

Scientific paper

Binding of Cetyl- and Dodecylpyridinium Cations by Poly (L – Glutamic Acid)[†]

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[†]Dedicated to Prof. Dr. Jože Škerjanc on the occasion of his 70th birthday

Abstract

The cooperative binding of cetyl-(CPC) and dodecylpyridinium (DPC) chloride by poly (L – glutamic) acid (PGA) and their effect on the conformation of PGA in aqueous solution without added simple salts was studied by the potentiometric and circular dichroism (CD) measurements. The degree of neutralization of the polyelectrolyte solution was $\alpha = 0.25, 0.50, 0.75$ and 1.0 . The concentration of the polyelectrolyte solutions was 5.0×10^{-4} monomol/kg and the measurements were carried out at 25.0 °C. Binding isotherms have shown that while the binding of DPC to PGA is negligible, the opposite is true for CPC. The cooperative binding of CPC to the polyion starts at a total surfactant concentration below 5×10^{-4} mol/kg, which is almost two orders of magnitude lower than its cmc. This result is independent of the degree of neutralization of the polyelectrolyte. It was also found that the amount of binding β depends almost linearly on α . In the case of fully ionized polyelectrolyte, β is approximately 1. By extrapolating β to $\alpha = 0$ it was shown that there is some cooperative binding of CPC also by the unionized polyacid. CD measurements showed that both surfactants induce partial helical structure of the polyelectrolyte with DPC being somewhat more effective. This finding is attributed to a smaller size of the DPC micelle.

Keywords: Poly (L-glutamic acid), degree of neutralization, cetyl- and dodecylpyridinium cations, potentiometry, binding isotherms, circular dichroism, conformational change

1. Introduction

Since the work of Zimm and Rice, synthetic polypeptides have been extensively investigated due to their potency to serve as model systems for proteins, many of which are known to be partially helical. Poly (L – glutamic acid) (PGA) exhibits a transition from an uncharged helix at pH 4 to a charged random coil at pH greater than 8 in aqueous solution, with both conformations present in the transition region. In addition to the pH induced transition,^{2–5} the same conformational change can be induced also by e.g. varying the temperature,^{6,7} solvent composition and counterion species,^{8–12} all of which were studied by different experimental techniques.

Interactions between this anionic polyelectrolyte and oppositely charged ionic surfactants were also studied.^{13–20} These interactions are influenced by the charge density of the polyion, the hydrophobic character of the surfactant and possible additional forces between surfactant micelles and charged polyion. As a consequence of

all this, the binding of ionic surfactants to the polyion can start at a surfactant concentration that is much lower than its critical micelle concentration, cmc. In addition, surfactants can induce conformational transition in the case of polyelectrolytes which have secondary structure. These phenomena can be studied by potentiometric techniques with surfactant ion selective electrodes and by measuring circular dichroism spectra.

In the case of PGA it was shown for example that decylammonium chloride, dodecyltrimethyl- and octadecyltrimethylammonium chloride,^{13–15} induce conformational transition from coil to helix. The same holds for dodecylammonium chloride, while cetyltrimethylammonium chloride stabilizes random conformation of this polyelectrolyte.¹³ This effect depends on the chain length and on the head group of the surfactant. Surfactants with longer chain lengths and fewer methyl groups have stronger ability to induce helical conformation of PGA.^{16–18}

In the present work we studied binding of two cationic surfactants cetylpyridinium (CPC) and dodecylpyri-

dinium (DPC) chloride by PGA as a function of the degree of ionization of this polyelectrolyte. Binding isotherms were constructed for this purpose using potentiometric technique. In addition, the ability of these two surfactants to induce conformational transition of PGA was studied by measuring the circular dichroism spectra.

