in eq 17 and 18 of ref 2. The matrix Ψ_i defined in the ARS scheme is the equivalent of the diagonal $\mathbf{F}_i$ matrix defined in eq 1 (Chapter IV) of ref 1; in fact they both contain a representation of the function which must be averaged and occupy the same place in the matrix product which performs this average. In particular we are interested in the function defined by the rotation matrix $\mathbf{T}(\varphi_i)$ which transforms the components of any vector, expressed in the reference frame connected with the $(i + 1)$th bond, into those appropriate to the ith bond. In this case the Ψ_i matrix assumes the specific form defined in eq 23 of ref 2 and may be indicated with τ_i. With these definitions it is easy to prove that

$$\langle \mathbf{T}(\varphi_i) \rangle_{N+1} = Z_{N+1}^{-1}(2\pi)^{N-1}(\mathbf{J} \otimes \mathbf{E}_3) \times [\prod_{j=3}^{i}(\mathbf{J}_j \otimes \mathbf{E}_3)]\tau_i[\prod_{j=i+1}^{N}(\mathbf{U}_j \otimes \mathbf{E}_3)](\mathbf{J}^T \otimes \mathbf{E}_3) \quad (9)$$

A formal equivalence may be found again between eq 9 and 8 of ref 1 and we see that the matrix τ_i plays, in eq 9, the same role as $\|\mathbf{T}\|_i$ in eq 8 of ref 1. For sake of brevity we ignore intermediate manipulations and write an equation formally similar to eq 28 of ref 1

$$\langle M^2 \rangle_{N+1} = 2Z_{N+1}^{-1}(2\pi)^{N-1}\mathbf{A}\mathbf{G}_1\mathbf{G}_2 \ldots \mathbf{G}_{N+1}\mathbf{A}^* \quad (10)$$

where $\mathbf{A}$ is a row of $5(2n + 1)$ elements, only the first of them being 1, while the others are 0 and $\mathbf{A}^*$ is a column of $5(2n + 1)$ elements, where only the $(8n + 5)$th is 1 while the others are 0. $\mathbf{G}_i$ is a generator matrix formally similar to that defined in eq 24 of ref 1 but with $\|\mathbf{T}\|_i$ substituted by τ_i; $\mathbf{U}_1$, $\mathbf{U}_2$, and $\mathbf{U}_{N+1}$ are unit matrices and $\mathbf{G}_{N+1}$ has all elements 0 except those of the last pseudocolumn. Equation 10 may be used, as in the case of the RIS scheme, to describe chains of finite number $(N + 1)$ of bonds while the sequence of $\mathbf{G}_i$ matrices may be constructed in order to produce any given sequence of structural units, provided the corresponding $w(\varphi_{i-1},\varphi_i)$ function is known. Consequently copolymer chains with any composition may be treated.

In the case of a three-bond chain the average value of $\mathbf{T}(\varphi_2)$ is

$$\langle \mathbf{T}(\varphi_2) \rangle = (a_2^0)^{-1}(a_2^0\mathbf{T}_0 + \tfrac{1}{2}a_2^1\mathbf{T}_c + \tfrac{1}{2}b_2^1\mathbf{T}_s) \quad (11)$$

where a_2^0 is defined in eq 7, a_2^1 and b_2^1 are the coefficients of the cos φ_2 and sin φ_2 terms of the Fourier expansion of $w(\varphi_2)$, and $\mathbf{T}_0$, $\mathbf{T}_c$, and $\mathbf{T}_s$ are defined in eq 22 of ref 2.

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Glass Transition Temperature of Anionic Polyisoprene

J. M. WIDMAIER and G. C. MEYER*

Ecole d'Application des Hauts Polymères, 67000 Strasbourg, France. *Received August 11, 1980*

We are currently investigating the glass transition temperature (T_g) of ABA poly(styrene-*b*-isoprene) block copolymers (SIS) as a function of their molecular characteristics.[1] Generally, block copolymers exhibit two T_g's corresponding approximately to those of the parent homopolymers. When the T_g values of the block polymers differ from those of the homopolymers, this indicates a change in morphology due to an evolution in the miscibility of the two phases.

