Apparent Helix-Coil Transition for Poly(L-Alanine) as Measured by Nuclear Magnetic Resonance*

(doubles peaks in spectra/circular dichroism)

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ABSTRACT The previous contention by several groups that double peaks that arise in NMR spectra from NH and \( \alpha \)-CH protons of poly L-alanine are caused by the coexistence of helical and random-coil structures in solution is disputed.

Numerous reports have appeared showing that under certain circumstances many polypeptides exhibit NMR spectra with double peaks from NH and \( \alpha \)-CH protons (2-8). The doubling of peaks was attributed initially by Ferretti et al. to the coexistence of helical and random-coil structures in solution, in spite of the fact that dynamic measurements indicate that the average rate of the helix to coil transition (0.1-10 ms) is extremely rapid on the NMR time scale (9-12). Such rates should lead to a single averaged peak. Explanations for the doubled peaks have been offered based on polydispersity (13), slow nucleation of the transition (14), and protonation of the peptide bonds (15). Scheraga and his coworkers (16) speculated that the \( \alpha \)-helix peak should not be observable because of dipolar broadening. They suggested that double peaks arise from specific, slowly exchanging solvates of random-coil species in mixed solvents containing acids such as trifluoroacetic acid (CF\(_3\)COOH).

Recently, Bradbury and his associates (17) assigned the double peaks to the \( \alpha \)-helix and random coils on the basis of comparisons of the areas for \( \alpha \)-CH with the side-chain peaks. The Bradbury group concludes that rapid exchange between \( \alpha \)-helix and random coil takes place, with the double peaks arising from polydispersity. NMR spectra of poly(L-alanine) were analyzed by Jardetzky (8), Bradbury (18), and Ferretti (3) and their associates. The second two groups found double peaks for the \( \alpha \)-CH at 220 MHz. One peak at 4.5 ppm was attributed to the \( \alpha \)-helix, while the other peak at 4.7 ppm was assigned to the random coil contribution of the polypeptide chain.

In this paper, we present results indicating that the double peaks seen for poly(L-alanine) may not arise from the helix to coil transition, and that the above explanations may not apply to the observed phenomena. We believe the low-field peak arises from the \( \alpha \)-CH of oligomeric peptides. The high-field peak represents a single averaged peak of helix and random coil. We performed measurements using a 220-MHz Varian spectrometer and very low concentrations of L-alanine-derived peptides, necessitating time-averaging to improve the signal-to-noise ratios in solvents composed of deuterochloroform (CDCl\(_3\)) and CF\(_3\)COOH. We varied the molecular weight of the polymers, sample concentrations, solvent compositions, and temperature at which the experiments were performed. Although the spectra do not exhibit good signal-to-noise ratios (even after time-averaging), we were able to obtain clear and interpretable results.

Double peaks† at about 4.6 and 4.3 ppm are observed for poly(L-alanine)\(_{22}\) [average degree of polymerization (DP)37], as is shown in Fig. 1, which contains results for the \( \alpha \)-CH region of the NMR spectrum as a function of solvent composition. Spectra were measured at a sample concentration of 0.1% with (CH\(_2\))\(_3\)Si as an internal standard. The curves were obtained in a solvent composed of mixtures of CDCl\(_3\) and CF\(_3\)COOH. Curves A-F (A = 5%, B = 10%, C = 15%, D = 20%, E = 25%, F = 30% CF\(_3\)COOH, respectively) exhibit broadened peaks, a result that is indicative of some aggregate

† Under certain circumstances, we observed double peaks for poly(L-alanine) and poly(DL-alanine) at 4.3 and 3.8 ppm. The peak at 3.8 ppm comes from water contamination. Protonation (15) does not appear to be involved since, after scrupulous drying of our solvents and samples, we can eliminate the high-field resonance at 3.8 ppm.

* This paper represents paper 42 in our series on Conformational Aspects of Polypeptide Structure. The previous paper in this series is ref. 1.
From the entire series of these spectra, we see that the high-field peak is continually moving to a lower field as the proportion of CF₃COOH in the solvent increases. Ultimately, the high-field peak overlaps the low-field peak (curves O, P, and Q).

