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ORIGINAL CONTRIBUTION

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Effect of the secondary structure of poly-L-lysine on the adsorption at the water/dodecane interface

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Abstract The adsorption of poly-L-lysine of different conformation (α-helix, β-sheet and random coil) at the water/dodecane interface has been studied by interfacial tension measurements using the drop volume method. The experimental adsorption isotherms provide information about a critical aggregation concentration, the maximum interfacial tension depression $\Delta\gamma_{max}$ and the minimum area A occupied by adsorbed molecules at the interface. Differences in $\Delta\gamma_{max}$ exist between α-helix and β-sheet and, moreover, in the area values between random-coil on the one side and α-helix and β-sheet on the other hand.

Key words Polypeptide – secondary structure – interfacial tension – adsorption isotherm – circular dichroism

Introduction

Poly-L-lysine is a useful model substance to study different secondary structures of polypeptides. The poly-L-lysine is a homopolymer, which means it consists of only one kind of amino acid residue. Moreover, in aqueous solution it can exist in an α-helix, in a β-sheet or random coil state. It is essential that the α-helix and the β-sheet conformation can exist under exactly the same conditions (pH, temperature, ion strength) [1].

The packing of different polypeptides at the air/water interface was already studied by different authors [2–4], while investigations at the oil/water interface are very scarce so that only little is known at the molecular level [5]. In many investigations globular proteins are only used and differences in the secondary structure are not of crucial interest. On the other hand, the secondary structure of polypeptide chains is of general interest as adsorption process of peptides and proteins at fluid hydrophobic/hydrophilic interfaces occur in nature and play an important role in numerous technological applications.

The aim of the present paper is to demonstrate the effect of the secondary structure of poly-L-lysine on the adsorption behavior at the water/dodecane interface. The adsorption is determined from interfacial tension measurements with the drop volume technique.

Materials and methods

The poly-L-lysine hydrobromides were purchased from SIGMA Chemical Corp. The following two samples were used (data from SIGMA):

Average molecular weight from viscosity (M_w)	Degree of polymerization from viscosity (n)	Polydispersity from LALLS (M_w/M_n)
288 400	1380	1.12
40 760	195	1.23

The *n*-dodecane was purchased from SIGMA and used without further purification.

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Preparation of poly-L-lysine solutions

The aqueous solutions were made with doubly distilled water. The weighing loss of poly-L-lysine was 2.39 10^{-2} M of amino acid residue equal for all of the samples with different molecular weight (stock solutions). In these stock solutions the secondary structure was established according to the directions by Greenfield et al. [6, 7]:

α-helix type	pH = 11.5	T = 22 °C
β-sheet type	pH = 11.5	solution heated for 25 min at 50 °C and then cooled down to 22 °C
random coil type	pH = 4.5	T = 22 °C

The pH = 11.5 was adjusted with 0.1 M NaOH, the pH = 4.5 with 0.1 M HCl. After the preparation, all solutions were kept at 22 °C for 3 h before use. The stock solutions were diluted with doubly distilled water to the concentration for each of the measurements.

Measurements of circular dichroism

The circular dichroism (CD) measurements have been used to assess the extent of the different secondary structure formation by poly-L-lysine in water solution which were performed with a Jasco J 600 spectrometer at 22 °C. The poly-L-lysine concentration was 0.02 wt-%, the layer thickness was 0.1 cm.

Interfacial tension measurements

Adsorption behavior was studied by interfacial tension measurements using the dynamic version of the drop volume tensiometer TVT1 from LAUDA. Details about the handling are given elsewhere [8, 9]. The drop volume technique is advantageous for studies at liquid/liquid interfaces. Moreover, if only small amounts of substance are available the drop volume technique is one of the methods of choice. As capillaries those with a conical shape made of Teflon were chosen. The radius of the capillary was 0.668 mm. Measurements with steel failed because of indefinite wetting which led to interfacial tension values being sometimes even higher than expected for the pure water/dodecane system (52.8 mN/m [10]). All measurements were carried out at 22 °C. Every interfacial tension value is a mean value consisting of three measurements and has an accuracy of 0.1 mN/m.

