Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2024, **34**, 348–350

Mendeleev Communications

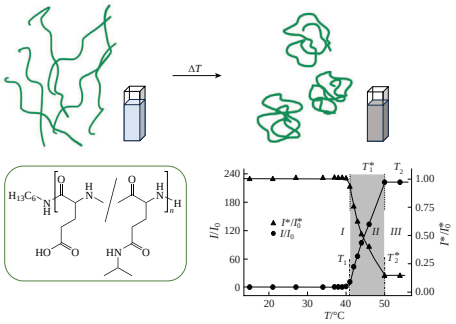
A novel thermoresponsive polypeptide: synthesis and characterization

Irina I. Tarasenko, Natalya N. Zakharova, Elena N. Vlasova and Evgenia G. Korzhikova-Vlakh*

Institute of Macromolecular Compounds, Russian Academy of Sciences, 199004 St. Petersburg, Russian Federation. E-mail: vlakh@hq.macro.ru

DOI: 10.1016/j.mencom.2024.04.012

Polypeptide consisting of γ-*N*-isopropyl glutamine and glutamic acid (90 : 10, mol/mol) was synthesized by post-polymerization modification of poly(γ-methyl glutamate). The obtained polypeptide exhibited thermoresponsive properties with the lowest critical solution temperature in the range of 41–54 °C depending on the medium and polymer concentration.



Keywords: polypeptide, thermoresponsive polymer, poly(γ-methyl glutamate), poly(γ-*N*-isopropyl glutamine), phase transition temperature.

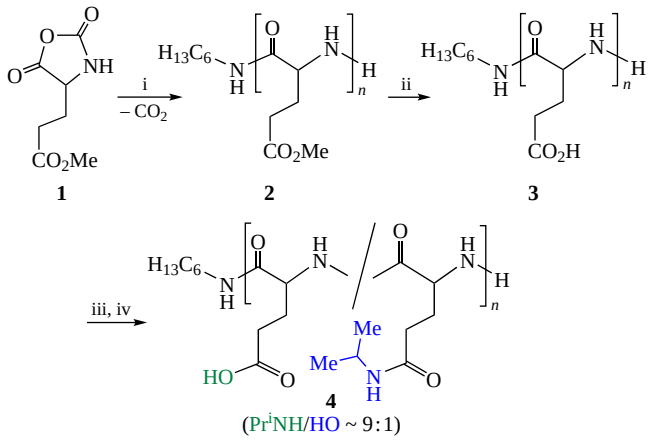
In the last two decades, a growing number of thermoresponsive polymers have been reported for a variety of applications, including drug delivery, cell technologies, tissue engineering, membrane transfer, smart clothing, shape memory polymers, *etc*.[1–4] Among thermoresponsive polymers, poly(*N*-isopropylacrylamide) (PNIPAM) has the lowest critical solution temperature (LCST) at 32–33 °C in aqueous media.[5] This temperature is close to that of human body making PNIPAM widely used for the development of thermosensitive biomedical materials. In order to adjust the thermosensitive properties of PNIPAM and to obtain thermoresponsive biomaterials, it was copolymerized with other natural[6] and synthetic[7] polymers. Besides PNIPAM, various polyoxazolines and their copolymers are capable of thermosensitive behavior.[8–10] Varying the molecular weight and composition of the copolymers allows the cloud point to be adjusted. For example, the LCST of copolymers of 2-ethyl-2-oxazoline and 2-*n*-propyl-2-oxazoline could be tuned from 25 to 100 °C.[11] Similar to polyoxazolines, cloud point of poly(oligoethylene glycol methacrylates) in water solutions can be precisely controlled over a range of 20–90 °C by the polymer length changing or by copolymerization of different oligoethylene glycol methacrylate-based monomers.[12]

