Biophysical aspects on interaction between DNA and the food dye – amaranth (azorubine S, E123)

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This paper reports the investigation of the interaction between the food additive amaranth (azorubine S, E123) and methylene blue-marked-DNA, monitored by UV-Vis absorption and fluorescence emission spectroscopy, as well as cyclic voltammetry. The binding constants (Kb) for the amaranth with marked-DNA was estimated to be 2×10^7 M^{-1} through absorption spectroscopy, and 1.1×10^7 M^{-1} through fluorescence analysis. The cyclic voltammetry method showed that both anodic and cathodic current peaks of amaranth decreased upon addition of the DNA. These findings could be exploited to design innovative DNA-based materials for biosensing and biophotonic applications.

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Keywords: Amaranth dye (azorubine S, E123), DNA, Methylene blue, Spectral and electrochemical characterization

1. Introduction

The "versatile world of nucleic acids" has been intensive exploited by the scientists in the last years, in various practical applications, such as nanotechnology, biosensing, or electronic devices [1]. Due to its unique 3D structure consisting of two polynucleotide chains self-assembled into a double helix, through hydrogen bonds, the "smart" biomolecule DNA gained interest as "green" material for biomedicine, optoelectronics and biophotonics [2-5].

DNA is the key *informational molecule of life* that encodes the genetic instructions for development and functioning of all living organisms. Organic materials such as dyes could enter into the biological systems by different ways, and could interact with DNA damaging its structure, and altering DNA function and genetic expression [6]. One kind of these dyes are azo dyes that are organic compounds possessing chromophoric azo bonds -N=N-, with the general chemical formula: R1–N=N–R2, where R1 and R2 are aromatic groups that can be substituted by sulphonated groups [7]. It has been reported that azo dyes are harmful to the human health as well as to the environment, having toxic, mutagenic and carcinogenic effects [7-8]. Due to their low-cost, excellent water solubility, high stability and low microbiological contamination, the azo dyes are intensively used as textile colorants and food dyes [9], and also in nonlinear optics [10], in colored optical devices and windows shutters [11], or in holographic recording materials [12].

Long-term exposure to food colorants even in low doses has potential risks to human health, and can cause serious adverse health effects such as allergies, hyperactivity disorder, and restlessness in children, and also genotoxicity and carcinogenicity [9]. On the other hand, the toxicological properties of dyes are generally related to their binding nature to the bio-macromolecules such as nucleic acids [6] and proteins [13], hence, the study on the interaction of these biomolecules with azo dyes are of great significance.

Amaranth dye (AM) (IUPAC name: trisodium (4E)-3-oxo-4-[(4- sulfonato-1-naphthyl)hydrazono]naphthalene-2,7-disulfonate) [14], also known as Azorubine S, E123, Acid Red 27, AR27 Compound, C.I. Acid Red 27, C.I. Food Red 9) is a reddish or brownish azo dye (see Fig. 1) widely used as food additive in various beverages, cake mixes, ice creams, cereals, candies, jelly, jams, chocolates, sausages, and other food products, as well as in cosmetics and pharmaceuticals [9]. It should not be confused with the flowering plant *Amaranthus retroflexus* named also "amaranth". As an azo colorant, amaranth dye was reported to possess potential toxicity to humans [13], owing to the presence of an azo group that could be reduced in organisms to aromatic amines which are highly harmful for human health [6, 15]. Although the *World Health Organization* (WHO) and *Food and Agriculture Organization* (FAO) have limited the daily amaranth intake to 0.5 mg/kg [16], it induces serious health problems in sensitive persons and in children.

There are few studies on the interaction of synthetic azo- dyes with DNA. Upon our knowledge, there is no biophysical reports on the association of food additive amaranth with DNA, only studies regarding the binding of

this dye to bovine serum albumin (BSA) [13] and human haemoglobin [18].

This paper reports biophysical aspects on interaction between DNA and the food dye amaranth, under physiological conditions. Spectral and electrochemical investigations were performed in order to gain deep insights into the association of this dye with DNA molecule, for potential use of amaranth colorant to develop DNA-dye complex for biosensing or biophotonic applications.

