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References and Notes

(1) Poland, D. C.; Scheraga, H. A. *Biopolymers* **1965**, *3*, 305.
(2) Poland, D. C.; Scheraga, H. A. *Biopolymers* **1965**, *3*, 335.
(3) Poland, D.; Scheraga, H. A. "Theory of Helix–Coil Transitions in Biopolymers"; Academic Press: New York, 1970; sections 9H and 9I.
(4) Finkelstein, A. V.; Ptitsyn, O. B. *J. Mol. Biol.* **1976**, *103*, 15.
(5) Ptitsyn, O. B.; Finkelstein, V. A. *Q. Rev. Biophys.* **1980**, *13*, 339.
(6) Lim, V. I. *FEBS Lett.* **1979**, *89*, 10.
(7) Matheson, R. R.; Scheraga, H. A. *Macromolecules* **1978**, *11*, 819.
(8) Skolnick, J. *Macromolecules* **1983**, *16*, 1069.
(9) Skolnick, J. *Macromolecules* **1983**, *16*, 1763.
(10) Skolnick, J. *Macromolecules* **1984**, *17*, 645.
(11) Zimm, B.; Bragg, J. *J. Chem. Phys.* **1959**, *31*, 526.
(12) Ananthanarayanan, V. S.; Andreatta, R. H.; Poland, D.; Scheraga, H. A. *Macromolecules* **1971**, *4*, 417.
(13) Platzer, K. E. B.; Ananthanarayanan, V. S.; Andreatta, R. H.; Scheraga, H. A. *Macromolecules* **1972**, *5*, 177.
(14) Alter, J. E.; Taylor, G. T.; Scheraga, H. A. *Macromolecules* **1972**, *5*, 739.
(15) Van Wart, H. E.; Taylor, G. T.; Scheraga, H. A. *Macromolecules* **1973**, *6*, 266.
(16) Alter, J. E.; Andreatta, R. H.; Taylor, G. T.; Scheraga, H. A. *Macromolecules* **1973**, *6*, 564.
(17) Maxfield, F. R.; Alter, J. E.; Taylor, G. T.; Scheraga, H. A. *Macromolecules* **1975**, *8*, 479.
(18) Scheule, R. K.; Cardinaux, F.; Taylor, G. T.; Scheraga, H. A. *Macromolecules* **1976**, *9*, 23.
(19) Dygert, M. K.; Taylor, G. T.; Cardinaux, F.; Scheraga, H. A. *Macromolecules* **1976**, *9*, 794.
(20) Matheson, R. R.; Nemenoff, R. A.; Cardinaux, F.; Scheraga, H. A. *Biopolymers* **1977**, *16*, 1567.
(21) van Nispen, J. W.; Hill, D. J.; Scheraga, H. A. *Biopolymers* **1977**, *16*, 1587.
(22) Hill, D. J.; Cardinaux, F.; Scheraga, H. A. *Biopolymers* **1977**, *16*, 2447.
(23) Konishi, Y.; van Nispen, J. W.; Davenport, G.; Scheraga, H. A. *Macromolecules* **1977**, *10*, 1264.
(24) Kobayashi, Y.; Cardinaux, F.; Zweifel, B. O.; Scheraga, H. A. *Macromolecules* **1977**, *10*, 1271.
(25) Hecht, M. H.; Zweifel, B. O.; Scheraga, H. A. *Macromolecules* **1978**, *11*, 545.
(26) Skolnick, J.; Holtzer, A. *Macromolecules* **1982**, *15*, 812.
(27) Holtzer, M. E.; Holtzer, A.; Skolnick, J. *Macromolecules* **1983**, *16*, 173.
(28) Skolnick, J.; Holtzer, A. *Macromolecules* **1982**, *15*, 303.
(29) Schellman, J. A. *C. R. Trav. Lab. Carlsberg.*, Ser. Chim. **1955**, *29*, 230.
(30) Flory, P. J. *J. Am. Chem. Soc.* **1956**, *78*, 5222.
(31) Jacobson, H.; Stockmayer, W. H. *J. Chem. Phys.* **1950**, *18*, 1600.
(32) Crothers, D.; Kallenbach, N. *J. Chem. Phys.* **1966**, *45*, 917.
(33) Mayer, J.; Mayer, M. "Statistical Mechanics"; Wiley: New York, 1940; Chapter 9.
(34) Zimm, B. *J. Chem. Phys.* **1960**, *33*, 1349.
(35) u_ϕ also contains the ratio, q_ϕ, of the volume of configuration space spanned by the internal degrees of freedom in the helical block in the hairpin to that in the noninteracting state. Since q_ϕ is unknown, we arbitrarily set it equal to unity.
(36) de Gennes, P. G. "Scaling Concepts in Polymer Physics"; Cornell University Press: Ithaca, NY, 1979.
(37) The reader interested in the specific details should write the author.
(38) Holtzer, M.; Holtzer, A. *Macromolecules* **1972**, *5*, 294.
(39) Chou, K. C.; Nemethy, G.; Scheraga, H. A. *J. Chem. Phys.* **1983**, *87*, 2869.
(40) Phillips, G. N. *Biophys. J.* **1984**, *45*, 392a.
(41) Skolnick, J.; Holtzer, A. *Macromolecules*, in press.
(42) Mattice, W. L.; Srinivasan, G.; Santiago, G. *Macromolecules* **1980**, *13*, 1254.
(43) Privalov, P. L. *Adv. Protein Chem.* **1979**, *33*, 167.
(44) J. Skolnick, *Macromolecules*, in press.
(45) Hodges, R.; Saund, A.; Chong, P.; St. Pierre, S.; Reid, R. *J. Biol. Chem.* **1981**, *256*, 1214.
(46) Karplus, M.; Weaver, D. L. *Nature (London)* **1976**, *260*, 404.
(47) Karplus, M.; Weaver, D. L. *Biopolymers* **1979**, *18*, 1421.
(48) Weaver, D. L. *Biopolymers* **1982**, *21*, 1275.