The above-mentioned thermoresponsive (co)polymers are biocompatible and can be used as part of biomedical materials, including drug delivery ones. The collapse of polymer coils at LCST to globules promotes the enhanced drug release. This is a result of the disorder in the hydrophilic/hydrophobic balance of a copolymer in aqueous solutions at temperatures above LCST leading to dehydration of the polymer, its phase separation and drug excretion. The only disadvantage of these copolymers is their non-biodegradable nature, which may limit their elimination in insoluble form from the body when applied *in vivo*. In this case, the use of biodegradable thermoresponsive polymers is more favorable. Among biodegradable thermoresponsive polymers, polypeptides[13,14] and polypeptoids[15] capable of degrading to water-soluble metabolites (free amino acids) are one of the promising candidates for developing various drug delivery systems (nanoparticle-based or implantable delivery systems).[16,17]

In this study, we propose a synthesis of novel thermoresponsive polypeptide and studies of its properties in different aqueous media. Similar to PNIPAM, we reasoned that the presence of isopropylamide moiety in the polypeptide main chain could lead to dehydration of chains at heating and change the polymer phase state. In addition, we hypothesized that the concentration of the polymer in solution and the medium could allow the LCST of the synthesized polypeptide to be regulated.

Ring opening polymerization (ROP) of *N*-carboxy anhydrides (NCAs) of amino acids and their derivatives with the use of primary amines as initiators is known to be one of the simplest methods for the synthesis of polypeptides with low dispersity.[17,18] Primary amines are inexpensive and provide the so-called normal amine mechanism of ROP. In the absence of side reactions, polypeptides with molecular weight close to a theoretical one can be obtained. A limitation for the versatile synthesis of some polypeptides by ROP NCAs is the unavailability of commercial monomers since they generally have low stability due to their high sensitivity to moisture. Because of the high sensitivity and reactivity of NCAs, the synthesis of some monomers is complicated by numerous side reactions (hydrolysis, deprotection with HCl release, *in situ* polymerization initiated by the α-amino group of an amino acid or some impurities, *etc*.) that drastically reduce the product yield. Given this, an alternative way to obtain some polypeptides is their post-polymerization modification[19,20] provided the corresponding monomers synthesized in good yields.

Here, the strategy for the synthesis of the target polypeptide containing isopropylamide moieties in the side chains included several steps (Scheme 1): (i) ROP NCA of glutamic acid γ-methyl ester **1** with *n*-hexylamine to synthesize poly(γ-methyl



Scheme 1 *Reagents and conditions*: i, *n*-$C_6H_{13}NH_2$ (0.01 equiv.), 1,4-dioxane (for 4% solution of **1**), 22 °C, 96 h; ii, NaOH (1 M in dioxane/water, 6:1, v/v), 22 °C, 1 h, then dialysis; iii, HOBt/DCC/CO_2H (2:1:1), DMF (for 10% solution), 22 °C, 45 min; iv, Pr^iNH_2 (4 equiv. per CO_2H), DMF, 22 °C, 20 h.

glutamate) **2**; (ii) alkaline removal of methyl protective groups to obtain poly(glutamic acid) **3**; (iii) activation of the γ-carboxylic groups in **3** and their capping with isopropylamine to obtain γ-*N*-isopropyl glutamine units in the target polymer **4**.

Monomer for ROP **1** was synthesized and purified prior to the polymerization using a common procedure[21] (for all experimental details, see Online Supplementary Materials). The obtained polymer **2** was analyzed by size exclusion chromatography (SEC). According to SEC, its number average molecular weight (M_n) and dispersity ($Đ$) values were 8700 and 1.22, respectively.[†] After removal of the protective methyl group and capping of the γ-carboxy groups of **3** with Pr^iNH_2, the final substance **4** was analyzed by static light scattering in dilute HEPES [4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid] buffer (pH 7.2). The weight average molecular weight (M_w) of modified polymer **4** was found to be equal to 8200 (the hydrodynamic radius $R_{h\text{-}D}$ value was 1.1 nm).