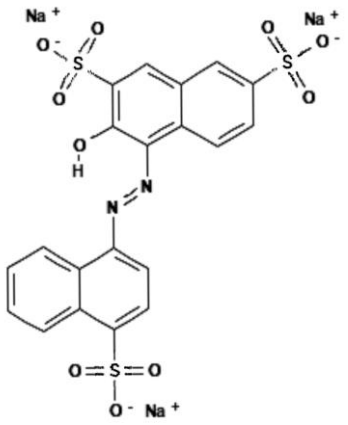

Fig. 1. Chemical structure of amaranth dye (adapted upon [17])

2. Experimental part

2.1. Materials

Potassium dihydrogen phosphate (KH_2PO_4) and sodium hydrogen phosphate (Na_2HPO_4) were supplied from Merck (Germany). Herring DNA was purchased from Fluka (Switzerland), and the methylene blue (MB, $C_{16}H_{18}ClN_3S$, M=315.5 g/mol) from Loba Fein Chemie. Azorubin S (amaranth dye, $C_{20}H_{11}O_{10}N_2S_3Na_3$, M =604.48 g/mol) and sodium chloride (NaCl) were supplied from Sigma Aldrich (Germany).

Herring DNA was labelled with methylene blue (MB) as described in [19]. The concentration of herring DNA solution prepared in PBS pH 7.4, was determined from UV absorption spectrum by using the Lambert-Beer law (the molar absorption coefficient for DNA: ε_{260} = 6600 $M^{-1}cm^{-1}$ [20]).

Methylene blue – labelled DNA (MB-DNA) samples were incubated with amaranth dye (in various concentrations) for 3 hours, before spectral analyses. All experiments were performed under physiological conditions, in phosphate saline buffer (PBS, KH_2PO_4-Na_2HPO_4-NaCl) at pH 7.4, and at 37°C.

2.2. Characterization methods

UV-Vis absorption spectra were recorded at the resolution of 1 nm, on a double beam spectrophotometer Lambda 2S Perkin Elmer, operating from 200 to 800 nm, with 1 nm slit width and 0.3 nm/s scan rate.

The fluorescence emission spectra of MB in samples were collected in the wavelength range of 670-800 nm, on a LS55 Perkin Elmer fluorescence spectrometer, at 662 nm excitation wavelength.

Cyclic voltammetry measurements were performed using a Voltalab 40 system (Radiometer Analytical) adapted for screen-printed carbon electrodes (SPCEs). SPCE DS-110 (Metrohm DropSens, ceramic substrate L33 x W10 x H0.5 mm, electric contacts: silver) is designed with 3 separate electrodes: (i) auxiliary electrode (counter)-carbon, (ii) working electrode (4 mm diameter)-carbon and (iii) reference electrode-silver. Potentials were recorded within the range (−0.4 to 1) V at a scan rate of 20 mV/s.

3. Results and discussions

3.1. Photophysical investigation of amaranth – DNA interaction

In order to investigate the interaction of amaranth dye (AM) with DNA molecule, the methylene blue (MB) was used as a spectral probe for DNA, being intercalated between DNA nitrogenous bases as previously described [19].

The effect of gradual addition of AM to MB-DNA samples was monitored firstly by UV-Vis absorption spectroscopy (Fig. 2).

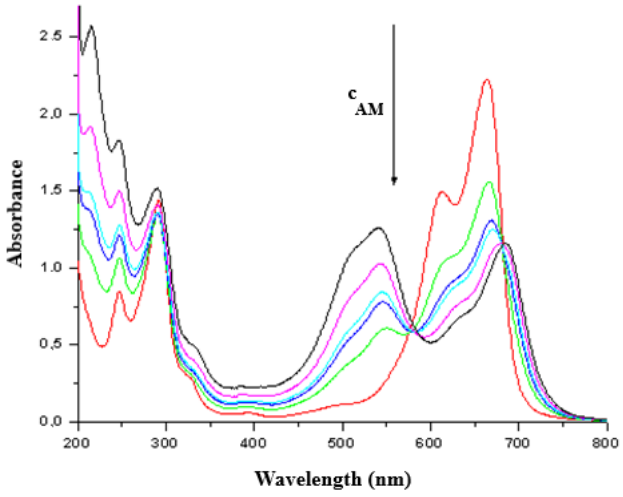

Fig. 2. Effect of gradual addition of AM, on the absorption spectra of MB-DNA in PBS pH 7.4

The MB characteristic UV-Vis absorption bands are observed in the visible region: a strong band at 665 nm attributed to MB monomer, and another one at 611 nm assigned to MB dimer [21].

By increasing AM concentrations, significant changes in the DNA-MB absorption spectrum occurred. Thus, a

new strong absorption band in the visible region, at 522 nm wavelength appeared, characteristic to amaranth dye, and responsible for its red colour [18].

