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Spectroscopic Investigation of Polypeptide Plane Brushes

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Summary: Fourier transform infrared spectroscopy (FTIR) was used for determination of orientational and conformational characteristics of plane polypeptide brushes with different density of polypeptide chains grafted to modified silicon surface. The determination of grafting density of polypeptide chains in brushes, which depends on chemical structure of spacer groups, was carried out using ultraviolet (UV) spectroscopy. The influence of chemical structure of modifying spacer groups and outer conditions on the characteristics of plane polypeptide brushes was established. The peculiarities of α-helical structure (random coil transfer of polymer chains in brushes under the action of denaturants) were investigated.

Keywords: FTIR; monolayers; orientation; polymer brushes; polymer chain

Introduction

The polypeptides bound by the one end of the chain to polymer surfaces are good models to investigate the orientational and conformational characteristics of plane polypeptide brushes. Strongly pronounced electro-optical and piezoelectric properties of macromolecules in brushes make them promising materials for magnetic switches and sensor systems.[1] However, there is little information on the possibility of varying properties of polypeptides in brushes. It is clear, that chemical structure of polypeptides in brushes as well as the method of brush preparation have a strong influence on their properties. According to theoretical simulations, the main factors determining the orientational and conformational properties of macromolecules in brushes are density of their grafting, molecular weight of polymer used and outer conditions.[2–4]

Among the approaches allowing to optimize these parameters there is a change of chemical structure of spacer used for grafting polymer moieties to the surface.

In our work, transmission FTIR was used as the main method of investigation of orientational and conformational characteristics of plane polypeptide brushes. UV spectroscopy was used for determination of grafting density of spacers bound to surfaces.

Materials and Methods

The N-carboxy anhydride (NCA) of γ-benzyl-L-glutamate was synthesized by triphosgene method.[5] (3-aminopropyl)-triethoxysilane (APES) and (3-aminopropyl)-trimethoxysilane (APMS) were purchased from Sigma-Aldrich Chemicals.

All chemicals were purified before use.

Double-polished silicon wafers were used as the substrates. Their surface was cleaned before use with a freshly prepared mixture of concentrated sulfuric acid and 30% hydrogen peroxide (70/30, v/v) at 120 °C for 30 min. After that, they were washed with a large amount of distilled water and subsequently rinsed with acetone. The substrates were dried under a stream of nitrogen and immediately used in the next steps.[6,7] Silicon plates were

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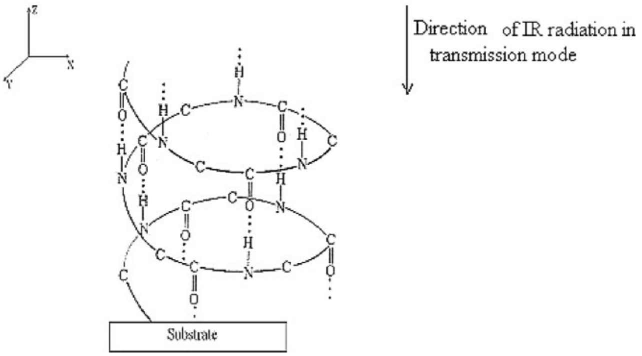

Figure 1.
Ideal geometry of polypeptide chain in brush.

incubated at 100 °C. Surface-Initiated Vapor Deposition Polymerization (SIVDP) of NCAs was carried out according to the previously described techniques.[6–9] FTIR spectra were acquired on IFS – 88 and Vertex 70 (Bruker, Germany) instruments. Transmission technique (resolution 4 cm^{-1}, 50 scans) was used for spectra registration. The homogeneity of polymer density in brushes was checked by IR-microscope technique (resolution 4 cm^{-1}, 3000 scans).

The conformational characteristics of the polypeptides in brushes were determined by means of IR spectroscopy data (the positions of absorption bands characteristic for amide groups Amide A, Amide I and Amide II (see Figure 1 and Table 1)).

Taking into account the direction of dipole moments of vibration, the qualitative evaluation of α-helix polypeptide brushes orientation (Figure 1, Table 1) becomes possible. This evaluation is based on relative intensities of Amide A, Amide I and Amide II absorption bands.