In order to establish these changes, it is useful for comparison purposes to know the relationship between T_g and the molecular weight of the parent homopolymers. Such a relationship was first established by Fox and Flory[2] as follows:

$$T_g = T_g^\infty - K\bar{M}_n^{-1}$$

in which T_g^∞ is the glass transition temperature of a polymer with infinite molecular weight, $\bar{M}_n$ is the number-average molecular weight, and K is a constant characteristic of a given polymer. For polystyrene and some other polymers, the K values are well-known.[3]

However, the literature contains little information about the molecular weight–T_g relationship of polyisoprene (PI). Different microstructures exist for this polymer which also play a role in the T_g value. Wood[4] found T_g's varying from −74 to −69 °C, but the molecular weights of his samples were not given. Essel[5] noted an increase in T_g with the overall 3,4- and 1,2-configuration content for a PI ($\bar{M}_n$ = 160 000). Dannis[6] and Haas et al.[7] found a difference ΔT_g of about 15 °C between cis-1,4 and trans-1,4 isomers. Morgan et al.[8] confirmed Haas' findings with T_g values of −60 and −42 °C, respectively. Cowie[9] reported data on samples with different chain lengths: for *trans*-1,4-polyisoprenes, in the molecular weight range 17 000–110 000, a constant T_g of −66 °C was found.

In this note, we report T_g values for a series of PI with molecular weights ranging from 3000 to 75 000 and of known microstructure. These polymers were obtained by anionic synthesis in benzene at 50 °C, with *n*-butyllithium as the initiator. Mass distribution was examined by gel permeation chromatography (GPC, Waters chromatograph, Styragel columns, THF). Two different calibration curves were used: one was the universal calibration curve,[10] and the other was deduced from the polystyrene curve. Since in THF, the polystyrene and polyisoprene curves are parallel,[11] a fixed molecular ratio of 1.35 can be used for the two polymers. Concordance was satisfactory. Polydispersity ranges from 1.10 to 1.22. The number of isomeric units in polyisoprene was determined by ^{1}H NMR in benzene-d_6 solution (Cameca 250-MHz spectrometer). As olefinic proton signals at 5.25 and 4.82 ppm, due, respectively, to the 1,4 and 3,4 units,[12] do not overlap, their ratio could be determined directly from the spectrum. The cis-1,4 and trans-1,4 content was calculated by Assioma's method.[13] Glass transition temperatures were determined by using a Perkin-Elmer DSC-1 differential scanning spectrometer, calibrated with *n*-octane. T_g values were measured at several heating rates (v = 2, 4, 8, 16 and 32 °C/min). From a plot of T_g vs. $v^{1/2}$,[14] the extrapolated zero heating rate value was taken as being the value of T_g. Its accuracy was ±2 °C.

Table I and Figure 1 show the T_g values of PI with high 1,4 content (over 85%) and various molecular weights. As a first approximation, all T_g's fall within a −70.5 to −64.5 °C range. The difference between these limits is very small, about 6 °C, and not systematic. Thus, Flory's law does not apply to this particular molecular weight range. Our values confirm those found by Wood[4] and Cowie.[9]

Table II and Figure 2 give T_g's in terms of the microstructure of the polyisoprene samples. Some PI of known microstructure that are listed in Table I also appear in

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Table I
Molecular Characteristics and Glass Transition Temperature of High-1,4-Content Polyisoprene

sample	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	T_g, °C
130	3 000	1.18	−69
142	6 400	1.19	−70.5
144	14 100	1.19	−68
141	15 300	1.20	−68
509	30 300	1.14	−66
123	34 000	1.16	−70
523	37 000	1.21	−69
124	58 100	1.22	−68.5
110	63 000	1.15	−68.5
132	68 200	1.15	−65
102	74 700	1.19	−64.5