Moreover, it was observed a hypochromic effect and a red shift of the band at 665 nm. An isosbestic point is observed suggesting binding of the food additive to DNA [21]. On the other hand, a pronounced hyperchromic effect occurred for the band at 260 nm, characteristic for DNA, revealing the damage of the DNA double-helix structure, by addition of the food colorant. These findings suggest that the food dye binds to DNA molecule by an intercalative mode.

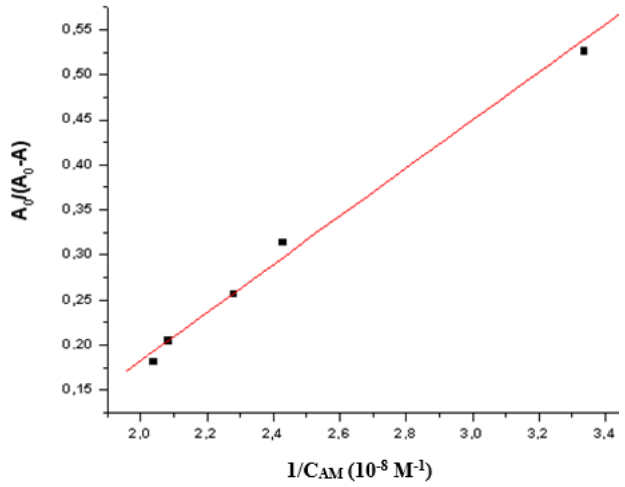

Fig. 3. Determination of binding constant (K_b) of amaranth with MB-DNA (correlation coefficient R^2=0.9729)

The binding constant K_b was calculated from the linear plot in Fig. 3, at a value of 2×10^7 M^{-1}. This value clearly suggested the formation of the complex between DNA and AM, in which the food dye binds to MB-DNA by an intercalative mode.

Furthermore, fluorescence emission spectroscopic studies revealed the fluorescence quenching effect of AM addition on MB-DNA spectra (Fig. 4). The binding constant (K_b) of AM to DNA labeled with MB, was calculated by using the modified Stern-Volmer equation according to the literature [22] and found to be 1.1×10^7 M^{-1} (Fig. 5). This value is in close agreement with that obtained from the UV-Vis absorption spectroscopic analysis.

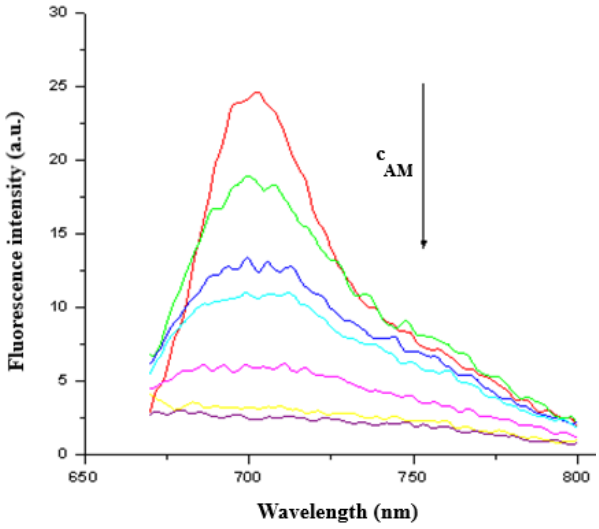

Fig. 4. The effects of AM on the fluorescence emission spectra of MB labelled DNA, in PBS pH 7.4 (λex = 662 nm; C_{AM} = (0÷6)×10^{-8} M)

Rasouli and co-workers [13] reported a binding constant of association of amaranth dye to BSA protein of 3.4×10^7 M^{-1} (by fluorescence spectroscopy) which is of the same order of magnitude as obtained in our experiments.

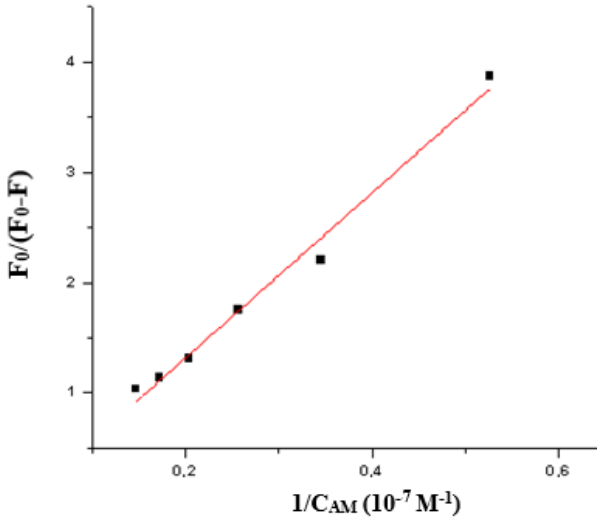

Fig. 5. Determination of binding constant (K_b) of amaranth with MB-DNA from plot of AM quenching effect on fluorescence of MB-DNA (correlation coefficient R^2=0.9573)

