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MEHMET BAHATTİN BAYSAL

ÇİĞDEM KARADAĞ

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Quasielastic light scattering studies of polypeptides: evidence for chain extension in solution

Bahattin M. BAYSAL,[1,2*] **Çiğdem KARADAĞ**[2]

[1]Department of Chemical Engineering, Boğaziçi University, Bebek, İstanbul, Turkey
[2]TÜBİTAK–Marmara Research Center, Energy Institute, Gebze, Kocaeli, Turkey

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Abstract: Dynamic light scattering and viscosimetric methods were used to study polypeptides including polyleucine, poly (γ-benzyl-L-glutamate), poly (α-L-glutamic acid), and polyproline II. Chain extension showing rod-like and flexible conformations occurred in poly(γ-benzyl-L-glutamate) and polyproline II solutions as these solutions were diluted in glacial acetic acid or in a mixed solvent of acetic acid and dichloromethane. The elongation of chains in poly(γ-benzyl-L-glutamate) and polyproline II solutions was studied by dynamic light scattering and viscosimetric methods. This nonenzymatic growth of peptide chains provides data on primordial synthesis of protein molecules in the absence of cell machinery and for this reason these reactions may yield valuable data for further studies.

Key words: Polypeptides, poly(γ-benzyl-L-glutamate), polyproline II, dynamic light scattering, hydrodynamic radius, intrinsic viscosity, chain extension

1. Introduction

The transition of a flexible macromolecular chain from a random coil conformation in the θ-state to a globular compact form in a collapsed state (before ultimate precipitation) has been the subject of extensive studies. We reviewed the works on various theoretical studies of single chain collapse and experimental studies on coil-globule transition of homopolymers, which were carried out on polystyrene, poly(methyl methacrylate), and poly(N-isopropyl acrylamide), as well as transitions of copolymers and polyelecrolytes.[1] In this work, we studied the dimensional properties of polypeptides as a logical next step.

Recently, rod-coil block copolymers showing interesting aggregation behaviors have received great attention for biomaterial applications. Aggregation of rod-coil block copolymers, for example microphase separation in solid state, and aggregation in solution were studied previously.[2,3] For biomedical application, in particular, synthetic block copolymers composed of both polypeptide and polydiene blocks would be expected to be useful in respect of biocompatibility.

Poly-L-leucine, poly(γ-benzyl-L-glutamate), poly(α-L-glutamic acid), and poly-L-proline were included in our dimensional and conformational studies. Poly-L-leucine belongs to the class of poly(amino acids) having bulky hydrophobic side chains. Conformational studies on poly-L-leucine are very limited.[2] This polyamino acid is incorporated as a chiral catalyst in asymmetric synthesis.[2–5] We were not able to dissolve our rather high molecular weight polyleucine sample in any common solvent or solvent mixture.

*Correspondence: bmbaysal@hotmail.com
Dedicated to the late distinguished organic chemist, Prof Ayhan Sıtkı Demir

PBLG is a liquid crystalline material and its synthesis,[6] dimensional characterizations,[7–11] and conformational helix-coil transitions[8,9] were reported.

The solution properties of PBLG were studied in nearly all typical organic solvents. This polypeptide chain exists in an α-helical rigid rod form in helicogenic solvents such as dioxane, benzene, chloroform, ethylene dichloride, and dimethyl formamide.[7–9] In dichloroacetic acid solution, this rod-like configuration has been partially or completely replaced by a random coil configuration.[12–17]

The synthetic polypeptide poly-L-proline shows 2 stable secondary structures: the poly-L-proline type I conformation (PPI) and the poly-L-proline type II conformation (PPII).[18–28] PPI is a right-handed helix containing all *cis* peptide bonds having specific rotation, $[\alpha]^{25D}$, $+50^{\circ}$. On the other hand, PPII is a left-handed helix with all *trans* peptide bonds having a specific rotation, $[\alpha]^{25}$D, -540°.[2,28,29] The PPI helix is compact, having a helical pitch of 5.6 Å/turn and 3.3 residues/turn. The extended PPI helix shows 9.3 Å/turn and 3.0 residues/turn.[30]

Conformational properties of poly-L-proline in concentrated salt and dilute solutions were studied in detail.[31–33] Studies were published on the information related to the morphological evidence for folding of PPII helices, aggregation of PPII,[29,30,34,35] and cooperative intramolecular transition of PPI to PPII forms.[4,23] Extensive work is also available on solution properties of this polypeptide.[24,25,28]

In this work, we studied the dimensional properties of poly(γ-benzyl-L-glutamate), poly(α-L-glutamic acid), and poly-L-proline in various solvents by viscometric and dynamic laser light scattering (DLS) spectrometric methods. We observed and quantitatively described the chain extension process for 2 polypeptides (PLBG and PPII) in dilute viscosimetric solutions. The observation of peptide elongation without the requirement of enzymes suggests that these reactions have biological importance.