Results and discussion

All three secondary structures of the poly-L-lysine samples with two different degrees of polymerization were prepared by the given procedures (Fig. 1). The CD spectra are given in Fig. 2. In some cases the formation of the specific structure is not complete and depends on the degree of polymerization. A comparison of the CD spectra with those of Greenfield and Fasman [7] is given in Table 1. From the results obtained it follows that the largest amount of α-helix of poly-L-lysine is formed at $n = 1380$. Using an empirical formula given in [7], the content of α-helix (Fig. 2, curve 1) can be calculated to be

$$\frac{[\Theta]_{208} - 4.0}{33.0 - 4.0} * 100 = 89.7\% \,, \tag{1}$$

where $[\Theta]$ is the ellipticity per amino acid residue.

Under the same conditions, at $n = 195$ poly-L-lysine forms a mixture of 64% α-helix, 16% β-sheet and 20%

Table 1. Circular dichroism spectra of the three secondary structures of poly-L-lysine

Data from Fig 2			Data from ref. [7]		
Figure/curve	Wavelength λ [nm]	$[\Theta]$ 10^{-3} deg cm²/dmole	Wavelength λ [nm]	$[\Theta]$ 10^{-3} deg cm²/dmole	Conformation
2/1	191.0	68.0	191.0	76.9 ± 8.4	100% α-helix
	208.5	− 30.0	208.0	− 32.6 ± 4.0	
	221.5	− 34.0	222.0	− 35.7 ± 2.8	
2/2	193.0	35.0	195.0	31.9 ± 5.0	100%
	215.0	− 18.0	217.0	− 18.4 ± 1.8	β-sheet
2/3	196.0	− 42.0	197.0	− 41.9 ± 4.0	100%
	217.0	4.0	217.0	4.6 ± 0.5	random coil
2/4	191.0	48.0	191.0	43.6	64% α-helix
	206.5	− 24.0	208.0	− 22.0	+ 16% β-sheet
	220.5	− 23.5	220.0	− 24.0	+ 20% r.coil
2/5	195.0	10.0	193.0	13.0	24% α-helix
	218.5	− 12.0	218.0	− 12.0	+ 36% β-sheet
					+ 40% r.coil

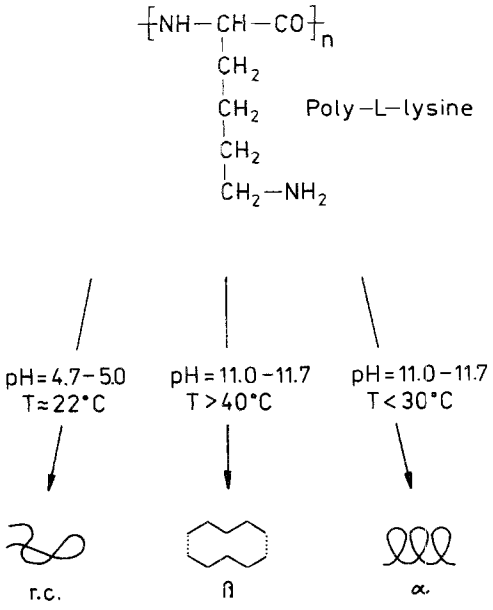


Fig. 1 Scheme of the formation of the three different secondary structures α-helix, β-sheet and random coil

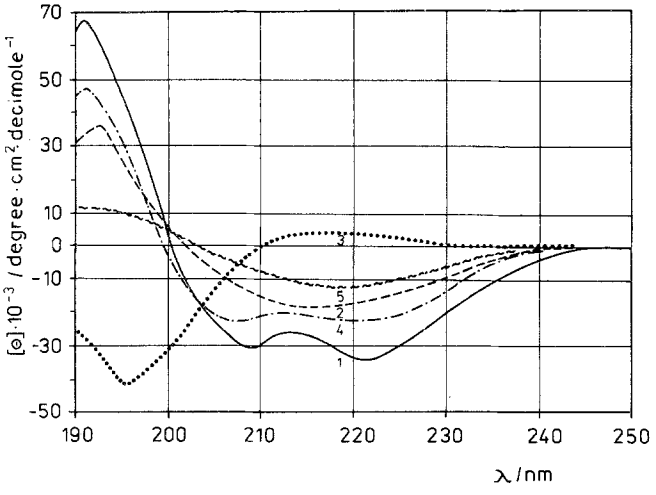


Fig. 2 Circular dichroism of poly-L-lysine (1) $n = 1380$, pH = 11.5, $T = 22\,°C$ (2) $n = 195$, pH = 11.5, $T = 50\,°C/25$ min → $T = 22\,°C$ (3) $n = 195$, pH = 4.5, $T = 22\,°C$ (4) $n = 195$, pH = 11.5, $T = 22\,°C$ (5) $n = 1380$, pH = 11.5, $T = 50\,°C/25$ min → $T = 22\,°C$

random coil (cf. Fig. 2, curve 4; Table 1). At $n = 1380$ the β-sheet type is formed only to an amount of 36%, mixed with 24% α-helix and 40% random coil structures (cf. Fig. 2, curve 5; Table 1). In contrast, at $n = 195$ poly-L-lysine exclusively forms β-sheets (cf. Fig. 2, curve 2, Table 1). Under the corresponding conditions, the same sample completely forms the random coil structure (cf. Fig. 2, curve 3; Table 1).