These results suggest the harmful effect of amaranth dye on the structure of DNA biomolecule. The binding constants calculated by UV-Vis absorption and fluorescence emission spectroscopy are on the order of 10^7 M^{-1} characteristic for classical intercalators [23].

3.2. Electrochemical aspects amaranth – DNA interaction

Fig. 6 shows the electrochemical behaviour of amaranth dye with different agents (MB-DNA and MB) and is compared to the electrochemical response showed when it stands alone in the solution. The indication is that the behaviour remains the same, but the intensity of the signal (expressed as current density) increases when MB is introduced in the solution. However, when the DNA-MB is present, the signal shows a decrease in the intensity and also a shift towards more positive potentials. This could mean that a stronger bond is formed between amaranth-DNA-MB, which needs higher potential to be oxidized.

For each sample the scan was performed five times and the third scan was chosen. Also, in order to present the graph for DNA on the same graph as all the samples, its intensity was divided by 10. The position and the height for the observed peaks are presented in Table 1. The results show that the interaction between Amaranth-DNA-MB is the strongest between all the samples (the peak position shifts to more positive values and the shift is +29 mV compared to amaranth dye). The cyclic voltamogram for the DNA-MB shows a peak value at 99 mV, with a height of 0.362 mA/cm^2. In comparison, the graph for Amaranth–MB indicates a peak position closer to Amaranth (234 mV as compared to 241 mV for pure sample Amaranth) and the highest current density for this peak among all the samples (0.529 mA/cm^2). Considering all the above, the sample containing Amaranth-DNA-MB can be used as sensor for Amaranth dye: the peak position and peak height (very similar to the height of the Amaranth sample) can be used for qualitative measurements.

Table 1. The main characteristics of the cyclic voltammetry for the sample presented

Sample name	Peak position (mV)	Peak height (mA/cm^2)
Amaranth	241	0.4
Amaranth-DNA-MB	270	0.423
Amaranth-MB	235	0.529
DNA-MB	99	0.362

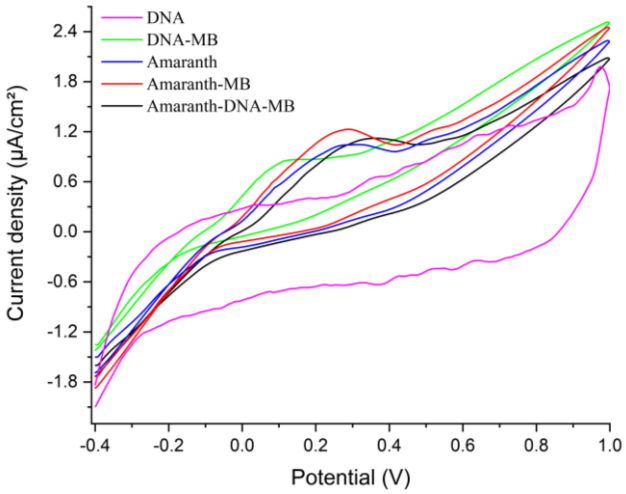

Fig. 6. Electrochemical behaviour of amaranth - MB-DNA as compared to that of DNA, MB-DNA, MB, amaranth dye, and dye mixture

4. Conclusions

This paper reported biophysical insights on interaction between DNA molecule and a food colouring agent – amaranth dye. The gradual addition of this synthetic colorant to DNA marked with methylene blue, resulted in the conformational changes in the DNA- double helix revealed by UV-Vis absorption and fluorescence emission spectroscopy. The absorption data are in good correlation with those of fluorescence and demonstrate that stable complex between DNA and food dye has been achieved.

Taking into account the values obtained for binding constants (Kb) for association of the food dye with marked-DNA: 2×10^7 M^{-1} through absorption spectroscopy, and 1.1×10^7 M^{-1} through fluorescence studies, it could be concluded that amaranth interacted with herring DNA by an intercalative mode. The electrochemical studies confirmed the formation of strong complex between this dye and DNA.

These results could be exploited to develop new DNA-based materials for biosensing or biophotonic applications.

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