Further Comparison with Experiment of the Calculated Results Obtained from Semiempirical and Quantum Mechanical Conformational Energy Maps Appropriate to Random-Coil Polypeptides

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ABSTRACT: The average vicinal nmr coupling constant $J_{N\alpha}$ between amide and α-protons, the mean-square unperturbed end-to-end distance $\langle r^2\rangle_0$ for randomly coiling polypeptides with side chains R of the type R = CH_2R', and the mean-square unperturbed dipole moments $\langle \mu^2\rangle_0$ of the zwitterionic diastereoisomers of the tri- and tetrapeptides of alanine are calculated from the conformational energy map reported to be appropriate to these random-coil polypeptides according to PCILO quantum mechanical molecular orbital calculations. These results are compared with experiment and with the corresponding quantities calculated from the conformational energy maps obtained by using semiempirical potential energy functions. Both conformational energy maps predict the correct vicinal coupling $J_{N\alpha}$ observed for three different random-coil polypeptides in solution. However, the PCILO map leads to dimensions $\langle r^2\rangle_0$ [$(\langle r^2\rangle_0/n_p l_p^2)_{n\to\infty} = 1.92$], almost identical with those calculated for a polypeptide chain with free rotation about the N–C^α and C^α–C backbone bonds, and predicts the mean-square dipole moments of the diastereoisomeric tri- and tetrapeptides of alanine to increase with decreasing stereoregularity and to be virtually independent of stereosequence, respectively, in disagreement with the experimental observations. The semiempirical map yields calculated dimensions and predicts the effect of stereosequence on the dipole moments of the zwitterionic tri- and tetrapeptides in excellent agreement with experiment. On this basis, and because other quantum mechanical calculations (extended Hückel type) conform very closely to the semiempirical energy maps, it appears that the PCILO map is in error and does not satisfactorily describe the conformational characteristics of randomly coiling alanine-type polypeptides as claimed.

Semiempirical potential energy functions[1-10] have been used to calculate the conformational characteristics of polypeptides in solution and in the crystal. These potential functions partition the conformational energy into bond torsional strains, van der Waals repulsions, London attractions, and electrostatic interactions (dipole–dipole or monopole–monopole) between nonbonded atoms and groups. In conjunction with statistical mechanical methods,[11] these conformational energy estimates have successfully predicted the dimensions[6,12] (the mean-square end-to-end distance $\langle r^2\rangle_0$) and molecular dipole moments[8] of random-coil polypeptide chains of varying amino acid sequence and composition and of varying stereoregularity. The correct signs of the rotational strengths[13,14] of the optically active electronic transitions and the observed vicinal nmr coupling[15,16] between amide and α-protons in random-coil polypeptides are also predicted. In addition, the most stable helical conformation found[5,17,18] for most poly(L-peptides) by the same potential energy estimates, when they are extended to include hydrogen bond interactions and steric and electrostatic interactions longer in range than those included in the random-coil energy maps, is the commonly observed right-handed Pauling–Corey α helix.

Recently, the conformational energy maps appropriate to randomly coiling polyglycine and poly(L-alanine) have been recalculated using quantum mechanical methods. Two different molecular orbital approaches have been taken, those of the extended Hückel type[19] and those based on perturbative configuration interaction[20] (PCILO) used in the CNDO/2 approximation. Both quantum mechanical calculations yield conformational energy maps for random-coil polyglycine which are in substantial agreement with the glycine map obtained from the semiempirical potential energy functions (compare Figure 1 in ref 19 and Figure 1 in ref 20 to the energy map in Figure 8 of ref 6). However, the conformational energy map for random-coil poly(L-alanine) obtained by Maigret, *et al.*,[20] using the PCILO method differs substantially from the semiempirical and extended Hückel maps, which are in close agreement.

