Biohybrids based on DNA and bio-inspired lipid membranes: design and characterization

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This paper described the design of materials based on DNA and biomimetic membranes fluorescently labelled with an optically active phytomolecule: chlorophyll *a*. The formation of DNA-constructs was investigated by UV-Vis absorption and fluorescence emission spectroscopy, by using the spectral fingerprint of chlorophyll. Dynamic Light Scattering (DLS) measurements revealed the nano-scaled size of the developed materials. The chemiluminescence method demonstrated the antioxidant properties of these DNA-based constructs. These findings highlighted the potential use of the obtained hybrids to develop new structures for biophotonics' applications.

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1. Introduction

The nowadays discoveries in nanotechnology are widely used in various fields such as nanomedicine (drug delivery systems, nanotherapy, cancer diagnosis, biomarking) or optoelectronics (nanomachines and photon nanoscale devices for delivery of therapeutic agents) [1].

A very interesting material often used in nanotechnological applications are *liposomes*, which are spherical-shaped entities consisting of one or more bilayered membranes ─ structures very similar with the biological lipid membranes. Liposomes containing fluorescent dyes were applied in optical and voltammetric immunoassays [2]. On the other hand, biomimetic membranes loaded with chlorophyll, a natural fluorophore, were used to develop bio-based materials with high biological value: antioxidant, antimicrobial and anti-proliferative activities [3-7].

An interesting and unique natural material is deoxyribonucleic acid (DNA), the genetic information -coding, -storing and -transmitting biopolymer. Since its discovery, this biomolecule has fascinated the scientists due to its unusual properties determined by its three-dimensional conformation consisting of double-strand DNA helical structure stabilized by base pairing through hydrogen bonding (G with C, and A with T). Yatsunyk and co-workers pointed out the DNA "nano-oddities" [8], coming from its ability for self-recognition and self-assembly, enabling the interactions with itself or with other biomolecules (lipids, proteins) or nanoparticles giving rise to unusual materials having various advantages over conventional double-helix DNA, such as enhanced sensitivity to chemical stimuli and thermal stability. Thus, DNA has been used in many applications including: biomedicine [9-11], bioelectronics and biophotonics [12-15]. Rau *et al*. [16] highlighted the richness of the possible functionalization of DNA with photosensitive and charge transporting molecules giving rise to novel eco-friendly materials with applications in photonics and in electronics.

The aim of this paper is the preparation of materials based on DNA and bio-inspired membranes labelled with a photo-active molecule: chlorophyll *a* (Chl*a*), with potential applications in photonics and in biomedical field. This natural porphyrin was used in previous studies to monitor the bioconstruction of various optical active biohybrid materials [3-6]. The use of porphyrins in optoelectronics was also reported by research team of Anderson [17] which achieved DNA–porphyrin adducts that can potentially be used for construction of efficient long-range energy transfer systems.

On the other hand, DNA-based nanostructures are valuable drug delivery systems due to their intrinsic biocompatibility, uniformity and versatility [18]. Moreover, lipid-DNA complexes are useful in biomedical field as effective gene delivery systems [19] or in optoelectronics to achieve DNA lipoplex-based light-harvesting antennae [20].

The DNA-based materials developed in this study, were characterized by UV-Vis absorption and fluorescence emission spectroscopy; their size was estimated by Dynamic Light Scattering (DLS) measurements. The antioxidant capacity of these bioconstructs was evaluated through chemiluminescence method.

2. Experimental part

2.1. Materials

Luminol (5-amino-2,3-dihydro-phthalazine-1,4-dione), Tris (hydroxymethylaminomethane base), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), sodium hydrogen phosphate (Na_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) were supplied from

Merck (Germany). Dimyristoyl phosphatidylcholine (DMPC, T_c = 23^{0}C) and sodium chloride (NaCl) were purchased from Sigma Aldrich (Germany), and herring DNA from Fluka (Switzerland).

2.2. Preparation of Bio-inspired lipid membranes labelled with Chlorophyll *a*

Bio-inspired lipid membranes (liposomes) were obtained by hydration of a thin film of DMPC, according to method previously described [21]. The artificial lipid bilayers were labeled with a natural optical probe – chlorophyll *a*, (lipid:Chl*a* molar ratio of 100:1). Chl*a* was isolated from spinach fresh leaves, in our laboratory as described in [22]. The lipid vesicles (0.5 mM) were suspended in a physiological phosphate saline buffer solution: KH_2PO_4-Na_2HPO_4-NaCl (PBS, pH 7.4).

