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Helix to coil transition in poly(γ -benzyl-L-glutamate)

Refractometric and sedimentation studies in chloroform-dichloroacetic acid systems

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With 2 figures

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1. Introduction

The helix-coil transition of poly(γ -benzyl-L-glutamate) (PBLG) in solution can be accomplished either by increasing the temperature or by varying the solvent composition. This conformational change can be detected by a variety of techniques, i.e. light scattering (1), ORD (2), NMR (3), viscometry (4), dilatometry (5). In chloroform-dichloroacetic acid (CHCl_3 -DCA) mixtures at 25 °C, PBLG exists as a coil when the amount of DCA exceeds 68% (6). In several polypeptides the transition causes changes in the refractive index increment (dn/dc) (7, 8) and the sedimentation constant (8). However, such data have not yet been published for PBLG. We now wish to report that the helix-coil transition of PBLG in CHCl_3 -DCA mixtures is also accompanied by a change in both the refractive index increment and the sedimentation properties.

2. Experimental

γ -Benzyl-L-glutamate was prepared following the method of Guttman and Boissonas (9).

γ -Benzyl-L-glutamate N-carboxy anhydride (NCA) was synthesized from γ -benzyl-L-glutamate and phosgene according to Blout and Karlson (10).

The NCA was crystallized three times from a mixture of ethyl acetate and hexane (1:1).

Polymerization of NCA (2.0 g) was carried out in dioxane (100 ml) during 94 h, using triethylamine as an initiator (NCA/initiator ratio, A/I = 100). The polymer was purified by dissolution in dioxane and precipitation in ethanol.

Dioxane was purified by Fiesers procedure (11), stored over sodium, and distilled immediately before use. Triethylamine was refluxed with sodium for 8 h and fractionally distilled. Ethyl acetate, reagent grade was dried over anhydrous MgSO_4 and distilled from calcium hydride. Hexane was dried over molecular sieves and fractionally distilled from sodium. Absolute ethanol reagent grade, was used for the polymer precipitation.

The molecular weight ($M_v = 530,000$) of PBLG was determined from results of viscometry in an Ubbelohde type viscometer in DCA at 25.00 ± 0.01 °C by use of Dotys (12) relation: $[\eta] = 2.78 \cdot 10^{-5} M^{0.87}$.

Refractive index measurements were performed at 25.00 ± 0.01 °C in a Brice-Phoenix differential refractometer at 546 nm.

Sedimentation coefficients of 0.5% solutions were obtained with a Spinco-Beckmann analytical ultracentrifuge, equipped with Schlieren optics.

DCA was distilled twice while reagent grade CHCl_3 was used.

3. Results and discussion

The refractive index increment (dn/dc) of PBLG versus the composition of the solvent mixture is plotted in fig. 1. A sudden change $dn/dc = 0.006 \pm 0.002$ ml/g is observed in the helix-coil transition region.

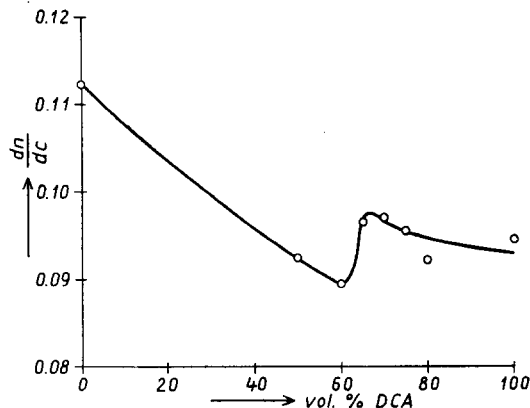


Fig. 1. Refractive index increment dn/dc (in ml/gram) versus volume % DCA in DCA- CHCl_3 mixtures