2. Experimental

2.1. Materials

Sodium salt of the poly(L – glutamic acid) (Na – PGA) was used (DP = 480, Sigma Chemical Company) without further purification. A dilute water solution of the sample of Na – PGA was passed through the cation exchange column with an excess of resin in the H⁺ form (Amberlite IR 200, Merck) in order to remove Na⁺ ions. Poly (L – glutamic acid) (PGA) thus obtained was neutralized with CO₂ – free solution of NaOH from the initial pH of about 4 (at $\alpha = 0$, helix) to the desired degree of neutralization as described previously.⁵ The neutralization followed immediately after ion exchange since the precipitation occurs in the PGA solution with $\alpha = 0$ on storage. In present experiments, the degrees of neutralization of the polypeptide were $\alpha = 0.25, 0.50, 0.75$ and 1.0. The solution of acid, HPGA, alone ($\alpha = 0$) was not used in the experiments since very soon the unionized polymer precipitates out of the solution.

The concentrations of the polymer solutions, expressed as molalities, m_p (i.e. monomols of carboxylic acid groups per kg of solvent) were determined potentiometrically under N₂ atmosphere with CO₂ – free solution of 0.100 M NaOH as the titrant. The concentration of the polyelectrolyte solution used in the experiments was 5.0×10^{-4} monomol/kg.

N-Dodecylpyridinium chloride DPC (Merck-Schuchardt, C₁₇H₃₀NCl, cmc = 1.52×10^{-2} M in water at 25 °C), and N-Cetylpyridinium chloride CPC (Kemika Zagreb, C₂₁H₃₈NCl, cmc = 6.3×10^{-4} M in water at 25 °C) were used after purification by repeated recrystallization from acetone.

Distilled water with electrical conductivity less than $1 \times 10^{-6} \Omega^{-1} \text{cm}^{-1}$ was used throughout solution preparation and experiments.

2.2. Methods

Binding isotherms were determined by using the potentiometric technique. PVC membrane electrodes¹⁹ selective for DP⁺ and CP⁺ ions were used. The potential difference between the surfactant and a suitable reference electrode was measured in surfactant solutions in the absence and in the presence of the polyelectrolyte (with various degrees of neutralization that were kept constant throughout the experiment). The concentrations of the surfactant solutions were in the range from 1.0×10^{-6} to

approximately 1.0×10^{-3} mol/kg. The concentration of the polyelectrolyte solution was kept constant at 5.0×10^{-4} monomol/kg. Temperature was $25.0 \text{ °C} \pm 0.1 \text{ °C}$.

CD measurements were carried out with the AVIV Circular Dichroism Spectrometer 62A DS. The optical path was 0.100 cm and the temperature was $25.0 \text{ °C} \pm 0.1 \text{ °C}$. Specific molar ellipticity at 222 nm, $(\Phi)_{222}$, was calculated and the helix content in the presence of surfactant was estimated from these measurements.

3. Results and Discussion

In order to obtain the amount of surfactant binding to polyelectrolyte, the potential difference, E , of the surfactant electrode against the reference calomel electrode was measured as a function of the surfactant concentration (molality). Potentiometric measurements were performed first in surfactant solutions without added polyelectrolyte to determine the calibration curve (Fig. 1). The response of both surfactant electrodes was Nernstian from around 1×10^{-6} mol/kg up to the surfactant's cmc (note that above the cmc the calibration curve shows a break). Next,

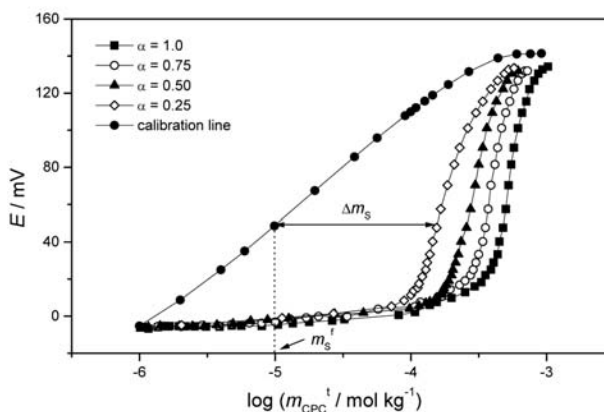


Figure 1. Plots of E of the cell with the CPC surfactant electrode as a function of the total surfactant concentration m_{CPC}^t in solutions with added poly(L – glutamic acid) with different degree of neutralization α . The concentration of the polyelectrolyte solution was 5.0×10^{-4} monomol/kg; m_s^t is the equilibrium concentration of the free CP⁺ ions and Δm_s is the amount of CP⁺ ions, bound by the polyelectrolyte PGA.

potentiometric curves were measured in solutions in the presence of poly (L – glutamic acid) with different degrees of neutralization. Plots of E versus the total surfactant concentration, m_s^t , for different degrees of neutralization of the polyelectrolyte show characteristic deviations from the calibration curve. This is clearly seen in the CPC case, indicating substantial binding of this surfactant by PGA (see Fig. 1). However, in the case of DPC such measurements indicate that there is only a negligible amount of binding of DPC by PGA (Fig. 2).