The FTIR examination in transmission mode shows the increase of Amide A and Amide I absorption bands intensities in plane oriented brushes and increase of Amide II absorption band intensity in homeotropic orientation. The degree of homeotropic orientation was determined according to formula (1):[11]

$$(A_{AMII}/A_{AMA})_{order}\cdot\frac{1.5\,S\sin^{2}\theta_{AMA}+(1-S)}{1.5\,S\sin^{2}\theta_{AMII}+(1-S)} = (A_{AMII}/A_{AMA})_{disorder} \qquad (1)$$

where $(A_{AMII}/A_{AMA})_{order}$ and $(A_{AMII}/A_{AMA})_{disorder}$ are the ratios of optical densities of Amide A and Amide II bands in spectra of ordered and disordered samples, respectively. The value of $(A_{AMII}/A_{AMA})_{disorder}$ has been defined from the spectrum of dilute solvent. Θ_{AMA} and (Θ_{AMII}) are the angles between transi-

Table 1.
Spectral characteristics of main amide bands in polypeptides.

Absorption band	Main vibrations of amide group	Frequency	Direction of dipole moments towards the bond θ [10]
		α-helix	
Amide A	NH- stretching vibrations	3292–3305 cm^{-1}	28°
Amide I	C=O stretching vibrations	1648–1662 cm^{-1}	34.5°
Amide II	NH- deformation vibrations	1531–1558 cm^{-1}	85°

tion moments of Amide A (Amide II) vibration and helix axis, and S – the parameter of order.

The average angle γ between helix axis and Z axis has been defined according to the following formula (2):

$$S = 0.5\,(3\overline{\cos}^2\gamma - 1) \qquad (2)$$

Results and Discussion

The preparation of plane brushes of poly-γ-benzyl-L-glutamate (PBLG) according to SIVDP approach allows increasing thickness of PBLG layers grafted to the surface (that is, to increase the degree of NCA polymerization). The thickness of brushes was about 1000–5000 Å.[7]

Positions of absorption bands Amide A, Amide I and Amide II indicate that PBLG brushes have α-helical conformation (Figure 2a and 2b). Ratios of intensities of these bands confirm that the orientation of brushes bearing APMS spacer group is homeotropic, and indicate the absence of any orientation in brushes with APES spacer group.

Treatment of brushes with chloroform leads to reduction of C=O band intensity of PBLG side chains in brushes prepared with APMS due to washing out of macromolecules not connected to the surface and subsequent change of orientation from homeotropic to plane. (Figure 2a, 2b)

The following placement of brushes into chloroform which is a ‘‘good’’ solvent for PBLG leads to the appearance of homeotropic orientation for brushes with APES spacer and to conservation of plane orientation for brushes with APMS spacer. The placement of brushes into the ‘‘bad’’ solvent (toluene) does not lead to rebuilding of homeotropic orientation in both cases. The parameters of orientation and angles between macromolecules axes and Z axis are presented in Tables 2 and 3.

According to the theoretical prediction [2], rigid polymers connected to the surface in the case of enough density of grafted polymers and their high molecular weight must show homeotropic orientation towards the surface.

We may suggest two reasons for changing orientation. Firstly, homeotropic orientation is stipulated by steric interaction of chains. Secondly, PBLG layers are capable to interact strongly with the surface due to the large dipole moment of macromolecules.

This interaction results in plane orientation of macromolecule.[12] It should be noted that chloroform weakens interaction between polymer chains and the surface.