2. Results and discussion

In this work the experimental results are organized as follows:

1. The results on poly(γ-benzyl-L-glutamate) are given in various solvents depending on the α-values of the Mark–Houwink relation: $[\eta] = \mathrm{K\ M}^{\alpha}$ (a coil conformation for $\alpha < 1$, and a rod conformation for $\alpha > 1$).
2. Viscosimetric measurements on the chain extension of PBLG in dilute solutions are given.
3. A short report on the poly(α-L-glutamic acid) in a buffer solution is presented.
4. The results on polyproline II are described in detail.
5. The radius of gyration values of these 2 polypeptides for various times and dilutions are reported.

2.1. Poly(γ-benzyl-glutamate)

We obtained the following intrinsic viscosity, $[\eta]$, values in various solutions of our PBLG samples: dichloromethane 3.24 dL/g, trichloroacetic acid 0.327 dL/g, and dimethylformamide 3.24 and 4.75 dL/g (Figure 1).

Poly(γ-benzyl-L-glutamate) is soluble in most of the common solvents, including dichloromethane, and dichloro- and difluoroacetic acid, but it is not soluble in acetic acid (AA) or propionic acid. A mixture of AA

and dichloromethane (DCM) was used to observe the dimensional changes in this polypeptide. After a few trials using various compositions of these solvents, we worked with an AA/DCM (40/60 v/v) mixed solvent.

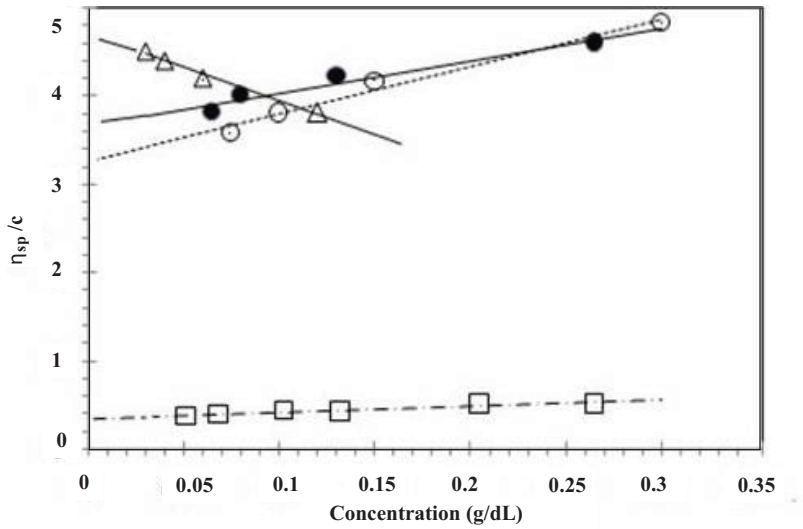

Figure 1. The concentration dependence of the reduced specific viscosity of poly(γ-benzyl-L-glutamate) at 25 °C. (◯) in dichloromethane, (●) in dimethyl formamide (sample 1), (■) in dimethyl formamide (sample 2), (△) in trichloroacetic acid.

It was shown earlier that poly(γ-benzyl-L-glutamate) exists in a random coil conformation in dichloroacetic acid and in a rigid, rod-like helical conformation in a number of organic solvents.[7,8] The helix-coil transition in PBLG was dependent upon the temperature and solvent composition, while the sharpness of the transition depended upon the molecular weight of the polypeptide.[8,9] For PBLG, the helix-coil transition in a solvent mixture of ethylene dichloride–dichloroacetic acid (30/70 v/v) was studied in detail and good agreement was obtained between the experimental results and theoretical calculations.[8,9]

Optical rotation of the solutions of PBLG was used as a measure of the fraction of residues in the helical form for long helices. For this polypeptide chain, $[\alpha]_D$ 25 $= +14°$ was associated with helical configuration, whereas $[\alpha]_D$ 25 $=\sim -15°$ was reported for coils.[8,9]

For the solution used in this work (PBLG in AA/DCM, 40/60 v/v), the optical rotation values were measured as $[\alpha]_D = +2.18°$ at 25 °C (steady for a period of 7 h) and $[\alpha]_D = +4.5°$ and $+12.2°$ at 28 °C and 35 °C, respectively. Considering similarities of the mixed solvents used in our work and the work mentioned in the literature,[8,9] we can assume that the fraction of helical residues at 25 °C is nearly half of the total chain in our chain extension experiments.

