Circular Dichroism of Polypeptides in Helix-disrupting Media. The "218 nm Band"

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Summary Data on several polypeptides, dissolved in a variety of helix-disrupting solvents, reveal the ubiquity of the peptide n–π^* band in their c.d. spectra.

IT has recently been claimed[1] that the two-band c.d. spectra [205 nm (−ve); 218 nm (+ve)] of charged polyelectrolytes such as poly-L-glutamic acid (PLGA) (pH 7—12) and poly-L-lysine (PLL) (pH <7) are characteristic of an extended chain conformation with threefold left-handed helical sequences (EH) which are stabilised by electrostatic repulsion between adjacent charged side-chains; and that a truly unordered, random-coil, form (U), characterised by a single negative c.d. band near 205 nm (no 218 nm band), is achieved upon addition of 4M-Ca^{2+} or Li^+ ions to the

solution. Our results reveal (Table) that the c.d. spectra of polypeptides with non-ionisable side-chains, dissolved in a variety of non-aqueous helix-disrupting media, are similar to the c.d. spectra of charged PLGA and PLL in salt-free aqueous media.

Polypeptide c.d. data—the "218 nm *band*"[a]

Polypeptide	Solvent	Temp.	λ_{max} (nm)	$\Delta\epsilon$
(γ-Methyl-L-glu)$_n$	H_2SO_4	25°	218	0·72
"	HFPD	50°	216	0·76
"	5FKPD	50°	216	0·74
"	HFPD, 0·54H_2O	25°	215	0·3
"	HFIP:HFPD[b]	25°	215	0·38
(L-Histidine)$_n$	H_2SO_4	"	217	0·9
(L-Leucine)$_n$	"	"	219	0·14
(L-Valine)$_n$	"	"	218	0·60
(γ-Methyl-D-glu)$_n$	"	"	220	−0·57
(γ-Ethyl-L-glu)$_n$	"	—	220	0·68[c]
"	CH_3SO_3H	—	220	1·65[c]
(Cyclohexyl-L-ala)$_n$	CH_3SO_3H	—	220	0·44[d]
(L-Phe)$_n$	"	—	221	2·7[d]

[a] In all these cases, the more intensive negative band appears below 205 nm.
[b] 48:52 w/w. This composition is non-helicogenic.
[c] J. Steigman, E. Peggion, and A. Cosani, *J. Amer. Chem. Soc.*, 1969, **91**, 1822.
[d] E. Peggion, L. Strasorier, and A. Cosani, *ibid.*, 1970, **92**, 381.

If the c.d. band near 218 nm can be used unambiguously as a criterion for the EH conformation then it would seem that coulombic repulsions between charged side-chains are not unique in generating such structures. It could be argued, however, that protonation of the polypeptide chain in H_2SO_4 and CH_3SO_3H† could give the EH by repulsion between protonated sites along the chain.

Surprisingly, the c.d. spectra of (γ-methyl-L-glu)$_n$ in hexafluoropropane-2,2-diol (HFPD); HFPD,0·54H_2O; pentafluoromonochloropropane-2,2-diol (5FKPD) and in hexafluoroisopropyl alcohol (HFIP) containing HFPD all have a positive band near 218 nm and are qualitatively similar to the spectra of *all* the polypeptides examined in H_2SO_4. The fluoro gem-diols are non-helicogenic solvents,[2] and being weak acids (pK_a *ca.* 6·5) would not be expected to protonate polypeptides.[3]‡

If the EH conformation is assumed by (γ-methyl-L-glu)$_n$ in the fluoro-alcohols, it seems very unlikely that it is stabilised by electrostatic forces. Chain expansion could result from specific polymer–solvent interactions involving hydrogen-bond formation.

Alternatively, the 218 nm band may be common to both the U and EH conformation and its disappearance on addition of Ca^{2+} and Li^+ ions to aqueous solutions of PLGA and PLL may be a result of specific ion-binding to the peptide chromophore.

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† It has been established that H_2SO_4 and CH_3SO_3H do not cause appreciable covalent damage to poly-γ-ethyl-L-glutamate over several hours (J. Steigman, E. Peggion, and A. Cosani, *J. Amer. Chem. Soc.*, 1969, **91**, 1822. The results reported here are reliable from that point of view.
‡ We have established that the fluoro-alcohols do not cleave side-chain ester bonds.

[1] M. Lois Tiffany and S. Krimm, *Biopolymers*, 1969, **8**, 347 and earlier references cited therein.
[2] D. Balasubramanian and R. S. Roche, *Polymer Preprints*, 1970, **11**, 127, 132.
[3] J. Steigman, A. S. Verdini, C. Montagner, and L. Strasorier, *J. Amer. Chem. Soc.*, 1969, **91**, 1829; F. A. Bovey, *Pure and Appl. Chem.*, 1968, **16**, 417; D. Balasubramanian, *Biochem. Biophys. Res. Comm.*, 1967, **29**, 538.