Table II
Molecular Characteristics, Microstructure, and Glass Transition Temperature of Polyisoprene

			microstructure					
sample	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	1,2	3,4	1,4 cis	1,4 trans	1,4 total	T_g, °C
590	3 800	1.10	0	23.3	46.9	29.8	76.7	−59
142	6 400	1.19	0	10.6	50.3	39.1	89.4	−70.5
592	7 700	1.12	0	37.8	35.4	26.8	62.2	−47
111	8 700	1.12	0	17.2	53.9	28.9	82.8	−63.5
144	14 100	1.19	0	12.1	36.0	51.9	87.9	−68
141	15 300	1.20	0	13.6	56.8	29.6	86.4	−68
600	17 800	1.10	0	19.2	48.7	32.1	80.8	−61.5
618	19 300	1.16	0	29.0	35.9	35.1	71.0	−51.5
559	20 100	1.17	0	49.3	21.8	28.9	50.7	−36
573	22 100	1.21	0	31.6	32.1	36.4	68.4	−50
615	24 600	1.11	0	35.6	40.5	23.8	64.4	−44.5
616	29 800	1.11	0	30.9	37.6	31.5	69.1	−51.5
523	37 000	1.21	0	9.0	70.5	20.5	91.0	−69
548	38 500	1.18	0	44.5	30.3	25.2	55.5	−43
116	49 300	1.21	0	27.1	36.8	36.1	72.9	−56

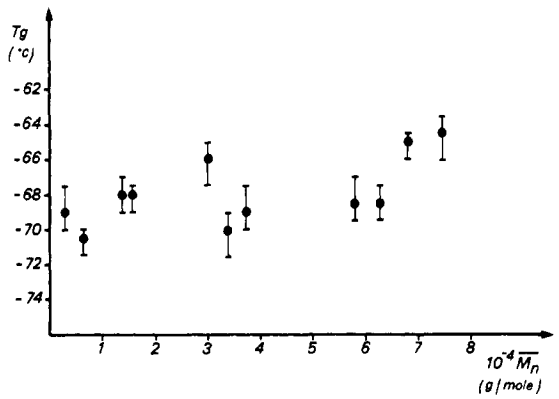

Figure 1. Glass transition temperature vs. molecular weight for high-1,4-content polyisoprene.

Table II. Note that PI prepared with lithium metal or lithium derivatives does not yield a 1,2 chain configuration.[15] Molecular weights range from 3800 to 49 300. It can be seen that T_g actually depends on microstructure. It increases linearly with 3,4 content and hence decreases with total 1,4 content. Similar results were found by Essel.[5] Again, the molecular weight does not seem to affect T_g since all samples fall on the same line.

It is more difficult to differentiate the respective roles of cis- and trans-1,4 structures. In all but a few samples, the trans-1,4 content is rather constant (Table II). This seems to indicate that the cis-1,4 configuration is the determining element in the observed T_g decrease.

Several factors relating to the chemical structure of polymers are known to affect the glass transition temperature. The most important of these is chain stiffness,

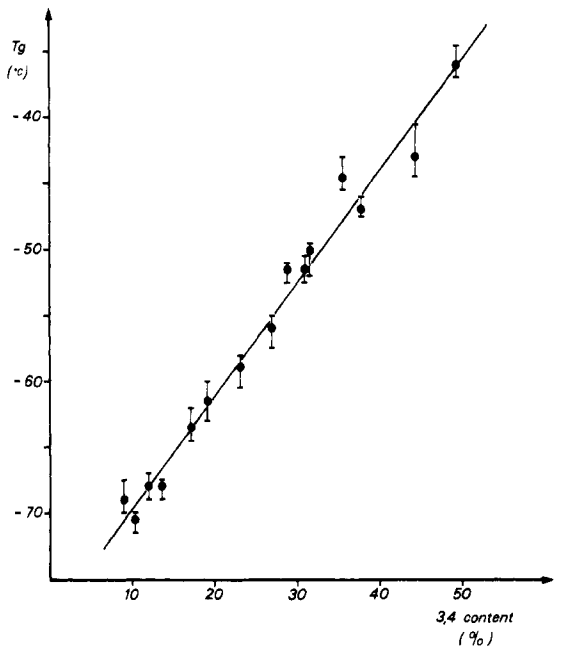

Figure 2. Glass transition temperature as a function of polyisoprene microstructure.

