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Synthesis and Study of New Copolymers Capable of Forming Molecular Complexes with DNA

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Summary: The possibility of creating the copolymers capable of specific interactions with DNA was studied. The copolymers of acrylamide and synthesized N-substituted acrylamides were obtained by free-radical copolymerization in aqueous medium. Using atomic force microscopy it was demonstrated that some of the obtained copolymers form molecular complexes with the bacteriophage lambda DNA. Potentially, the copolymers can be used for detection of DNA in sensing applications.

Keywords: atomic force microscopy (AFM); biomaterials; copolymerization; N-substituted amides of acrylic acid

Introduction

The development of sensitive and selective sensors is of high importance for medicine and biotechnology. The operation principals of many promising sensors and diagnostic systems are based on specific molecular interactions.[1–3] In this connection the synthesis of new copolymers, capable of specific interaction with biomacromolecules, in particular, with deoxyribonucleic acid (DNA), is of great interest.

In this paper, atomic force microscopy (AFM) was appliedto study the interaction between DNA and the copolymers. AFM has significant advantages in the investigation of biological objects. AFM has been applied successfully to the study of bacterial cells[4] and viruses.[5] It is actively used for the study of nucleic acids interactions with proteins,[6] dendrimers[7] and polymers.[8–11] These investigations are important for the development of gene therapy of diseases. It has been shown that DNA can form complexes with the polymers in the form of rods, toroids, and nonspherical aggregates.[12] NH_2-functionalized polymer films have been used to align and immobilize DNA molecules on a Si substrate.[13] The results of numerous studies show that AFM is very informative tool for the investigation of specific molecuar interactions.

Experimental Part

Synthesis of N-Substituded Amides of Acrylic Acid

In order to create the copolymers interacting with biomacromolecules the synthesis of N–substituted acrylamides with various functional groups has been realized (Figure 1).

All experiments were carried by used standard chemical reagents, glass and equipment. Organic solutions were concentrated on a rotary vacuum evaporator (Laborota 4000, Heildolph). Column chromatography was performed using silica gel (60, Merck). LC/MS spectra were recorded on LC/MS system Thermo Scientific (Finnigan). Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Bruker Model ARX (300 MHz) spectrometer. Abbreviation: s.- singlet, d.- doublet, tr.- triplet, q.- quartet.

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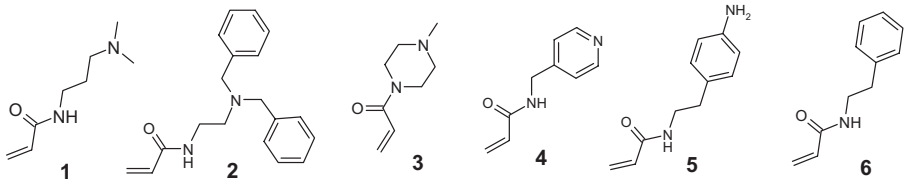

Figure 1.
1. N-(2-dimethylamino-ethyl)-acrylamide, **2**. N-(2-dibenzylamino-ethyl)-acrylamide, **3**. 1-(4-methyl-piperazin-1-yl)-propenone, **4**. N-pyridine-4-yl-methyl-acrylamide, **5**. N-[2-(4-aminophenyl)-ethyl]-acrylamide, **6**. N-phenethyl-acrylamide.

General Procedure for the Synthesis of N-Substituded Amides of Acrylic Acid (1–6)

To solution of acrylic acid (10 mmol), amine (12 mmol) and triethylamine (20 mmol) in dichloromethane (DCM) (50 ml) was added N,N,N',N'-tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate (12 mmol). The reaction mixture was stirred for 12–24 h at room temperature (r.t.). The organic solution was washed with water and brine, dried over anhydrous sodium sulfate and concentrated. The crude residue was purified via silica gel column chromatography (eluent ethylacetate-hexane or DCM-ethanol).

All obtained compounds gave satisfactory spectral data consistent with their structures.

Selected Data

N-(3-dimethyl-aminopropyl)-acrylamide (**1**) was obtained in yield 14.7%.

LC/MS: $[M + H^+]$ 157.