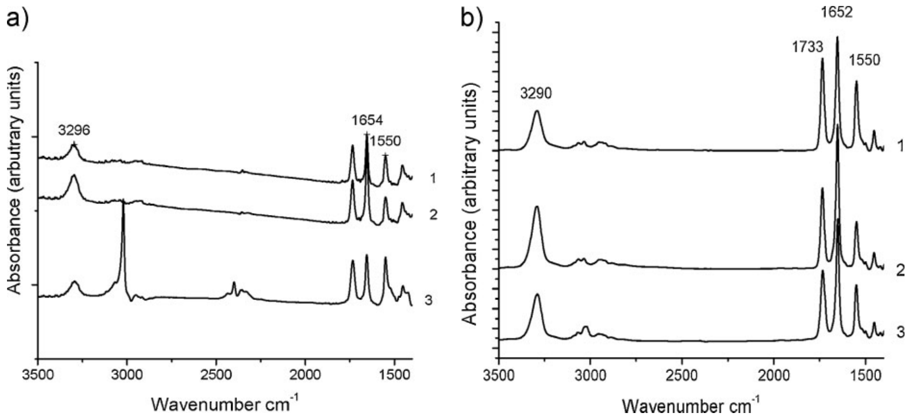

Figure 2.

a) IR absorption spectra of PBLG brushes prepared using APES spacer and SIVDP: fresh brush (1), washed with chloroform (2), placed in chloroform (3). b) IR absorption spectra of PBLG brushes prepared using APMS spacer and SIVDP: fresh brush (1), washed with chloroform (2), placed in chloroform (3).

Table 2.
Orientation parameters of brushes with APMS spacer prepared in gaseous phase.

NO	Fresh brushes		Washed brushes	
	S	Angle between macromolecules axis and Z axis (γ°)	S	Angle between macromolecules axis and Z axis (γ°)
1	0.4	40	−0.5	90°
2	0.61	31	−0.5	90°
3	0.69	27	−0.5	90°

Table 3.
Influence of solvents on orientation parameters of brushes with APES spacer prepared in gaseous phase.

NO	Washed brushes		Brushes in $CHCl_3$ solution		Brushes in toluene solution	
	Type of orientation	Angle between macro-molecules axis and Z axis (γ°)	Type of orientation	Angle between macro-molecules axis and Z axis (γ°)	Type of orientation	Angle between macro-molecules axis and Z axis (γ°)
1	plane	90°	homeotropic	43°	plane	90°
2	plane	90°	homeotropic	32°	plane	90°
3	plane	90°	homeotropic	32°	plane	90°

Differences of brushes behavior in chloroform and the dependence of the behavior on the type of spacer may be caused by different grafting densities, as well as by different molecular weights of polymer moiety in brushes. The density of PBLG bound to surface depends on the quantity of NH_2 groups on the silicon plate. These quantities were determined using UV spectroscopy.[13] Quartz plates transparent to UV radiation were used in the measurements as the base plates. They were modified with APES and APMS as described earlier. Terminal amino groups were modified with *p*-nitrobenzaldehyde as a chromophore group.

Taking into account the value of absorption coefficient of band at the wavelength of 295 nm, the quantity of NH_2 groups on the surface can be found.[13] The data are given in Table 4.

Taking into account that the diameter of α-helical PBLG is equal to 20 Å, we may suppose that the number of NH_2 groups on the surface will be sufficient for compact grafting of polymer chains to the surface.

The length of PBLG chain was calculated from the optical density of C=O band (1730 cm^{-1}) of ester group randomly oriented with respect to helical axis, according to the formula:

Table 4.
The influence of structure of spacer and duration of its modification on the density of NH_2 groups on the silicon surface.

Spacer type/Duration of modification, 100 °C	Number of NH_2 groups
APMS/1 h	3 NH_2/100 Å^2
APMS/5 h	3 NH_2/100 Å^2
APES/1 h	5 NH_2/100 Å^2
APES/5 h	5 NH_2/100 Å^2

$$D_{1730} = k_{1730} \cdot c \cdot l \qquad (3)$$

where k_{1730} is the absorption coefficient of the oscillation band of ester group C=O in the spectra of PBLG chloroform solution, c is the polymer concentration in solution, l is the layer depth.

It was assumed that monolayer of PBLG on the surface is a residual after washing of freshly prepared brushes with chloroform (24 h) and with dimethylformamide (12 h).