α-Helix-to-Random-Coil Transition of Two-Chain, Coiled Coils. Light Scattering Experiments on the Thermal Denaturation of α-Tropomyosin†

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ABSTRACT: Light scattering experiments on solutions of α-tropomyosin in benign buffer near neutral pH are reported as a function of temperature. The Rayleigh ratio for all cases is almost independent of scattering angle, as expected for optically clear solutions whose constituent particles have a greatest dimension barely greater than one-tenth the wavelength. Terms in the virial expansion beyond the first are shown to be negligible, and the absolute value of the relevant function of the zero-angle excess Rayleigh ratio, $(Kc/R_0)^{-1}$, at 20 °C agrees satisfactorily with the known molecular weight of the two-chain, coiled-coil, native molecule. At the highest temperatures (≥60 °C), the molecular weight is half that value, indicating dissociation into two, separate polypeptide chains. Comparison of the full thermal course of weight-average molecular weight with studies of helix content (by circular dichroism) indicates that chain dissociation and cooperative loss of helix occur in the same temperature domain. Thus, it is likely that the two processes are closely coupled.

The native tropomyosin molecule comprises two right-handed α-helical polypeptide chains set side-by-side in parallel and in register and given a slight, left-handed supertwist, a structure that can be called a two-chain, coiled coil.[1] In the absence of interchain cross-links, the molecule is supposedly converted at elevated temperature to two separated chains of very low helix content, i.e., to

† We dedicate this paper to Prof. Paul Doty in this year of his 65th birthday.

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essentially random coils. This thermal transition has been under experimental study for some time, but attention has hitherto been given almost exclusively to measurement of chiroptical properties such as optical rotatory dispersion (ORD) or circular dichroism (CD), which measure helix content.[2-19] From a strictly experimental viewpoint, it is also desirable to characterize the transition by measuring the temperature dependence of average molecular weight in order to reveal a possible relationship between the helix content and chain dissociation. To our knowledge, such measurements have not been reported hitherto for the thermal transition. Molecular weight measurements (by equilibrium ultracentrifugation) indicating chain dissociation are extant for the guanidinium-induced transition at room temperature.[9]

Recently, a preliminary, statistical mechanical theory for thermal transitions has been developed and implemented for several types of coiled coils.[16,17,20-22] Measurements of helix content by CD were used to fit the thermal data on α-tropomyosin to the theory, whence quantitative predictions were generated for the extent of dissociation (and therefore any type of molecular weight average) vs. temperature.[16] These predictions aver that the observed cooperative loss of helix content (as seen in CD) be closely accompanied by chain dissociation. The two should go essentially hand-in-hand. Therefore, molecular weight measurements can not only provide an independent test of the validity of such specific theories but also clarify our broad, qualitative picture of the molecular events underlying these transitions.

