Synthesis of Poly-(L-prolyl-L-prolylglycyl) of Defined Molecular Weights

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A major problem in polypeptide synthesis is the formation of products with a wide range of molecular weights. As a step toward the total synthesis of natural protein, an attempt was made to synthesize a sequential polypeptide with a defined molecular weight. Thus, poly-(L-prolyl-L-prolylglycyl) with ten or twenty repeating units was synthesized by the step-by-step addition of AOC-L-prolyl-L-prolylglycine (I)[1] using the solid-phase procedure.[2] The synthesis was started from glycine-anchored polystyrene (100—200 mesh, copolymerized with 2% divinylbenzene; 14 g, 0.1 mmol/g), to which AOC-L-prolyl-L-proline (1.4 g)[1] was coupled in methylene chloride with dicyclohexylcarbodiimide. The AOC-group was then removed with 2N hydrogen chloride in acetic acid, and the tripeptide-cycle was extended with I (1.7 g per cycle) and dicyclohexylcarbodiimide (0.9 g per cycle); each coupling reaction was carried out in methylene chloride for 3 hr at room temperature. After nine reaction cycles, when ten repeating units were expected to have been formed, a part of the resin was dried (wt 7 g) and placed in an HF-reaction cylinder;[3] anisole (7 ml) was added, and then anhydrous hydrogen fluoride (HF, 60—70 ml) was introduced over the resin by means of the HF-reaction apparatus.[3] After one hour at 0°C, the excess HF was removed, and the peptide liberated was extracted with water. The water extract was treated with IR-45 (OH^- form), and then dialyzed against distilled water for 3 days. The final solution was lyophilized (II, wt 0.74 g). Found: C, 53.49; H, 6.96; N, 15.54%. Calcd for $(C_{12}H_{17}O_3N_3)_{10}\cdot 10H_2O$: C, 53.52; H, 7.11; N, 15.60%.

The polypeptide-resin, which was expected to include the eicosamer (wt 6g), was also synthesized from the rest of the decamer-resin by ten more reaction cycles, and the resin was treated as has been described above. The lyophilizate (III, wt 0.67 g) thus obtained dissolved poorly in water, in contrast to the reported findings of Engel *et al.*,[4] but it was soluble in dilute aqueous acetic acid or 50% ethanol. Found: C, 53.40; H, 7.01; N, 15.62%. Calcd for $(C_{12}H_{17}O_3N_3)_{20}\cdot 20H_2O$: C, 53.52; H, 7.11; N, 15.60%.

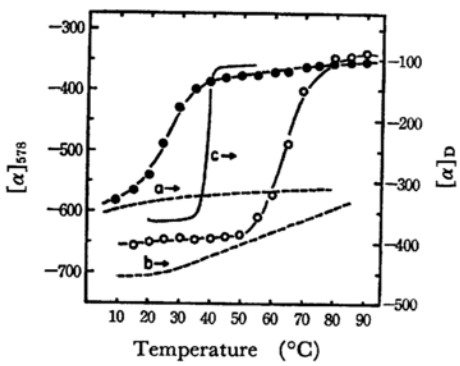

Fig. 1. Temperature dependence of specific optical rotation of $(Pro\text{-}Pro\text{-}Gly)_n$; (—●—), II, $n=10$, $c=0.187$ in 0.1 M NaCl solution; (—○—), III, $n=20$, $c=0.183$ in 10% acetic acid solution. The broken lines are cited from the data published by Engel *et al.*[4] for $(Pro\text{-}Gly\text{-}Pro)_n$, which were plotted by corrected data; a, mol wt 2950; b, mol wt 6900. The continuous line (c) is cited from the data published by Sakai *et al.*[5] for natural collagen.

The weight-average molecular weight of each polymer was determined by the sedimentation-equilibrium technique in the presence of 5M guanidine hydrochloride (0.73 was assumed for the partial specific volume). Found: 2300 (calcd for II: 2531). Found: 5300 (calcd for III: 5044). In these cases, plots for the logarithm of the concentration of the solute against the square of the radial distance, which was measured from the axis of the rotor, were completely linear. This indicates that the distribution of molecular weights was practically monodispersed in each case. The temperature-dependence of the optical rotation change was measured with the samples II and III. As is shown in Fig. 1, the behavior was much more similar to that of natural collagen[5] than those of the same type of polypeptides with a random molecular weight, $(L\text{-prolylglycyl-L-prolyl})_n$.[4] Furthermore, it was found that the two regular polypeptides, II and III, each showed a different transition temperature, depending on their molecular weight.

Thus, a new route for the synthesis of regular polypeptides has been developed.

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