In view of the disparity in the descriptions of the conformational characteristics of random-coil poly(L-alanine) provided by the semiempirical (or the extended Hückel) and the PCILO methods of calculation, it seems useful to compare the results calculated from both energy maps with each other and with experiment. Such a comparison was attempted by Pullman, *et al.*,[21] who concluded that the conformational

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energy map for random-coil poly(L-alanine) (and other polypeptides with R groups of the type R = CH_2R') obtained[20] by the quantum mechanical PCILO method is to be preferred over the semiempirical and extended Hückel maps. Their preference is based solely on the comparison of the crystallographically determined conformations of the residues in crystalline lysozyme,[22] myoglobin,[23] and several smaller peptides[24] with the conformationally accessible portions of the semiempirical and PCILO random-coil maps and upon the coincidence of the lowest energy conformation predicted by the PCILO calculation with the internally hydrogen-bonded solution conformation proposed by Bystrov, *et al.*,[25] for alanine dipeptides.

In view of the dangers[11,26] inherent in comparisons of the conformations of residues in space-filling protein chains packed into ordered crystals with the conformations predicted for random-coil polypeptides in dilute solution, we do not feel that the preliminary comparison reported by Pullman, *et al.*,[21] is entirely appropriate or conclusive. Consequently, the average nmr vicinal coupling, $J_{N\alpha}$, between the amide and α-protons, the unperturbed dimensions $\langle r^2\rangle_0$ of randomly coiling poly(L-alanine), and the unperturbed dipole moments $\langle \mu^2\rangle_0$ of the zwitterionic diastereoisomers of the tri- and tetrapeptides of alanine are calculated here from the PCILO map and are compared with experiment and with the same quantities obtained from the semiempirical map for an alanine residue. Since the experiments used for comparison are performed on randomly coiling polypeptides in solution and not on crystalline proteins, we feel the present comparison of the calculated and experimental conformational characteristics of random coil polypeptides to be more cogent than that reported by Pullman, *et al.*[21]

Method of Calculation

The calculation of the average vicinal coupling $J_{N\alpha}$ and the unperturbed dimensions and dipole moments of random-coil polypeptides has been fully described elsewhere.[1,6,8,15,16] Briefly summarizing, $\langle J_{N\alpha}\rangle$ is obtained by averaging a "Karplus-like" relation[25,27] connecting the dihedral angle φ' and the vicinal coupling between the amide and α-protons over all conformations, *i.e.*, over all pairs of φ and ψ (see Figure 1), allowed by the potential energy estimates. (The angles of rotation φ, ψ, and ω (see Figure 1) are taken[28] as zero in the all-trans or planar-zigzag conformation and are measured in a right-handed sense. All peptide bonds are assumed to be planar trans, thus $\omega = 0$. The dihedral angle φ' between N–H and C^α–H^α is directly related to the angle of rotation φ about N–C^α, $\varphi' = |240° - \varphi|$. Recently a new convention defining the backbone rotations in peptides has been proposed.[29] The new convention assigns the planar-zigzag or all-trans conformation to $\varphi = \psi = \omega = 180°$. To minimize confusion, the values of the rotation angles as defined in the new convention are given in parentheses.)

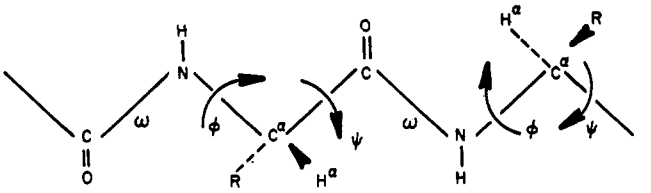

Figure 1. A schematic representation of a portion of a poly(L-peptide) in the planar trans conformation.

$$J_{N\alpha} = 8.5\cos^2\varphi \quad (0° \le \varphi' \le 90°)$$

$$J_{N\alpha} = 9.5\cos^2\varphi \quad (90° \le \varphi' \le 180°) \qquad (1)[27]$$

$$J_{N\alpha} = 8.9\cos^2\varphi' - 0.9\cos\varphi' + 0.9\sin^2\varphi' \qquad (2)[25]$$

The averaging of eq 1 and 2 is accomplished[15,16] by varying φ and ψ in small increments and multiplying the value of $J_{N\alpha}$ corresponding to a particular (φ, ψ) by its Boltzmann factor ($\exp^{-V(\varphi,\Psi)}/RT$) obtained from the conformational energy map. The $J_{N\alpha}$'s are then summed and divided by the corresponding sum of Boltzmann factors to obtain $\langle J_{N\alpha}\rangle$.

