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Biophysics

Study of DNA Interaction with Polyamidoamine (PAMAM) Dendrimers Using Spectroscopic and Thermodynamic Methods

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(Presented by Academy Member Tengiz Zaalishvili)

Abstract. One of the significant nanomaterials for health applications is PAMAM (polyamidoamine) dendrimers as drug delivery systems, which makes the study of PAMAM dendrimers' interaction with DNA highly relevant. Using spectroscopic and thermodynamic methods, we investigated the interaction of PAMAM dendrimers of different generations and functional groups (G2(NH_2), G4(NH_2), and G4(OH)) with DNA molecules. Based on comparative analysis of absorption spectra, melting curves and fluorescence resonance energy transfer of DNA-PAMAM complexes, it was established that the interaction is determined by the surface functional groups of PAMAM dendrimers. Dendrimers with NH_2 groups on their surface increase DNA melting temperature by 20°C and energy transfer efficiency by 18%, while dendrimers with OH groups increase DNA melting temperature by 4°C and energy transfer efficiency by 5%. The strong interaction of PAMAM dendrimers with NH_2 functional groups is caused by electrostatic interactions. © *2025 Bull. Natl. Acad. Sci. Georg.*

Keywords: DNA, PAMAM dendrimers, FRET, spectroscopy, nanomaterials

Introduction

One of the main challenges in nanomedicine is the targeted delivery of drugs to disease or tumor sites, which can be achieved through the use of various nanosystems. In this regard, it is interesting to study PAMAM dendrimers as drug delivery nanosystems.

Dendrimers have a large surface area; spherical shape; water-filled cavities between branches, which serve as a kind of trap for drug transport. In addition, they have a defined molecular size and shape, have a large number of functional groups on the surface (Kesharwani et al., 2014; Tag et al., 2014; Kharwade et al., 2020) and are characterized by a high ability to penetrate cells (Fox et al., 2018; Parajapati et al., 2016; Kumar et al., 2014; Giorgadze et al., 2020). There are more than ten generations of PAMAM dendrimers, which differ from each other in size. In our study, we used two small-sized second and fourth generation (G2 and G4) PAMAM dendrimers with two different functional groups (NH_2 and OH) (Fig. **1**). Due to their

small size, PAMAM dendrimers have the ability to easily penetrate cells and, most importantly, the cell nucleus, whose membrane pores do not exceed 8-9 nanometers.

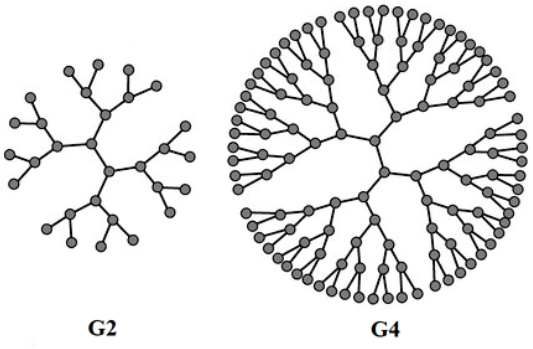

Fig. 1. Structure of G2 PAMAM and G4 PAMAM dendrimers.

Besides to drug delivery, PAMAM dendrimers are used in many other fields, such as: gene transfer, imaging, and the creation of antimicrobial agents. In all these fields, PAMAM dendrimers require certain specificity, which can be acquired through binding with DNA (Fox et al., 2018; Tarach et al., 2021; Fana et al., 2020). Considering this, it is relevant to study the interaction of DNA with PAMAM dendrimers (Ainalem et al., 2011; Perico et al., 2016).

The aim of the presented work is to study the interaction of PAMAM dendrimers of different generations and functional groups (G2(NH_2), G4(NH_2), and G4(OH)) with calf thymus DNA using spectrophotometric, spectrofluorimetric, and thermodynamic methods.

Materials and Methods

The the calf thymus DNA (40% GC), obtained from "Sigma-Aldrich" was used in the tests. The concentration of nucleic acids was determined by UV absorption using molar extinction coefficients ($\varepsilon = 6600\ cm^{-1}\ M^{-1}$ at $\lambda = 260$ nm).

