in the Elastin Backbone during Pulling/Releasing Cycles. Table 2 shows the main-chain (ϕ, ψ) fluctuations on a repeating peptide basis, that is, as Val 1-Pro 2-Gly 3-Val 4-Gly 5. Overall, pulling the molecule resulted in an increase in the (ϕ, ψ) fluctuations in short extension cycles (pull 1 at both 10 and 42 °C, Table 2), while there was an inverse trend in the long extension cycle (pull 2 at 42 °C). Here, short extension means ∼50% extension based on the radius of gyration (Table 1). In a recent mechanical study of elastin, the aortic circumference was assumed to expand 30% upon inflation from 0 to 13.7 K Pa,[31] such that our short extension cycles are in line with the degree of extension experienced by elastin physiologically. In addition, although the ϕ value of Pro 2 had the lowest

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Table 1. Average Properties over the Final Nanosecond of Different Portions of the Elasticity Simulations[a]

MD name	Rg[b] (Å)	SASA[c] ($Å^2$)	nonpolar SASA[c] ($Å^2$)	side-chain contacts[d]	no. of protein H-bonds[e]	no. of waters in hydration shell[f]
			elasticity simulations at 42 °C			
control	12.9 ± 0.2	5661 ± 137	4103 ± 122	53 ± 4	16 ± 2	376 ± 14
pull 1	19.7 ± 0.3	6260 ± 181	4663 ± 145	40 ± 4	14 ± 2	449 ± 16
release 1	13.8 ± 0.2	5625 ± 164	4082 ± 139	54 ± 5	13 ± 2	346 ± 15
pull 2	24.9 ± 0.4	6736 ± 111	4898 ± 92	38 ± 3	9 ± 1	494 ± 14
release 2	14.0 ± 0.3	6052 ± 148	4325 ± 122	51 ± 4	14 ± 2	415 ± 14
			elasticity simulations at 10 °C			
control	21.0 ± 0.5	6922 ± 131	5107 ± 114	33 ± 3	4 ± 1	526 ± 15
pull 1	28.6 ± 0.5	7686 ± 206	5713 ± 191	28 ± 4	0 ± 0	598 ± 20
release 1	23.1 ± 0.8	7283 ± 188	5339 ± 157	35 ± 2	2 ± 1	553 ± 19

[a] The data were averaged over the last ns of the designated time periods (see Figure 2) and values after "±" are the standard deviations. [b] Radius of gyration based on the positions of the α-carbons. [c] All solvent accessible surface areas were calculated with the program NACCESS,[27] which employs Lee and Richard's algorithm with a probe radius of 1.4 Å.[28] [d] Side-chain contacts were counted when two aliphatic carbon atoms were within 5.4 Å of each other. Atoms in neighboring residues were not considered. [e] Hydrogen bonds were tabulated as intact when the hydrogen and the acceptor were within 2.6 Å and the hydrogen bonding angle was within 35° of linearity. [f] Water in water shell 1 was defined as water molecules within 4.5 Å from any elastin heavy atoms.