2.1.1. DLS studies of poly(γ-benzyl-L-glutamate) (PBLG) in various solvents

2.1.1.1. PBLG in dichloroacetic acid (DCAA)

Mark–Houwink relations at 25 °C in this solvent were given earlier as[7,11]

$$[\eta] = 2.78 \times 10^{-3} M^{0.87} \tag{1}$$

$$[\eta] = 8.8 \times 10^{-3} M^{0.77} \tag{2}$$

which indicate a coil conformation of this polypeptide in this solvent. The hydrodynamic radius (R_h) values measured with DLS at various temperatures are given in Table 1. The molecular weight of this PBLG sample was 230 $\times$ 10^3 g/mol. The R_h values of the coils are rather small and they do not change significantly with increasing temperature.

Table 1. Hydrodynamic radius of PBLG in different solvents.

Solvent Temperature	(°C)	R_h (nm)
DCAA	25	11.1
	25	10.3
	35	12.2
	35	14.2
	45	13.3
	45	12.6
DCAA/H	25	14.9
(70/30 v/v)	35	15.5
	45	19.6
DCAA/H	25	19.9
(65/35 v/v)	35	23.1
	45	29.6

To work with dichloroacetic acid is difficult because this solvent is a highly corrosive liquid. Therefore, PBLG was studied in DCAA–heptane (H) solutions. The following relations were reported at 21 °C:[36]

$$[\eta] = 116 \times 10^{-3} M^{0.53} in DCAA/H(55/45 v/v) \quad (3)$$

$$[\eta] = 25.4 \times 10^{-3} M^{0.68} in DCAA/H(90/10 v/v) \quad (4)$$

The molecular weight of our PBLG sample was rather high; it was not dissolved in (DCAA/H (55/45 v/v) solution. We carried out DLS experiments in 2 DCAA/H solutions and reported the R_h values in Table 1. In DCAA and DCAA/H solutions, the PBLG chains were in the coil conformation ($\alpha < 1$). The hydrodynamic radius values of PBLG in DCAA and in these mixed solvents are rather small, and increase slightly with temperature.

2.1.1.2. PBLG in halogenic solvents

PBLG is known as a liquid crystalline material and numerous articles have been published on its synthesis,[24] dimensional characterizations,[25,29] and conformational helix-coil transitions.[26,27]

The solution properties of PBLG have been studied in nearly all typical organic solvents. It is established that in helicogenic solvents such as dioxane, benzene, chloroform, ethylene dichloride, and dimethyl formamide this polypeptide chain exists in helical rigid rod form.[25–27]

Later it was proposed that in these solvents a more or less flexible rod would be a better presentation of the shape of PBLG.[28,29] In dichloroacetic acid, this rod-like conformation has been partly or completely replaced by a random coil conformation.[30–33]

PBLG molecules are in the rigid rod conformation in most common solvents. The hydrodynamic radius values of a PBLG sample in DCM, tetrahydrofurane (THF), and dioxane (D) at 25 °C are reported in Table 2.

Table 2. Hydrodynamic radius of PBLG in halogenic solvents.

Solvent	Temperature (°C)	R_h (nm)
DCM	25	53.7
THF	25	40.3
D	25	27.8
DMF	25	29.6[a]
DMF	25	47.5[b]
DMF	25	79.9[c]
DMF	25	240.10[d]

[a]after preparation of the solution in DMF, [b]after 1 day of solution preparation, [c]after 1 week of solution preparation, [d]after 3 months of solution preparation

The following Mark–Houwing relations were reported for this polypeptide in dimethyl formamide (DMF) at 25 °C.[7,11]

$$[\eta] = 0.29 \times 10^{-3} M^{1.70} \tag{5}$$

$$[\eta] = 5.60 \times 10^{-3} M^{1.45} \tag{6}$$

At room temperature, PBLG molecules are in the rod conformation ($\alpha > 1$).

The intrinsic viscosity of our PBLG sample as measured in DMF at 25 °C is $[\eta] = 4.60$ dL/g (Figure 1). The molecular weight of this polypeptide is calculated as 287×10^3 g/mol from Eq. (6).

Molecular parameters of a PBLG molecule having $M_w = 299 \times 10^3$ g/mol were calculated in a quasielastic light scattering study at 25 °C in DMF, and the following values were reported:[37] length L = 205 nm, diameter b = 2.2 nm, radius of gyration of this chain $R_g = 57.5$ nm.

For a rod-like chain the following relation is reported:[38]

$$(R_h/R_g) = 3^{1/2}/(\ln(L/b) - \gamma), \tag{7}$$

where L is rod length, b is rod diameter, and $\gamma \approx 0.3$.