The $G4(NH_2)_{64}$ PAMAM (Polyamidoamine) dendrimers, with molecular sizes 4.5 nm, molecular weight 14215, $G2(NH_2)_{16}$ PAMAM dendrimers with molecular sizes 2.9 nm, molecular weight 3256, and $G4(OH)_{64}$ PAMAM dendrimers with molecular sizes 4.5 nm, molecular weight 14279 were used in the tests. They were purchased from "Sigma-Aldrich".

Intercalators – Acridine orange (AO) and Ethidium bromide (EB) were purchased from "Sigma-Aldrich". The concentrations of the intercalators were determined colorimetrically using the molar extinction coefficients: $\varepsilon_{AO} = 68500\ cm^{-1}M^{-1}$ at $\lambda = 492$ nm, and $\varepsilon_{EB} = 5600\ cm^{-1}M^{-1}$ at $\lambda = 480$ nm.

All experiments were performed in 10^{-2} M NaCl, at pH=6.8. The pH was controlled by a pH-meter HANNA instruments HI98103.

Absorption spectra were carried out by compact, mobile, small power consumption optical fiber CCD spectrometer AvaSpec ULS 2048-USB2. Experimental results were processed by Origin software. Deuterium lamp in the ultraviolet area and quartz halogen incandescent lamp in the visible area were used as a source of light. The melting of DNA–PAMAM dendrimer complexes were performed from 40 to 95°C at 260 nm.

Light scattering spectra were registered using a CCD spectrometer. By the method of turbidimetry, the size of the condensed particles formed after adding the PAMAM dendrimers to the DNA was measured.

Fluorescence spectra of binary DNA-AO and ternary DNA-AO-EB complexes were carried out in 1 cm quartz fluorescent cell; the volume of studied samples was 2 ml. For fluorescence excitation of AO diode laser ($\lambda = 457$ nm) was used. For real time fluorescence spectra registration CCD spectrometer was used (with integration time of CCD detector 8 ms).

Fluorescence resonance energy transfer: the proposed laser-induced FRET method allows an estimation of the concentration of double helix areas with a high-quality stability applicable for intercalation in DNA after it was subjected to the stress effect. The approach was based on intercalated in DNA acridine orange (donor) and ethidium bromide (acceptor) molecules (Bregadze et al., 2016).

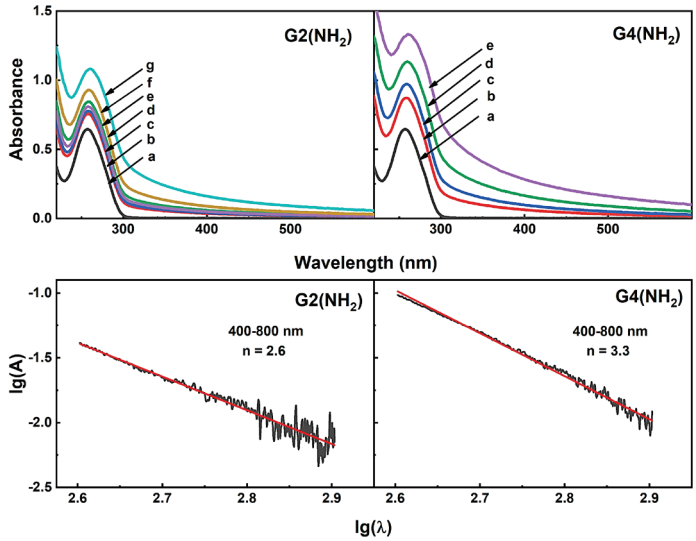

Fig. 2. Absorption spectra of DNA and DNA-PAMAM complexes. DNA titration was performed with PAMAM dendrimers: G2(NH_2) and G4(NH_2). Concentration of DNA is same in all experiments: [DNA]=$5 \cdot 10^{-5}$ M (bp), concentrations of PAMAM dendrimers are: (a) 0; (b) $1.7 \cdot 10^{-5}$ M; (c) $2.2 \cdot 10^{-5}$ M; (d) $2.5 \cdot 10^{-5}$ M; (e) $2.7 \cdot 10^{-5}$ M; (f) $2.9 \cdot 10^{-5}$ M; (g) $3.1 \cdot 10^{-5}$ M. The spectra below are in logarithmic coordinates in the 400-800 nm interval for a 1 PAMAM/2.5 bp ratio.