All obtained polymer products **2**–**4** were analyzed by Fourier-transform infrared (FTIR) and ^{1}H NMR spectroscopy. According to FTIR spectra, the bands at 2960 and 1732 cm^{-1} characteristic of methyl ester **2** disappeared after alkaline hydrolysis. In the spectrum of polyacid **3**, broad band with a maximum at 3280 cm^{-1} for the OH vibrations of glutamic acid moiety and the valence vibrations for the NH bond of the amide group appeared. The shoulder at 1708 cm^{-1} is characteristic of the C=O vibrations of the glutamic acid group while characteristic bands at 1650 and 1550 cm^{-1} correspond to the amide bond when the polypeptide chain exists in the α-helical conformation. In comparison to spectrum of **3** [see Online Supplementary Materials, Figure S2(*b*)], that for substance **4** contains a band at 2960 cm^{-1} corresponding to the CH_3 group of isopropylamide moiety [Figure S2(*c*)].

^{1}H NMR spectroscopy indicated the disappearance of the signal for the protective methyl group at 3.6 ppm (polymer **2**) and appearance of signals for isopropylamide group (polymer **4**) at δ_H 1.22 (C*H*$_3$), 3.85 (C*H*Me$_2$) and 7.71 (N*H*Pr^i) (Figure S3). According to the integral intensities of the characteristic signals, the modification degree was 90%. Thus, the in fact synthesized polypeptide **4** represents a copolymer of γ-*N*-isopropyl glutamine (90 mol%) and glutamic acid (10 mol%) (P[Gln(Pr^i)-*co*-Glu]).

The thermosensitive behavior of aqueous solutions of **4** was investigated by light scattering and turbidimetry methods.[‡] The temperature and concentration dependences of the scattered light intensity (I), optical transmittance (I*), and hydrodynamic radii (R_h) of scattering objects in solutions were determined. All the results discussed below were obtained under the conditions of the 'equilibrium' state of the system, *i.e.*, when the characteristics of the solutions do not change in time (for more details, see Online Supplementary Materials).

With the aim to investigate the thermosensitive behavior of polymer **4** depending on its concentration, the temperature dependence of the polypeptide in deionized water (pH 6.0) was measured at concentrations of 0.04 and 0.10 g dl^{-1} throughout heating. The dependence presented in Figure 1 is typical of a termoresponsive polymer. At low temperatures, I and I* are independent on concentration. At temperature T_1, the light scattering intensity begins to increase with simultaneous decrease in the optical density, which is due to the appearance of large supramolecular structures in the solution (see Figure 1). The value of I* reaches zero at temperature T_2. Between T_1 and T_2, the values of hydrodynamic radius (R_h) increase, reflecting the growth of aggregates. Above T_2, light scattering is not classical (zone *III*).

The determined phase transition temperatures are summarized in Table 1. As can be seen, cloud point (T_1) decreases and the ΔT narrows with increase in the polymer concentration. The sharper phase transition at higher polymer concentration can be explained by the important role of hydrophobic interactions in the self-organization of macromolecules during phase separation. An increase in polymer concentration leads to the formation of more