The characteristic ratio

$$\left(\frac{\langle r^2\rangle_0}{n_p l_p^2}\right)_{n_p\to\infty}$$

of the mean-square unperturbed end-to-end distance $\langle r^2\rangle_0$ to the number n_p of planar, trans peptide units times the square of the length l_p between adjacent α-carbons (in polypeptides with the usual[1] valence angles and bond lengths and with all-trans peptide bonds, the distance (3.8 Å) between adjacent α-carbons is invariant to the rotations φ and ψ) can be calculated[1] for long polypeptide chains according to

$$\left(\frac{\langle r^2\rangle_0}{n l_p^2}\right)_{n_p\to\infty} = [(E + \langle T\rangle)(E - \langle T\rangle)^{-1}]_{11} \qquad (3)$$

where E is the identity matrix of order 3, $\langle T\rangle$ is the statistical mechanical average (taken as described above for $\langle J_{N\alpha}\rangle$) of the matrix T which transforms the representation of a vector in the coordinate system of a given virtual bond connecting adjacent α-carbons to its representation in the similarly defined coordinate system of the preceding virtual bond (see ref 1), and the subscript 11 indicates the (1, 1) element of the enclosed matrix product.

The unperturbed mean-square dipole moment of a randomly coiled polypeptide chain of $x + 1$ residues can be calculated[8] from

$$\langle \mu^2\rangle_0 = [2\ 0\ 0\ 0\ 0]\left(\prod_{i=0}^{x+1} G_i\right)\begin{bmatrix}0\\0\\0\\0\\1\end{bmatrix} \qquad (4)$$

where

$$G_i = \begin{bmatrix}1 & \mathbf{p}^T\langle T\rangle & p^2/2\\ \mathbf{0} & \langle T\rangle & \mathbf{p}\\ 0 & \mathbf{0} & 1\end{bmatrix}_i \qquad (5)$$

with $i = 0, 1, \ldots, x, x + 1$; the $\mathbf{0}$'s are null matrices; and $\mathbf{p}$, $\mathbf{p}^T$, and p^2 are the residue dipole moment, its transpose,

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TABLE I
COMPARISON OF CALCULATED AND EXPERIMENTAL COUPLING CONSTANTS AND DIMENSIONS

Method	$\langle J_{N\alpha} \rangle$, Hz	$\left(\frac{\langle r^2 \rangle_0}{n_p l_p^2}\right)_{n_p \to \infty}$
Experimental[15,29]	6.3–7.0	9.0 ± 0.5
Semiempirical[15]	6.1 (eq 1), 6.7 (eq 2)	9.3 (2.0)[a]
PCILO[20]	6.6 (eq 1), 6.7 (eq 2)	1.92
Free rotation[b]	4.5 (eq 1), 4.9 (eq 2)	1.93

[a] Calculated for polyglycine. [b] Calculated assuming free rotation about the N–C^α and C^α–C backbone bonds in each residue.

and the square of its magnitude, respectively. For a polypeptide terminated with NH_3^+ and CO_2^- groups (zwitterion), $\mathbf{p}_0$ and $\mathbf{p}_{x+1}$ do not include the peptide group dipole moment, $|m| = 3.7$ D, but simply the terminal plus and minus charges, respectively.

The coupling constant $J_{N\alpha}$ and the transformation matrix T were averaged at 25° over all (φ, ψ) of the PCILO map[20] for L-alanine within 5.0 kcal/mol of the minimum-energy conformation, using 20° increments in φ and ψ. The transformation matrix for an interior L-alanine residue averaged over the PCILO map[20] is

$$\langle T \rangle_L = \begin{bmatrix} 0.066 & -0.68 & -0.13 \\ -0.34 & -0.42 & 0.32 \\ -0.29 & 0.064 & -0.39 \end{bmatrix} \quad (6)$$

where $\langle T \rangle_D$ is identical with $\langle T \rangle_L$ except for the signs of elements (1, 3) (2, 3), (3, 1), and (3, 2). The average transformation matrices for the terminal residues T_0 and T_x in the zwitterionic peptides were taken from Flory and Schimmel,[8] with T_{x+1} not defined, since only elements in the last column of G_{x+1} are required in the product of eq 5.