Results

Figure 2 shows the absorption spectra of complexes of DNA with PAMAM dendrimers of different generations (G2(NH_2) and G4(NH_2)) and different PAMAM/DNA ratios. Also Fig. 2 shows spectra in logarithmic coordinates in the 400-800 nm interval for a 1 PAMAM/2.5 bp ratio.

Figure 2 shows that dendrimers with NH_2 functional groups cause scattering of the DNA absorption spectrum upon DNA interaction, which is caused by aggregate formation. Dendrimers with OH functional groups practically do not change the DNA absorption spectrum.

The sizes of aggregates formed for G2(NH_2) and G4(NH_2) dendrimers at a 1 PAMAM/2.5 bp ratio were calculated using the turbidimetry method to determine the particle size. The scattering equation is expressed by Güller's formula (1):

$$A_{\lambda=\lambda_{max}} = k\lambda^{-n}, \qquad (1)$$

where A is the optical density, while k and n are constants

$$\log A = \log k - n \log \lambda. \qquad (2)$$

Experimental data in logarithmic coordinates represent a straight line in the wavelength region where light attenuation is caused only by scattering. Güller experimentally constructed a calibration curve using electron microscopic methods (Frolov, 1982; Bregadze et al., 2020), which expresses the dependence of *n* (presented in Güller's formula) on aggregate size, with the approximation that these aggregates are spherical in shape.

Based on the above, using the *n* values obtained from the insets in Fig. 2 and applying Güller's curve, the aggregate sizes for a 1 PAMAM/2.5 bp ratio: G2(NH_2)-DNA ~ 150 nm and G4(NH_2)-DNA ~ 100 nm were calculated.

Melting of DNA complexes with G2(NH_2), G4(NH_2), and G4(OH) PAMAM dendrimers at 260 nm was spectroscopically monitored (Fig. 3). Fig. 3 shows that the melting temperature of native DNA is 70.5°C, while upon interaction of dendrimers with NH_2 functional groups with DNA, specifically at a 1 PAMAM/10 bp ratio, two transitions are observed on the DNA melting curves. For G2(NH_2)-DNA and G4(NH_2)-DNA complexes, the melting temperatures of the first transition on the

melting curves are in the 73-76°C interval, while the melting temperatures of the second transition reach 90°C. At a 1 PAMAM/5 bp ratio, one transition is observed on the DNA melting curves and the melting temperatures are ~90°C. It should be noted that during melting of G2(NH_2)-DNA and G4(NH_2)-DNA complexes, the hypochromic effect of DNA decreases by approximately 10%, which demonstrates that complete DNA denaturation does not occur. As for the G4(OH) PAMAM dendrimer, it stabilizes DNA by 3-4°C (see Table).

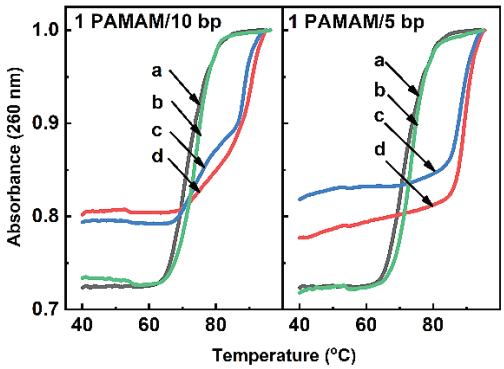

Fig. 3. Melting curves of DNA and DNA-G2(NH_2), DNA-G4(NH_2) and DNA-G4(OH) complexes at λ=260 nm, for different concentrations of PAMAM. The melting temperature is determined from the derivative of the curve. Melting was recorded in the 40-95°C temperature interval. (a) DNA, (b) G4(OH)-DNA, (c) G4(NH_2)-DNA, (d) G2(NH_2)-DNA. [DNA]=$3\cdot10^{-5}$ M (bp).