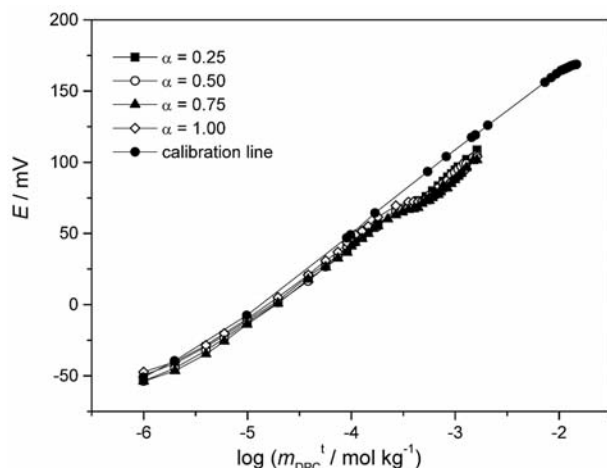


Figure 2. Plots of E of the cell with the DPC surfactant electrode as a function of the total surfactant concentration m_{DPC}^t in solutions with added poly(L – glutamic acid) with different degree of neutralization α . The concentration of the polyelectrolyte solution was 5.0×10^{-4} monomol/kg.

From these plots, the amount of cationic surfactant, Δm_s , bound per ionic group of poly(L – glutamic acid) was determined. Δm_s is the difference between the total surfactant concentration in solution, m_s^t , and the corresponding concentration of the free surfactant, m_s^f , which was obtained from the calibration curve as shown in Fig. 1. The amount of binding β of the CPC to PGA was calculated according to the well known relation:

$$\beta = \frac{\Delta m_s}{m_p} = \frac{m_s^t - m_s^f}{m_p} \quad (1)$$

In this equation, m_p is the molality of the polyelectrolyte solution. Parameter β gives the fraction of the functional groups on the polyion that are occupied by surfactant ions.

Interactions between surfactant and polyelectrolyte can be best viewed from the binding isotherms, i.e. plots of the amount of binding β vs. molal concentration of the free surfactant m_s^f . Fig. 3 represents the binding isotherms for the binding of the surfactant CPC by PGA at different α values.

Binding isotherms for the CPC binding to PGA that are plotted in Fig. 3 clearly show the initial steep rise of β in a narrow region of the free surfactant concentration. This type of behavior is characteristic for the cooperative binding of surfactant cations to polyions. In addition to electrostatic forces there are obviously also hydrophobic interactions between the bound surfactant cations that are responsible for this process.

It is evident that the binding of CPC to PGA starts at approximately the same free surfactant concentration, i.e. at 2×10^{-6} mol/kg, for all α . The total surfactant concentration at this point is 1.0×10^{-5} mol/kg, which is almost

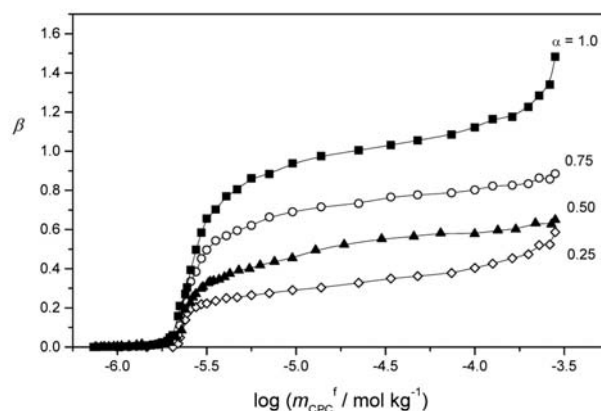


Figure 3. Binding isotherms for the binding of the CP^+ cations by PGA with different degrees of neutralization α at 25.0°C . The concentration of the polyelectrolyte solution was 5.0×10^{-4} monomol/kg.