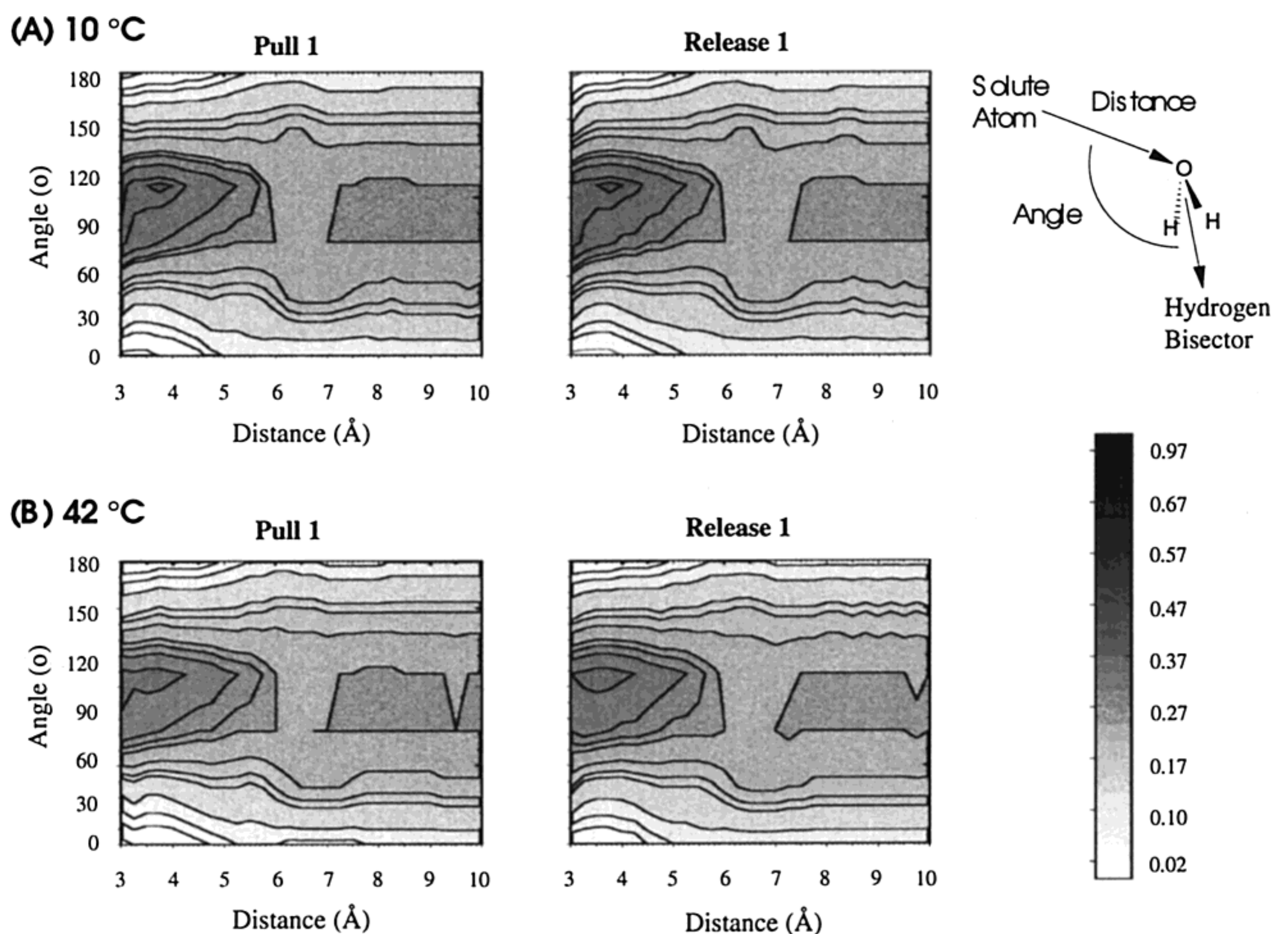

Figure 3. The radial and angular distribution of water around elastin's hydrophobic surface (carbon atoms) at (A) 10 and (B) 42 °C. The angular distributions are given as percentages for each radial distance.

fluctuation, Pro 2 ψ and Gly 3 ϕ had the highest values (Table 2). Correspondingly, many crankshaft-like motions of the Pro 2−Gly 3 peptide plane were observed in both the current and previous elastin simulations.[16] Also, as expected, glycine exhibited larger (ϕ, ψ) fluctuations than proline and valine (Table 2).