Based on the analysis of absorption spectra and melting curves, it can be concluded that dendrimers with NH_2 functional groups interact more strongly with DNA than dendrimers with OH functional groups. The electronic charge distribution on NH_2 and OH groups has been calculated (Renugopalakrishnan et al., 1971) and the charge on the NH_2 group is +0.04 and on the OH group is -0.11. This accounts for the electrostatic interaction between negatively charged DNA and PAMAM dendrimers with NH_2 groups.

Using the nanoscale laser-induced fluorescence resonance energy transfer (FRET) method in a donor-acceptor intercalator pair, the efficiency of energy transfer in DNA was assessed quantitatively. The dyes acridine orange (AO) as donor and ethidium bromide (EB) were used as acceptor, which intercalate into the DNA double helix and do not interact with PAMAM dendrimers. The energy transfer efficiency quantitatively determines the reduction in the concentration of DNA double helix sites that are suitable for subsequent intercalation.

Figure 4 shows the effect of dendrimers at different ratios G2(NH_2), G4(NH_2) and G4(OH) on the DNA double helix. The spectra presented in the figure are normalized to the fluorescence of the AO-DNA complex.

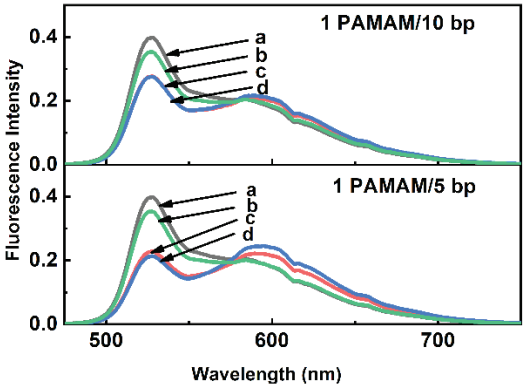

Fig. 4. Fluorescence spectra of energy transfer for DNA-AO-EB and DNA-AO-PAMAM-EB complexes for different concentrations of PAMAM. [DNA] = $1.4\cdot10^{-4}$ M (bp), [AO] = $7\cdot10^{-6}$ M, [EB] = $7\cdot10^{-6}$ M. (a) DNA-AO-EB, (b) DNA-AO-G4(OH)-EB, (c) DNA-AO-G4(NH_2)-EB, (d) DNA-AO-G2(NH_2)-EB.

Inductive-resonance energy transfer from AO donor to EB acceptor intercalated in the DNA double helix is described by the following formula:

$$\frac{q_{AO}}{q_{oAO}} = \left[1 + \left(\frac{R_0}{R}\right)^4\right]^{-1}, \qquad (3)$$

where q_{0AO} is the quantum yield of fluorescence of donor molecules in the absence of acceptor, when the distance between donor and acceptor $R \to \infty$, while q_{AO} is the quantum yield of fluorescence of donor molecules when the acceptor is at distance R. An important characteristic of the energy transfer process is the Forster distance R_0. At this distance, half of the donor molecules decay by energy transfer and the other half decays by the usual

radiative and non-radiative rates (Bregadze et al., 2016), experimental value of R_0 is 3.75 ± 0.3 nm.