2.3. Preparation of DNA-based biohybrids

Two types of supramolecular entities containing herring DNA and liposomes were prepared by ultrasound-assisted method.

The concentration of herring DNA solutions prepared in PBS, at physiological pH (7.4), was determined through UV absorption spectroscopy by using the Lambert-Beer law, and the molar absorption coefficient: ε_{260} = 6600 $M^{-1}cm^{-1}$ for DNA [23].

Appropriate amounts of herring DNA were mixed with liposome suspensions (5 mM) in a volume ratio V_{DNA}/V_{Lip} of 100:1 and 10:1, and then subjected to ultrasounds for 5 min in an ultrasonic bath (BRANSON 1210), resulting the biohybrids DNA-Lip1 (n_{DNA}: n_{Lip} = 0.112) and DNA-Lip2 (n_{DNA}: n_{Lip} = 0.017), respectively (see Table 1).

The experiments carried out in darkness. The samples were coded as displayed in Table 1.

Table 1. The abbreviations of the samples

Sample	Code
Chl*a*-DMPC Liposomes (0.5 mM)	Lip
Phosphate saline buffer (KH_2PO_4-Na_2HPO_4-NaCl) pH 7.4	PBS
Lip:PBS (1:100, v/v)	Lip1
Lip:PBS (1:10, v/v)	Lip2
DNA:Lip1 biohybrid (100:1, v/v)	DNA-Lip1
DNA:Lip2 biohybrid (10:1, v/v)	DNA-Lip2

2.4. Characterization methods

The UV-Vis absorption spectra were obtained using a double beam spectrophotometer Lambda 2S Perkin Elmer, in the wavelength range of 200-800 nm.

The fluorescence emission spectra of DNA/liposomes were collected in the wavelength range of 600-800 nm, on a LS55 Perkin Elmer fluorescence spectrometer, by illuminating the samples with 430 nm excitation light.

Particle size analysis: $Z_{average}$ (Z_{av}, the particle diameter plus the double-layer thickness) and polydispersity index, Pdl (the indicative of the width of the size distribution; 0<Pdl<1) of each system was performed by Dynamic Light Scattering (DLS) technique (Zetasizer Nano ZS, Malvern Instruments Ltd., U.K.), at 25ºC temperature and at a scattering angle of 90º. The samples were analyzed as described in literature [24]: a volume of 0.1 mL of each sample was diluted in 25 mL of ultrapure distilled water. The average diameters (based on Stokes–Einstein equation) and polydispersity indexes were calculated from three individual measurements, and the mean value ± standard deviation was reported for each sample.

The *in vitro* antioxidant activity (AA%) of the DNA/liposomes hybrids was evaluated by chemiluminescence (CL) method, on a Chemiluminometer Turner Design TD 20/20 (USA), using a generator system for free radicals, containing: luminol (1 mM), H_2O_2 (10 μM) in Tris-HCl buffer solution (pH 8.6). The AA% value was estimated by the expression:

$$AA\% = [(I_0\text{-}I)/ I_0] \cdot 100\% \quad (1)$$

where I_0 is the maximum CL intensity at t = 5 s, for the standard, and I is the maximum CL intensity for sample at t = 5 s [25]. The mean value of AA% was calculated from three individual experiments, with standard deviation (SD) procedure. The data were presented as mean ± standard deviation; SD was calculated as the square root of variance using STDEV function in Excel 2010.

3. Results and discussions

3.1. Spectral characterization of biohybrids

UV-Vis absorption and fluorescence emission spectra provide deep insight into the formation of the developed materials.

Spectral patterns of Chl*a* were used to monitor the interaction between Chl*a*-loaded liposomes and herring DNA in aqueous solution at physiological pH.

The UV-Vis absorption spectra of Chl*a*-DMPC liposomes in the absence or presence of increasing amounts of DNA were displayed in Fig. 1.

