Research Article

Polyamide Dendrimers and Spermine Interact with DNA

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Abstract

Polyamines and polyamide dendrimers based on tetraethyl-1,1,3,3-propanetetracaroxylate positively charged organic cations under physiologic ionic- and pH conditions and therefore interact with negatively charged sites at double stranded Deoxyribo nucleic Acid(dsDNA). The interaction of these dendrimers with the DNA double helix has been studied with two generations (G): G_2-NH_2, G_4-NH_2. The binding properties of the two generations were examined by ethidium bromide (EtBr) displacement using fluorescence spectroscopy. It has been shown that G_2-NH_2 and G_4-NH_2 dendrimers can interact with dsDNA through an intercalative binding mode as its displaced EtBr from the dsDNA-dye complex. The electrostatic interaction is the main mode of polyamine-nucleic acid interactions. In conclusion, the synthetic dendrimer polymers appear most interesting and highly promising candidates as DNA-interactive drugs that have antitumor activity.

Keywords: DNA-interactive agents; Dendrimers polymer, Chemotherapeutic intervention, Ethidium bromide

Introduction

DNA represents a major target for chemotherapeutic strategy in human cancers, particularly for those where elevated proliferation rates of some tumor cell types have resulted in sensitivity to drugs, which obstruct transcription and replication of their DNA [1]. Plenty of research projects are endeavored designing new molecules that are able to interact with the double stranded DNA (dsDNA) of tumor cells. Among DNA recognizing small molecules, intercalators represent an important group of potential anticancer drugs [2,3]. Ethidium bromide is a cationic dye and can interact with ds DNA by intercalation between the base pairs [4-7]. The fluorescent complex between ethidium bromide and DNA was reported previously [4-7]. A large increase in fluorescence is observed when ethidium bromide intercalates DNA making it a useful probe to measure drug–DNA interactions [8-10].There are two binding sites where ethidium bromide can bind to the DNA: a primary site, which has been interpreted as intercalation between base pairs, and a secondary site, which is considered to serve for electrostatic interactions, between the cationic ethidium bromide and the anionic phosphate groups located at the DNA surface [11,12].

The polyamines, spermine and spermidine and their diamine precursor putrescine are naturally occurring, polycationic alkylamines that are essential for the cell growth. The need of polyamines is often dysregulated in cancer and other hyperproliferative diseases, it therefore appears eligible making polyamine function and metabolism an important target for therapeutic intervention [13-15]. Many cationic polymers such as polylysine, polyethylenimine and fractured dendrimers can interact with DNA and form a complex. These polymers interact with DNA through electrostatic interaction forming a unit structure with toroidal morphology. The level of aggregation of the unit structures in solution depends on the features of the individual polymer [16]. Dendrimers are a class of polymers which distinguishes them
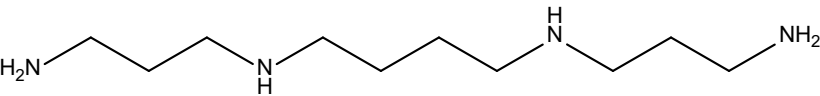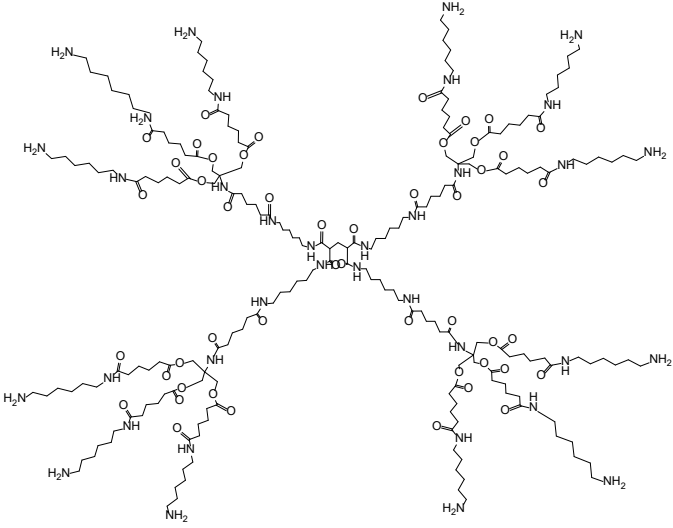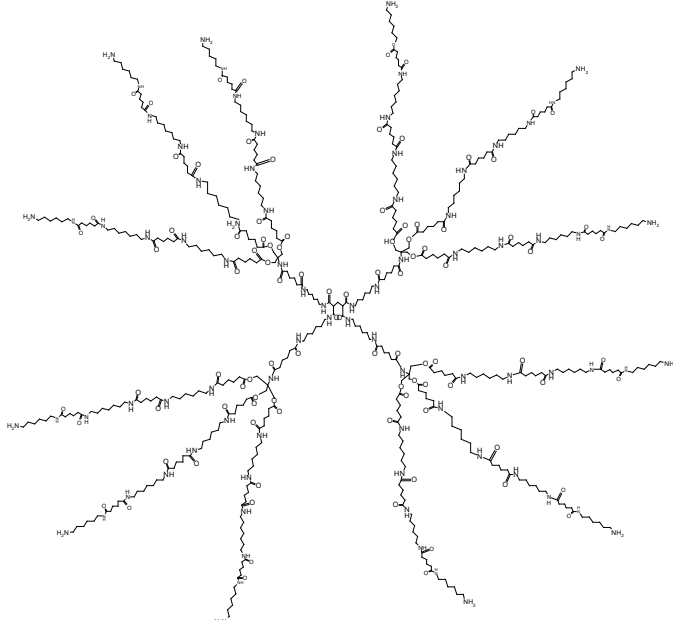
from linear polymers by their remarkable chemical and physical properties, which can be controlled during their synthesis. They are normally synthesized from a central polyfunctional core, branching segment and a surface called periphery. The periphery segments are the most effective parts in the dendrimers, they control the solubility and bioactivity. The potential of using dendrimers as vessels or hosts for other molecules was strikingly demonstrated by many researchers [17-20]. Amine terminal groups adds positive charge to the surface of dendrimersthat can be used for electrostatic drug interactions. Polyamide dendrimer based on tetraethyl-1,1,3,3-propanetetracaroxylate synthesized through repeating amide formation of 1,6-diaminohexane with adipoyl chloride,