We have also calculated the generalized order parameter (S^2) for the N−H vectors of the main-chain atoms and the $C_\beta-C_\gamma$ vectors of the valine side-chain atoms. We and others have shown that MD-derived S^2 order parameters can be in good agreement with S^2 values derived from NMR relaxation data.[32] For these S^2 order parameters, the values approaching 1 suggest that the peptide is rigid and those near 0 reflect unrestricted motion. The data in Table 3 show that elastin is quite dynamic at both temperatures and in both states. Moreover, the S^2 values in the extended states are slightly lower in short extension cycles, indicating slightly greater mobility for both the main-chain and side-chain atoms of elastin in the extended state. These results are in good agreement with the larger main-chain (ϕ, ψ) fluctuation values in the extended states in short extension cycles (Table 2). As a result, in the case of short extensions, the

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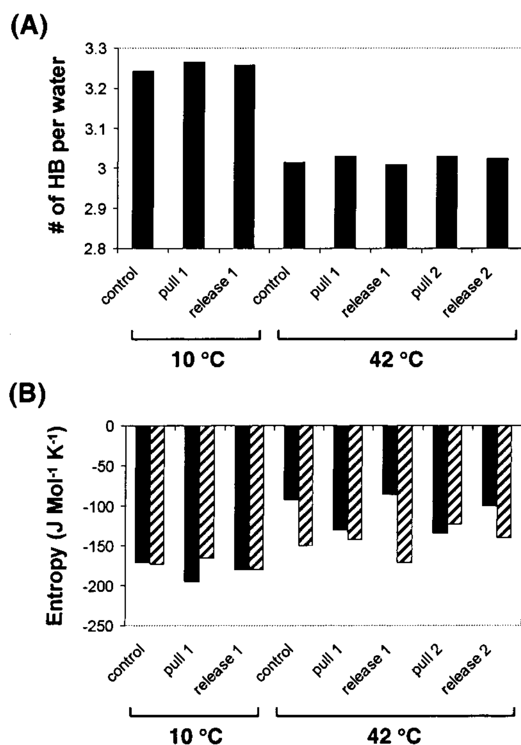

Figure 4. The average properties of water shell 1 (within 4.5 Å of any elastin heavy atom) of elastin during the final nanosecond time period. (A) The average number of hydrogen bonds per water molecule in shell 1. (B) The orientational entropy of water in shell 1 for hydrophobic side-chain hydration (solid bar) and main-chain hydration (cross-hatched bar).

extended peptide chain is more dynamic, has more configurational states accessible to it, and therefore has greater entropy. Thus, the peptide itself does not seem to provide the main source of entropy-based elasticity, instead the entire system (elastin plus its hydration waters) must be considered to explain elasticity.

When the distance of the selected pair of atoms reached the designated length, it was held constant for 3 ns to simulate the extended state of elastin (holding process). The holding process from the 42 °C pull 1 simulation is illustrated in Figure 5. Residues Pro 67 and Val 71 formed dynamic hydrophobic interactions with other residues, and the Pro 67-Val 71 segment joined the main hydrophobic core of elastin. Since the Pro 12-Gly 78 $C_\alpha-C_\alpha$ distance was constant, the Pro 72-Gly 78 segment became more extended in this holding process (Figure 5). The straight chain of Pro 72-Gly 78 was unfavorable due to the exposure of hydrophobic side chains to water and the fewer main-chain conformations available to extended states, therefore this process only occurred because the main hydrophobic core gained more favorable energy/entropy to overcome the unfavorable effect. In fact, after the Pro 67-Val 71 segment joined the main hydrophobic core (~7.8 ns), more dynamic hydrogen bonds formed and the hydrogen bonding network was very fluid (Figure 6). Such a dynamic hydrogen-bonding network (Table 4) provides elastin with many low-energy states, allowing it to populate a range of conformations (high entropy). Moreover, benefiting from the extremely dynamic features of elastin, our 3 ns holding processes were on par with the experimentally determined relaxation times.[33,34] Therefore, the larger hydrophobic globule formed at 42 °C during the holding process (Figure 5) may represent the extended state of elastin. Also, in the current paper, we compared only the average properties over the last nanosecond for the pulling/releasing simulations (Figures 1 and 3 and Table 1) to get a more reasonable comparison of the extended/relaxed state of elastin (elasticity).