Calculated Results and Their Comparison with Experiment

The calculated and experimentally observed average amide to α-proton couplings $J_{N\alpha}$ in random-coil poly(L-peptides), whose side chains R are of the type R = CH_2R', are compared in Table I. The range of experimental values[15,30] corresponds to measurements performed on polypeptides with side chains R = CH_3 (alanine), $CH_2CH_2SCH_3$ (methionine), and $CH_2CO_2CH_2C_6H_5$ (β-benzyl aspartate). Both conformational energy maps (semiempirical and PCILO quantum mechanical) lead, according to eq 1 or 2, to average couplings that are in excellent agreement with experiment.

On the other hand, the comparison of calculated and experimental dimensions[12] of random-coil poly(L-peptides) (R = CH_2R') also presented in Table I reveals a serious discrepancy between the dimensions calculated from the PCILO map and those obtained experimentally or calculated from the semiempirical map. The dimension measurements[12,31] were performed on random-coil poly(L-peptides) with R = $CH_2CO_2CH_2C_6H_5$ (β-benzyl aspartate), $CH_2CH_2CO_2H$ (glutamic acid), $(CH_2)_4NH_2$ (lysine), and $CH_2CH_2CO_2CH_2C_6H_5$ (γ-benzyl glutamate).

In Table II a comparison is made between the experimentally observed[32] and calculated[8] dipole moment dependence on stereosequence of the zwitterionic tri- and tetrapeptides

TABLE II
COMPARISON OF CALCULATED AND EXPERIMENTAL DIPOLE MOMENTS OF THE ZWITTERIONIC DIASTEREOISOMERS OF THE TRI- AND TETRAPEPTIDES OF ALANINE

Alanine peptide	Relative values of the dipole moments: Semiempirical[a]	Exptl[b]	PCILO[c]
LLL	1.0	1.0	1.0
LLD	0.97	0.93	1.05
DLL	0.91	0.89	1.10
DLD	0.81	0.85	1.11
LLLL	1.0	1.0	1.0
DLLL	0.92	0.95	1.02
LLLD	0.97	0.93	1.02
DDLL	0.91	0.91	0.95
DLLD	0.90	0.85	1.04
LLDL	0.86	0.85	0.98
LDLL	0.79	0.81	1.01
LDLD	0.74	0.77	1.04

[a] Reference 8. [b] Reference 32. [c] This work.

of alanine. The experimentally observed[32] decrease in the dipole moment with decreasing stereoregularity is closely reproduced by the calculations[8] performed using the semiempirical energy map. However, the PCILO map yields calculated dipole moments which increase with decreasing stereoregularity and are virtually independent of stereosequence for the tri- and tetrapeptides of alanine, respectively.

Discussion of Results and Conclusions

The conformational energy maps appropriate to random-coil poly(L-alanine) and obtained by the use of semiempirical[6] and quantum mechanical (PCILO)[20] energy estimates are reproduced and superimposed in Figure 2. One immediately notes the following differences: (i) the disappearance of the allowed region centered at (φ, ψ) = [240° (60°), 240° (60°)] and the appearance of an allowed region centered at (φ, ψ)

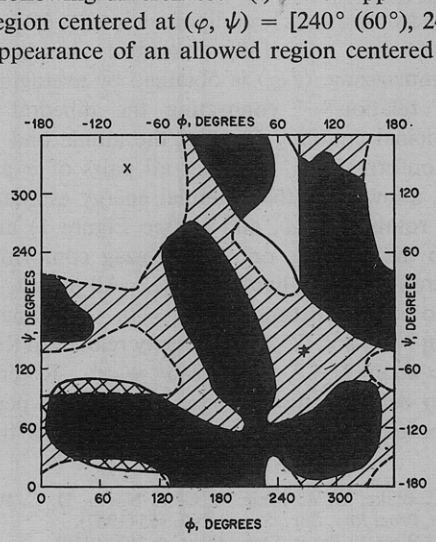

Figure 2. Superimposed conformational energy maps appropriate to random-coil poly(L-alanine) and calculated using semiempirical potential functions[6] (---) and the PCILO molecular orbital method[20] (—). Those portions of the map within the contours (outside the cross-hatched and shaded areas) are within 6.0 kcal/mol of residue of the minimum-energy conformation (× for semiempirical and ‡ for PCILO map). The shaded, ////, solid, and cross-hatched, ×××, areas correspond to the energy-forbidden conformations calculated by the semiempirical, PCILO, and both methods, respectively. The left ordinate and lower abscissa are labeled according to the former convention,[28] while the right ordinate and upper abscissa are labeled in accordance with the latest convention.[29]