two orders of magnitude below the cmc value for this surfactant ($\text{cmc} = 6.3 \times 10^{-4}$ M in water at 25°C). Compared to other polyelectrolytes, the binding of CP^+ to PGA starts at approximately 10 and 100 times higher free surfactant concentration as in the case of poly(acrylic acid), PAA, or sodium polystyrenesulfonate, respectively.²⁰ Plots of binding isotherms for the DPC case are not shown. However, it can be deduced from the curves in Fig. 2 that the situation is similar as in the CPC/PGA system: appreciable binding of DPC to PGA starts at around 2×10^{-4} moles DPC/kg, irrespective of α . It is somewhat surprising that this concentration threshold does not depend on the charge density of the polyelectrolyte, as would be expected according to the Manning theory,²¹ especially at higher values of α . On the other hand, it has been found also for the binding of CPC by PAA²⁰ that the onset of binding does not depend on α . This feature could be explained by taking into account nonelectrostatic forces in addition to the pure electrostatic ones.⁵

The initial slopes of binding isotherms and the amount of binding at higher free CP^+ concentrations do depend on α . At higher free surfactant concentration the curves in Fig. 3 level off. Values of β in the plateau region as well as the initial slopes of binding isotherms, increase with increasing α . This point is further stressed in Fig. 4, where the plateau values of β at the free surfactant concentration of 2.2×10^{-5} mol kg^{-1} are plotted as a function of α .

As seen, the amount of binding β depends almost linearly on α . In the case of the fully ionized polyelectrolyte ($\alpha = 1.0$), β is approximately 1, indicating that all carboxylic groups on PGA are occupied by the CP^+ surfactant cations. The same holds also for example in the case of decylammonium chloride binding to the fully ionized PGA. Among other polyelectrolytes, CP^+ cations bind completely for example to sodium polystyrenesulfonate, NaPSS, whereas in the case of polyacrylate anion, PA^- , with $\alpha = 1$, the value of β is 0.79, which means that

approximately 21% of the charged groups on PA^- are not associated with surfactant cations.²⁰

Extrapolation of the curve in Fig. 4 to $\alpha = 0$ gives the value of β approximately 0.2. Unfortunately this value cannot be verified experimentally since the solution of PGA at $\alpha = 0$ is not stable long enough for accurate measurements. However, this value of β implies that around 20% of the functional groups are occupied by surfactant also in the case of the unionized PGA.

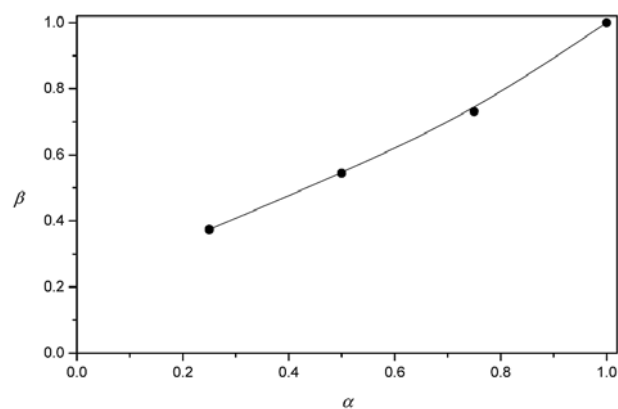


Figure 4. The amount of binding β of the CP^+ cations in the plateau region of binding isotherms (at a free surfactant concentration $m_{\text{CPC}}^f = 2.2 \times 10^{-5} \text{ mol kg}^{-1}$) as a function of the degree of neutralization α of the poly(L – glutamic acid).