Discussion

Comparing the Contributions of Elasticity: Hydrophobic Hydration vs Backbone Mobility. Although elasticity is well-known to be linked with entropy,[12] it is not clear whether this entropy arises from the conformational entropy of the protein backbone[11] or from the change in the hydration of hydrophobic side chains.[9] Our results indicate that the water orientational entropy for the hydrophobic hydration waters, but not the main-chain polar hydration waters, increases in all relaxed states for all pulling/releasing cycles (Figure 4B). In addition, the structure of the hydration shell does not change much upon extension (Figure 3), but there are more "ordered" hydration waters in the extended than in the relaxed state (Table 1). These results suggest that hydrophobic hydration of elastin contributes to elasticity and the release of hydration waters plays a dominant role. Experiments have also shown that removal of only 10% of elastin's hydration waters has a profound effect on its elasticity modulus, suggesting that hydration is critical to elasticity.[35]

On the other hand, our main-chain (ϕ, ψ) fluctuation values and order parameter data suggest that the peptide is more dynamic in the extended state during short extension cycles but more dynamic in the relaxed state during long extension cycles (Tables 2 and 3). These results suggest that the conformational entropy of elastin actually decreases when elastin is released from the extended state over short extension cycles, which means that the conformational entropy would not appear to be the source of elasticity in this case. As mentioned above, these short extension cycles are consistent with the degree of elastin's physiological extension.[31]

Gosline's microcalorimetry study supports this idea: the overall conformational entropy of the peptide chain actually increases when elastin is stretched and the polymer becomes more "random".[9] Furthermore, he found that the conformational entropy of elastin does not contribute to elastic force at extensions of less than ~70%.[9] In the more extreme and nonphysiological extension ranges, the main-chain conformational entropy does contribute to the elasticity (Tables 2 and 3). This is in accordance with meanfield work[36−38] that predicts a maximum in the number of conformations at intermediate radii of gyration. However that number tails off for very extended or compact polymer molecules. Therefore, both experimental and our simulation results suggest that the conformational entropy of the elastin polymer itself is not the main source of elasticity, at least in the case of short extension cycles. Instead, the increase in entropy originates from the hydrophobic collapse of the chains and the concomitant expulsion of waters from the nonpolar surfaces leading to an increase in solvent entropy, which appears to be the major determinant of elasticity.

The Roles of Elastin's Hydration Waters. Previously, Gosline and French found that elastin has similar elastic character both in a thermodynamic open system (swelling equilibrium with water) and in a thermodynamic closed system (constant water content, 0.46 g of water/g of protein).[35] In

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Table 2. Main Chain (ϕ, ψ) Fluctuations for the Final Nanosecond of Different Portions of the Elasticity Simulations

MD name	Val 1		Pro 2		Gly 3		Val 4		Gly 5	
	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ
elasticity simulations at 42 °C										
control	17.48	13.26	9.96	21.51	29.47	29.80	18.88	14.58	22.00	24.71
pull 1	19.50	14.59	9.96	27.50	29.57	30.07	18.74	14.58	22.29	27.67
release 1	20.57	14.23	10.06	22.25	30.34	25.86	20.52	16.36	21.86	24.85
pull 2	22.48	15.01	10.01	19.78	27.38	29.85	17.01	13.95	20.66	27.45
release 2	26.53	15.67	9.93	19.98	31.64	32.98	23.99	16.14	24.73	31.01
elasticity simulations at 10 °C										
control	20.49	13.39	9.36	18.08	25.96	27.34	19.18	14.92	21.08	23.22
pull 1	22.52	13.76	9.66	19.37	31.29	31.09	19.82	14.12	23.53	25.54
release 1	16.51	13.32	9.56	18.06	26.87	28.37	17.35	13.55	21.22	26.27

Table 3. Calculated Order Parameters (S^2) over the Final Nanosecond of Different Portions of the Simulations[a]

MD name	main chain –NH	side chain $-CH_3$ for Val
elasticity simulations at 42 °C		
control	0.51 ± 0.21	0.47 ± 0.21
pull 1	0.42 ± 0.19	0.40 ± 0.22
release 1	0.49 ± 0.14	0.45 ± 0.18
pull 2	0.44 ± 0.20	0.45 ± 0.22
release 2	0.41 ± 0.19	0.40 ± 0.21
elasticity simulations at 10 °C		
control	0.51 ± 0.16	0.46 ± 0.20
pull 1	0.40 ± 0.14	0.37 ± 0.15
release 1	0.50 ± 0.14	0.49 ± 0.18