Here, we report on experiments in which the temperature dependence of light scattering was determined on solutions of α-tropomyosin. This protein has been amply demonstrated to have the coiled-coil structure.[2,5,23] Furthermore, it comprises two identical chains, each having 284 amino acid residues in known sequence.[24] Each chain has one free sulfhydryl at position 190; the two-chain pair is readily air oxidized to form a disulfide cross-link which interferes with the experiment.[14] Two standard strategies were employed to prevent such interference. Some experiments were done by using α-tropomyosin whose adventitious disulfides were reduced with dithiothreitol (DTT) and protected from reoxidation by excess DTT. Other experiments were done on samples whose sulfhydryls were blocked by carboxyamidomethylation with iodoacetamide.[14,25,26] As expected, reduced and blocked samples denature in the same way; this slight modification at one site per chain has a very small effect, if any, on the transition as measured by CD.[14,27]

Materials and Methods

Preparation of α-tropomyosin from rabbit cardiac muscle and general manipulation of the protein were as previously described.[16] Sulfhydryls were kept reduced or were blocked with iodoacetamide as described earlier.[25,26] Protein concentrations were determined routinely by using absorbance at 277 nm with an extinction coefficient of 0.314 cm^2 mg^{-1}.[5] We specify complex aqueous solvent media by giving the chemical formula of each solute in parentheses with its millimolarity as subscript, followed by the pH in parentheses. Expressed in that notation, the solvent used was $(NaCl)_{1000}(NaP_i)_{50}(DTT)_{20}(7.4)$ (reduced samples) or $(NaCl)_{500}(NaP_i)_{50}(7.4)$ (blocked samples), against which the protein solution had been exhaustively dialyzed. The intensity of light scattered by solvent was unaffected by the DTT. The temperature dependence of the CD is insensitive to NaCl concentration in the range employed.

For measurement of light scattering, a Fica 50 automatic light scattering photometer was used with unpolarized light of 436-nm wavelength over the angular range 30–150°. This photometer has a built-in thermostat bath which controls temperature to ±0.1 °C up to ~50 °C and to ±0.25 °C above 50 °C. Measurements were made over the range 20–70 °C in these experiments. A cylindrical scattering cell was used that requires a minimum volume of 5.0 mL. The absolute calibration was determined by using benzene at 25 °C, for which the accepted value of the Rayleigh ratio of 4.65×10^{-5} cm^{-1} sr^{-1} was employed.

Solutions were optically clarified by passage, using a positive air pressure, through a double thickness of 0.22-μm Millipore filter. Use of positive N_2 pressure with an ultrafine fritted glass filter (nominal pore size ~1 μm) constructed as described earlier[28] also yielded clean solutions, but this method is slow and cumbersome, largely because of the time required to acid wash the filter. Nucleopore and Millipore filters of pore size 0.45 μm allow rapid filtration under gravity, are easily manipulated and give clean solvent, but the protein solutions gave every evidence (including high dissymmetry of scattering) of the presence of large particles. Ultracentrifugation is time-consuming and requires a high-speed refrigerated centrifuge, which was not available.

Unfortunately, the feasible range of protein concentration (~0.3–1.2 mg cm^{-3}) was not wide enough to demonstrate the effect of mass action on chain dissociation.[16] Given the unavoidable experimental error, this would require measurement over an approximately 100-fold range. This is not possible at present, since higher concentrations become hard to filter and lower ones scatter too little light (in excess of solvent) to provide reliable data.

