Notes

Nuclear Magnetic Resonance Study of Hydrophobic Bonding in (D,L-Lysine)$_m$–(L-Alanine)$_n$–(D,L-Lysine)$_m$[1]

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Ingwall, *et al.*,[3] studied the helix–coil transition of poly(L-alanine) in water by using water-soluble block copolymers of the type (D,L-lysine)$_m$–(L-alanine)$_n$–(D,L-lysine)$_m$. They found that a sample with n = 160 (Ala-160) exhibited similar transition curves in the absence and presence of salt, but that the helical form of a sample with n = 450 (Ala-450) was more stable in salt than in water; the difference in stability was attributed[3] to the folding of the long poly(L-alanine) α helix of Ala-450 in hairpinlike fashion, stabilized by methyl–methyl side-chain hydrophobic bonds. This salt effect was also demonstrated by laser Raman spectroscopic studies of these same samples.[4] The present paper reports evidence from nuclear magnetic resonance spectra of the identical Ala-160 and Ala-450 samples, which supports the earlier[3] conclusion that the side-chain methyl groups of Ala-450 are involved in hydrophobic bonds.

Samples Ala-160 and Ala-450 of the previous paper[3] and poly(D,L-lysine·HBr) (Miles Laboratories Lot No. LY114A, molecular weight 15,000–40,000) were dissolved in D_2O (International Chemical and Nuclear Corp., 99.8% isotopic purity) and in 0.2 N NaCl in D_2O, respectively. The apparent pD's were measured at 24° with a Radiometer Model PHM 4c pH meter using a Model GK2302B combined glass and saturated calomel electrode. Proton magnetic resonance spectra of these solutions were obtained at 100 MHz under *saturating* conditions with a Varian Associates HA-100 nmr spectrometer in two temperature regions, 29–32° and 70–76°, *i.e.*, at moderate and low helix content, respectively.

The nmr spectrum of Ala-160 in D_2O is shown in Figure 1. The region between δ 1.3 and 2.2 ppm is assigned to the resonances of the β-, γ-, and δ-CH_2 protons of the D,L-lysine residues[5] and of the β-CH_3 protons of the L-alanine residues.[5] The region at δ 3.0–3.3 ppm is assigned to the resonance of the ϵ-CH_2 protons of the D,L-lysine residues,[5] and that at δ 4.3–4.7 ppm to the resonances of the α-CH protons of both the D,L-lysine and the L-alanine residues.[5]

Since the resonances of the β-CH_3 protons of L-alanine (those of primary interest here) overlap with those of the β-, γ-, and δ-CH_2 protons of D,L-lysine, it was assumed that the *total area* (measured with a K & E compensating polar planimeter) of the complex set of peaks between δ 1.3 and 2.2 ppm results from additive contributions from the alanine and lysine protons; *i.e.*, it is assumed that the spin–lattice and spin–spin relaxation times (T_1 and T_2, respectively) of the alanine protons are not affected by the lysine protons. The effect of changes in solvent and temperature on T_1 and T_2 of the D,L-lysine residues is assessed by observations on poly(D,L-lysine) (see Table I). Thus, since we cannot obtain the line widths (which would be a measure of T_2) of the peak from the β-CH_3 protons, we shall consider changes in the *ratio* of the total area of the complex peak to the area of the ϵ-CH_2 peak of the *same* spectrum (under *saturating* conditions) as arising from changes in the relaxation times of the β-CH_3 protons because of structural changes in the (L-alanine)$_n$ block.[6]

Figure 2 shows the spectra of poly(D,L-lysine), Ala-160, and Ala-450 on an expanded scale under various conditions. Changes in the state of bonding of the β-CH_3 protons are assessed in terms of the variation in the ratio of the total area of the complex peaks at 1.3–2.2 ppm to that of the ϵ-CH_2 protons at 3.0–3.3 ppm; this procedure is valid, since the relative area is essentially constant (independent of temperature, salt concentration, and pD in the acid range) for poly(D,L-lysine)