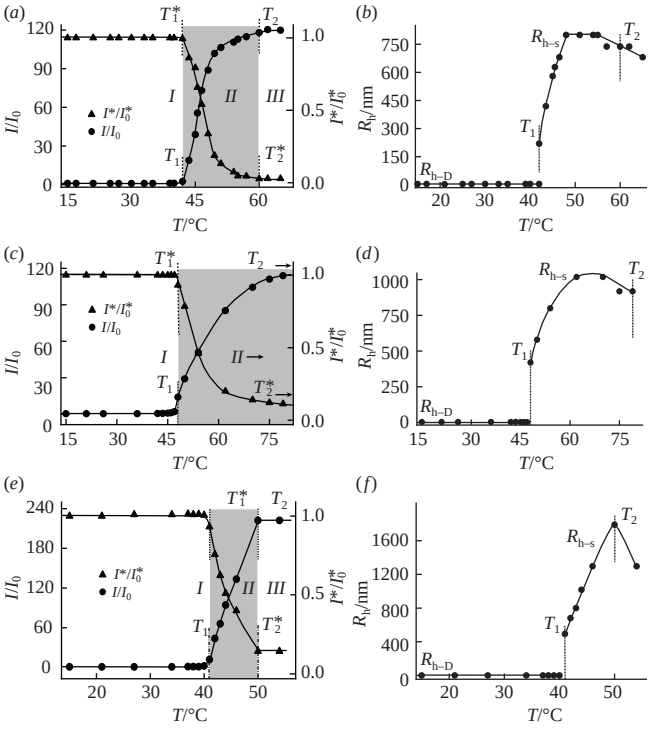


Figure 1 Dependences of (*a*), (*c*), (*e*) relative values of scattered light intensity, optical transmittance and (*b*), (*d*), (*f*) hydrodynamic radius (R_h) on temperature for polypeptide **4** aqueous solutions under different conditions. I_0 and I_0* are the values of scattered light intensity (I) and optical transmittance (I*) at 20 °C; T_1 and T_2 are the temperatures of the beginning and end of the transition. Conditions: (*a*), (*b*) deionized water, polypeptide concentration is 0.10 g dl^{-1}; (*c*), (*d*) deionized water, polypeptide concentration is 0.04 g dl^{-1}; and (*e*), (*f*) 0.01 M HEPES buffer solution (pH 7.2), polypeptide concentration is 0.10 g dl^{-1}.

[†] SEC was performed in DMF containing 0.1 mol dm^{-3} LiBr at 40 °C using a Shimadzu LC-20 HPLC system (Japan) with refractometric detection. Molecular weights were calculated regarding to calibration plot built for poly(methyl methacrylate) standards.

[‡] A Photocor Complex unit equipped with a Photocor-PD sensor for recording the intensity of transmitted light was used for analysis.

Table 1 Thermoresponsive properties of polypeptide P[Gln(Pr^i)-*co*-Glu] **4** (90 : 10, mol%) under different conditions.

Medium	pH	Concentration/ g dl^{-1}	Phase transition		
			T_1/°C	T_2/°C	ΔT/°C
Deionized water	6.0	0.10	43	55	14
		0.04	47	71	24
Deionized water + 0.1 M NaOH	9.7	0.10	54	>70	>16
0.01 M HEPES buffer solution	7.2	0.10	41	50	9

hydrophobic contacts between isopropylamide groups of the polymer. As a result, phase separation in concentrated solutions is faster than in the diluted polymer solutions.

Taking into account that polypeptide **4** contains about 10 mol% of carboxylic groups, it can be sensitive to pH of the medium. Indeed, a study of the thermoresponsive behavior of the polypeptide under basic conditions revealed an increase in cloud point (see Table 1). This effect may be related to the improved solubility of the polypeptide due to better ionization under alkaline conditions. The rather wide phase transition region may be a result of the non-uniform modification of polypeptide chains with Pr^{i}NH units affecting their different solubility/ insolubility in the medium.

In addition, the thermoresponsive properties of polypeptide **4** have been determined in the presence of salts, namely, in 0.01 M HEPES buffer solution. In this solution, the temperature dependence was similar to that in water but indicated a lower cloud point (41 °C, and the narrowest transition area, see Figure 1). The faster phase transition in the buffer medium can probably be attributed to the easier micellization of the polypeptide in the presence of salts due to poorer thermodynamic quality of the solvent for the polymer.

In conclusion, polypeptide P[Gln(Pr^i)-*co*-Glu] **4** (90 : 10, mol%), synthesized by combination of ROP and post-polymerization modification, exhibits thermosensitivity in the close to neutral values of pH and undergoes a reversible phase transition. At the same time, the phase transition temperatures decrease with increasing the polypeptide concentration in the solution. Increasing the concentration of salt in the buffer solution also leads to a decrease in the T_1 and T_2 values and a narrowing of the phase transition interval. The obtained information is a necessary fundamental basis for consideration of the new polymer in biomedical applications, *e.g.* as a drug delivery system.

According to the literature, increasing the molecular weight of the polymer or changing the nature of the side substituent can decrease the phase transition temperature.[11,12] The synthetic approaches used in this work, namely, ROP NCA accompanied with post-modification by amines, allow varying the molecular weights of polypeptides and their degree of modification by amines with short aliphatic chains. All these issues require further investigation for the polypeptide proposed in this study.

This study was performed within the framework of the State Assignment for IMC RAS (124013000730-3).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.04.012.