Protein concentration for a light scattering solution of blocked protein concentrated enough for the absorbance at 277 nm to be precisely measured was determined after the run by removing the recooled solution from the scattering cell. Thus, possible effects from loss of protein on the filter were avoided. Concentration of a light scattering solution of blocked protein too dilute for an absorbance determination was determined by comparison of its excess scattering at 20 °C with a more concentrated solution run in the same series of experiments. That is $c_{dil} = c_{conc}[R_{dil}(20\ °C)/R_{conc}(20\ °C)]$. This procedure is valid since the second virial coefficients of blocked and of nonblocked tropomyosins under these conditions are known to be zero.[5,6]

Determination of the concentration of the native (nonblocked) protein creates a different problem because the presence of unknown amounts of cyclized DTT, itself an absorbing species, precludes measurement of protein absorbance. However, in benign medium at 20 °C, any tropomyosin, blocked or unblocked, reduced or cross-linked, must show the same light scattering per unit concentration, since only intact, two-chain species are present. Hence, comparison of two solutions, with and without DTT, allows the concentration of the former to be calculated as $c_{+DTT} = c_{-DTT}[R_{+DTT}(20\ °C)/R_{-DTT}(20\ °C)]$.

The refractive increment used was 0.187 cm^3 g^{-1}. This value was previously measured at 25 °C for a slightly different solvent medium, one in which the supporting electrolyte was KCl instead of NaCl.[5] An estimate using the known refractive increments of the two salts and the Gladstone–Dale equation shows that the effect of this change in solvent is completely negligible. The effect of temperature on dn/dc can also be ignored, as shown by the data of Perlmann and Longsworth.[29] Their data on several proteins show rather similar behavior (as well as absolute values) for all, namely a modest decline with temperature from near-freezing to room temperature. This decline levels out near room temperature. The data do not extend above 25 °C. Examination of the data for, say, bovine serum albumin (chosen because it has one of the larger observed temperature coefficients) suggests a temperature coefficient near room temperature of -0.8×10^{-4} cm^3 g^{-1} $°C^{-1}$. At that rate, dn/dc at 60 °C would be 0.184 instead of our value, 0.187. Use of 0.187 would thus yield a molecular weight that is too low by ~3%, well within the experimental uncertainty. Since the dn/dc vs. t curves seem to be leveling off, even this is probably an overestimate. In fact, even if one were to use the steepest slope shown (near 0 °C), which is $\sim 2 \times 10^{-4}$ cm^3 g^{-1} $°C^{-1}$, this would only change dn/dc to 0.180 at 60 °C, still necessitating only an 8% correction, which is just beginning to be comparable to the experimental uncertainty. The intrinsic temperature dependence of dn/dc therefore cannot be significant. It might be argued that since these dn/dc measurements did not extend to higher temperatures, they leave the effect of conformation changes out of account. However, nonchiral electronic properties such as dn/dc are notoriously oblivious to such changes; indeed, bovine

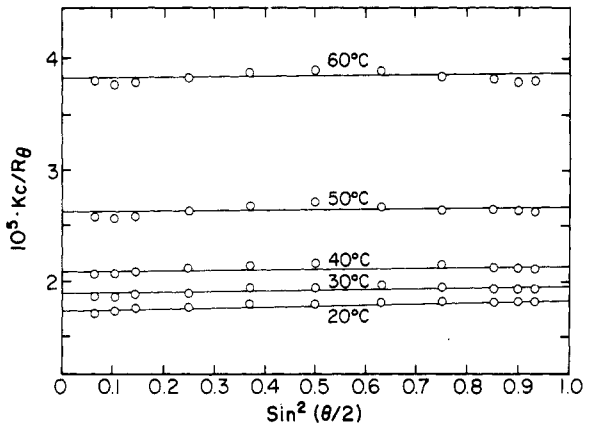

Figure 1. Angular scattering envelopes for α-tropomyosin. Plot is the customary Kc/R_θ vs. $\sin^2(\theta/2)$. K is the usual optical constant, c is the protein concentration (g cm^{-3}), and R_θ is the excess Rayleigh ratio at scattering angle θ. Data for blocked sample of protein concentration: 0.33 mg cm^{-3}. Temperature as marked.

serum albumin, a protein differing vastly in conformation from tropomyosin, has almost the same dn/dc.[29] Proteins, except for those with extraordinary amino acid composition, differ very little in this property.