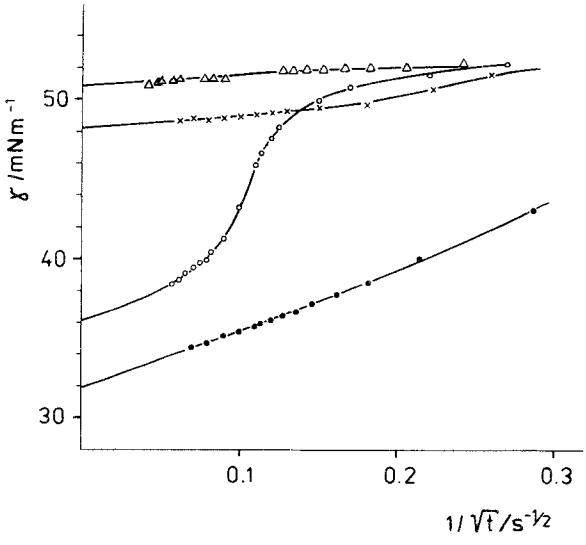
Hence, solutions with polypeptide having a complete secondary structure do not exist for one and the same degree of polymerization. Therefore, the following samples are used for the adsorption studies of the effect of the different secondary structure:

poly-L-lysine, $n = 1380$, forms 90% α-helix in solution at 22 °C and pH = 11.5
poly-L-lysine, $n = 195$, forms 100% β-sheet in solution at 22 °C and pH = 11.5
poly-L-lysine, $n = 195$, forms 100% random coil in solution at 22 °C and pH = 4.5

The polypeptide adsorption was determined from measurements of the interfacial tension γ as a function of bulk concentration. As the adsorption of the polypeptide requires longer time the equilibrium data are extrapolated from dynamic interfacial tensions obtained in the time interval between 10 and 250 s following the procedure proposed in [11]. Examples of these extrapolations are given in Fig. 3. The behavior of the γ-plots in the midrange of the bulk concentration (for example $6.76\,10^{-4}$ mole residue) is proved by a diffusion controlled adsorption. The course of the $\gamma(1/\sqrt{t})$-dependencies is known also for surfactants and is in agreement with the theory [11].

The time dependence of the interfacial tension of a pure water/dodecane system is also shown in Fig. 3. Although the values are lower than those given in the literature, the time dependence does not show any remarkable decrease within the time interval of the experiments. Therefore, aging effects as observed with poly-L-lysine solutions can

Fig. 3 Interfacial tension of aqueous poly-L-lysine ($n = 195$) solutions at the water/dodecane interface as a function of drop formation time t at different concentrations: C = 0.0 (△). $6.76\,10^{-5}$ mole residue (×), $6.76\,10^{-4}$ mole residue (○), $2.24\,10^{-3}$ mole residue (●) per liter



be attributed only to the poly-L-lysine adsorption at the interface.

The extrapolated equilibrium interfacial tension data for poly-L-lysine of all three secondary structures are summarized in Fig. 4. From these isotherms the maximum interfacial tension depression $\Delta\gamma_{max}$, a critical aggregation concentration (cac), and the minimum area of an adsorbed segment of the molecules can be deduced. The lowest interfacial tension is observed for the α-helix. The critical aggregation concentrations for α-helix and β-sheet are similar, whereas that for the random coil structure is higher (cf. Table 2).