The calculated value of hydrodynamic radius for the above-mentioned chain ($M_w = 299 \times 10^3$) from Eq. (7) is $R_h = 23.9$ nm. In this work, the experimental value of hydrodynamic radius for a similar chain is measured as $R_h = 29.6$ nm (Table 2). The difference between the calculated and experimental values of hydrodynamic radius can be attributed to chain extension in PBLG rods in DMF solution.

We observed a chain extension of PBLG in DMF solutions at room temperature with time (Table 2). Hydrodynamic radius was measured with laser light scattering after preparation of PBLG solution in DMF as 29.6 nm. The next day it was 47.5 nm. Hydrodynamic radius was observed to be about 8 times greater after 3 months. For a chain extended sample in DMF solution a large polydispersity value observed in laser light scattering measurements indicates a very heterogeneous system.

2.2. Viscosimetric measurements on the chain extension of PBLG in dilute solutions

The increase in the η_{sp}/c values of PBLG solutions in a mixed solvent of AA-DCM with dilution is shown in Figure 2A–D. In these 4 sets of experiments, the initial polypeptide concentrations and dilution procedures were slightly different.

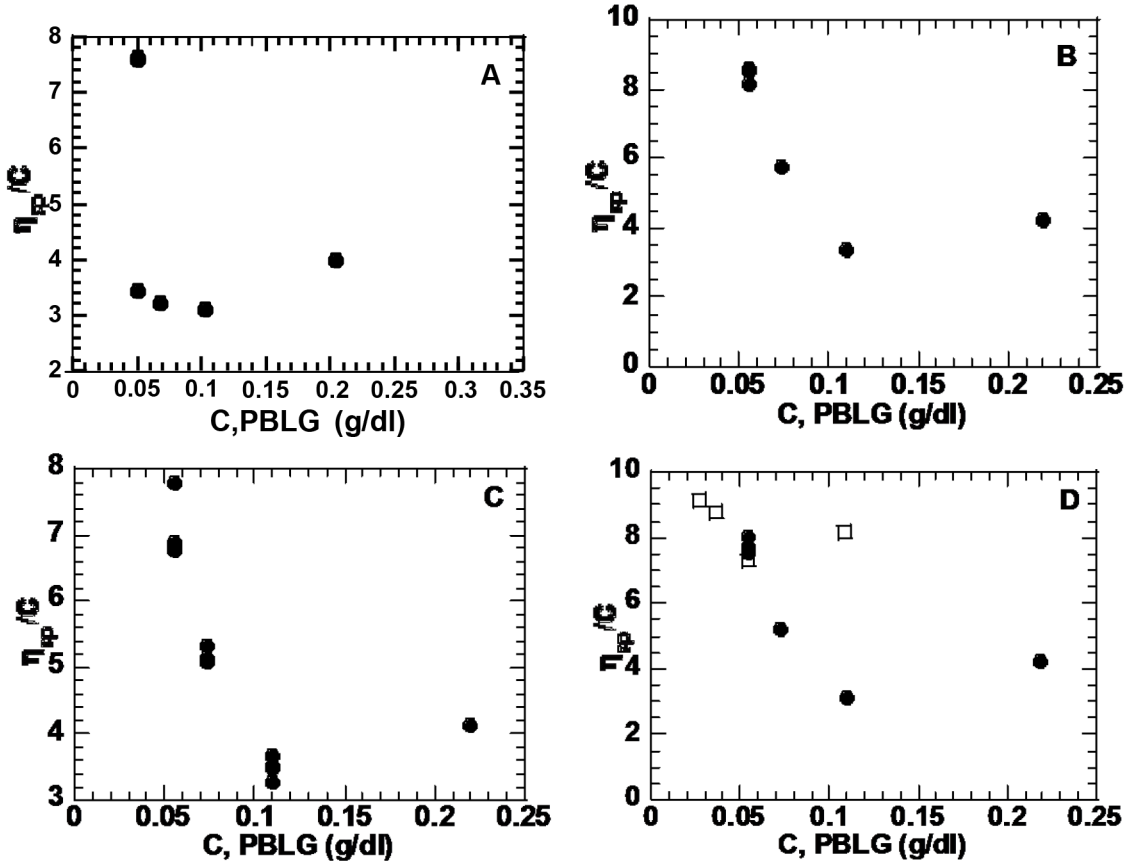

Figure 2. (A–D) (●) chain extension of poly(γ-benzyl-L-glutamate) with dilution in an acrylic acid–dichloromethane solution, (2D) (□) conformation of chain extension process (see text).