Results and Discussion

Angular scattering envelopes for one of the most dilute solutions (0.33 mg cm^{-3}) measured are shown on Figure 1. These form the most stringent test of the quality of the data, because failure to obtain dust-free solutions has drastic consequences at low angles in this dilute range. The data show no evidence of such difficulties. The experimental scatter is normal. The slopes are virtually indistinguishable from zero, as expected for a particle which, even in the native, two-chain state, has a longest dimension (~40 nm)[23] that is at most about 12% of the wavelength (in the solution) of light being used.

Although absolute determination of molecular weight is not an aim of this study, measurement of the protein concentration as well as the scattering at 20 °C (where the second virial coefficient is zero) allows such an estimate to be made. The data of Figure 1, being for a very dilute solution of blocked protein, were calculated by using a concentration determined (see "Materials and Methods") relative to the more concentrated solution. The more concentrated solution, whose concentration was determined by absorbance, was found to give at zero angle and 20 °C $[R_0(20\ °C)/Kc] = M_w(20\ °C) = 5.8 \times 10^4$. This absolute value compares favorably with the known value of 6.5×10^4, the difference of 11% being just about at the tolerable uncertainty level for an absolute determination on a protein.[30]

The primary purpose here is to elucidate the *change* in weight-average molecular weight with temperature in order to characterize the dissociative nature of the transition. Data for all solutions are therefore shown on Figure 2 as $2R_0(t)/R_0(20\ °C)$. With the assumptions (deemed adequate here) of constant dn/dc and zero second virial coefficient, this should equal $2M_w(t)/M_w(20\ °C)$, the weight-average number of chains per molecule (weight-average aggregation number), a quantity that should vary from 2 to 1 in traversing a transition that results in a halving of the molecular weight. Results for all the experiments are given in the same terms in Table I.

Close examination of Figure 2 suggests that there may be a slight difference between reduced and blocked samples. However, if such a difference exists, it is barely outside experimental error. The light scattering data thus

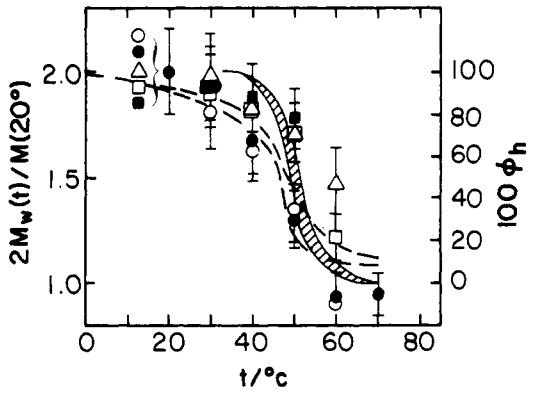

Figure 2. Weight-average aggregation number of α-tropomyosin vs. Celsius temperature. Left ordinate scale gives the weight-average number of chains per molecule (aggregation number), i.e., $2M_w(t)/M(20\ °C)$. Medium is $(NaCl)_{1000}(NaP_i)_{50}(DTT)_{20}(7.4)$ (reduced samples) or $(NaCl)_{500}(NaP_i)_{50}(7.4)$ (blocked samples). Blocked samples: open circles, 0.33 mg cm^{-3}; filled circles, 0.56 mg cm^{-3}. Reduced samples: open squares, 0.38 mg cm^{-3}; triangles, 1.04 mg cm^{-3}; filled squares, 1.17 mg cm^{-3}. Error bars show ±10%. Hatched band is weight-average aggregation number predicted from the primitive statistical mechanical theory for the range 0.33–1.17 mg cm^{-3}. For comparison, right ordinate scale and dashed curves show percent helix from CD using experimental data from ref 18 covering a comparable concentration region. Lower dashed curve, 0.104 mg cm^{-3}; upper dashed curve, 5.2 mg cm^{-3}.