The minimum area A occupied by an adsorbed molecule can be calculated using Gibbs' adsorption equation [12–14],

$$\Gamma_\infty = -\frac{1}{RT}\left(\frac{d\gamma}{d\ln c}\right)_{c\to cac}, \quad (2)$$

from which the area A per molecule or residue results,

$$A = 1/N_L\Gamma_\infty . \quad (3)$$

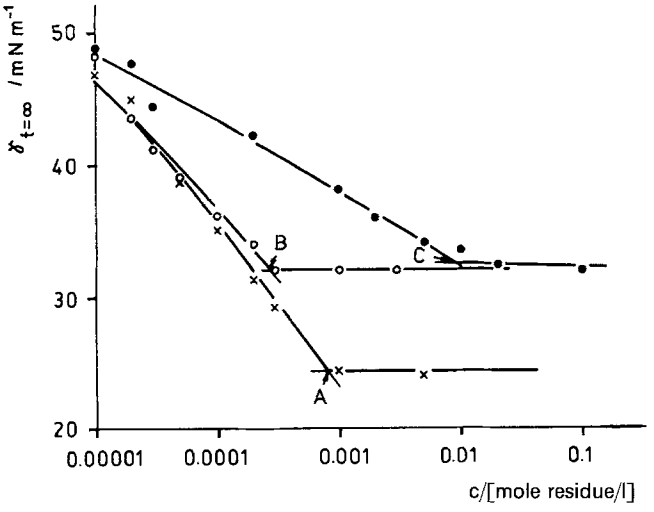


Fig. 4 Interfacial tension isotherms of poly-L-lysine at the water/dodecane interface (extrapolated values, cf. text) for different secondary structures: α-helix (×), β-sheet (○), random coil (●); A, B, and C mark the respective critical aggregation concentrations (cac), solid lines below the cac – fitted Langmuir isotherm

Table 2. Adsorption parameters for the three secondary structures of poly-L-lysine obtained from the respective adsorption isotherms (Fig. 4)

Parameter	α-Helix	β-Sheet	Random coil
$\Delta\gamma_{max}$ [mN/m]	27	19	19
cac [mole residue/l]	$1.58\ 10^{-4}$	$4.79\ 10^{-4}$	$1.26\ 10^{-3}$
Γ_∞ [mole residue/cm^2]	$2.44\ 10^{-10}$	$2.11\ 10^{-10}$	$1.04\ 10^{-10}$
A[Å^2/residue]	68	79	160

N_L is the Avogadro number. The values of A are similar for α-helix and β-sheet while again the value for the random coil structure differs remarkably.

Conclusions

At the water/dodecane interface the poly-L-lysine α-helix and β-sheet areas are 68 and 79 Å^2 per residue, respectively. Compared with other polypeptides, for example, with β-lactoglobulin at the water/air interface, studied with a Langmuir film balance, the area per residue amounts to 23.1 and 21.0 Å^2 at 3 and 5 mN/m surface pressure, respectively [15]. That is of the same order of magnitude.

The poly-L-lysine random coil has a maximum coverage density significantly lower than the poly-L-lysine α-helix or the β-sheet, which is certainly caused by electrostatic repulsion between the random coil molecules because the ε-amino side chain groups of the poly-L-lysine are protonated at pH = 4.5. At pH = 11.5 this repulsion disappears as the pK is about 10.0 [16]. Thus, the random coil state cannot be directly compared with the α-helix and the β-sheet state. A direct comparison is possible for the α-helix and the β-sheet state, where the secondary structures are established under exactly the same bulk conditions (pH, ion strength, temperature), Also, the conditions in the adsorption layer are very similar as one can see from the shape of the adsorption isotherms (cf. Fig. 4). But there are essential differences: a) The area per residue is almost 10 Å^2 smaller by the α-helix as by the β-sheet. b) The α-helix conformation yields a greater interfacial tension depression as the β-sheet conformation. For the area difference the more compact structure of the α-helix could be responsible, provided that the poly-L-lysine secondary structure was not destroyed by adsorption at the interface. But this is an open question. When a polypeptide or protein reaches an oil/water interface, the molecules unfold into a chain conformation in which nonpolar domains are predominantly directed toward the oil phase and polar domains to the aqueous phase [17]. Loop and tail formation occur most probably [5]. The chain conformation at the interface depends also on the nature of the polypeptide. Norde and Favier [18] have shown that some polypeptides are rather rigid, and adsorb from aqueous solutions onto silica as they are, while others are softer and may unfold to some extent. Other workers report [19] that polypeptides form viscoelastic films at interfaces due to interactions between neighboring molecules. This mechanical property is considered to be responsible for differences in the adsorption layer structure rather than the reduction in surface tension. On the other hand, the differences in poly-L-lysine states α-helix and β-sheet significantly affect the structure of the interfacial layers. The significantly

lower interfacial tension and the lower area per residue is not fully understood so far. The interpretation of differences in the structure of polypeptides on the formation of interfacial layers at the oil/water interface and the additional influence of mechanical interfacial properties are currently under study using interfacial rheological experiments.

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