The equilibrium for the formation of the complex between Chl*a*-liposomes and DNA is given by the following equation:

$$\text{Chl}a\text{-liposomes} + \text{DNA} \overset{K_{app}}{\rightleftharpoons} \text{Chl}a\text{-liposomes}\cdots\text{DNA} \quad (2)$$

The value of the apparent association constant (K_{app}) was calculated as previously described [26] based on the Benesi & Hildebrand equation from the absorption spectra of the liposome–DNA complex:

$$1/(A_{obs} - A_0) = 1/(A_C - A_0) + 1/[K_{app}\cdot(A_C - A_0)\cdot C_{DNA}] \quad (3)$$

where A_0 is the Chl*a*-liposome maximum absorbance in the absence of DNA and A_C is the recorded absorbance at 672 nm for the Chl*a*-liposomes at different DNA concentrations.

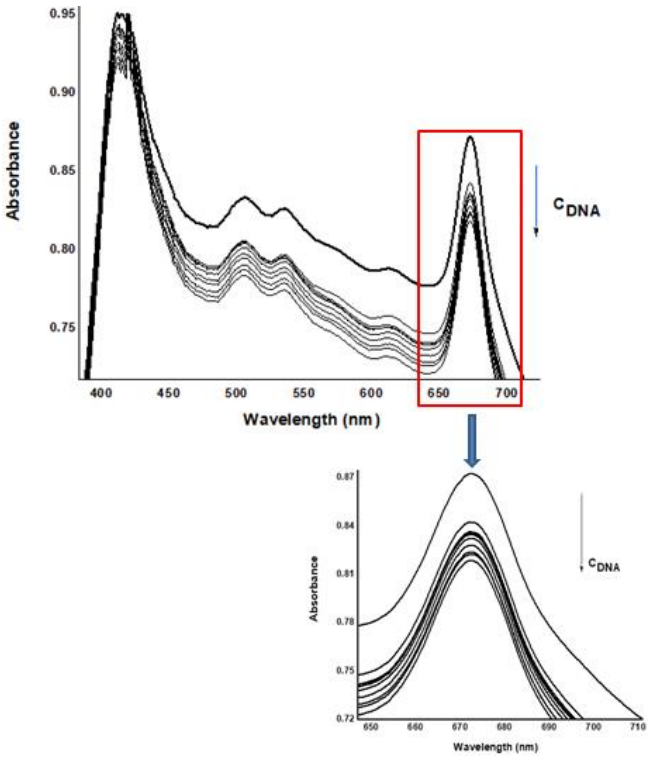

Fig. 1. The changes in the UV-Vis absorption spectra of Chla - DMPC liposomes (0.5 mM) after addition of increasing amounts of herring DNA (0÷8.8)×10^{-4} M

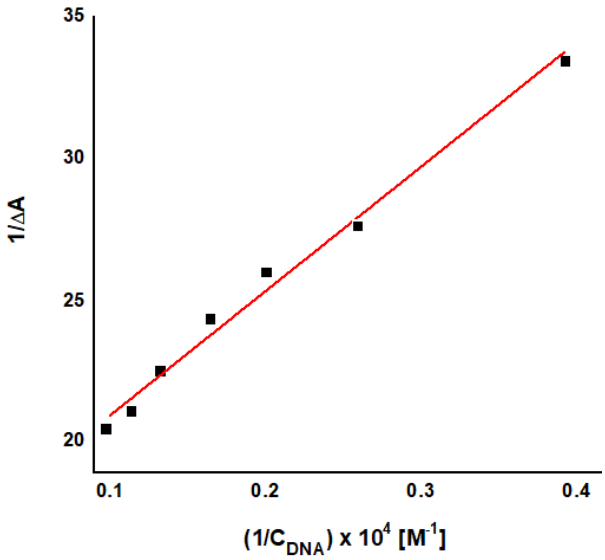

Fig. 2. Determination of apparent association constant (K_{app}) of Chla-DMPC liposomes with herring DNA (correlation coefficient R^2=0.9455)

As seen in Fig. 1, by addition of increasing DNA amounts to lipid vesicles, the value of the absorbance of chlorophyll *a* from its characteristic maximum (672 nm) decreased.