Table I
Weight-Average Aggregation Number[a] from Light Scattering on α-Tropomyosin

	concn/(mg cm^{-3})				
t/°C	0.33[b]	0.38	0.56[b]	1.04	1.17
20	2.00	2.00	2.00	2.00	2.00
30	1.82	1.92	1.94	1.98	1.94
40	1.66	1.82	1.68	1.84	1.88
50	1.32	1.72	1.30	1.72	1.78
60	0.90	1.22	0.92	1.48	
70			0.94		

[a] Weight-average aggregation number = $2M_w(t)/M_w$ (20 °C). [b] Carboxyamidomethylated (sulfhydryl blocked) sample.

confirm the CD measurements, which show a transition that is insensitive to blocking and to NaCl concentration in the relevant range.[14,27]

Qualitatively, the experimental points on Figure 2 clearly show the expected behavior. Purely empirical comparison with experimental thermal curves of helix content,[16,18] moreover, show that cooperative loss of helix and chain dissociation occur in the same temperature region. For comparison, curves of helix content are also shown on Figure 2 (dashed curves). At a middling concentration (~0.1 mg cm^{-3}), the helix content data show a midpoint in the range 45–50 °C, as do the light scattering data of Figure 2. Although closer examination of the data is needed, and will be given below, it is perhaps worth noting in advance that such closer examination only reinforces the initial qualitative conclusion drawn from a purely empirical point of view.

The high-temperature limit bears a closer look. In principle, that limit should, on Figure 2, be exactly unity. The five highest temperature points shown agree well and average to 1.09 ± 0.11 (SE). This is within experimental error of unity. The concentration range covered is unfortunately too narrow to reveal mass actional differences in chain dissociation. This is expected for the range covered.[16] For technical reasons (see "Materials and Methods"), it is not possible to extend it with our present methods.

The agreement of the various concentrations at the highest temperatures strongly suggests that the second virial coefficient is indeed near zero there as well as at room temperature. However, even an unrealistically high second virial coefficient would not be sufficient, at these concentrations, to force a material change in these results. Even if the second virial coefficient at, say, 60 °C were as high as it is at room temperature in 5 M guanidinium chloride (7.13×10^{-4} mol cm^3 g^{-2}),[5] it would increase the molecular weight given in Figure 2 for the sample of highest concentration by less than 6%. To produce a change comparable to the experimental error, a second virial coefficient of $\sim 14 \times 10^{-4}$ mol cm^3 g^{-2}—an unheard of value—would be required. The assumption that the second virial coefficient can be ignored over the entire temperature range is clearly justified.

The hatched band drawn on Figure 2 shows the previously published (Figure 7 of ref 16) prediction of weight-average aggregation number made from a statistical mechanical theory of the helix–coil transition; this prediction is shown as corrected for mass action to the concentration range of our light scattering experiments. Although recent advances in the theory[31-33] make it unwise to make a detailed comparison of our data with this relatively unsophisticated earlier version, it seems safe to say that the principal qualitative theoretical prediction—that loss of helix content and chain dissociation go hand-in-hand—is strikingly borne out. This close linkage is by no means generally accepted at present; at least two laboratories much concerned with these matters would center the dissociation at rather higher temperature than the loss of helix.[13,19] Whether the present data are compatible in more quantitative detail with the current, more sophisticated form of the theory remains to be seen. In any event, we now have two independent physical characteristics of the transition, helix content and weight-average aggregation number, to guide our understanding.