References

1 M. A. Ward and T. K. Georgiou, *Polymers*, 2011, **3**, 1215.
2 M. Sponchioni, U. Capasso Palmiero and D. Moscatelli, *Mater. Sci. Eng. C*, 2019, **102**, 589.
3 R. Purwar and R. Sachan, in *Advances in Functional and Protective Textiles*, eds. S. Ul-Islam and B. S. Butola, Elsevier, 2020, pp. 37–62.
4 A. A. Belyaeva, I. V. Tretyakov, A. V. Kireynov, Y. A. Nashchekina, V. I. Solodilov, E. G. Korzhikova-Vlakh and S. M. Morozova, *J. Colloid Interface Sci.*, 2023, **635**, 348.
5 Q.-T. Pham, Z.-H. Yao, Y.-T. Chang, F.-M. Wang and C.-S. Chern, *J. Taiwan Inst. Chem. Eng.*, 2018, **93**, 63.
6 I. M. Pilipenko, V. A. Korzhikov-Vlakh, N. V. Zakharova, A. Urtti and T. B. Tennikova, *Carbohydr. Polym.*, 2020, **248**, 116764.
7 Y. Zhao, H. Gao, G. Liang, F. Zhu and Q. Wu, *Polym. Chem.*, 2014, **5**, 962.
8 A. N. Blokhin, A. B. Razina, M. P. Kurlykin, A. V. Kashina and A. V. Tenkovtsev, *Mendeleev Commun.*, 2020, **30**, 799.
9 A. I. Amirova, T. U. Kirila, A. N. Blokhin, A. B. Razina, A. E. Bursian, A. V. Tenkovtsev and A. P. Filippov, *Mendeleev Commun.*, 2020, **30**, 502.
10 S. Nemati Mahand, S. Aliakbarzadeh, A. Moghaddam, A. Salehi Moghaddam, B. Kruppke, M. Nasrollahzadeh and H. A. Khonakdar, *Eur. Polym. J.*, 2022, **178**, 111484.
11 R. Hoogenboom, H. M. L. Thijs, M. J. H. C. Jochems, B. M. van Lankvelt, M. W. M. Fijten and U. S. Schubert, *Chem. Commun.*, 2008, 5758.
12 B. Trzebicka, R. Szweda, D. Kosowski, D. Szweda, Ł. Otulakowski, E. Haladjova and A. Dworak, *Prog. Polym. Sci.*, 2017, **68**, 35.
13 S. S. Patkar, Y. Tang, A. M. Bisram, T. Zhang, J. G. Saven, D. J. Pochan and K. L. Kiick, *Angew. Chem., Int. Ed.*, 2023, **62**, 1331.
14 A. A. Vdovchenko, A. V. Hubina, E. G. Vlakh and T. B. Tennikova, *Mendeleev Commun.*, 2017, **27**, 153.
15 D. Liu and J. Sun, *Polymers*, 2020, **12**, 2973.
16 N. N. Sudareva, I. I. Tarasenko, D. N. Suslov, O. M. Suvorova, K. A. Kolbe, G. Y. Yukina, M. L. Tyndyk, Yu. G. Zmitrichenko and E. G. Korzhikova-Vlakh, *Mendeleev Commun.*, 2024, **34**, 18.
17 M. Stepanova, A. Nikiforov, T. Tennikova and E. Korzhikova-Vlakh, *Pharmaceutics*, 2023, **15**, 2641.
18 J. Cheng and T. J. Deming, in *Peptide-Based Materials*, ed. T. Deming, Springer, Berlin, Heidelberg, 2012, vol. 310, pp. 1–26.
19 T. J. Deming, *Chem. Rev.*, 2016, **116**, 786.
20 A. Yu. Dzhuzha, I. I. Tarasenko, L. I. Atanase, A. Lavrentieva and E. G. Korzhikova-Vlakh, *Int. J. Mol. Sci.*, 2023, **24**, 1049.
21 R. Wilder and S. Mobashery, *J. Org. Chem.*, 1992, **57**, 2755.

Received: 16th January 2024; Com. 24/7371