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= [260° (80°), 140° (−40°)] in the PCILO map, (ii) the difference in the locations of the minimum-energy conformation in both maps, and (iii) the greater number (one-half *vs.* one-third of the conformations lie within the 6.0-kcal contour) and the increased symmetry about (φ, ψ) = [180° (0°), 180° (0°)] of the allowed conformations in the PCILO map.

The PCILO map for L-alanine is similar to the symmetric map calculated for glycine by the PCILO,[20] the extended Hückel,[19] or the semiempirical methods.[6] It is just this increased symmetry about (φ, ψ) = [180° (0°), 180° (0°)] in the L-alanine map calculated by the PCILO method which leads[6] to calculated dimensions characteristic of polyglycine, whose dimensions also approximate the value obtained assuming free rotation,[11] and to dipole moments for the stereoisomeric tetrapeptides of alanine which are virtually independent of stereosequence, both results in disagreement with extensive experimental data.

The minimum-energy conformation in the PCILO map, (φ, ψ) = [260° (80°), 140° (−40°)], and its symmetry-related conformation, (φ, ψ) = [100° (−80°), 220° (40°)], are similar to those proposed by Bystrov, *et al.*,[25] and Mizushima, *et al.*,[33] respectively for the conformations of *N*-acetyl-L-alanyl-L-alanyl methyl ester, acetylsarcosine-*N*-methylamide, and acetyl-*N*-methyl-DL-norleucine-*N*-methylamide in solution. Both conformations are thought to involve a seven-membered intramolecular hydrogen bond between the acetyl oxygen and the amide hydrogen nearest the C terminal. (Infrared spectroscopic evidence[25,33] obtained in solution indicates the partial existence of the seven-membered intramolecular hydrogen bond described above. However, recent[34] nmr studies of the same dipeptide in solution cast some doubt on its existence. The temperature coefficients of the chemical shifts of both amide protons are nearly the same and not zero in $CDCl_3$ and DMSO. Deuterium exchange occurs much more slowly for one of the amide protons in DMSO, but no difference is observed in the exchange of both amide protons in $CDCl_3$. The exchange study is in opposition to Bystrov, *et al.*,[25] who conclude that the intramolecular hydrogen-bonded conformation is more prevalent in $CDCl_3$ than in the more polar solvents DMSO and H_2O.)

The suggested hydrogen bond in both conformations is characterized by an O-to-H distance of 1.97 Å, an angle of 90° between C=O and O···H, and an angle of 26° between N—H and N···O. If the semiempirical potential function is modified, following the method of Brant,[18] to include the favorable contribution made by intramolecular hydrogen bonds, then the energies of both proposed hydrogen-bonded conformations {(φ, ψ) = [260° (80°), 140° (−40°)] and (φ, ψ) = [100° (−80°), 220° (40°)]} are lowered by 4.5 kcal/mol of residue compared to the energies corresponding to these conformations shown in the semiempirical map in Figure 2. Even after inclusion of the favorable hydrogen bond interactions,[18] both conformations remain at least 5.0 kcal/mol of residue above the minimum-energy conformation[6] at (φ, ψ) = [90° (−90°), 330° (150°)], and therefore should play only a minor role in the conformational characteristics of random-coil poly(L-alanine).

The major difference between the results of the semiempirical and PCILO calculations is the favorable interaction assigned to the proposed[25,33] seven-membered hydrogen bond {(φ, ψ) = [260° (80°), 140° (−40°)] and [100° (−80°), 220° (40°)]} by the PCILO method. It appears that the PCILO calculation[20] overestimates the intramolecular attractions involved in these conformations. The extent of the deviation from linearity and planarity of the proposed hydrogen bond (98° between C=O and O···H and 26° between N—H and N···O) indicates a weak attraction at best based on the observed[35] geometry of hydrogen-bonded crystals and a nonempirical quantum mechanical (LACO-MO-SCF calculation) study[36] of the hydrogen bond between peptide units.