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References and Notes

(1) Fraser, R.; MacRae, T. "Conformation in Fibrous Proteins"; Academic Press: New York, 1973; pp 419–468.
(2) Cohen, C.; Szent-Györgyi, A. G. *J. Am. Chem. Soc.* **1957**, *79*, 248.
(3) Noelken, M. Ph.D. *Thesis*, Washington University, 1962.
(4) Noelken, M.; Holtzer, A. In "Biochemistry of Muscle Contraction"; Gergely, J., Ed.; Little, Brown and Co.: Boston, 1964; pp 374–378.
(5) Holtzer, A.; Clark, R.; Lowey, S. *Biochemistry* **1965**, *4*, 2401–2411.
(6) Olander, J.; Emerson, M.; Holtzer, A. *J. Am. Chem. Soc.* **1967**, *89*, 3058–3059.
(7) Olander, J. *Biochemistry* **1971**, *10*, 601–609.
(8) Woods, E. F. *Int. J. Protein Res.* **1969**, *1*, 29–43.
(9) Pont, M.; Woods, E. F. *Int. J. Protein Res.* **1971**, *3*, 177–183.
(10) Woods, E. F. *Aust. J. Biol. Sci.* **1976**, *29*, 405–418.
(11) Woods, E. F. *Aust. J. Biol. Sci.* **1977**, *30*, 527–542.
(12) Halsey, J.; Harrington, W. *Biochemistry* **1973**, *12*, 693–701.
(13) Tsong, T.; Himmelfarb, S.; Harrington, W. *J. Mol. Biol.* **1983**, *164*, 431–450.
(14) Lehrer, S. *J. Mol. Biol.* **1978**, *118*, 209–226.
(15) Betteridge, D.; Lehrer, S. *J. Mol. Biol.* **1983**, *167*, 481–496.
(16) Holtzer, M. E.; Holtzer, A.; Skolnick, J. *Macromolecules* **1983**, *16*, 173–180.
(17) Holtzer, M. E.; Holtzer, A.; Skolnick, J. *Macromolecules* **1983**, *16*, 462–465.
(18) Isom, L.; Holtzer, M. E.; Holtzer, A. *Macromolecules* **1984**, *17*, 2445–2447.
(19) Potekhin, S.; Privalov, P. *J. Mol. Biol.* **1982**, *159*, 519–535.
(20) Skolnick, J.; Holtzer, A. *Macromolecules* **1982**, *15*, 303–314.
(21) Skolnick, J.; Holtzer, A. *Macromolecules* **1982**, *15*, 812–821.
(22) Skolnick, J.; Holtzer, A. *Macromolecules* **1983**, *16*, 1548–1550.
(23) Caspar, D.; Cohen, C.; Longley, W. *J. Mol. Biol.* **1969**, *41*, 87–107.
(24) Mak, A.; Lewis, W.; Smillie, L. *FEBS Lett.* **1979**, *105*, 232–234.
(25) Angeletti, R.; Bradshaw, R.; Wade, R. *Biochemistry* **1971**, *10*, 463–469.
(26) Holtzer, M. E.; Breiner, T.; Holtzer, A. *Biopolymers* **1984**, *23*, 1811–1833.
(27) Holtzer, M. E.; Holtzer, A., unpublished work.
(28) Hawkins, R. Ph.D. Thesis, Washington University, 1967.
(29) Perlmann, G.; Longsworth, L. *J. Am. Chem. Soc.* **1948**, *70*, 2719–2724; see especially Figure 1 and Table III.
(30) Lowey, S.; Kucera, J.; Holtzer, A. *J. Mol. Biol.* **1963**, *7*, 234–244.
(31) Skolnick, J. *Macromolecules* **1983**, *16*, 1069–1083.
(32) Skolnick, J. *Macromolecules* **1983**, *16*, 1763–1770.
(33) Skolnick, J. *Macromolecules* **1984**, *17*, 645–658.

Carbon-13 Nuclear Magnetic Resonance Chemical Shifts of Poly(vinyl alcohol)

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ABSTRACT: ^{13}C NMR chemical shifts are calculated for the carbon nuclei in poly(vinyl alcohol) (PVOH) to the pentad and hexad levels of stereosequence for the methine and methylene carbons, respectively. This is achieved through utilization of the conformationally sensitive γ-gauche effect method which accounts for the magnetic shielding of those carbons in a gauche arrangement with their γ substituents. The RIS model of PVOH developed by Wolf and Suter is employed to calculate the frequencies of γ-gauche arrangements between methylene and methine carbons and between hydroxyl groups and methine carbons. Calculated chemical shifts are compared to the 100-MHz ^{13}C NMR spectra reported for PVOH in D_2O and dimethyl-d_6 sulfoxide by Ovenall. The relative orders of the observed methine pentad and methylene hexad ^{13}C resonances agree with the calculated chemical shifts, in addition to agreement between the overall chemical shift dispersions measured and predicted for the methylene carbons. However, the 3–4 ppm spread observed for the methine resonances is severalfold larger than the dispersion in chemical shifts calculated for the methine carbons.

Introduction

The microstructural dependence of the ^{13}C NMR spectra of vinyl polymers has been demonstrated to have a conformational origin.[1] Polymer microstructure affects the local conformations[2-5] of the polymer chain, which in turn determine the magnetic fields experienced by its carbon

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