The plot of $1/\Delta A$ vs. $1/C_{DNA}$, where $\Delta A=A_{obs}-A_0$, is linear and K_{app} was estimated to be 0.4×10^4 M^{-1} (Fig. 2) from the ratio of the intercept to the slope [27]. The obtained value of K_{app} clearly suggested the formation of the liposome–DNA complex.

The fluorescence emission spectra of Chl*a*-DMPC liposomes (Fig. 3) illustrated a decrease in fluorescence intensity of Chl*a* incorporated into biomimetic bilayers, upon addition of increasing DNA amounts, Chl*a* detecting the possibility of formation of DNA–liposomes bioconstructs. The spectral fingerprint of Chl*a* located at 679 nm slightly shifted to 681 nm after DNA addition to liposomal suspensions. The binding constant (K) of DNA to liposomes, was calculated according to the literature [26] and found to be 10^4 M^{-1}; the length of intercept on Y-axis equals to logK from the linear plot log $[(F_0-F)/F]$ vs. log(C_{DNA}) (Fig. 4).

The spectral data showed that DNA could interact with liposomes by means of Chl*a*, resulting in quenching of the Chl*a* fluorescence intensity. On the other hand, according to Tajmir Riahi and co-workers [28], that porphyrins like chlorophyll and its derivatives are known to be strong DNA binders, so Chl*a* could facilitate the formation of Chl*a*-liposomes···DNA complex in these experiments.

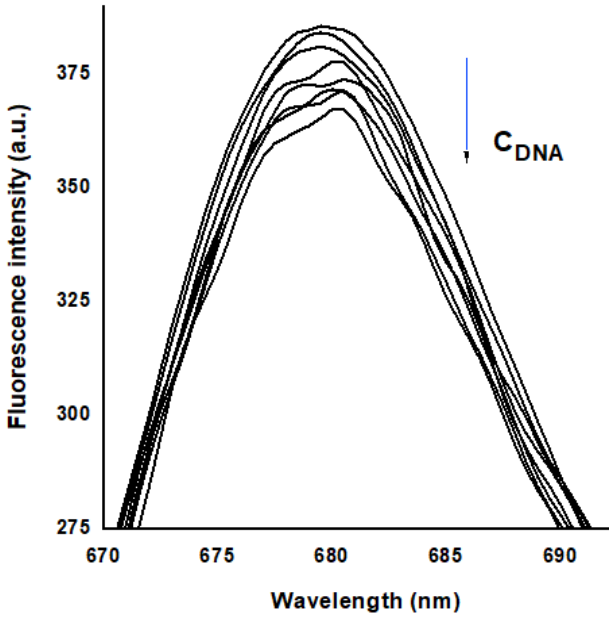

Fig. 3. Fluorescence quenching spectra of Chla-DMPC liposomes by herring DNA; λ_{ex} = 430 nm; C_{Lip} = 5×10^{-4} M; C_{DNA} = (0÷8.8)×10^{-4} M

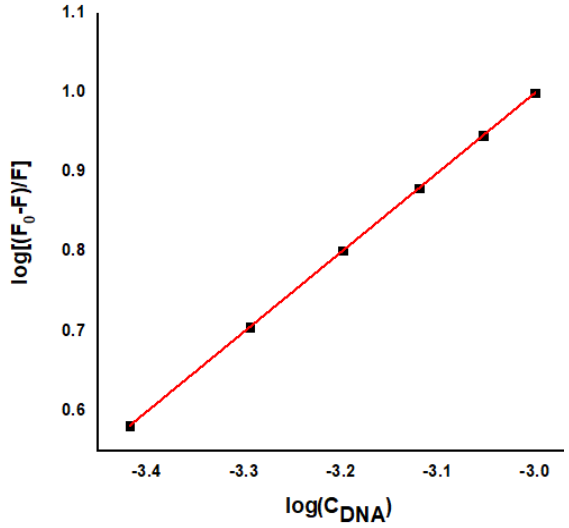

Fig. 4. Calculation of binding constant (K) from plot of herring DNA quenching effect on fluorescence of Chla-DMPC liposomes (correlation coefficient R^2=0.9999)

The absorption data correlate with those of fluorescence and demonstrate that quite stable supramolecular entities have been obtained.