^{1}H-NMR (DMSO – d $_6$): NH ($\delta =$ 8.14,1H, tr., $J_{CH2NH} = 6$ Hz), =CH ($\delta = 6.24$, 1H, dd, $J_{cis} = 9.9$ Hz, $J_{trans} =$ 17.1 Hz), $=CH_2$ ($\delta = 6.09$, 1H, dd, $J_{trans} = 17.1$ Hz, $J_{gem} = 2.4$ Hz; $\delta = 5.61$, 1H, dd, $J_{cis} = 9.9$ Hz, $J_{gem} = 2.3$ Hz); $HNCH_2$ ($\delta = 3.16$, 2H, q., $J_{CH2CH2} = 7$ Hz, $J_{CH2NH} = 6$ Hz); CCH_2C ($\delta = 1.61$, 2H, m.) NCH_2 ($\delta =$ 2.32, 2H, tr., $J_{CH2CH2} =$ 7 Hz).

N-(2-dibenzylamino-ethyl)-acrylamide (**2**) was obtained in yield 32.2%.

LC/MS: $[M + H^+]$ 295.

^{1}H-NMR ($CDCl_3$): NH ($\delta = 5.87$, 1H, s.), =CH ($\delta = 6.05$, 1H, dd, $J_{cis} = 10.2$ Hz, $J_{trans} = 17.0$ Hz), $=CH_2$ ($\delta = 6.23$, 1H, dd, $J_{trans} = 17.0$ Hz, $J_{gem} = 1.4$ Hz; $\delta = 5.62$,1H, dd, $J_{cis} = 10.2$ Hz, $J_{gem} = 1.4$ Hz); $HNCH_2$ CH_2N ($\delta = 3.42$, 2H, q., $J_{CH2CH2} = 7$ Hz, $J_{CH2NH} = 6$ Hz; $\delta = 2.68$, 2H, tr., $J_{CH2CH2} =$ 7 Hz); $N(CH_2)_2$ ($\delta = 3.66$, 4H, s.); $(Ph)_2$ (δ 7.48 ÷ 7.30, 10H, m.).

1-(4-methyl-piperazin-1-yl)-propenone (**3**) was obtained in yield 16.4%.

LC/MS: $[M + H^+]$ 155.

^{1}H-NMR ($CDCl_3$): =CH ($\delta = 6.61$, 1H, dd, $J_{cis} = 11.0$ Hz, $J_{trans} = 16.8$Hz), $=CH_2$ ($\delta = 6.33$; 1H, dd, $J_{trans} = 16.8$ Hz, $J_{gem} = 2.2$ Hz; $\delta = 5.74$, 1H, dd, $J_{cis} =$ 11.0 Hz, $J_{gem} = 2.2$ Hz); $CON(CH_2)_2$ ($\delta =$ 3.76, 2H, m.; 3.65, 2H, m.); $N(CH_2)_2$ ($\delta = 2.46$, 4H, tr., J =7.0 Hz); NCH_3 ($\delta = 2.36$, 3H, s.).

N-(pyridyl-4-methyl)-acrylamide (**4**) was obtained in yield 9.4%.

LC/MS: $[M + H^+]$ 163.

^{1}H- NMR (DMSO-d_6) δ, ppm: 8.77 (NH,1H, t., J_{CH2NH} =5.8 Hz); 8.53, 7.29 (NCH arom., 2H, d., CCH arom., 2H, d., $J_{CHCH} = 6.1$ Hz); 6.35 (=CH, 1H, dd., J*cis* = 10.4 Hz, $J_{trans} = 17.2$ Hz); 6.18, 5.71 ($=CH_2$, 1H, dd., $J_{cis} = 10.4$ Hz, 1H, dd., $J_{trans} = 17.2$ Hz, J_{gem} =2.5 Hz); 4.42 (NCH_2, 2H, d., J_{CH2NH} =5.8 Hz).

N-[2-(4-aminophenyl) ethyl)]-acrylamide (**5**) was obtained in yield 12.2%.

LC/MS: $[M + H^+]$ 191.