This overestimate does not seriously affect the PCILO energy map of the glycyl residue due to the symmetry of both proposed hydrogen bonded conformations about (φ, ψ) = [180° (0°), 180° (0°)]. In addition, more recently the PCILO method has been applied[37,38] to the calculation of the energy maps of an L-alanine residue preceding L-prolyl, of an L-prolyl residue preceding L-prolyl, and of an isolated L-prolyl residue. Each of these three maps closely approximates the results obtained[7,9] by the semiempirical method. Severe steric overlaps or geometrical restraints imposed by the pyrrolidine ring in proline are present in these residues at (φ, ψ) = [260° (80°), 140° (−40°)] and [100° (−80°), 220° (40°)], and as a result, the overestimate of the seven-membered hydrogen bond strength by the PCILO method is inconsequential in these residues. (In the map for the isolated L-prolyl residue,[38] however, the barrier to rotation about the C^{α}–C backbone bond at ψ = 230° (50°) between the two allowed conformations centered at ψ = 150° (−30°) and $\psi \approx$ 340° (160°), respectively, is said to be substantially lower than the barrier at ψ = 60° (−120°) (35.5 *vs.* 75.0 kcal/mol) due to the presence of the proposed seven-membered hydrogen bond at ψ = 230° (50°). The authors state that this barrier can be reduced further to 9.0 kcal/mol if the peptide bond is allowed to rotate −40° from the trans conformation (see Figure 1). Calculations show that the hydrogen bond at ψ = 230° (50°) and ω = −40° (140°) [with φ = 122° (58°)][38] is characterized by an O-to-H distance of 2.1 Å, an angle of 112° between C=O and O···H, and an angle of 38° between N—H and N···O. These parameters correspond to an even weaker hydrogen bond than that proposed by Bystrov, *et al.*,[25] Mizushima, *et al.*,[33] and Maigret, *et al.*,[20] at (φ, ψ) = [260° (80°), 140° (−40°)] or [100° (−80°), 220° (40°)], and casts doubt on their[38] calculated reduction in the energy of this barrier.)

In light of the above comparison with experiment of several calculated results obtained from the semiempirical[6] and PCILO quantum mechanical[20] conformational energy maps appropriate to random-coil poly(L-peptides) with a β-CH_2 group in their side chains, it is clear that the semiempirical map is superior to the PCILO map in its ability to predict the conformational characteristics of random-coil polypeptides, and in the small amount of computer time required to complete these calculations. The PCILO map fails to predict the dimensions and their dependence upon stereoregularity for random coil polypeptides. (Since the mean-square dipole moments of the tri- and tetrapeptides of alanine arise[8] overwhelmingly from the separation of the charged termini, comparison of calculated and experimental dipole moments reduces to a comparison of the calculated and experimental

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dimensions.) A clue to the origin of this failure may lie in the observation that the extended Hückel[19] (quantum mechanical) map for alanine agrees rather closely with the semiempirical map, indicating that the quantum mechanical estimation of the conformational energy of peptides is sensitive to the particular molecular orbital method employed. Since it would be valuable to have a reliable quantum mechanical method for estimating the validity of, or for refining the parameters used in the semiempirical potential energy functions applied to polypeptides, a comparison and analysis of the assumptions adopted and the methods used in both the extended Hückel and PCILO molecular orbital calculations and their effects on the calculation of conformation dependent energies appears to be desirable.

Though the PCILO method goes beyond the SCF–MO–CNDO/2 molecular orbital scheme[39] by incorporating some electron correlation, the PCILO method involves the use of localized orbitals. To determine the importance of electron correlation and the use of localized orbitals to the calculated conformational energies, it becomes important to recalculate the alanine map using the SCF–MO–CNDO/2 method. The feasibility of such a calculation is presently under study.

Acknowledgment. The author is grateful to Dr. R. Ditchfield for several helpful conversations.