tris(hydroxymethyl) aminomethane was introduced as branching point. The main aim of our study was to explore the ability of polyamines and polyamidedendrimers polymers to displace the ethidium bromide binding dye from the genomic DNA giving the opportunity to create new effective substances for cancer therapy.

Materials and Methods

Table 1 summaries the chemical structures of the compounds used in the study. Spermine dihydrate was purchased from Sigma Aldrich, cat No. 85588. The G_2-NH_2 and G_4-NH_2 were obtained from Dr. Ali Elejmi's team (Chemistry Department, Tripoli University) (Table 1).

Table 1: Chemical structures of spermine, G2-NH2 and G4-NH2 molecules.

Name	Chemical Structure
Spermine	
G_2-NH_2	
G_4-NH_2	

DNA binding properties

To study how competently the synthesized compounds, interact with genomic DNA (G-DNA), we investigated their DNA binding

ability using fluorescence emission spectra. All experiments were conducted in Tris buffer (0.01M Tris, 0.1M NaCl, at pH 7.4). Glass-distilled deionized water and analytical grade reagents were used

throughout experiments. pH values of solutions were measured with a calibrated Jenway pH-meter model 3510 (Staffordshire, UK). All buffer solutions were filtered through Millipore filters (Millipore, UK) of 0.45 mm pore diameter.

Absorbance spectra

Absorbance spectra were measured on a Jenway UV-visible spectrophotometer, model 6505 (London, UK) using quartz cells of 1.00 cm path length. The UV-Vis absorbance spectra were recorded in the 200-500 nm range and a spectral bandwidth of 3.0 nm. For the final spectrum of each solution analyzed baseline subtraction of the buffer solution was performed. Genomic DNA was used in a concentration of 75 µg/ml. DNA was extracted from peripheral lymphocytes of anticoagulated blood (EDTA) sampled by Proteinase K digestion and phenol/chloroform extraction [21]. The purity was determined by measuring the absorbance at 260/280nm indicating that the sample was free from protein contamination [21]. The concentration was assayed spectrophotometrically using 6600M-1cm-1 as a molar extinction coefficient at 260 nm.

Fluorescence spectra and DNA-binding studies

Fluorescence emission and excitation spectra were measured using a Jasco FP-6200 spectrofluorometer (Tokyo, Japan) using fluorescence 4-sided quartz cuvettes of 1.00 cm path length. The automatic shutter-on function was used to minimize photo bleaching of the sample. The selected excitation wavelength for ethidium bromide was 480 nm. The emission spectrum was corrected for background fluorescence of the buffer. Ethidium bromide (EB) fluorescence displacement experiment were performed by sequential addition of aliquots of 1790 µl Tris buffer, 10 µl EB (final concentration of 72 µM), 100 µl G-DNA from stock solutions (1.5 mg/ml) and finally 10 µl of compounds (spermine, G_2-NH_2 and G_4-NH_2 final concentration of 50µM). Emission spectra were recorded for each system using excitation wavelengths of maximum fluorescence intensity determined for the systems at to be480 nm using a slit width of 5 nm to examine alterations in emission spectra resulting from the complex construction of both systems. After completion of the full systems, the system was allowed to equilibrate for 30 minutes at room temperature and emission spectra (500-730nm) were recorded to monitor changes in EB intensity.