To confirm these assignments, we determined the chemical shift dependence of (l-alanine)₆ and poly(l-alanine)₃₋₆. In Fig. 2, we show that these materials exhibit only single peaks over the entire range of solvent composition from 5 to 100% CF₃COOH. The chemical shift for the oligomer [(l-alanine)₆] follows an identical solvent dependence as observed for the low-field peak of poly(l-alanine)₆. The chemical shifts of the high polymer poly(l-alanine)₆ turn out to be identical to those observed for the high-field peak of poly(l-alanine)₆ seen in Fig. 2. With solvents containing low concentrations of CF₃COOH, there are deviations from the chemical shift dependence of the low-field peak of poly(l-alanine)₆ that derive from broadened resonances.

In Fig. 2 there appears to be a sharp break in the chemical shift dependence for the high-field peak at a solvent composition of about 75% CF₃COOH-25% CDCl₃. The effect is the same for both poly(l-alanine)₆ and poly(l-alanine)₃₋₆. We believe that the helix random-coil transition occurs at about this solvent composition. The low-field peak for poly(l-alanine)₆ as seen in Fig. 1, and its chemical shift dependence seen in Fig. 2, provide evidence for the presence of oligomeric peptides of molecular weights sufficiently low so as not to form helices under these conditions. We believe that (l-alanine)₆ obtained by polymerization of l-alanine α-amino acid N-carboxyanhydride under standard conditions contains such oligomers.

In order to show the effect of oligomers on the NMR spectrum of a high polymer such as (l-alanine)₃₋₆ we added (l-alanine)₆ to (l-alanine)₆ and artificially reproduced the curves observed for the (l-alanine)₆. These results are shown in Fig. 3, where curve A indicates the single observable α-CH peak for (l-alanine)₆, while curve B contains the spectrum for (l-alanine)₆ to which an equal amount of (l-alanine)₆ was added. Curve C shows the α-CH region of the NMR spectrum for (l-alanine)₆.

If the polymer is heated to 60° in 80% CF₃COOH-20% CDCl₃ irreversible changes in the α-CH resonances occur that are indicative of degradation of the peptide chain to form oligomers. Such short-chain peptides should exhibit their α-CH resonances in the same downfield region as was established for (l-alanine)₆. That this is the case can be seen from Fig. 4. We also studied the temperature dependence of the α-CH peaks for the same polymer in 10% CF₃COOH-90% CDCl₃. No change in chemical shifts was observed between 52
served for poly(L-alanine) arise from a low-field peak assignable to oligomer content and a high-field peak that can arise from a helix or a rapidly exchanging helix and random coil. At sufficiently high concentrations of CF$_3$COOH, the random coil for high molecular weight polymer and the oligomer exhibit their $\alpha$-CH resonances at the same frequency. We established that poly(L-alanine) exists only in the random-coil form in 100% CF$_3$COOH. Our investigations bring into question the contention that the double peaks arise from separate helix and random coil resonances for poly(L-alanine). We are currently examining other polymers to test the generality of our findings for poly(L-alanine).

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FIG. 5. CD spectra for poly(L-alanine) in various CF$_3$COOH–CHCl$_3$ solvents (% of CF$_3$COOH is indicated) at 0.10–0.15% sample concentration; path length = 0.01 mm. (a) (L-alanine)$_{50}$; (b) (L-alanine)$_{500}$.

and 9°. However, severe broadening of these peaks was noted below 30°, a result that indicates that aggregation occurs.

To clarify the large change in chemical shifts encountered between 70 and 90% CF$_3$COOH, we measured the circular dichroism (CD) spectra for poly(L-alanine) under identical conditions to our NMR measurements. In Fig. 5a, we show that (L-alanine)$_{50}$ exists primarily as an $\alpha$-helix in solvents containing 70% or less CF$_3$COOH. Above this concentration of CF$_3$COOH, random-coil structures are easily observable. At 100% CF$_3$COOH, the polymer appears to be completely random. Fig. 5b contains our results for (L-alanine)$_{500}$. Broad troughs are seen for the spectra obtained in 30, 50, and 70% CF$_3$COOH, a result that is indicative of aggregation. From the shapes of these curves, we deduced that (L-alanine)$_{500}$ exists as a mixture of $\alpha$-helix and some aggregates under these conditions. With larger fractions of CF$_3$COOH in the solvent (80, 90, and 100%), spectra for random coils are observed. These results are identical to those we obtained for (L-alanine)$_{500}$ described above.

From this work, we conclude that the double peaks ob-

$\dagger$ These measurements were made with a special Teflon holder with a 0.01-mm cell path with a Cary 61 spectropolarimeter. We will report elsewhere on the design of this novel cell holder for circular dichroism measurements.