The Nuclear Magnetic Resonance Spectrum and Conformation of Poly-γ-benzyl-L-glutamate in Solution

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THE n.m.r. spectrum of poly-γ-benzyl-L-glutamate (PBLG) was first studied by Bovey, Tiers, and Filipovich,[1] who observed a normal spectrum in trifluoracetic acid (TFA), in which the molecule exists in the random coil conformation, but no proton bands in trichloroethylene, in which the molecule is in the helical conformation;[2] they further observed that the addition of a small amount of TFA to the solution in trichloroethylene resulted in the appearance of peaks corresponding to all the different types of proton in the molecule. Similar observations were made with the corresponding ethyl ester in mixtures of TFA and trifluoroethanol by Goodman and Masuda,[3] who suggested that n.m.r. spectroscopy could be used for studying helix–coil transitions in polypeptides in solution.

We have now studied the n.m.r. spectrum and optical rotatory dispersion of PBLG in mixtures of deuterochloroform and TFA. Our results are summarised in the Table; the assignments of the bands observed in 100% TFA are as follows:— I, peptide NH; II, benzyl C_6H_5; III, benzyl CH_2; IV, α-CH; V, side-chain C_2H_4. In 100% deuterochloroform the only detectable band is a broad one corresponding to the C_6H_5 protons; the other bands appear successively as TFA is added to the system.

It will be seen that the α-CH and peptide NH bands appear almost simultaneously at a TFA concentration between 20 and 30%, in good agreement with the sudden change in the specific rotation and the Moffitt[4] parameter, b_0, over this range of solvent composition. Clearly it is the helix–random coil transition which is responsible for the sudden appearance of these two bands; the seeming appearance of the α-CH band before the NH band is probably due to masking of the latter by the closely adjacent large C_6H_5 band.

Of greater interest are the changes in the n.m.r. spectra which precede the breakdown of the helical structure of the polypeptide and which are not paralleled by marked changes in optical rotation. It will be seen from the Table that bands corresponding to the various types of side-chain proton appear successively as more TFA is added to the system; the benzyl C_6H_5 protons appear first,

TABLE

N.m.r. spectrum and optical rotatory dispersion of poly-γ-benzyl-L-glutamate in $CDCl_3$–TFA

N.m.r. bands (10% solution; 33·5°)

	I (NH)		II (C_6H_5)		III (CH_2)		IV (α-CH)		V (C_2H_4)		O.r.d. (1—6% solution)	
%TFA	τ	Relative* intensity	τ	Relative intensity	τ	Relative† intensity	τ	Relative† intensity	τ	Relative intensity	$[\alpha]_{600}^{33\cdot5}$	b_0‡
0**	—	—	2·75	—	—	—	—	—	—	—	+8·69°	−625°
5	—	—	2·75	5·00	4·94	1·45	—	—	7·65	1·75	—	—
10	—	—	2·76	5·00	4·93	1·60	—	—	7·60	2·25	+12·23	−645
20	—	—	2·72	5·00	4·90	2·00	5·2	0·40	7·54 7·90	3·25	+ 8·55	−579
30	2·11	0·95	2·70	5·00	4·88	2·00	5·28	1·05	7·53 7·87	4·20	−29·06	+60
40	2·12	1·00	2·67	5·00	4·87	2·00	5·36	1·05	7·53 7·86	4·30	−35·40	+75
100	2·01	1·00	2·65	5·00	4·80	2·00	5·20	1·35	7·46 7·74	4·45	−49·20	+57

* Integrated area relative to that of band II = 5·00.
† When bands III and IV are both present, the intensity of band IV has been taken as 2·00.
‡ For $\lambda_0 = 212$ mμ.
** 5% solution.

[1] F. A. Bovey, G. V. D. Tiers, and G. Filipovich, *J. Polymer Sci.*, 1959, **38**, 73.
[2] See P. Urnes and P. Doty, *Adv. Protein Chem.*, 1961, **16**, 401.
[3] M. Goodman and Y. Masuda, *Biopolymers*, 1964, **2**, 107.
[4] W. Moffitt, *J. Chem. Phys.*, 1956, **25**, 467; *Proc. Nat. Acad. Sci. U.S.A.*, 1956, **42**, 736.

followed next by the benzyl CH_2 protons and finally by the side-chain C_2H_4 protons, the latter being fully developed only at the stage of complete breakdown of the helix. All the bands are broad and featureless at their first appearance but sharpen as the amount of TFA increases, the side-chain C_2H_4 band first clearly splitting into two bands corresponding to the two methylene groups of 30% TFA.

Bovey and his co-workers[1] ascribed the absence of bands in the n.m.r. spectrum of PBLG in trichloroethylene to the complete rigidity of the polypeptide molecules and it seems clear that the gradual development of the various bands, which we have observed with changing solvent composition, is due to the "unfreezing" of the side-chains from the periphery of the molecule inwards. No doubt TFA is particularly effective in the present case owing to its capacity for interacting with the ester side-chains of PBLG. What we have observed is, in fact, the breaking down, first of the interactions, both inter- and intra-molecular, of the side-chains, analogous to the hydrophobic bonds now ascribed[5] an important role in the adoption of specific conformations by protein molecules in solution, and then of the hydrogen bonds involved in the helical structure of the peptide backbone.

It seems, therefore, that n.m.r. spectroscopy may be a powerful tool for the study in polypeptides, not only of helix–coil transitions, but also of side-chain interactions not readily amenable to investigation by other means. We are now engaged on similar studies with other polypeptides in other solvent systems, notably water-soluble polypeptides in aqueous solution.

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[5] *Cf.* W. Kauzmann, *Adv. Protein Chem.*, 1959, **14**, 33; C. Tanford, P. K. De, and V. G. Taggart, *J. Amer. Chem. Soc.*, 1960, **82**, 6028; I. M. Klotz and J. S. Franzen, *ibid.*, 1962, **84**, 3461; C. Tanford, *ibid.*, p. 4240; J. F. Brandts, *ibid.*, 1964, **86**, 4291, 4302.