In one of the chain extension experiments (Figure 2A), the initial concentration of PBLG was (c = 0.205 g/dL), and decreased specific viscosity was measured as η_{sp}/c = 3.99 dL/g. The experimental points reported for dilute solutions indicate an initial slow decrease in reduced specific viscosity. A chain extension of PBLG was observed after 4-fold dilution over 4 days, and in the following period the reduced specific viscosity was measured as 7.65 dL/g. In a second run, after 3 successive dilutions performed in a 2-h period, reduced specific viscosities reached 8.16, 8.19, and 8.53 dL/g (Figure 2B). In Figure 2C, several values of reduced specific viscosity were reported for the same dilution in 2-h intervals. A final constant value of the reduced viscosity (η_{sp}/c = 7.79 dL/g) was observed after 4 days of dilution.

In one of the chain extension experiments of PBLG, reduced specific viscosity was measured as (η_{sp}/c) = 8.07 dL/g (Figure 2D). This solution was placed in a hood to evaporate its solvent and the polypeptide was dried in a vacuum oven at 50 °C for about 10 h to reach a constant weight. The reduced specific viscosity of this sample (c = 0.1090 g/dL) was measured as 8.15 dL/g (Figure 2D open square). After 3 successive dilutions to a final concentration (c = 0.0273 g/dL), a 10% increase in reduced specific viscosity was observed, (η_{sp}/c) = 9.12 dL/g (Figure 2D open square).

The following limiting specific viscosity values were observed from Figure 2 A–D: 7.6, 8.2, 7.8, and 8.1 dL/g.

Figure 3 was obtained by plotting the existing literature intrinsic viscosity values for PBLG in dichloroacetic acid at 25 °C.[7,10] By using the above limiting viscosity values of chain extended PBLG samples in Figure 3, we estimated from extrapolated lines the molecular weights of our chain extended samples to be within the range of 2.0 × 10^6–3.2 × 10^6 g/mol. These results indicate that the molecular weight of ∼300 × 10^3 was increased about 7- or 10-fold after successive dilutions.

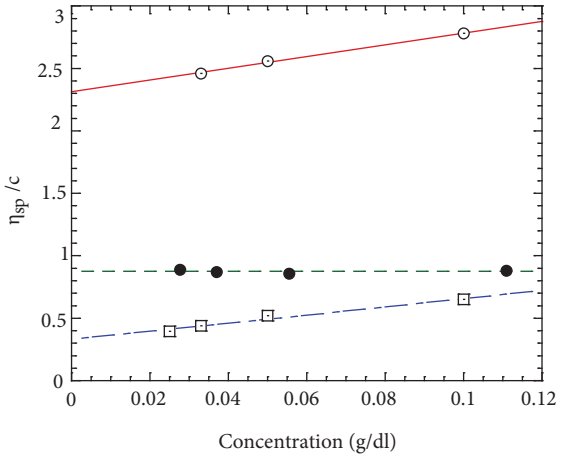

Figure 3. For poly(γ-benzyl-L-glutamate), the double logarithmic plot of $[\eta]$ vs. M_w at 25 °C in dichloroacetic acid, (●) Ref. 25, (◯) Ref. 28, (×) chain extended samples in a mixed solvent (AA/DCM).

2.3. PLGA in a buffer solution

For poly(α-L-glutamic acid), the following relation in sodium phosphate buffer solution was reported:[39]

$$[\eta] = 1.55 \times 10^{-3} M^{0.96} \tag{8}$$

The intrinsic viscosity of this PLGA sample was $[\eta]$ = 0.47 dL/g and the calculated molecular weight was 303 × 10^3 g/mol. The hydrodynamic radius in this buffer solution was R_h = 30.3 nm.

2.4. Chain extension of PP II in solution by viscosimetric studies

In order to characterize our PPII sample, we determined the intrinsic viscosities ($[\eta]$) of this polypeptide in water, sodium acetate, and AA solutions at 30 °C by plotting the reduced specific viscosities (η_{sp}/c) against concentration (c, g/dL). Intrinsic viscosities were obtained in Figure 4 as $[\eta]$ = 0.921 dL/g (in H_2O), 0.330 dL/g (in AcONa), and 2.31 dL/g (in glacial AA). The results indicate that glacial AA is a good solvent for PPII. In these experiments, specific viscosities were measured on the same day by successive dilution of the PPII solutions immediately prior to measurements in the viscosimeter.

Figure 5 is plotted by using the literature values of intrinsic viscosities and molecular weights for poly-L-proline in AA at 30 °C.[10,33] The estimated molecular weight of the PPII sample ($[\eta]$ = 2.31 dL/g) that we used in this work was 90 × 10^3 g/mol (Figure 5).