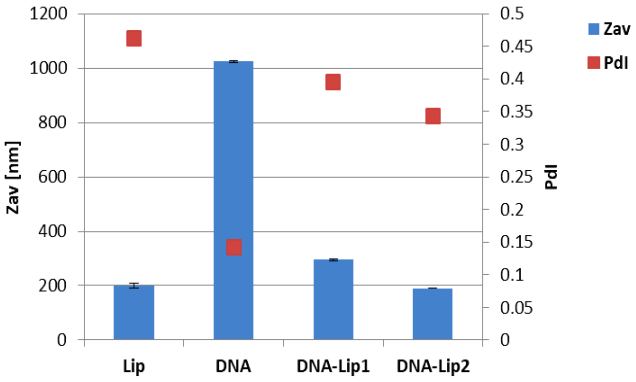

Fig. 5. Mean particle size and polydispersity index of Liposomes-DNA biohybrids, by DLS technique

Particle size analysis of developed DNA/lipid materials (Fig. 5) showed that the DNA-Lip2 presented smaller particle dimension and also small value of polydispersity index, due to a better nano-biointeraction between lipid matrix and biopolymer. Thus, DNA-Lip1 presented a mean diameter of 295±3.8 nm being larger than those of DNA-Lip2 (Z_{av} = 190±1.3 nm) which it was prepared with a lipid amount 6.71 times higher. These findings showed that the increase in liposome content (related to DNA amount) in hybrid systems resulted in decrease in dimension of DNA-Liposomes biohybrids, due to a reorganization in system.

For internalization pathway of particles through the cell membrane, the preferred particle size is 100–200 nm to allow gene or drug delivery [29], so DNA-Lip2 bio-particles could be used in biomedical applications as vehicles for transportation of genes or various therapeutic agents.

3.2. Estimation of antioxidant properties of Liposomes-DNA biohybrids

The *in vitro* antioxidant properties of Liposomes-DNA biohybrids were evaluated through chemiluminescence method (Fig. 6).

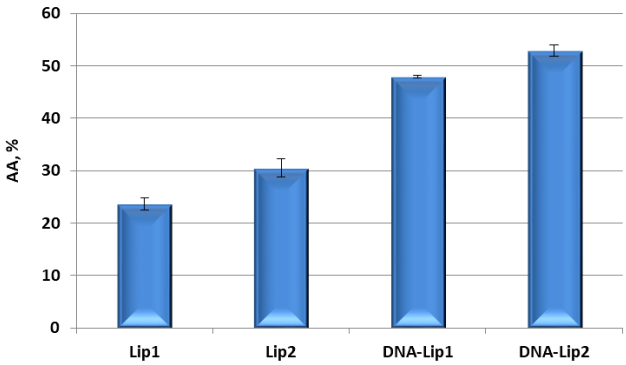

Fig. 6. The antioxidant activity of the developed lipid-based systems

The DNA/liposomes systems showed medium antioxidant activity values (Fig. 6). The biohybrids DNA-Lip2 presented AA% value of 53% greater than DNA-Lip1 (AA = 48%). The enhanced capacity of DNA-Lip2 biohybrids to scavenge short-life free radicals is due to the presence of Chl*a* inside their structure, which is a recognised natural antioxidant [30], but also to the nanoscaled size of this complex offering more reaction centers for free radical scavenging.

4. Conclusions

This work reports the design and characterization of hybrid materials containing DNA and bio-inspired lipid membranes labeled with a natural fluorophore: chlorophyll *a*.

Photophysical studies of DNA–Chl*a*/liposomes bioconstructs illustrated that UV-Vis absorption spectra showed a pronounced hypochromism in absorption peaks of Chl*a*, and the emission spectra presented a fluorescence quenching, after addition of increasing DNA amounts to liposomal suspension. These spectral changes indicated that chlorophyll detected the formation of liposome-DNA complexes, and the bio-interaction between DNA and liposomes was facilitated by means of Chl*a*.

The obtained materials presented nano-scaled dimension and antioxidant properties. The biohybrids with low DNA:liposomes ratio, DNA-Lip2, presented AA% value of 53% greater than the biohybrids with high DNA:liposomes ratio, DNA-Lip1 (AA = 48%).

These developed materials based on DNA and artificial cell membranes could be applied in biomedical field and in biophotonics.

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