^{1}H- NMR (DMSO-d_6) δ, ppm: 8.18 (NH,1H, tr.,J_{CH2NH} =6.0 Hz); 6.7 (C_6H_4arom., 4H, q., $J_{CHCH} = 7.2$ Hz), 6.24 (=CH, 1H, dd., $J_{cisc} = 10.2$ Hz, $J_{trans} = 17.0$ Hz), 6.12, 5.58 ($=CH_2$, 1H, dd., $J_{trans} = 17.0$ Hz, $J_{gem} = 2.5$ Hz, 1H, dd., $J_{cis} =$ 10.2 Hz, $J_{gem} = 2.5$ Hz); 4.90 (NH_2, 2H, broad s.); 3.28, 2.6 ($HN(CH_2)_2$, 2H,

q., $J_{CH2CH2} = 7.0$ Hz, $J_{CH2NH} = 6.0$ Hz, 2H under the DMSO signal).

N-phenethyl-acrylamide (**6**) was obtained in yield 26.4%.

LC/MS: $[M + H^+]$ 176.

^{1}H- NMR ($CDCl_3$) δ, ppm: 7.39 - 7.26 (C_6H_5 arom., 5H, м); 6.33 (=$CH_{2(Htrans)}$, 1H, dd., J_{gem} =1.4 Hz, J_{trans} =17.0 Hz); 5.69 (=$CH_{2(Hcis)}$,1H, dd., J_{gem} =1.4 Hz, J_{cis} = 9.8 Hz); 6.08 (=CH, 1H, dd., J_{trans} = 17.0 Hz, J_{cis} = 9.8 Hz); 5.60 (NH,1H, broad signal); 3.67 (NCH_2, 2H, q., J_{NHCH} =6.0 Hz, JcHCH = 6.0 Hz); 2.92 (CCH_2, 2H, tr., JcHCH = 6.0 Hz).

Synthesis of Copolymers

The copolymers of acrylamide and synthesized N-substituted acrylamides have been obtained by free-radical copolymerization in aqueous medium using red-ox system of ammonium persulfate - tetramethylethylenediamine (APS - TMED) at 65°C. The solutions of polymers have been dialyzed against low molecular weight (up to 10 kDa) fractions, and then freeze-dried.

The ratio of components has been determined by NMR spectroscopy. Mixing ratio of copolymer of acrylamide and N-(2-dimethylamino-propyl)-acrylamide (**I**) is 95:5 respectively. Mixing ratio of copolymer of acrylamide and N-(2-dibenzylamino-ethyl)-acrylamide (**II**) is 97:3 respectively. Mixing ratio of copolymer of acrylamide and 1-(4-methylpiperazin–1-yl)-propenone (**III**) is 90:10 respectively.

The calculation of ratio of the components was done on the basis of CH_2 protons of the chain.

Copolymer of Acrylamide and N-(2-Dimethylamino-propyl)-acrylamide (I)

To the solution of 0.3 g (0.0042 mol) acrilamide and 0,12 g (0.7680 mol) N-(3-dimethyl-aminopropyl)-acrylamide (**1**) in water (9.5 ml) was added 12 μl TMED. The reaction mixture was heated to 50 °C and the solution of 20 mg (0.0876 mmol) APS in water (0.5 ml) was added. The polymerization was conducted under stirring for one hour at 50 °C. The reaction mixture was cooled to r.t. The solution of copolymer was dialyzed against low molecular weight (up to 10 kDa) fractions, and then freeze-dried. Copolymer **III** was obtained in yield 70%.

Copolymer of Acrylamide and N-(2-Dibenzylamino-ethyl)-acrylamide (II)

To the solution of 0.15 g (0.0021 mol) acrilamide 0,11 g (0.0004 mmol) N-(2-dibenzylamino-ethyl)-acrylamide (**2**) in water (4.5 ml) was added 12 μl TMED. The reaction mixture was heated to 65 °C. The reaction mixture was acidified with 0.1N HCl to pH 5–6 and the solution of 20 mg (0.0876 mmol) APS in water (0.5 ml) was added. The polymerization was conducted under stirring for one hour at 65 °C. The reaction mixture was cooled to r.t. The solution of copolymer was dialyzed against low molecular weight (up to 10 kDa) fractions, and then freeze-dried. Copolymer **II** was obtained in yield 19%.