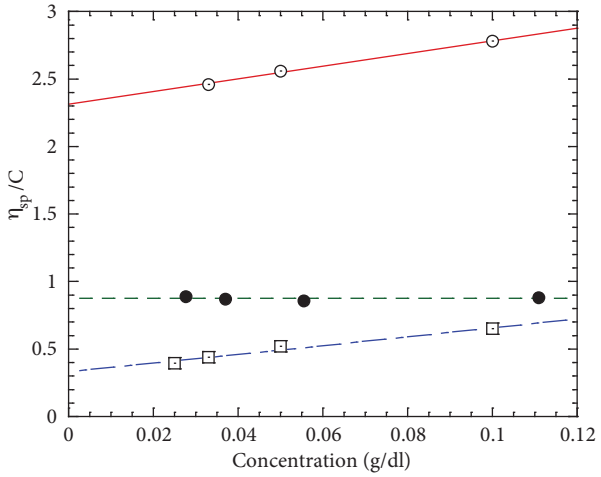

Figure 4. The concentration dependence of the reduced specific viscosity of polyproline II at 30 °C. (□) in acrylic acid, (◯) in water, (●) in sodium acrylate.

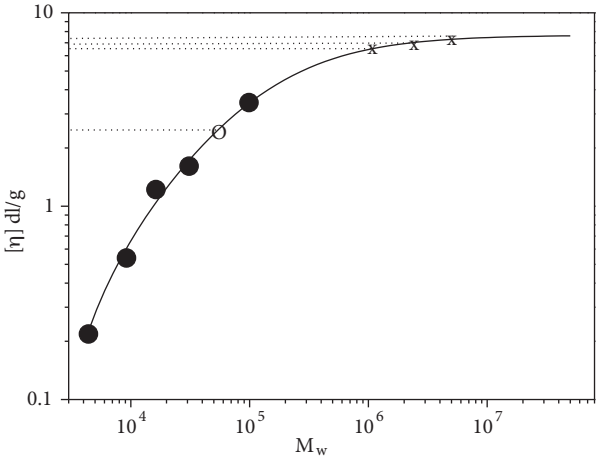

Figure 5. For polyproline II, the double logarithmic plot of $[\eta]$ vs. M_w at 30 °C in acrylic acid. (●) Ref. 9, 16, (◯) sample used in this work, (□) chain extended samples (see text).

Figure 6A–D shows the increase in the η_{sp}/c values of PP II solutions in glacial AA by dilution. In these 4 sets of experiments, solutions of PPII were prepared in glacial AA and kept at 50 °C for 5 h. Viscosity measurements were carried out several days after preparation of this solution at 30 °C. The initial reduced specific viscosities of these solutions were measured as $(\eta_{sp}/c) = \sim 2.10$ dL/g (Figure 6A). In one of these chain extension experiments (Figure 6A), PPII was dissolved in glacial AA (c = 0.2 g/dL) and kept at 50 °C for 5 h. Reduced specific viscosity was measured the next day as $\eta_{sp}/c = 2.20$ dL/g. This value was constant for the next 3 experiments performed with 1-h intervals. This solution was diluted (c = 0.10 g/dL) and reduced specific viscosities were measured at 1-h intervals as 4.10, 4.22, 4.27, and 4.35 dL/g. Concentration of the solution was again reduced (c = 0.067 g/dL), and reduced specific viscosity values were measured the next day again at 1-h intervals as 5.15, 5.36, 5.41, and 5.52 dL/g. The reduced specific viscosity value reached 7.24 dL/g with further dilution of the solution to 0.05 g/dL.

In another of the above-mentioned chain extension experiments (Figure 6B), a reduced specific viscosity value of $(\eta_{sp}/c) = 6.95$ dL/g was attained with dilution of the solutions in 2 days. This chain extended solution was kept in an oven at 50 °C for 48 h. The specific viscosity of this sample was measured as 6.67 dL/g after this procedure.

Figure 6C shows the increase in the reduced specific viscosities from 2.08 to 3.70 dL/g in 4 successive dilution experiments carried out at 2-h intervals. The next day, the (η_{sp}/c) value of this diluted solution was measured as 6.52 dL/g.

We postulate that the increase in (η_{sp}/c) values observed upon dilution of PPII solutions is a result of chain extension of these polypeptide molecules. To eliminate the possible occurrence of a polyelectrolyte effect in dilute polyelectrolyte solutions,[37] the following experiments were performed. After observing the highest value of reduced specific viscosity, 7.17 dL/g (Figure 6D), the solution in the viscosimeter was used for characterization of this chain extended PPII sample. The solvent (AA) in this dilute sample was evaporated in a hood and the polypeptide was dried in a vacuum oven at 50 °C for about 10 h to reach a constant weight of the PPII residue.