This result is not surprising, since similar observation is reported also for example in the case of surfactant binding by polyacrylic acid.²⁰ This effect is observed even in the case of binding by uncharged water soluble polymers²² and can be attributed to hydrophobic interactions between surfactant and nearly uncharged PGA in the present case. Besides, it has been demonstrated that the addition of an oppositely charged surfactant increases the degree of ionization of unneutralized or partly neutralized polyacrylic acid,²³ and atactic and isotactic poly(methacrylic acid).²⁴ Similar influence of surfactant on self-ionization can be expected also in the PGA case.

Additional insight into the process of binding is possible with the aid of Fig. 5, which shows the degree of binding, b , as a function of the total surfactant concentration for different α values. The value b is related to the value of β by the following simple equation:

$$b = \frac{\Delta m_s}{m_s^t} = \beta \frac{m_p}{m_s^t} \quad (2)$$

From Fig. 5 one can see the effect of the polyelectrolyte charge density on CP^+ binding by PGA. In the case of the fully ionized polyelectrolyte ($\alpha = 1$) the binding is 100% in a broad concentration region up to the equivalence point, where the polyelectrolyte and total surfactant

concentration are equal (i.e. $5.0 \times 10^{-4} \text{ monomol/kg}$). This region becomes narrower with decreasing value of α . Thus, the degree of binding is only approximately 50% at the equivalence point in the case of $\alpha = 0.25$.

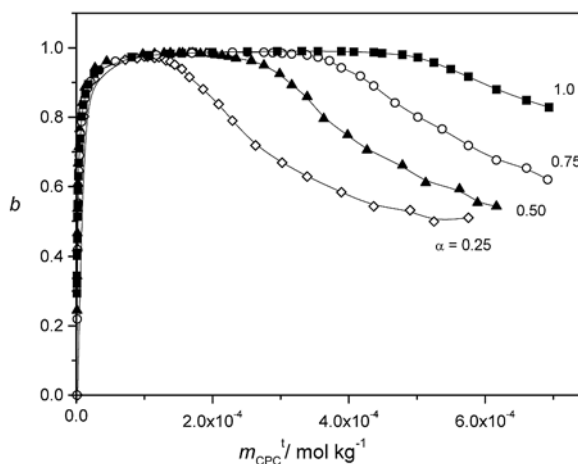


Figure 5. The degree of binding, b , of the CP^+ cations by poly(L – glutamic acid) with different degree of neutralization α , as a function of the total surfactant concentration, m_{CPC}^t .

It has been pointed out in the Introduction that the presence of surfactant ions can induce the conformational change of the polyelectrolyte if the latter has a secondary structure. Therefore, we investigated such possibility also in the case of PGA by measuring the CD spectra in the absence and in the presence of DPC and CPC. First we determined the CD calibration diagram for PGA, i.e. the mean residue ellipticity at 222 nm, $[\theta]_{222}$, as a function of the degree of neutralization α of this polyelectrolyte (see example of such diagram, Fig. 6).

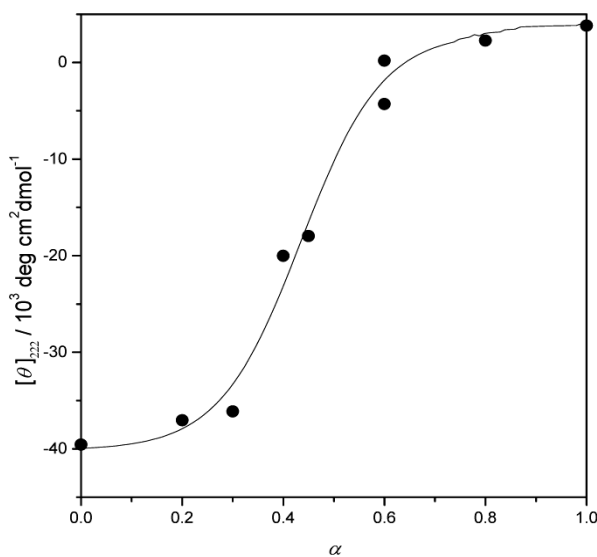


Figure 6. Mean residue ellipticity $[\theta]_{222}$ at 222 nm as a function of the degree of neutralization α of poly(L – glutamic acid) at 25.0 °C.