Copolymer of Acrylamide and 1-(4-Methylpiperazin-1-yl)-propenone (III)

To the solution of 0.3 g (0.0042 mol) acrilamide and 0,12 g (0.7781 mmol) 1-(4-methylpiperazin-1-yl)-propenone (**3**) in water (9.5 ml) was added 12 μl TMED. The reaction mixture was heated to 65 °C and the solution of 20 mg (0.0876 mmol) APS in water (0.5 ml) was added. The polymerization was conducted under stirring for one hour at 65 °C. The reaction mixture was cooled to r.t. The solution of copolymer was dialyzed against low molecular weight (up to 10 kDa) fractions, and then freeze-dried. Copolymer **III** was obtained in yield 71%.

AFM Measurements

The AFM measurements were performed using scanning probe microscope FemtoScan in tapping mode in air. Triangular cantilevers fpN20S with typical resonance frequency of 420 kHz and the tip radius of less than 10 nm were used. AFM images were processed and analysed in the software FemtoScan Online. Samples for AFM were prepared as follows. 2.5 μl of an

aqueous solution, containing 10 μg/ml of DNA (λ phage DNA, Fermentas) and 10 μg/ml of a copolymer, was deposited on the surface of a piece of freshly cleaved mica. Then the sample was dried in air. Samples of pure copolymers were prepared similarly using aqueous solutions with a copolymer concentration of 10 μg/ml. The control sample of DNA was prepared as follows. Cleaved mica was incubated in a solution containing 1 μg/ml of DNA, 5 mM of $MgSO_4$, and 20 mM of NaCl for 10 minutes. Then the sample was washed with bidistilled water and dried in air.

Results and Discussion

AFM Study of Interaction between the Copolymers and DNA

On the mica surface the aqueous solutions of copolymers **I** and **II** after drying form smooth films with roughness (0.4 ± 0.3) nm for copolymer **I** and (0.15 ± 0.05) nm for copolymer **II**. The films contain pores whose depths are less than 1 nm for copolymer **I** and less than 0.6 nm for copolymer **II**. It can be assumed that the maximum pore depth is equal to a copolymer film thickness. Probably, lower film thickness of copolymer **II** means its better wetting of the surface, as the solutions with the same concentrations are used for the films preparation.

The average DNA height in AFM images was 0.6 nm (Figure 2 a). In Figures 2 b and 2 c typical images of DNA complexes with copolymers **I** and **II**, respectively, are shown. In the AFM image of copolymer **I** drops with height of up to 16 nm and diameter of up to 1 μm are visible. They are interconnected by filamentous structures. We can distinguish thin filamentous structures with height of (0.65 ± 0.20) nm and thick filamentous structures with height of (1.95 ± 0.35) nm. Planar structures are found in the intersections of the filamentous structures, as well as around the droplets. Their height coincides with the height of the thick filamentous structures. It is assumed that the thin filamentous structures correspond to fragments of double-stranded DNA, which has typical height of (0.7 ± 0.1) nm on the mica surface.[14] The thick filamentous structures can be interpreted by the formation of DNA complexes with the copolymer. It is quite possible that the complexes contain several DNA strands. The formation of droplets, consisting of the copolymer and DNA, can be attributed to stronger interaction between copolymer **I** and DNA than between copolymer **I** and the substrate.

In the images of copolymer **II** and DNA, a grid formed by filamentous structures with different thickness from 0.5 nm to 2.3 nm is visible. In some areas of the mica surface, compact flat nest-like structure with average height of 0.8 nm and diameter of up to 800 nm are found. Similar structures are observed for DNA complexes with dendrimers.[7] The increasing of the thickness of the filamentous structures can be related to the formation of complexes between DNA and copolymer **II**. In the case of copolymer **II** DNA molecules are more evenly distributed over the mica surface. Positively charged copolymer **II** compensates the negative charge of mica surface and contributes to the DNA adsorption.

The film of copolymer **III** on the mica surface has a globular structure. The height of the globules ranges from 0.3 nm to 1.1 nm. Larger aggregates of up to 80 nm can be found on the surface. The complexes between DNA and the copolymer **III** consist of the core with height from 5 nm to 11 nm and diameter from 100 nm to 270 nm (Figure 2 d). DNA loops are observed around the core of the complexes.