This material was dissolved in AA to obtain a dilute solution of polypeptide having a concentration c = 0.0209 g/dL. The reduced specific viscosity of this solution was measured as (η_{sp}/c) = 7.18 dL/g (Figure 6D open square). After 3 successive dilutions of this solution and reaching a concentration of 0.005 g/dL, we observed that the reduced specific viscosity value was slightly increased and reached 8.05 g/dL (Figure 6D, open squares).

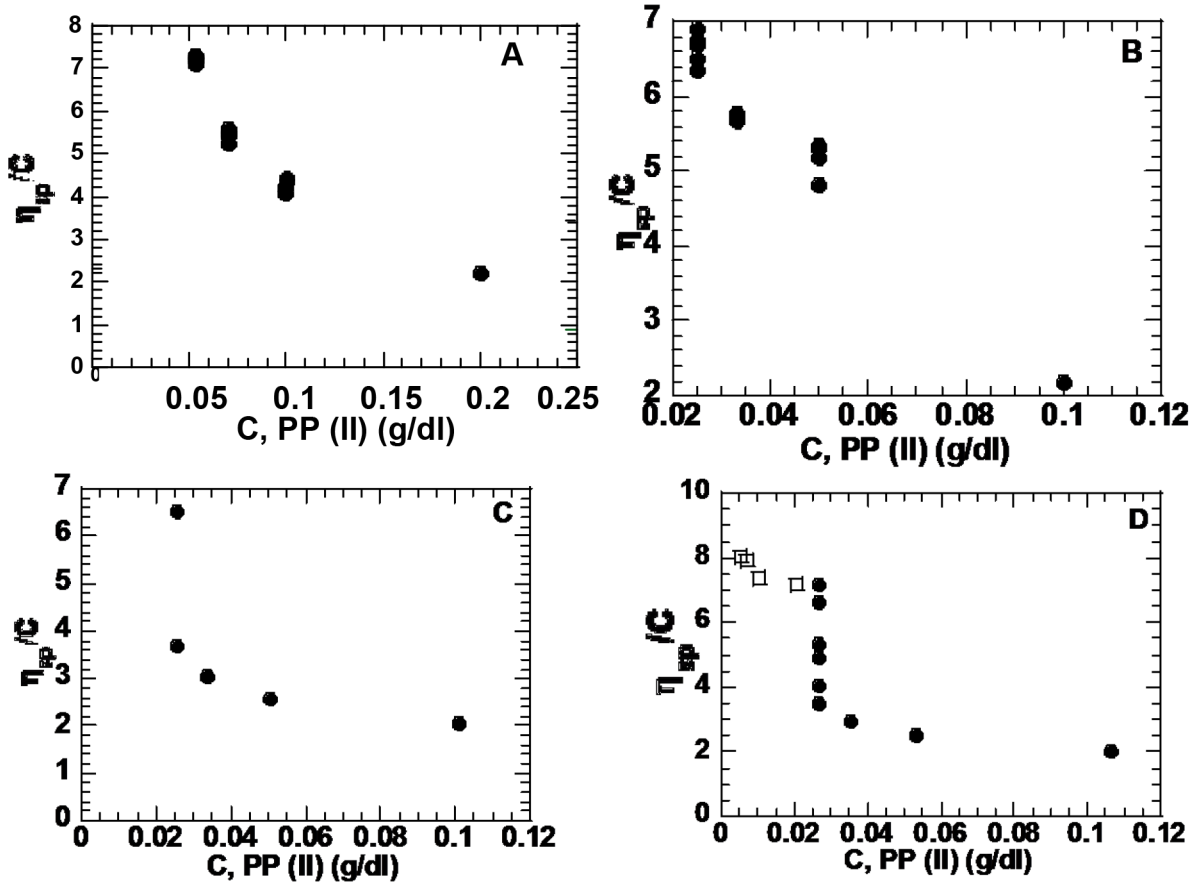

Figure 6. (A–D) (●) chain extensions of polyproline II with dilution in acrylic acid, (6D) (□) confirmation of chain extension process (see text).

Optical rotations of PPII samples in glacial AA were measured in a polarimeter. The specific optical rotation $[\alpha]_D^{25} = -540^\circ$ did not change during the chain extension process.[18,21,28] The highest reduced specific viscosity values obtained in 4 sets of chain extension experiments (Figure 6A–D), (η_{sp}/c) = 7.25, 6.95, 6.60, 7.25 dL/g, were used to calculate the molecular weights of these chain extended polypeptides. Assuming that the reduced specific viscosity values in these dilute solutions were close to intrinsic viscosities, we obtained high molecular weight values in the range of 1.1×10^6–1.8×10^6 by using the extrapolated curve in Figure 5.