[a] The values after "±" are the standard deviations.

contrast, removing only 10% of elastin's hydration waters can greatly influence elasticity.[35] Our hydration water content in the simulation (waters in water shell 1) is in very good agreement with the experimentally observed values.[35,39] Both the experiments and our simulations suggest that it is the hydration waters of elastin, but not the bulk waters, that contribute to elasticity.

On the basis of our simulations, the hydration waters of elastin play at least the following roles in the elastin–water system: First, the orientational entropy of elastin's hydrophobic hydration layer decreases as the molecule is pulled, but increases upon release. This entropy change is suggested to be an important source of elasticity. Then, hydration waters form hydrogen bonds with elastin's main-chain atoms as opposed to elastin-forming intramolecular hydrogen bonds as with globular proteins, which makes elastin fully dynamic in both the extended and relaxed states. Similarly, previous atomic force spectroscopy[40] and molecular dynamics simulations[41] suggest that water drastically affects the elastic properties of poly(ethylene-glycol) (PEG) through its dynamic hydrogen bonding to PEG's main-chain atoms. Finally, the unfavorable interaction between waters and elastin's hydrophobic side-chain atoms results in hydrophobic collapse with concomitant expulsion of waters from nonpolar surfaces, which is the source of both the inverse temperature transition and elasticity of elastin.

Elastin's Dynamic Peptide Chain. Both experimental[42] and simulation[18] studies indicate that the fluctuating turns (mainly β-turns) and distorted β-strands are the main components of elastin's secondary structure. These secondary structural features are closely related to elastin's ability to reversibly deform.

Several groups have suggested that the turns in elastin might contribute to elasticity. Arad and Goodman's NMR and CD studies of elastin-like peptides suggest that an equilibrium exists between a β-turn and a γ-turn, resulting in a structure that combines flexibility with strong conformational preferences.[43] Wasserman and Salemme's simulations revealed a kind of crankshaft motion in the elastin peptide, which results in a reorientation of the central peptide bond with little deformation of the overall structure of elastin.[16] We also have observed such crankshaft motions of the Pro 2-Gly 3 peptide bond in all the trajectories (data not shown).

However, the dynamic β-turn is only part of the story of elastin and elasticity. Experimental results[42] suggest that elastin has ~45% β-strand structure, which translates into a remarkable amount of nonlocal hydrogen bonds. In contrast, the previous elastin models neglected nonlocal hydrogen bonds, as they were considered harmful for elasticity.[10,44] The current simulations might provide a reasonable explanation of this disagreement. On the one hand, only ~10–20% of elastin's main chain polar groups are involved in main-chain/main-chain hydrogen bonds (Table 1). This percentage is much lower than the average value for globular proteins of ~50%,[45] and as a result, elastin is more dynamic.[18] On the other hand, both experimental[42] and simulation[18] studies suggest that elastin's β-structure is distorted. As shown in Table 4, a pair of peptide segments can form exchanging hydrogen bonds with each other among elastin's distorted β-strands. In this way, elastin can form energetically favorable nonlocal hydrogen bonds, while maintaining its plasticity and populating more conformations.

Elastin consists mainly of hydrophobic amino acids and almost all of the lysines in elastin are involved in cross-linking.[1] Therefore, it has almost no polar groups other than main-chain NH/O groups. The primary sequence of a globular protein and its interaction with solvent determine its 3-dimensional structure. A globular protein uses side-chain polar groups and some of its main-chain NH/O groups to interact with water, and the bulk of the main-chain polar groups form secondary structural main-chain/main-chain hydrogen bonds. Although elastin can undergo a hydrophobic collapse,[46] similar to that of globular proteins,[47] its main-chain NH/O groups seem to be used mainly for interactions with water and they form relatively few main-chain/main-chain hydrogen bonds. As a result, elastin is extremely dynamic in water.