All copolymers are positively charged. They can form complexes with negatively charged double-stranded DNA due to electrostatic interaction. We can see the formation of compact complexes in the case of the interaction of copolymers **I** and **III** with DNA, while we observe an uniform grid in the case of deposition of aqueous solution of DNA and copolymer **II** on mica surface.

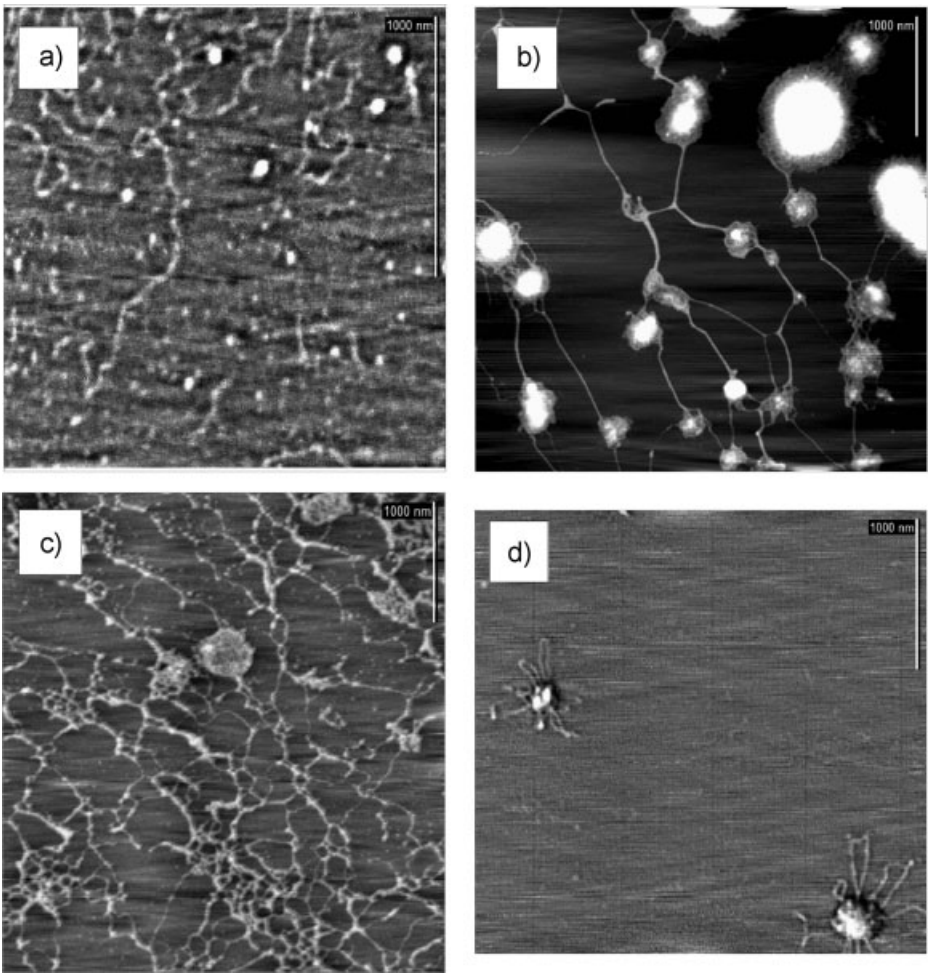

Figure 2.
AFM images: a) DNA; b) DNA and copolymer **I**; c) DNA and copolymer **II**; d) DNA and copolymer **III**.

Conclusion

N-substituted amides of acrylic acid with various functional groups have been synthesized. The copolymers **I-III** of acrylamide and N-(2-dimethylamino-propyl)-acrylamide (**1**), N-(2-dibenzylamino-ethyl)-acrylamide (**2**), 1-(4-methylpiperazin-1-yl)-propenone (**3**) have been obtained. Using AFM it was shown that all copolymers contribute to the adsorption of DNA on the negatively charged mica. Copolymers **I** and **III** are able to interact strongly with DNA forming compact complexes.

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