which depends upon the molecular weight and configuration of the polymer. In the case of polyisoprene, molecular weight does not seem to play a major role and different T_g's correspond to different microstructures. One might explain the increase of T_g with the 3,4 content by the steric hindrance from the side-chain vinyl group which contributes to a stiffening of the polymer chain. But, as the 3,4 content increases, the 1,4 content, i.e., the number of double bonds in the main chain, decreases, thus rendering the chain more flexible and reducing the T_g value. The observed increase of T_g with 3,4 content indicates that steric hindrance is more important than the presence of double bonds, even at low 3,4 contents.

One must also take into account the plasticizing role of the chain ends, which tends to decrease T_g. The shorter chains have a more pronounced effect.[16]

All of the parameters discussed above contribute to the glass transition behavior of polyisoprene, but their respective importance is difficult to determine as it varies from sample to sample. Thus, it is quite possible that the experimentally observed invariance of T_g with molecular weight is only a coincidence. If it had been possible to correct this so that we knew what the respective contribution of each factor was to the observed behavior, perhaps we would have been able to formulate a law similar to Flory's or even make use of this law itself.

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Nonrandom Structure in Thermally Unfolded Ribonuclease A

C. R. MATTHEWS* and C. L. FROEBE

Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802.
Received August 11, 1980

A complete understanding of the factors important in directing the rapid and efficient transition of a thermally unfolded protein to its native conformation requires information on the structure of the unfolded form. Spectroscopic evidence suggests that the thermally unfolded form of ribonuclease A (RNAase A) contains nonrandom structure;[1-3] however, detailed information on the structure of this form has not been obtained.

In a previous NMR study of thermally unfolded RNAase A,[4] we reported that two of the four histidine residues occupy a solvent-exposed environment while the remaining two appear to be associated with nonrandom structure. Only a partial assignment of the NMR resonances to specific histidine residues was obtained in that study, leaving incomplete our knowledge of the structure of this form. Recently, the application of a paramagnetic probe that forms tight complexes with histidine residues, $(NH_3)_5Ru^{III}$, to RNAase A[5,6] enabled us to obtain a derivative labeled at histidine-105.[7] By examining the proton NMR spectrum of the thermally unfolded form of this derivative, we can now complete the assignment of the histidine C-2 resonances in thermally unfolded RNAase A and thereby obtain more information on the structure of this form.

Experimental Methods

The synthesis, isolation, and characterization of the derivative of RNAase A in which a single $(NH_3)_5Ru^{III}$ complex is formed with the N-3 nitrogen of histidine-105 has been described.[5-7] As expected from model compound data,[8] this complex is extremely stable at moderate temperatures (0–50 °C) from pH 1 to 8. It decomposes at temperatures greater than 60 °C or at pH values above pH 11.[15] The samples were prepared for NMR study by two cycles of a process in which they were heated at 40 °C in 99.7% ^2H_2O (Merck Sharp and Dohme Isotopes) at pH* 2 (uncorrected meter reading) for 15 min and then lyophilized. This procedure serves to remove nitrogen protons which absorb in the same region as the histidine C-2 protons and to decrease the contribution from the solvent.

