

Conformational Changes of Polypeptides on Crystallization

We are interested in determining whether polypeptides retain their conformation on crystallizing from various solvent systems.¹⁻³ We give here a brief summary of some systematic infrared measurements on a series of five polypeptides. The spectra of solutions of the polypeptides in pure trifluoroacetic acid, (trifluoroacetic acid—TFA, *cis*-1,2-dichloroethylene—DCE, poly-L-alanine—PLA, poly-L-leucine—PLL, poly- γ -benzyl-L-glutamate—PBLG, poly-L-valine—PLV, poly- γ -methyl-L-glutamate—PMLG), and in a mixed solvent (trifluoroacetic acid plus dichloroethylene) were obtained, as were the spectra of the solids crystallized from these solvents. The amide I and II bands were used as the principal though not exclusive diagnostic tool.⁴

All solvents were the purest grade available and used as received from the manufacturers; synthetic polypeptides were purchased from Pilot Chemical Company. Infrared difference spectra were determined with a calibrated Perkin-Elmer 521 instrument using a demountable cell with 0.025-mm pathlength for the solution spectra (concentration 10 mg/ml). Solid samples were cast on watch glasses by controlled slow solvent evaporation at $23^\circ \pm 0.2^\circ\text{C}$; this procedure yielded a wide range of morphologies,²⁻⁴ which were reproducible for each polypeptide under the same casting conditions. Solid samples were usually prepared in KBr pellets or as films sandwiched between KBr discs. No solvent inclusion effects were observed and the spectra were found highly reproducible. The positions and accepted conformational assignments⁵⁻⁸ of the observed amide I and II maxima are summarized in Table I for two widely different solvent compositions. (The amide I and II regions of the PLL spectra, shown in Figure 1, will serve as an illustrative example.)

TABLE I
Observed Amide I and II Band Maxima of Polypeptides in Various
Solvents and in Solid States Crystallized from these Solvents

Poly-peptide	Solvent Composition TFA:DCE (v:v)	Solution			Solid State		
		Amide I (cm ⁻¹)	Amide II (cm ⁻¹)	Con- for- mation	Amide I (cm ⁻¹)	Amide II (cm ⁻¹)	Con- for- mation
PLL	25:75	1649, 1610	— ^a	α	1654	1544	α
	100:0	1624	1532	β^d	1656	1546	α
PLA	25:75	1656, 1619	1539 ^b	α	1656	1541	α
	100:0	1624	1532	β^d	1657	1543	α
PLV	25:75	1632	— ^a	β	1633	1543	β
	100:0	1625	1531	β	1628	1544	β
PMLG	35:65	1640	1539 ^c	β	1630	1530	β
	100:0	1636	1533	β^d	1624	1540	β
PBLG	25:75	1637	1534	β	1650	1543	α
	100:0	1640	1533	β^d	1650	1541	α

^a Broad band, very poorly resolved, but shifted to higher wavenumber than in pure TFA.

^b Poorly resolved.

^c Shoulder.

^d These polypeptides are assigned a random-coil conformation in TFA by most authors.⁸ It is difficult to reconcile our observed frequencies, which are in the range usually attributed to a β form,⁵⁻⁸ to this assignment. Absorption near 1630 cm⁻¹ has been used by others to indicate the presence of β conformation in solution;^{9,10} Bradbury and Rattle, however, refer to "the acid solvated coil frequency of the amide I" peak at ca. 1630 cm⁻¹ for PLA in TFA.¹¹

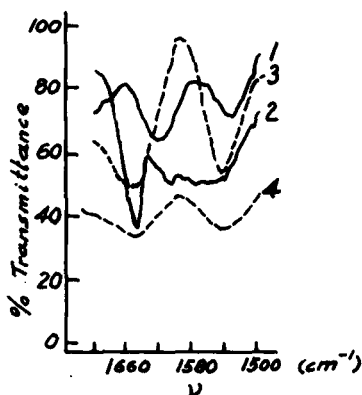


Fig. 1. Amide I and II regions of the ir spectrum of poly-L-leucine; curve 1—PLL in TFA; curve 2—PLL in 25:75 TFA:DCE; curve 3—PLL cast from TFA; curve 4—PLL cast from 25:75 TFA:DCE.

The solid-state conformations agree with those obtained previously by various experimental methods.¹²

One is struck by the fact that while the dominant conformation in solution appears, in six of the ten cases, to be largely retained on crystallization, α helix (or possibly a disordered conformation) is found in the solid state in the remaining four, even though the polypeptide appears to be predominantly in an extended form in solution (PLA, PLL, and PBLG, in pure TFA; PBLG in mixed solvent). Conformations thus may be quite different in the crystalline solid and in the dissolved material. Some similar observations have been made previously, for instance for poly(acetyl) hydroxyproline.¹³ These findings would suggest considerable caution in transferring solid-state conformational assignments (for instance based on crystal structures) uncritically to the dissolved state.

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