Mechanism of Elasticity. Only water-swollen elastin exhibits elasticity,[17] so both elastin and its hydration shell should be

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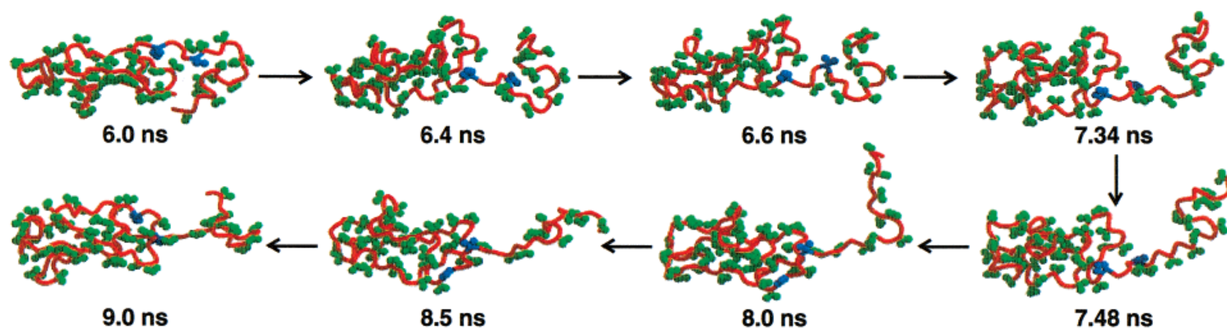

Figure 5. Hydrophobic collapse in the 42 °C *pull 1* holding process—the Pro 67-Val 71 segment joins the main hydrophobic core of elastin: red, elastin main chain; green, hydrophobic side-chain atoms; blue, Pro 67 and Val 71.

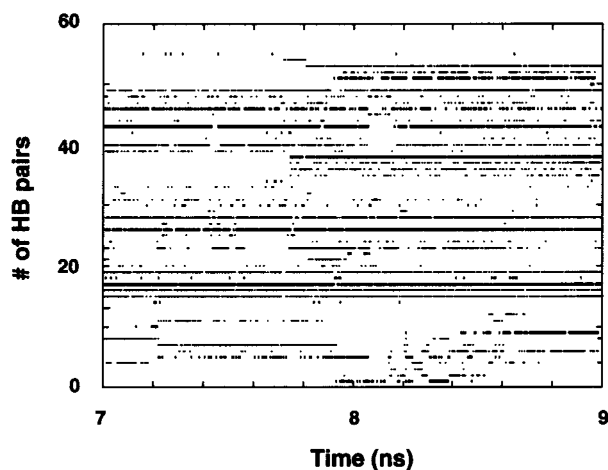

Figure 6. Dynamic main chain/main chain hydrogen bonds of elastin in the 42 °C *pull 1* holding process. Each number along the *Y*-axis corresponds to a unique hydrogen bond in elastin. The locations of hydrogen bonds 40−49 in this figure are shown in Table 4.

Table 4. Representative Example of Dynamic Hydrogen Bonds of the Distorted β-Sheets in the 42 °C Holding Simulation

HB sequence in Figure 6	HB donor	HB acceptor	total HB time (ps)	# of HB transitions	HB lifetime (ps)
40	63 NH	35 O	575	342	1.7
41	63 NH	36 O	21	21	1.0
42	64 NH	35 O	7	6	1.2
43	64 NH	36 O	1174	371	3.2
44	64 NH	37 O	48	41	1.2
45	65 NH	36 O	15	15	1.0
46	65 NH	37 O	374	257	1.5
47	65 NH	38 O	70	58	1.2
48	66 NH	37 O	112	79	1.4
49	66 NH	38 O	882	368	2.4