TABLE I

RATIO OF AREA OF COMPLEX PEAK AT 1.3–2.2 PPM TO THAT OF THE ϵ-CH_2 PROTONS AT 3.0–3.3 PPM

Polymer[a]	Solvent	pD[b]	Temp, °C	Ratio of areas
Poly(D,L-lysine)	D_2O	3.9	32	3.2
		7.2	28	3.2
		3.9	76	3.7
		7.2	70	3.6
	0.2 N NaCl	3.9	32	3.5
		3.9	70	3.6
Ala-160	D_2O	6.7	29	4.2
		6.7	70	5.5
	0.2 N NaCl	6.6	32	4.2
		6.6	70	5.2
Ala-450	D_2O	7.1	32	5.0
		7.1	76	6.0
	D_2O*		32	5.2
	0.2 N NaCl	7.0	32	4.0
		7.0	76	5.7
	0.2 N NaCl*		32	3.6

[a] Polymer concentration was 2% (w/v) in all cases except that noted by an asterisk, for which the concentration was 0.2% (to explore the concentration dependence of the area ratio). [b] Measured at 24°.

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[3] R. T. Ingwall, H. A. Scheraga, N. Lotan, A. Berger, and E. Katchalski, *Biopolymers*, 6, 331 (1968).

[4] A. Lewis and H. A. Scheraga, *Macromolecules*, 4, 539 (1971).

[5] E. M. Bradbury, C. Crane-Robinson, H. Goldman, and H. W. E. Rattle, *Biopolymers*, 6, 851 (1968).

[6] The area of a peak is proportional to[7] $\chi_0 H_1/(1 + \gamma^2 H_1^2 T_1 T_2)^{1/2}$, where χ_0 is the bulk magnetic susceptibility, H_1 is the rf amplitude, γ is the magnetogyric ratio, and T_1 and T_2 are previously defined relaxation times. Under nonsaturating conditions, the peak width at half of the maximum height depends on $1/T_2$. However, under saturating conditions (*i.e.*, in a range of H_1 and T_1T_2 where $\gamma^2 H_1^2 T_1 T_2$ is not negligible compared to unity), and at constant H_1, changes in the area reflect changes in the product T_1T_2, since χ_0 is constant for a particular polymer–solvent system. The use of saturating conditions must be distinguished from the conventional nmr experiment (nonsaturating conditions) in which the line width provides a measure of T_2. Since it is not possible to determine T_2 in our system because of the overlap of peaks, resort has been had to the method of modified progressive saturation at constant H_1 to detect changes in T_1T_2. Since T_2 cannot be measured, T_1 cannot be obtained from the product T_1T_2; however, changes in the observed area do reflect changes in T_1T_2 which, when combined with the information in Figure 9-2 on p 205 of ref 7, indicate the directions of changes in T_1 and T_2, respectively.

[7] J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, N. Y., 1959, pp 39, 205.

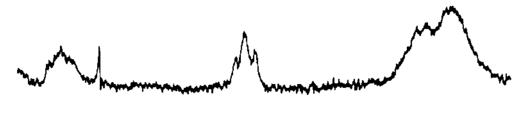

Figure 1. Nmr spectrum of 2% (w/v) Ala-160 in D_2O, at 29° at an apparent pD of 6.7 (measured at 24°) at a sufficiently high rf amplitude to ensure partial saturation. The chemical shifts were measured with respect to the coaxially inserted (external) standard tetramethylsilane. The peak at 4.25 ppm is a spinning side band of the HDO peak.

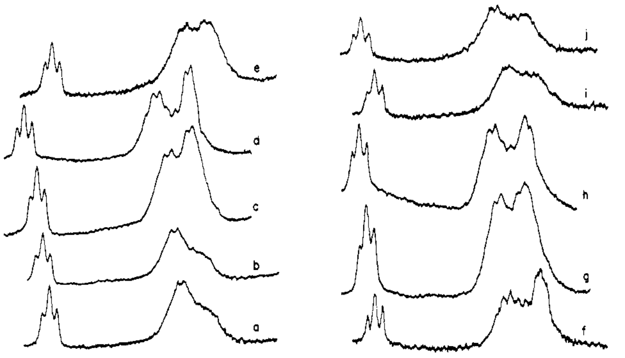

Figure 2. Nmr spectra (at partial saturation) in the ϵ-CH_2 and (β-, γ-, δ-CH_2 + β-CH_3) regions of Ala-160 and Ala-450 (all at 2% (w/v)) at various temperatures and salt concentrations, and at pD's given in Table I. Each spectrum is the result of the accumulation of at least 23 scans stored in a Varian Associates C-1054 Computer of Average Transients (CAT) to improve the signal-to-noise ratio. The chemical shifts vary slightly from curve to curve because they were each measured with respect to an external standard: (a) poly(D,L-lysine) in D_2O at 32°, (b) poly(D,L-lysine) in 0.2 N NaCl at 32°, (c) Ala-160 in D_2O at 29°, (d) Ala-160 in D_2O at 70°, (e) Ala-450 in D_2O at 32°, (f) Ala-450 in D_2O at 76°, (g) Ala-160 in 0.2 N NaCl at 32°, (h) Ala-160 in 0.2 N NaCl at 70°, (i) Ala-450 in 0.2 N NaCl at 32°, (j) Ala-450 in 0.2 N NaCl at 76°.