From the plot in Fig. 6 we estimated⁵ the fraction of the helical content, f_H , according to the following relation:¹³

$$f_H = \frac{[\theta]_{222}^C - [\theta]_{222}^H}{[\theta]_{222}^H - [\theta]_{222}^C} \quad (3)$$

In this equation, $[\theta]_{222}^C$ and $[\theta]_{222}^H$ are the values of the mean residue ellipticity for the polyelectrolyte in the fully ionized (coil) and in the helical conformation, respectively. Clearly, the content of helix increases with decreasing value of the mean residue ellipticity.

In the experiment, the solution of surfactant was gradually added to the 5.0×10^{-4} monomol/kg solution of the fully ionized polyion ($\alpha = 1$) and the mean residue ellipticity at 222 nm $[\theta]_{222}$ was determined from the measured CD spectra. Fig. 7 represents the dependence of $[\theta]_{222}$ on the surfactant/polyelectrolyte mixing ratio (m_s/m_p) for both surfactants, DPC and CPC, up to equivalence point. Higher mixing ratios were not considered because strong precipitation occurs for $m_s/m_p > 1$.

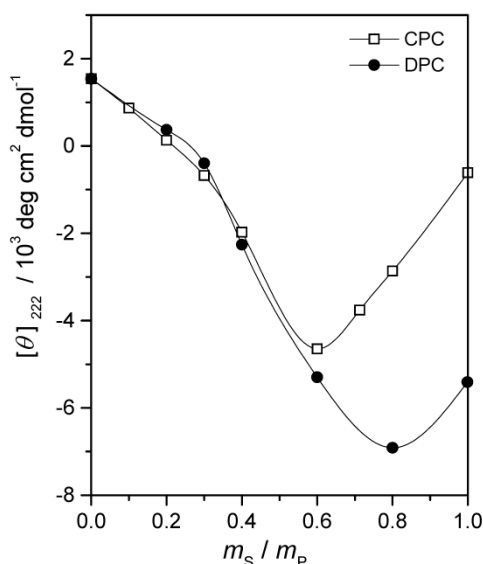


Figure 7. The dependence of the mean residue ellipticity, $[\theta]_{222}$, on the surfactant/polyelectrolyte mixing ratio, m_s/m_p , for CPC and DPC at 25.0 °C. Surfactant was added to the fully ionized poly(L-glutamic acid).

From $[\theta]_{222}$ the content of the helical conformation of the PGA in the presence of surfactant was estimated. It follows from Fig. 7 that both surfactants induce partial helicity of the polyion immediately after the addition. In the case of CPC, the largest portion of the helical content is around 16% for the sample with a mixing ratio 0.6. For DPC, this value is larger (around 24%) and occurs at a slightly higher mixing ratio (~ 0.8). This result is surprising since there is only very little cooperative binding of

DPC to PGA as indicated by potentiometric measurements (see Fig. 2). On the other hand, the inducing power of both surfactants is weak, and can be influenced by various factors. As known from the literature,¹⁷ the neutralization of the polyelectrolyte charge by surfactant binding alone is not enough to induce ordered helical conformation of the polyelectrolyte; hydrophobic interactions are also required for this process to occur. Cetyltrimethylammonium chloride for example stabilizes the random conformation of PGA, while dodecylammonium chloride stabilizes the ordered helical conformation at higher mixing ratios.¹³ In the latter case the transition was stepwise. Such stepwise change is indicated also in Fig. 7 for CPC and DPC at mixing ratio approximately 0.3. The same behaviour was found for dodecylmethylammonium chloride DDAC and dodecylmethylammonium chloride DMAC binding to PGA.¹⁷ Induction of the helix occurs also in the case of dodecyltrimethylammonium chloride DTAC,¹⁶ but only under very limited conditions. One of the possible reasons for the described behaviour in our case could be that CPC, with a longer hydrocarbon chain, forms bigger micelles than DPC.