Results and Discussion

In the previous 100-MHz NMR study,[4] it was shown that the histidine C-2 region of the proton NMR spectrum of thermally unfolded RNAase A consists of two equal-area peaks, each of which represents two histidine C-2 protons. The computer fits of the pH titration curves for these peaks to the Henderson–Hasselbach equation showed that the peak with pK = 5.96 at 69 °C should appear 0.01–0.02 ppm upfield from the peak with pK = 5.75 when the pH

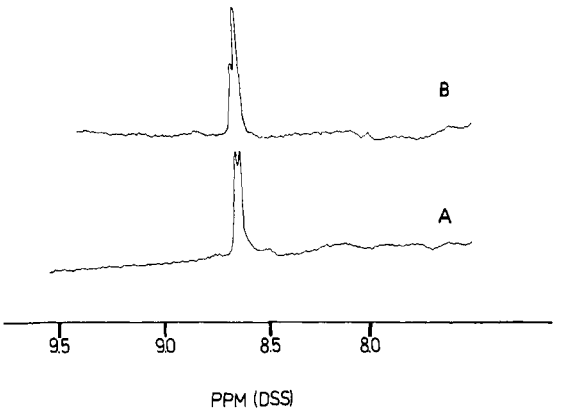

Figure 1. 200-MHz Fourier transform proton NMR spectra of unmodified RNAase A (A) and $(NH_3)_5Ru^{III}$-His-105 RNAase A (B) in ^2H_2O containing 20 mM glycine, pH* 2.3 (uncorrected meter reading), 0.2 M NaCl, 50 °C. The spectra were obtained by averaging transients of 32 768 data points for a spectral width of 3012 Hz. The acquisition time was 5.4 s and the digital resolution was 0.184 Hz/point. The spectra are the results of accumulating 1000 transients following 90° pulses. The delay between pulses, 5.4 s, was sufficient to permit complete recovery of the magnetization. The free induction decays were smoothed with an exponential function that resulted in 0.4-Hz line broadening. The chemical shifts are measured relative to the resonance of internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate. The protein concentration was ~10 mg mL^{-1}.

was less than 3 (Figure 1 in ref 4). Although these peaks could not be resolved at acidic pH at 100 MHz, they are easily resolved at 200 MHz (Figure 1). Also shown in Figure 1 is the effect of incorporating $(NH_3)_5Ru^{III}$ at histidine-105. The spectrum clearly shows that the downfield peak is reduced in amplitude by half when histidine-105 is labeled with $(NH_3)_5Ru^{III}$. Studies on a model compound, $(NH_3)_5Ru^{III}$-His, indicate that the resonances of the C-2 and C-4 protons of the histidine residue to which the ruthenium is bound are shifted away from the frequency at which they normally appear.[15]

To confirm that the histidine-105 C-2 proton can indeed be associated with the resonance whose pK value is 5.75 at 69 °C, we also obtained a spectrum of the labeled enzyme at pH 5.6, 69 °C. The results of the previous study had shown that both the resonances for the C-2 protons are shifted upfield due to deprotonation of the histidines under these conditions; however, the resonance with the lower pK value appears at higher field. In the spectrum at pH 5.6, 69 °C (data not shown), it was observed that the amplitude of the upfield peak was lower by a factor of 2 than that of the downfield peak. This result confirms the assignment of one of the two protons in the resonance whose pK is 5.75 at 69 °C to histidine-105. Since histidine-48 had already been assigned to this peak using selective deuteration techniques,[4] we conclude that histidines-48 and -105 have a pK of 5.75 in thermally unfolded RNAase A at 69 °C. By elimination histidines-12 and -119 have a pK of 5.96 at this temperature.

In the previous study,[4] the pK of histidine in the tripeptide Gly-His-Gly was found to be 6.03 at 69 °C. Assuming that this value is representative of a fully solvated histidine residue, it can be concluded that histidines-12 and -119 are freely exposed to solvent in thermally unfolded RNAase A; however, histidines-48 and -105 appear to be involved with some type of nonrandom structure. That the appearance of two peaks with differing pK values is due to nonrandom structure was demonstrated in the previous study[4] by the observation that the addition of guanidine hydrochloride to the thermally unfolded form

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