within the experiment error of ~15% (indicated in Table I), implying that T_1T_2 for poly(D,L-lysine) is essentially constant. Since *ratios* of areas are considered, any temperature dependence of χ_0 does not affect the result. These ratios, obtained from the spectra of Figure 2, at constant rf amplitude, are given in Table I.

The area ratios for Ala-160 and Ala-450 are larger than those for poly(D,L-lysine) because of the contribution from the β-CH_3 protons of alanine; the area ratio for Ala-450 is greater than that of Ala-160 because of the larger alanine content (Ala/Lys ratio) of the former polymer. Over the range of 2–0.2% (w/v) concentration, the area ratios are not dependent on the concentration within the experimental error; this, together with similar observations by Ingwall, *et al.*,[3] indicates that the polymers are not aggregated in this range of concentrations.

As the temperature increases, the relative area increases for both Ala-160 (by 31% in D_2O and 24% in 0.2 N NaCl) and Ala-450 (by 20% in D_2O and 42% in 0.2 N NaCl); this is a reflection of the increase in the random-coil content at higher temperatures.[8] When salt is added, the relative areas of Ala-160 are unaffected, but those of Ala-450 at low temperature decrease by 20% from 5.0 to 4.0 and by 31% from 5.2 to 3.6 in 2 and 0.2% solutions, respectively. The decrease in area is attributed[6] to an increase in T_1T_2 (because of an increase in T_1) arising from a stabilization of the hairpinlike conformations formed because of side-chain methyl–methyl hydrophobic bonds. Thus, these nmr data support the conclusion from ORD measurements[3] that hairpinlike bends of the α helix exist in the longer Ala-450 but not in the shorter Ala-160 polymer.

Further work is being carried out on similar block copolymers, which contain deuterated poly(D,L-lysine), to remove the lysine hydrogen resonances from overlap with those of the alanine protons, thereby permitting relaxation times to be determined from the peak widths.

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[8] As the helix is converted to coil, the area increases because T_1T_2 decreases; *i.e.*, the increase[6,7] in T_2 (which would be reflected in a decrease in line width, if the isolated peak could be observed) is more than offset by a decrease in T_1. Figure 9-2 of ref 7 would imply that the correlation time in our experiments is $>10^{-8}$ sec, since T_1T_2 decreases (because of the decrease in T_1) with an increase in mobility (when helix is converted to coil).

Thermodynamic Interactions in Polymer Systems by Gas–Liquid Chromatography. II. Rubber–Hydrocarbons

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The application of gas–liquid chromatography (glc) to the study of thermodynamic interactions in polymeric systems is attractive in its ability to generate data rapidly and to extend these into high polymer concentration ranges which are difficult to study by conventional methods. Several recent publications[2-4] have shown that glc data can produce thermodynamically meaningful results, our most recent work[4] showing very good agreement in a comparison of thermodynamic interaction parameters calculated from glc and equilibrium sorption data for poly(dimethyl siloxane) (PDMS) interacting with selected hydrocarbons. The demand for accuracy in thermodynamic data is considerable, if, for example, these are to be of use in the resolution of varying predictions drawn from theories of solution thermodynamics. It is therefore prudent to extend the test of the rapid method's usefulness by comparing thermodynamic data calculated from conventional equilibrium sorption results and from glc data for additional polymers. The system natural rubber–hydrocarbons lends itself for such a comparison and is the subject matter of this report.

Experimental Section

The gas chromatograph and experimental procedure for the determination of retention volumes have been described in detail in an earlier publication.[4] In the present case, natural rubber was used as the polymer stationary phase. The sample, kindly supplied and characterized[5] by Professor B. M. van der Hoff, University of Waterloo, was used without further purification. The polymer was dissolved in benzene and coated onto Chromosorb W support (60/80 mesh, acid washed, DMCS treated). The exact polymer weight supported was determined by combustion and ashing methods.[6]

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