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5. References

1. B. H. Zimm, S. A. Rice, *Mol. Phys.* **1960**, *3*, 391–407.
2. A. Wada, *Mol. Phys.* **1960**, *3*, 409–415.
3. J. H. Rai, W. G. Miller, *Biopolymers* **1973**, *12*, 845–856.
4. F. Bordi, C. Canetti, G. Paradossi, *J. Phys. Chem.* **1992**, *96*, 913–918.
5. A. Godec, J. škerjanc, *J. Phys. Chem. B* **2005**, *109* (27), 13363–13367.
6. G. Santiago, R. C. Maroun, E. R. Hawkins, W. L. Mattice, *Biopolymers* **1981**, *20*, 2181–2194.
7. A. Warashima, A. Ikegami, *Biopolymers* **1972**, *11*, 529–547.
8. G. D. Fasman, C. Lindblow, E. Bodenheimer, *Biochemistry* **1964**, *3*, 155–166.
9. G. Conio, E. Patrone, *Biopolymers* **1969**, *8*, 57–68.
10. M. Morcellet, C. Loucheux, *Biopolymers* **1980**, *19*, 2177–2190.
11. H. Daoust, D. St-Cyr, *Polymer J.* **1982**, *14*, 831–838.
12. M. Satoh, Y. Fujii, F. Kato, J. Komiyama, *Biopolymers* **1991**, *31*, 1–10.
13. W. L. Mattice, R. W. McCord, P. M. Shippey, *Biopolymers* **1979**, *18*, 723.
14. H. Maeda, H. Kato, S. Ikeda, *Biopolymers* **1984**, *23*, 1333–1346.
15. I. Satake, T. Gondo, H. Kimizuka, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 361–364.

16. H. Maeda, K. Fujio, S. Ikeda, *Colloid Polymer Sci.* **1988**, 266, 248–253.
17. K. Hayakawa, T. Nagahama, I. Satake, *Bull. Chem. Soc. Jpn.* **1994**, 67, 1232–1237.
18. H. Maeda, T. Nezu, K. Fukada, S. Ikeda, *Macromolecules* **1988**, 21, 1154–1158.
19. K. Hayakawa, J. C. T. Kwak, *J. Phys. Chem.* **1982**, 86, 3866–3870.
20. K. Kogej, J. Škerjanc, in: T. Radeva (Ed.): *Physical Chemistry of Polyelectrolytes*, Marcel Dekker, Inc., New York – Basel, **2001**, pp. 793–827
21. G. S. Manning, *J. Chem. Phys.* **1969**, 51, 924–933.
22. B. Cabane, *J. Phys. Chem.* **1977**, 81, 1639–1645.
23. K. Kogej, *J. Phys. Chem. B* **2003**, 107, 8003–8010.
24. N. Vlachy, J. Dolenc, B. Jerman, K. Kogej, *J. Phys. Chem. B* **2006**, 110, 9061–9071.

Povzetek

Raziskali smo kooperativno vezanje surfaktantov cetil- (CPC) in dodecilpiridinijevega klorida (DPC) na poliglutaminsko kislino (PGA) ter njun vpliv na konformacijo tega polielektrolita v vodnih raztopinah brez dodatka soli. Pri raziskavah smo uporabili potenciometrično titracijo in meritve cirkularnega dikroizma pri 25.0 °C. Stopnje nevtralizacije polielektrolita α so bile 0.25, 0.50, 0.75 in 1.0, koncentracija pa 5.0×10^{-4} mol/kg. Potenciometrične meritve so pokazale, da je v primeru DPC vezanje na PGA šibko, v primeru CPC pa močno. Kooperativno vezanje surfaktanta CPC na PGA se začne že pri totalni koncentraciji surfaktanta pod 5×10^{-4} mol/kg, kar je skoraj dva reda velikosti manj od vrednosti cmc za ta surfaktant. Ta začetek je tudi neodvisen od stopnje nevtralizacije polielektrolita. Stopnja vezanja β narašča skoraj linearno v odvisnosti od α . V primeru popolnoma ioniziranega polielektrolita znaša β okrog 1. Z ekstrapolacijo na $\alpha = 0$ smo ugotovili, da se kooperativno vezanje CPC pojavi tudi v primeru neioniziranega polielektrolita. Meritve cirkularnega dikroizma so pokazale, da oba surfaktanta inducirata delno heličnost polielektrolita, pri čemer je DPC za malenkost učinkovitejši. Tak rezultat smo pripisali manjšim micelam DPC.