considered. Our results suggest that hydrophobic hydration is an important source of elasticity, thereby supporting the hydrophobic mechanism.[9] Moreover, the current research provides some clues for the molecular basis of the relationship between the inverse temperature transition and elasticity of elastin. Elastin tends to form a larger hydrophobic globule during the holding processes above its transition temperature (Figure 5); however, it does not form similar hydrophobic interactions during the holding processes below its transition temperature (Figure 1). It seems that the thermodynamic features that cause the hydrophobic collapse and represent the driving force for the inverse temperature transition also make elastin form a larger hydrophobic core in the holding state (Figure 5). Moreover, collapse automatically occurs when the pulling force is removed (Figure 1) at physiological temperature. In this way, the hydrophobic hydration mechanism of elasticity we present here is probably most applicable to physiological temperatures. On the other hand, at temperatures below elastin's transition temperature, the hydrophobic side chains of elastin are already "dissolved" in water and the cross-linking elastin becomes swollen. Therefore, the contribution of hydrophobic hydration to elasticity is lower compared with that at physiological temperatures (Figure 4), and elastin does indeed show much less elasticity below its transition temperature.[48]

Previously, Urry's group provided another explanation of elasticity, the librational entropy mechanism of elasticity.[48,49] Their elasticity simulation[15] showed that the "suspended" part of elastin, the portion between the β-turns in Urry's β-spiral model,[10] had much larger (ϕ, ψ) fluctuations in the relaxed state than in the extended state; but the β-turn portion of elastin was unaffected. As a result, they proposed a "librational elasticity mechanism", which suggests that the primary mechanism of elasticity for elastin is the damping of the peptide backbone dihedral angle dynamics upon elongation.[10] However, their simulation was performed in vacuo and was only 100 ps long. Water is known to be a plasticizer of elastin and only water-swollen elastin displays elasticity.[17] Also, dielectric[33] and NMR[34] relaxation studies have shown that water-swollen elastin has a correlation time from several to tens of nanoseconds. Therefore, one might question whether the previous simulation[15] can represent elastin in its functional state.

Wasserman and Salemme[16] extended the previous simulation by including water. They stretched their elastin peptide by proportionally translating all atoms out from the center of mass in a direction parallel to the helix axis (similar to Chang and Urry's simulation) over 130 ps, and they obtained larger Gly 5 than Gly 3 (ϕ, ψ) fluctuations. Thus, their simulation supported Urry's librational elasticity mechanism. However, one cannot exclude the possibility that the larger Gly 5 (ϕ, ψ) fluctuations they observed are the result of the proportional transition protocol they employed.[16] Moreover, they did not discuss elastin's hydration properties and their relationship to elasticity.

In fact, the librational entropy mechanism depends heavily on the linear β-spiral structural model of elastin developed by Venkatachalam and Urry.[10] However, recent scanning force microscopy experiments suggest that elastin molecules form a disordered array of beaded filaments containing globular domains in the relaxed state, and the array of globules becomes more expanded and ordered but elastin does not adopt precisely ordered β-spiral conformations in the stretched state.[24] Also, our realistic simulations of elastin starting with the β-spiral conformation result in dynamic-compact amorphous structures at physiological temperature and these results are consistent with

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various experimental results.[18] Therefore, the β-spiral structure and the librational entropy mechanism might represent some local features of elastin, but they seem not to provide the best overall explanation of elasticity.

In summary, we have performed molecular dynamics simulations to investigate the mechanism of elasticity. It is the first time that the simulations are on par with the experimentally determined relaxation times of elastin.[33,34] Also, because solvent was present and its effect on the peptide were considered, the simulations provide detailed information for both the peptide chain and the role of its hydration waters in elasticity. At elastin's physiological temperature and degree of extension, the simulations indicate that hydrophobic hydration is an important source of its entropy-based elasticity. For all the simulations at both 10 and 42 °C, hydrophobic hydration has a favorable contribution but the elastin peptide itself has a negative contribution to elasticity at short, physiological extensions, while both the peptide and its hydration layer contribute favorably to elasticity at longer extensions. These results and the agreement between the simulations with various experimental observations suggest that the hydration of hydrophobic residues contributes significantly to elasticity, thereby supporting the hydrophobic mechanism of elasticity.

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