Result and Discussion

DNA binding properties

Measurement of the capability of ligands to displace ethidium bromide from genomic DNA is established as a suitable measurement of DNA binding ability for both intercalative and non-intercalative drugs. The displacement of ethidium bromide from DNA provides an indirect method of measuring the binding affinity of drugs that lack a chromophore as in case of spermine, G_2-NH2 and G_4-NH_2. To study how effective the three compounds interact with G-DNA, the DNA primary binding properties were explored by using fluorescence emission spectroscopy. Fluorescence emission experiments have been extensively used to study the spermine,

G_2-NH_2 and G_4-NH_2 compound-DNA interactions, in which the fluorescence emissions of ethidium-DNA complex was decreased significantly in the presence of these compounds, which is in good agreement with fluorescence results of other intercalators reported in the literature [22]. The results indicate that all three compounds can interact with G-DNA. It is well established in the literature that polyamines have multiple positive charges in vivo and in vitro due to protonation of the amine groups, they will interact with negatively charged polynucleic acids. It has been estimated that a large amount of polyamines available in cells are associated with DNA or RNA [23]. Polyamines have been shown to provoke DNA condensation and to stabilize compact forms of DNA [24]. Polyamines are also able to promote secondary structural transitions of DNA with the B–Z transition of alternating purine/pyrimidine oligonucleotides in solution [25]. All these features effect the fluorescence of ethidium bromide when it is displaced from the DNA by polyamines or polymers.

EB competition assay

The structural properties of the complex formed between genomic DNA and ethidium bromide using Raman microscopy equipped with near-infrared laser excitation was studied by Tsuboi, [26]. The study showed that the phenanthridinium plane is tilted by 35±5° from the plane perpendicular to the fiber (DNA helix) axis. The study also assumed coplanarity of the phenanthridiniumbyring and its immediate base neighbors at the intercalation site. Such bases would have a tilt angle closer to that of A-DNA (20°) compared to B-DNA (6°) and the average base tilt in stretches of DNA between intercalation sites remains that of B-DNA [26]. In our experiments the entrenched quenching assay based on the displacement of the intercalating dye, ethidium bromide (EB), from G-DNA was utilized to explore the interaction mode between the complexes and G-DNA. EB is an extremely helpful DNA probe, which exhibits a significant increase in fluorescence intensity when intercalating into the base pair of DNA. However, the increased fluorescence can be obviously quenched when a second complex can replace the bound EB or break the secondary structure of DNA [27,28]. It has been shown that the groove DNA binders possibly cause the decrease in EB emission intensities [29,30]. The entire compounds do not fluoresce with an excitation at 612 nm.
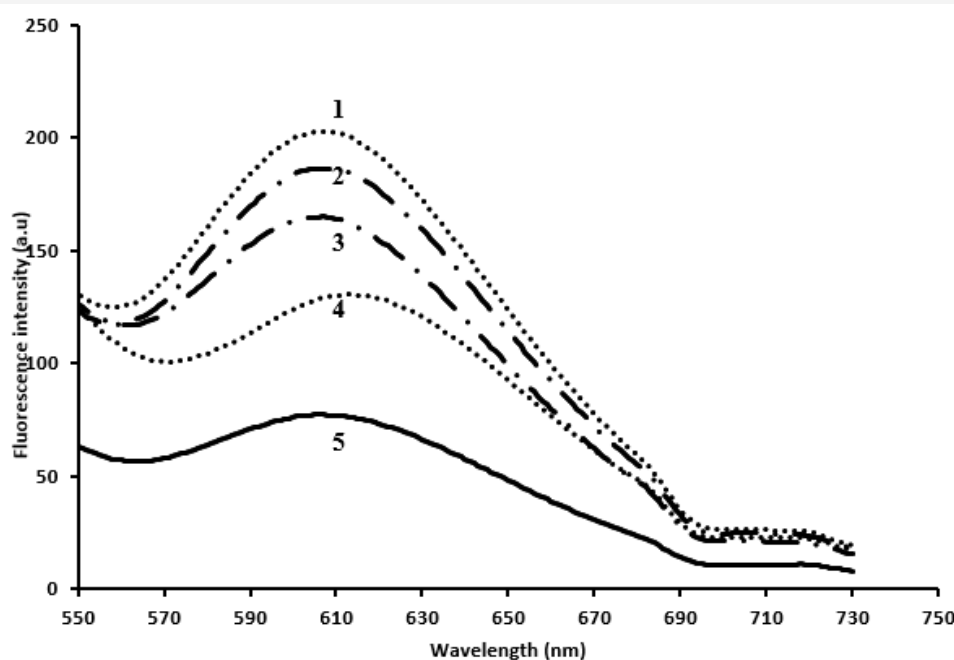
The EB competition assay results are shown in Figure 1. The fluorescence intensity of DNA-bounded EB at 612 nm decreased substantially in the presence of spermine, G_2-NH_2 or G4-NH_2. This decrease in fluorescence intensity may be due to the quenching of some EB molecules that were released from DNA into the solution after being substituted by spermine, G_2-NH2 and G4-NH_2 compounds. A similar phenomenon of fluorescence quenching caused by DNA - compound interactions has been reported previously [29,30] (Figure 1).

