Polymer Nuclear Magnetic Resonance Spectroscopy. XX. Helix–Coil Transitions in Poly(γ-benzyl D,L-glutamates)

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ABSTRACT: It is shown by high-resolution nmr that both statistical and alternating poly(γ-benzyl D,L-glutamates) undergo a transition in $CDCl_3$ upon addition of trifluoroacetic acid that is identical with that observed for α-helical poly(γ-benzyl L-glutamate) except that it occurs at much lower acid concentration. It is concluded both types of D,L polymer are capable of forming α-helical structures in $CDCl_3$.

The helix–coil transition in polypeptides has by now been extensively observed by nmr. Most of the work is of comparatively recent date (see ref 2 for a recent review), and the amount of information given by the spectra tends to be proportional to the frequency at which observations are made. It has been shown that the resonances of the α-CH and NH protons undergo significant and characteristic downfield and upfield shifts, respectively, when transition from the α helix to the random coil occurs,[3-6] and that in organic solvents the helix and coil exhibit separate resonances for these protons in the transition region. In this paper we employ this technique to examine the conformations of the regularly alternating and statistically distributed poly(γ-benzyl D,L-glutamates). It is concluded that polymers of both types are α-helical (and associated) in chloroform, and that they undergo very similar transitions to the random coil upon addition of trifluoroacetic acid.

Experimental Section

The preparation and optical properties of the regularly alternating polymers are described in the preceding paper.[7] The two types (see below) of statistical polymers have been previously described.[8] Because of relative scarcity of material, the polymer solutions were made up initially in $CDCl_3$ (5% w/v), and then precise additions of trifluoroacetic acid were metered into this same nmr tube, using a "Digipet" pipet (Manostat Corp., N. Y.), to levels of 1.0, 3.0, 5.0, and 10.0% (v/v). Hexamethyldisiloxane (HMDS, τ 9.94) was employed as internal reference.

All measurements were made using a Varian HA-100 100-MHz spectrometer. With the exception of a limited series of measurements at elevated temperatures, all measurements were made at 30°. (Earlier measurements on poly(γ-benzyl L-glutamate) were made at 50°,[5] but relatively rapid ester exchange makes the use of lower temperatures advisable.)

Results and Discussion

Three polypeptides were observed:[7] (1) alternating copoly(γ-benzyl D,L-glutamate) of DP *ca.* 100, containing approximately 53 D and 47 L residues,[7] (2) a statistical copoly(γ-benzyl D,L-glutamate) (designated A) prepared by polymerizing in benzene an equimolar mixture of the D- and L-*N*-carboxy anhydrides (this polymer appeared to be a random coil or a broken rod in dimethylformamide solution),[8] (3) a statistical copolymer prepared like A but in dioxane (designated B) (hydrodynamic measurements indicate a rod-like particle).[8]

The nmr spectra of sample B as a function of trifluoroacetic acid content are shown in Figure 1. Peak assignments are indicated on spectrum e. They are strongly reminiscent of those of poly(γ-benzyl L-glutamate) under similar conditions,[5] except that the half-way point of the transition (spectrum c) is at *ca.* 3% rather than *ca.* 20% trifluoroacetic acid. At this point, two α-H peaks and (somewhat less clearly) two NH peaks can be distinguished.[4,6] In chloroform alone, the peaks are all broadened, those of the α-CH and NH being scarcely observable. This is no doubt due to association to form a liquid–crystal mesophase, as is well established for poly(γ-benzyl L-glutamate) (see ref 5 and references therein).

The corresponding spectra of samples 1 and A are not shown because, within the probable experimental error, they cannot be distinguished from those in Figure 1.

Certain hypotheses concerning the conformations of these polymers are either ruled out or made improbable to varying degrees by these observations. In the polymerization of a mixture of D- and L-*N*-carboxyanhydrides one could imagine (as several authors have pointed out) that a growing chain end would prefer to add monomer of its own handedness. If this preference were strong, substantial blocks of right-handed α-helical L units and of left-handed α-helical D units would be formed. These enantiomorphic units would be expected to undergo the helix–coil transition in much the same manner, *i.e.*, at the same level of trifluoroacetic acid, as chains of a single chirality. It is clear that this is not the case, and that if any recognizable blocks are formed, they must be short. One must also recognize the possibility that the growing chain end may prefer to add monomer units of opposite handedness alternately. If this tendency were strong, the "alternating" and "random" polymers would be substantially identical. The nmr evidence does not rule out this possibility.

Tsuboi, *et al.*,[9] have concluded from X-ray and infrared measurements that fibers of random copoly(γ-benzyl D,L-glutamates) contain both an α-helical portion and a random-coil portion, the former tending to increase with molecular weight. Actually, the most striking finding of their study is the remarkably close similarity of the random polymers to

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(2) E. M. Bradbury and C. Crane-Robinson, *Nature (London)*, **220**, 1079 (1968).

(3) (a) W. E. Stewart, L. Mandelkern, and R. E. Glick, *Biochemistry*, **6**, 143 (1967); (b) K.-J. Liu, J. S. Lignowski, and R. Ullman, *Biopolymers*, **5**, 375 (1967).

(4) J. A. Ferretti, *Chem. Commun.*, 1030 (1967).

(5) F. A. Bovey, *Pure Appl. Chem.*, **16**, 417 (1968).

(6) J. A. Ferretti and B. W. Ninham, *Macromolecules*, **3**, 30 (1970).

(7) F. Heitz and G. Spach, *ibid.*, **4**, 429 (1971).

(8) G. Spach, *C. R. Acad. Sci.*, **249**, 543 (1959).

(9) M. Tsuboi, Y. Mitsui, A. Wada, T. Miyazawa, and N. Nagashima, *Biopolymers*, **1**, 297 (1963).