Thus, on assumption that the concentration of PBLG chains on the surface is equal to 100% and polymer molecules are compactly grafted in a monolayer, the minimal length of PBLG chains estimated

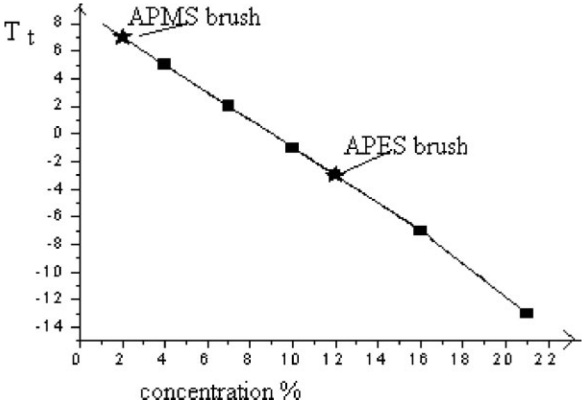

Figure 3.
The concentration dependence of temperature of α-helical structure to random coil semi-transition (T_t) in mixed solvent (40% chloroform – 60% dichloroacetic acid) under cooling.

as 1000 Å for APES, and 5000 Å in case of APMS.

To determine the real concentration of PBLG branches in brushes, we have used the data obtained from concentration dependence of inverted α-helical structure – random coil transition of PBLG in a mixed solvent (40% chloroform – 60% dichloroacetic acid) under cooling and transition of PBLG in a mixed solvent (20% chloroform – 80% dichloroacetic acid) under heating. These transitions demonstrate linear dependence on concentration.[14]

Then, the temperatures of semi-transition (T_t) for different concentrations of free PGBG (2–20%) and for brushes prepared with APMS and APES spacer groups under the same conditions were compared. It was found that polymer concentration in the brushes prepared with APMS is equal to 1–2%, and the brushes with APES contain 12% of PBLG (Figure 3). From the data obtained on real polymer concentration and helical parameters (pitch length and quantities of amino acids in a pitch) as well as from preliminary found length of polymer chain, molecular weight of polymer moiety in brushes was calculated:

Spacer	Minimum length	Concentration, %	MW
APES	1000 Å	~12	~700000
APMS	5000 Å	~2	~1300000

Ratio of intensities of Amide I and Amide II bands in a mixed solvent (40% chloroform – 60% dichloroacetic acid) shows the presence of homeotropically oriented branches in brush prepared with the use of APES. The threshold concentration of liquid crystalline order (LC) formation for free PBLG molecules in the same mixed solvent lies within the range of 21–23%. Therefore, grafting of PBLG chains to surface significantly diminishes concentration border of LC formation. Orientational characteristics defined using both differential and integrated relations of Amide I and Amide II bands intensities and the relation of AI_{calc} to AI_{exp} testify the beginning of helix disorientation with respect to the surface at the helix content of 50%. (Figure 4).

This observation can be explained by the fact that orientation stabilizes helix conformation since a 12% PBLG solution in a mixed solvent (20% chloroform – 80% dichloroacetic acid) at room temperature fully transformed α-helix to random coil, although in the brushes prepared with APES under the same conditions approximately 50% of helical conformation is retained.

Conclusion

Applicability of spectroscopic methods for determination of basic characteristics of

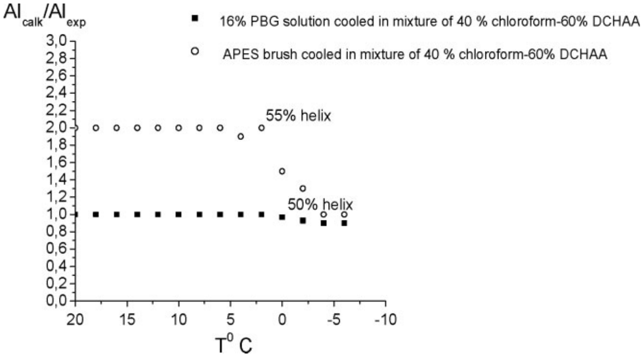

Figure 4.
Temperature dependence of AI_{calc}/AI_{exp} for PBLG and APES brush; AI_{calc} — calculated for corresponding content of random coil, AI_{exp} – observed under the corresponding temperature.

polypeptide brushes, such as density of polypeptide chains grafted to the surface, their orientational and conformational characteristics, was shown. It was found that grafting of rigid macromolecules to surfaces results in diminishing concentration border of LC formation as compared with the same parameter for polymer solution.

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