2.5. Dynamic light scattering results related to chain extension of PBLG and PPII in dilution

The results of dynamic light scattering experiments are given in Table 3. Our viscosity measurements on PPII were carried out in glacial AA, which is a difficult solvent for DLS measurements. Therefore, Z_{Ave} values were determined in a mixed solvent of AA and DCM (40:60 v/v) at 25 °C. The hydrodynamic radius increased from 24.1 nm to 58.1 nm subsequent to 4-fold dilution of the original solution.

Table 3. Dynamic light scattering results of chain extension of polyproline II in acrylic acid and poly(α-benzyl-L-glutamate) in a mixed solvent of (AA + DCM) at 25 °C.

Polyproline II		
C (g/dL)	Z_{Ave} (nm)	time*
0.200	22.4	-
0.100	24.1	-
0.050	28.1	-
0.100	31.9	2
0.100	40.2	7
0.025	58.1	5
Poly(γ-benzyl-L-glutamate)		
0.203	26.8	-
0.217	27.7	-
0.040	28.2	-
0.040	40.2	5
0.020	27.9	-
0.020	35.2	2
0.101	28.6	-
0.020	40.0	3
*time after preparation of the solution, days		

DLS measurements of PBLG were performed in the same mixed solvent at 25 °C. The Z_{Ave} value increased from 27.7 nm to 40.2 nm after dilution.

For both polypeptides, Z_{Ave} values increased with dilution and elapsed time after dilution. The molecular weights of the polypeptides used in this work were relatively small compared to those of synthetic polymers such as polystyrene and poly(methyl methacrylate), which were studied for dimensional measurements. Nevertheless, we observed rather large Z_{Ave} values ($\sim$ 22–27 nm) for both polypeptides used in this work, which indicates a rod-like shape of these polypeptides in this solution.[37] On the other hand, the limited increases observed in Z_{Ave} values compared to large increases in specific viscosities may be attributed to the increase in flexibility of the extended chains in dilute solutions.

There is limited research in the literature on the dimensional properties of polypeptides indicating a head-to-tail dimerization for PBLG in 1,2-dichloroethane by light scattering.[39,40] Therefore, the chain extension phenomenon by dilution for these 2 polypeptides reported in this work is a novel observation.

3. Conclusion

Polyelectrolytes play an important role in biosciences, chemical industries, and material sciences. Extensive work related to evaluation of viscosimetric results and intermolecular origin of polyelectrolyte effects was recently published.[41–44] The sharp increase in the reduced specific viscosity with decreasing polymer concentration is discussed as an intramolecular phenomenon. However, polyelectrolyte hydrodynamics describe this effect as purely electrostatic, caused by intermolecular forces.[44]

The polypeptides studied in this work do not exhibit the above-mentioned concentration effect in various solvents. The increase in specific viscosity is only observed in glacial AA (for PPII) and in an AA-DCM solution (for PBLG). The increases observed in hydrodynamic radius (Z_{Ave}) by dilution of polypeptide chains provide proof of the chain extension process.

The observation of peptide elongation without the requirement of enzymes suggests this reaction may be

of biological importance. The nonenzymatic growth of peptide chains may be utilized in primordial synthesis of protein molecules in the absence of cell machinery.

4. Experimental

4.1. Materials

All polypeptides used in this work were products of Sigma Chemical Co. Molecular weights, based on viscosity, were as follows: poly-L-leucine 100,000–150,000; poly-L-proline >30,000; poly(γ-benzyl-L-glutamate) 150,000–350,000; poly(α-L-glutamic acid) sodium salt 50,000–100,000. Polypeptides were dialyzed against water and recovered by lyophilization before use. Reagent grade solvents were used throughout this work.

4.2. Methods

4.2.1. Dynamic light scattering

DLS measurements were performed with a Malvern Autosizer 4800 Spectrometer operating with a Coherent INNOVA 70C Series Argon Ion Laser System. Dilute solutions ($\sim 2 \times 10^{-3}$ g/mL) of polypeptides were prepared with various solvents. Each solution was filtered carefully with 0.2-μm Millipore filters into DLS sample tubes. The viscosity and refraction indices of the solvents used in the light scattering experiments were entered into the data analysis program before each measurement.

4.2.2. Optical rotations

Measurements were performed with an Automatic Polarimeter AA-10R Angular Single Wavelength Model.

4.2.3. Refractive indices

ABBE–60 Refractometer, Bellingham Stanley Ltd.

4.2.4. Viscosity

Flow times were measured using a Cannon-Ubbelohde dilution viscometer in a thermostat with the temperature controlled to ± 0.1 °C. Kinetic energy corrections are not required with this viscometer. Flow times for solvents were 100–150 s/mL.

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