The efficiency of excitation energy transfer from donor to acceptor is expressed by the ratio of the quantum yields of donor fluorescence:

$$E_{ET} = 1 - \frac{q_{AO}}{q_{oAO}}, \quad (4)$$

Table. Efficiency of energy transfer E_{ET} from AO donor to EB acceptor, R distance between AO donor to EB acceptor, and T_M melting temperature of complexes at different ratios of PAMAM and DNA

	PAMAM/ DNA (bp)	E_{ET} (%)	R (nm)	T_M (°C)
DNA	-	60	3.4	70.5
G4(NH_2)-DNA	1/10	73	2.9	73.2
				88.3
	1/5	79	2.7	88.3
G2(NH_2)-DNA	1/10	73	2.9	75.8
				90.4
	1/5	78	2.7	89.2
G4(OH)-DNA	1/10	65	3.2	73.4
	1/5	65	3.2	74.5

From the fluorescence spectra (Fig. 4), the energy transfer efficiency and the distance between AO and EB were calculated for different ratios of G2(NH_2), G4(NH_2) and G4(OH) PAMAM dendrimers to DNA base pairs. The data are presented in Table, which also shows the melting temperatures of DNA-dendrimer complexes.

Conclusions

Absorption spectra of DNA-G2(NH_2) and DNA-G4(NH_2) complexes showed scattering. The aggregate sizes of these complexes at 1 PAMAM/2.5 bp ratio, we got 150 nm for DNA-G2(NH_2) and 100 nm for DNA-G4(NH_2) were calculated.

Analysis of the melting curves of DNA complexes with G2(NH_2), G4(NH_2), and G4(OH) PAMAM revealed that PAMAM dendrimers with NH_2 functional groups stabilize DNA by almost 20°C, while PAMAM dendrimers with OH functional groups stabilize it by 3-4°C.

Results obtained by the fluorescence resonance energy transfer method showed that upon interaction of PAMAM dendrimers with NH_2 functional groups with DNA, the efficiency of energy transfer from donor to acceptor increases by ~18%, while for PAMAM dendrimers with OH functional groups – by 5%.

Using spectrophotometric, spectrofluorimetric, and thermodynamic methods, the comparative binding affinity of different types of PAMAM dendrimers with DNA molecules based on their surface functional groups were studied. The results demonstrate that PAMAM dendrimers with NH_2 groups on the surface interact more strongly with DNA compared to dendrimers with OH groups, which is attributed to the positive surface charge of G2(NH_2) and G4(NH_2) dendrimers.

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ბიოფიზიკა

დნმ-ის ურთიერთქმედების შესწავლა პოლიამიდოამინი (PAMAM) დენდრიმერებთან სპექტრომეტრული და თერმოდინამიკური მეთოდების გამოყენებით

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(წარმოდგენილია აკადემიის წევრის თ. ზაალიშვილის მიერ)

სამედიცინო სფეროში წამლის გადამტანი ნანომასალებიდან ერთ-ერთ პერსპექტიულ გადამტანად განიხილება PAMAM (პოლიამიდოამინი) დენდრიმერები, რაც აქტუალურს ხდის PAMAM დენდრიმერების ურთიერთქმედების შესწავლას დნმ-თან. სპექტროსკოპიული და თერმოდინამიკური მეთოდების გამოყენებით, ჩვენ შევისწავლეთ PAMAM დენდრიმერების სხვადასხვა თაობისა და ფუნქციური ჯგუფების (G2(NH_2), G4(NH_2) და G4(OH)) ურთიერთქმედება დნმ-ის მოლეკულებთან. დნმ-PAMAM კომპლექსების შთანთქმის სპექტრების, დნობის მრუდების, ფლუორესცენტული რეზონანსული ენერგიის გადატანის შედარებითი ანალიზის საფუძველზე დადგენილია, რომ ურთიერთქმედებას განსაზღვრავს PAMAM დენდრიმერების ზედაპირული ფუნქციონალური ჯგუფები. დენდრიმერები, რომელთა ზედაპირზე NH_2 ჯგუფებია, დნმ-ის დნობის ტემპერატურას ზრდიან 20°C და ენერგიის გადატანის ეფექტურობას 18%-ით, ხოლო OH ჯგუფების მქონე დენდრიმერები დნმ-ის დნობის ტემპერატურას ზრდიან 4°C და ენერგიის გადატანის ეფექტურობას 5%-ით. NH_2 ფუნქციონალური ჯგუფების მქონე PAMAM დენდრიმერების ძლიერი ურთიერთქმედება გამოწვეულია ელექტროსტატიკური ურთიერთქმედებით.

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