Self-association and Solubility of Peptides. An Infrared Absorption Method for Quantitative Titration of the Extent of Self-association in Poly(ethylene glycol)-bound Peptides

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The disruption of the sparingly soluble, self-associated species (β-structure) of the polymer-bound, *N*-protected peptides Boc-L-Ala-Gly-L-Ile-(L-Ala)$_2$-NHPEG and Boc-(L-Ala)$_2$-Gly-L-Ile-(L-Ala)$_2$-NHPEG [PEG = poly(ethylene glycol)] in methylene chloride by increasing amounts of *N,N*-dimethylformamide or dimethyl sulphoxide has been monitored by following the disappearance of the intense amide I or A i.r. bands of the strongly intermolecularly hydrogen-bonded molecules; this suggests the use of this method for the quantitative titration of the extent of self-association in PEG-bound peptides.

There are often solubility problems in the synthesis of large peptides. In general, a minimum in solubility is observed for medium-chain lengths ($5 \leqslant n \leqslant 15$; n = number of amino acid units), where a transition from unordered, solvated species to ordered, strongly intermolecularly hydrogen-bonded species (β-structure) takes place.[1–4] The decrease in solubility is paralleled by a decrease in the coupling rates during peptide synthesis.

The liquid-phase method for peptide synthesis[5] reduces this difficulty by using PEG [poly(ethylene glycol)] as a solubilizing macromolecular protecting group for the *C*-terminal amino acid. Owing to its high solubility in a variety of

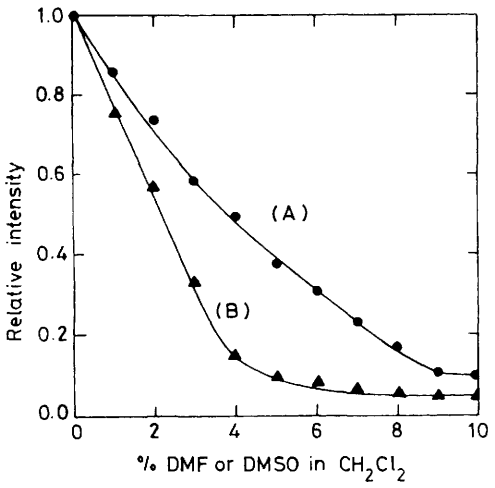

Figure 1. Relative intensity (area) of the 1630 cm^{-1} band in the i.r. spectra of Boc-(L-Ala)$_2$-Gly-L-Ile-(L-Ala)$_2$-NHPEG (5×10^{-3} M) in methylene chloride solution as a function of increasing percentage of (A) DMF and (B) DMSO (v/v), recorded on a Perkin-Elmer model 580 spectrometer, 0.1 mm path length cell, at 20 °C. The area of the 1630 cm^{-1} band in the absence of DMF or DMSO was taken as unity.

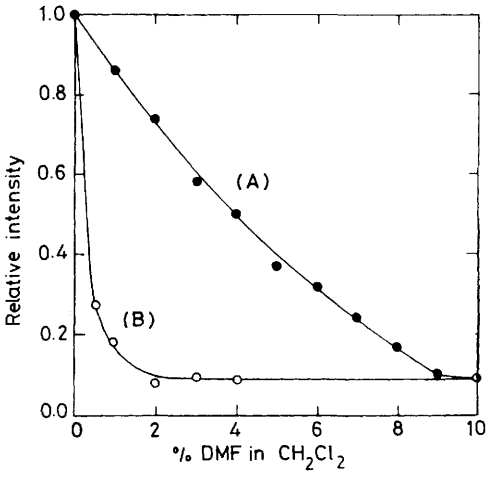

Figure 2. Relative intensity of the 1630 cm^{-1} band in the i.r. spectra of (A) Boc-(L-Ala)$_2$-Gly-L-Ile-(L-Ala)$_2$-NHPEG and (B) Boc-L-Ala-Gly-L-Ile-(L-Ala)$_2$-NHPEG in methylene chloride solution as a function of increasing percentage of DMF (v/v); concentration 5×10^{-3} M.

solvents, the PEG chain prevents the peptide from precipitation.[6] However, even in the case of PEG-bound peptides, coupling rates are still affected by self-association.

Schmitt and Mutter[7] have recently proposed that gel permeation chromatography can be used to detect the onset and stability of self-associated structures in PEG-bound peptides. We describe here a new method to titrate quantitatively the extent of self-association in PEG-bound peptides under the experimental conditions relevant to the coupling step.

We have studied two *N*-protected peptides, Boc-L-Ala-Gly-L-Ile-(L-Ala)$_2$-NHPEG and Boc-(L-Ala)$_2$-Gly-L-Ile-(L-Ala)$_2$-NHPEG (PEG is a bifunctional polymer, molecular weight 6000; Boc = t-butoxycarbonyl), recently synthesized in connection with a conformational investigation on potential β-structure forming peptides.[8] It was shown[9] that: (i) both the penta- and the hexa-peptide have a low solubility in deuteriochloroform, and (ii) the solid-state i.r. spectrum of the hexapeptide features the presence of an almost fully developed β-structure, characterized by an intense amide A band at about 3290 cm^{-1} (N–H stretching vibration) and an amide I band at about 1630 cm^{-1} (C=O stretching vibration).[10]

The disappearance of the 1630 cm^{-1} band upon addition of increasing amounts of either *N*,*N*-dimethylformamide (DMF) or dimethyl sulphoxide (DMSO) to methylene chloride solutions of the hexapeptide is shown in Figure 1. Methylene chloride is the solvent most frequently employed in peptide bond formation, while DMF as well as DMSO are known to compete effectively with the N–H · · · O=C peptide–peptide hydrogen bonds.[11] From Figure 1 it is evident that DMSO is more effective than DMF in disrupting the peptide aggregates.

DMF, being less effective, is capable of distinguishing between the stability of the β-structure of the pentapeptide and that of the hexapeptide (Figure 2). Clearly, much less DMF is needed to destroy the β-structure formed by the pentapeptide.

Alternatively, the disruption of the β-structure of PEG-bound peptides can be followed by monitoring the disappearance of the 3290 cm^{-1} band. However, except for high proportions of DMF, it is more accurate to use the 1630 cm^{-1} band than the 3290 cm^{-1} band (with high proportions of DMF, the short-frequency tail of the amide I band of the solvent overlaps the 1630 cm^{-1} absorption). Concentrations close to those usually employed in peptide synthesis (5×10^{-2}—10^{-1} M) and N^{α}-deblocked peptides in the presence or absence of added tertiary amine are also suitable for this study.

It has become increasingly clear that conformational transitions are important during chain elongation in peptide synthesis.[3,4] We have clearly shown that DMF or DMSO can completely disrupt the β-structure of the two PEG-bound peptides, increasing their solubility. However, with particularly stable β-structures, the addition of DMF, or even DMSO, may not totally disrupt the self-associated structures;[12] a deeper insight into the relationship between conformational preferences and physico-chemical properties of peptides is urgently needed.

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