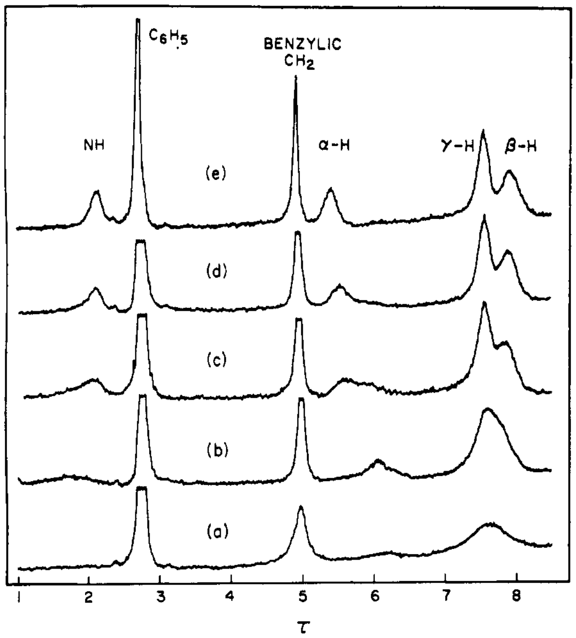

Figure 1. Spectra (100 MHz) of copoly(γ-benzyl D,L-glutamate) ("random" sample B) in (a) $CDCl_3$; (b) $CDCl_3$ + 1% trifluoroacetic acid; (c) $CDCl_3$ + 3% trifluoroacetic acid; (d) $CDCl_3$ + 5% trifluoroacetic acid; and (e) $CDCl_3$ + 10% trifluoroacetic acid (observed at 30°).

poly(γ-benzyl L-glutamate) in both X-ray and infrared properties. Bradbury, *et al.*,[10] however, accepted their conclusion and assumed it to hold in chloroform solution as well; they were then obliged to postulate that both α-helical and random-coil copoly(γ-benzyl D,L-glutamate) had α-CH peaks in the same position. In view of the existence of a clear-cut transition on adding trifluoroacetic acid, closely resembling that of poly(γ-benzyl L-glutamate) itself, we cannot accept this interpretation. It appears that polymer A may have more breaks in the α-helical conformation, possibly as a result of a somewhat different statistical distribution of D and L units than in B, and that this accounts for its hydrodynamic properties being more nearly those of a random coil in dimethylformamide solution.[8] It is somewhat surprising in this case, however, that A and B are equally stable upon addition of trifluoroacetic acid. (Upon addition of dichloroacetic acid to chloroform solutions, polymer A undergoes a sharp transition at about 15% acid, whereas B undergoes a broader transition at about 30%.)

Spach, *et al.*,[7,11] have concluded from optical measurements that the alternating poly(γ-benzyl D,L-glutamate) is mainly helical and left-handed; presumably the overall conformation is dictated by the slight predominance of D units. The nmr results support this idea, and supply the additional conclusion that in fact it is strongly helical. Thus, whether distributed in pairs or (presumably) at random, the coexistence of D and L units in the same chain is not incompatible with a helical conformation.

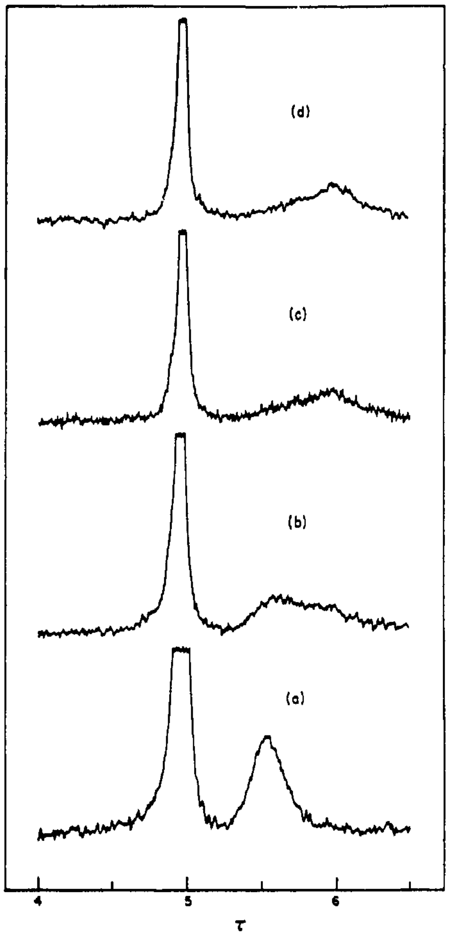

Figure 2. Spectra (100 MHz) of copoly(γ-benzyl D,L-glutamate) in $CDCl_3$ + 3% trifluoroacetic acid observed at (a) 8°; (b) 30°; (c) 52°; and (d) 65°.

The effect of temperature on the position of the helix–coil equilibrium is in the same direction as that long recognized from optical measurements and supported by nmr for poly(γ-benzyl L-glutamate).[5] In Figure 2 are shown the α-H (and benzylic) proton spectra of polymer B at four temperatures. It is evident that the equilibrium shifts toward the helix as the temperature is increased.

Acknowledgment. It is a pleasure to acknowledge several stimulating conversations with Professor Murray Goodman, who originally suggested that the problem of the stability of helices of mixed residue chirality be attacked by nmr.

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(10) E. M. Bradbury, R. G. Carpenter, C. Crane-Robinson, and H. W. E. Rattle, *Nature (London)*, 220, 69 (1968).

(11) G. Spach, A. Brack, and F. Heitz, *C. R. Acad. Sci., Ser. C*, 267, 809 (1968).