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Living Poly(2-vinylquinoline)

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ABSTRACT: Living poly(2-vinylquinoline) was prepared. Its spectrum as well as the spectra of the dead polymer and of the monomer are reported. Conductance studies of the sodium salt of living poly(2-vinylquinoline) in THF showed that its dissociation is very low, $-\Delta H_{diss} = 2.3$ kcal/mol, $\Delta S_{diss} = -43$ eu. These findings resemble those previously reported for the living poly(2-vinylpyridine) and indicate that the cation associated with the growing end is simultaneously coordinated with the first quinoline moiety of the polymer. Kinetic studies led to the determination of the propagation constant $k_{\pm}$, its activation energy (7.9 kcal/mol), and the temperature-independent factor (9.6×10^8 M^{-1} sec^{-1}). The free ~~VQ$^-$ carbanions seem to be not much more reactive than the ion pairs.

Pursuing our studies of anionic propagation, we investigated the kinetics of polymerization of living sodium 2-vinylquinoline in tetrahydrofuran.

Experimental Section

Preparation of 2-Vinylquinoline.[1] 2-(2-Hydroxyethyl)quinoline (19.9 g), 3.5 g of powdered KOH, and 0.1 g of *N*-phenyl-β-naphthylamine were slowly heated to 103° under a 12-mm vacuum. The monomer which distills over was fractionated *in vacuo;* the proper fraction was dried by stirring it for 4–5 days over CaH_2 and finally distilled under high vacuum into ampoules equipped with break-seals which eventually were sealed off.

The identity and purity of the monomer were established by gpc and nmr.

Preparation of Living Sodium Poly(2-vinylquinoline). The preparation of living poly(2-vinylquinoline) was accomplished in a high-vacuum system with break-seals instead of stopcocks, following the procedure described elsewhere.[2] Several initiators were used, namely, sodium naphthalene, the dianion of dimeric 1,1-diphenylethylene, α-methylstyrene tetramer, or metallic sodium. The preparations were performed at three different temperatures, *viz.*, −50, −20, and +20°. A four- to fivefold excess of the monomer was added to a stirred solution of the initiator in tetrahydrofuran (THF). The initiation was completed in a fraction of a second and the resulting living polymer appeared to be the same whatever method was used for its preparation. However, living polymer of low degree of polymerization, DP, rapidly "aged" when it was prepared and kept at room temperature, although the polymer was stable when prepared and kept at low temperature (< −20°). The aging manifested itself in spectral changes which could be noted after 2 hr of storage (see the next section).

Spectra of 2-Vinylquinoline and of the Living and Dead Poly(2-vinylquinoline). The spectrum of 2-vinylquinoline is depicted in Figure 1. Two sharp absorption maxima appear at 325 and 336 nm, the extinction coefficients being 5.1×10^3 and 3.14×10^3, respectively. The absorbance at 336 nm was utilized for monitoring the consumption of the monomer in kinetic experiments because the absorbence of the polymeric chain (excluding the contribution of the carbanions) is negligible at this wavelength. The dead polymer absorbs at shorter wavelengths (<330 nm), as shown in Figure 1. The sharp peak at 328 nm is attributed to the quinoline moiety; it also appears in the spectrum of the monomer albeit, as expected, at slightly longer wavelength.

The spectrum of the freshly prepared living poly(2-vinylquinoline) of DP >4 shows a broad maximum at 345 nm, ϵ 1.3×10^4 (see again Figure 1). At this wavelength the extinction coefficients of 2-vinylquinoline and of segments of poly(2-vinylquinoline) are 370 and 150, respectively. The concentration of living polymers before the addition of the monomer and after completion of the polymerization was determined by the optical density at 340–345 nm. Although the position of this flat maximum shifts slightly toward shorter wavelength (by 2–3 nm) as the DP of the polymer increases from 4 to 15, its extinction is hardly affected. Therefore, the determination of the concentration of the living polymer at the end of a run is not complicated by this shift.

Living poly(2-vinylquinoline) shows another broad absorption band at about 520 nm. The ratio of OD(520)/OD(345) ≈ 0.3; however, its value varies slightly in a haphazard way. The 520-nm absorption is sensitive to aging processes—the position of the maximum shifts considerably toward longer wavelength with time (within about a week) and the relevant optical density doubles. The aging only slightly changes the absorption in the 345-nm region; the maximum shifts toward a shorter wavelength, and this

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