In addition, it has been reported that polyamine binding to DNA in the presence of ethidium bromide forming a polyamine–DNA complex was not completely responsible for the release of ethidium bromide. Polyamines in high concentration induces DNA

bending leading to conformational changes within the double helix that helps the release of bound ethidium bromide [31,32]. Spermine, G_2-NH_2 and G_4-NH_2 have polybasic character. The three compounds bind strongly to genomic DNA molecules and stabilize them by neutralizing negative charges of phosphate groups and by decreasing the repulsion between complementary strands. The fluorescence quenching activity obtained in this study could be related to DNA condensation which generally depends on the positive charge of polyamines and this is consistent with reports in the literature [33-35]. In spermine the distance between amino groups attached to carbon atoms is nearly the same as the distance between phosphate anions in the DNA backbone. This makes

spermine an ideal molecule for creating a zwitterionic structure that often perk up target affinity to DNA. This may explain the pattern that G_2-NH_2 and G_4-NH_2 bind to DNA even they are big molecules.

The literature also confirms that polyamines and their polymers bind to the DNA. It is reported that both electrostatic and chemical interactions take place in polyamine-DNA binding using Raman spectroscopy [36,37]. In addition, modeling, x-ray diffraction, and solution studies have emphasized our understanding of interactions between polyamines and nucleic acids. Polyamines appear to ccupy specific sites, particularly the major and minor grooves, in different DNA conformations [38-40].

Figure 1: Fluorescence changes of ethidium bromide that contains (1) G-DNA-ethidium bromide complex, (2) G-DNA-ethidium bromide complex with 50 μm spermine (3) G-DNA-ethidium bromide complex with 50 μm G4-NH2(4) ethidium bromide alone and (5) G-DNA-ethidium bromide complex with 50 μm G2-NH2. Experiments were conducted in solutions containing: Tris buffer (0.01M Tris, 0.1M NaCl, at pH 7.4, λex = 480 nm. Genomic DNA was used in a concentration of 75 μg/ml and ethidium bromide 72 μM.

As shown is Figure 1, compound G_2-NH_2 had the highest quenching efficacy suggesting that compound G2-NH2 attaches most tightly to G-DNA - even more than spermine and G_4-NH_2. The differences in the fluorescence quenching ability between G_2-NH_2 and G_4-NH_2 could be related to the fact that the conformational change of the double stranded DNA molecule induced by G_2-NH_2 is more pronounced than by G_4-NH_2. A reason may be that the chain branch lengths in G_2-NH_2 are shorter than the branches lengths in G_4-NH_2 dendrimers. Thus G_4-NH_2 dendrimers are less flexible and bulky, as a result of the increase of hydrogen bonds as branches increase. This result is confirmed by other researchers [16]. Moreover, the results shed light on the periphery interaction of dendrimers with DNA rather than at interior segments.

Conclusion

In conclusion, the polyamine field, predominantly in relation to cancer and other hyperproliferative disorders, is at an exciting stage. The molecular mechanisms related to polyamine function are

now becoming more obvious, creating an enhanced understanding of the differential requirements for polyamines in tumor cells versus normal cells. In our study the DNA binding of the spermine, G_2-NH_2 and G_4-NH_2 was investigated by fluorescence spectral techniques and revealed an intercalative interaction between them and G-DNA. Among the investigated three compounds G2-NH2 showed the highest quenching affinity. Furthermore, the study provides evidence that the bulkiness of the molecules play role in the interaction with DNA.

Acknowledgement

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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