(dn/dc) can be related to a change in the partial specific volume of the polymer (\bar{v}). Okita (8) has shown that the quantity Ω , defined by

$$\Omega = \left(\frac{n_1^2 - 1}{n_1^2 + 2} \right) \bar{v} + \frac{6n_1}{(n_1^2 + 2)^2} \left(\frac{dn}{dc} \right) \quad [1]$$

where n_1 is the refractive index of the solvent, is a characteristic of a given polymer, regardless the environmental conditions and its molecular conformation. Substitution of literature data (13) for n_1 , \bar{v} and dn/dc yields

$\Omega = 0.264$ for 100% CHCl_3 as well as 100% DCA. Surprisingly, this value of Ω for PBGL equals the Ω value found by Okita (8) for poly-N⁵-(3-hydroxypropyl)-L-glutamine. Inserting the experimentally found values for Ω and dn/dc in equation [1] gives $\Delta(\bar{v}) = 2.4 \pm 0.8$ ml/mole monomer unit. $[\Delta(\bar{v})]$ corresponds to the helix-coil transition, achieved by varying the solvent composition].

In the literature (5, 14, 15) only $\Delta(\bar{v})$ values of helix to coil transition by temperature variation in 1,2-dichloroethane-DCA have been reported. Disagreeing values have been reported by Bradbury et al. (5). [$\Delta(\bar{v}) = -0.077$ ml/mole m. u.] and Gill et al. (14) [$\Delta(\bar{v}) = 0.6$ ml/mole m. u.]. Karasz et al. (15) have demonstrated that reinterpretation of Gills experiments results in the following value for $\Delta(\bar{v})$:

$\Delta(\bar{v}) = 0.93$ ml/mole m. u. Thus $\Delta(\bar{v})$ as a result of varying the CHCl_3 -DCA composition is about 2.5 times the value obtained by increasing the temperature.

Investigation by means of ultracentrifugation revealed that sedimentation properties of PBLG were also influenced by the helix-coil transition.

We used a reduced quantity $[S]$, defined by

$$[S] = \frac{S\eta}{(1 - \bar{v}d)}. \quad [2]$$

Here η and d denote the viscosity coefficient and density of the solvent respectively, and S the sedimentation coefficient of a 0.5% solution of PBLG.

Fig. 2 shows S versus the solvent composition (CHCl_3 -DCA). As can be seen $[S]$ changes from 3.6 (S. cpoise) (0% DCA) to 4.2 (S. cpoise) (100% DCA). In the transition region a rather complex behaviour is observed.

According to Elias et al. (16), PBLG of high molecular weight is not associated in CHCl_3 or DCA. Thus the observed behaviour

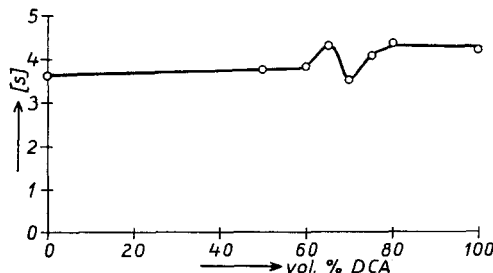


Fig. 2. Reduced sedimentation coefficient $[S]$ (in S. cpoise) versus volume % DCA in DCA- CHCl_3 mixtures

of both dn/dc and S cannot be related to association effects.

Summary

The helix-coil transition of poly(γ -benzyl-L-glutamate), $M_v = 530,000$, in mixture of chloroform (CHCl_3) and dichloroacetic acid (DCA) was studied by measuring refractive index increments (dn/dc) and reduced sedimentation coefficients ($[S]$) as functions of the solvent composition.

In the transition region a significant change in both dn/dc and $[S]$ was observed.

Zusammenfassung

Der Helix-Knäuel-Übergang von Poly(γ -benzyl-L-glutamat), $M_p = 530,000$, in Mischungen von Chloroform (CHCl_3) und Dichloressigsäure (DCA) wurden untersucht durch Messung der Zunahme des Brechungsindex (dn/dc) und des reduzierten Sedimentationskoeffizienten ($[S]$) mit Rücksicht auf deren Abhängigkeit von der Zusammensetzung des Lösungsmittels.

Im Übergangsgebiet wurden deutliche Änderungen von dn/dc und $[S]$ festgestellt.

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