Astaxanthin binding affinity to DNA: Studied by fluorescence, surface plasmon resonance and molecular docking methods

Farideh Ranjbary
Tabriz University of Medical Sciences

Farzaneh Fathi (f.fathi@arums.ac.ir)
Ardabil University of Medical Sciences

Somaiyeh Maleki
Tabriz University of Medical Sciences

Research Article

Keywords: Astaxanthin, DNA, Interaction, fluorescence, Surface plasmon resonance, Molecular docking

Posted Date: May 22nd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2946014/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Astaxanthin (Ax), as a novel food supplement, a pink-red pigment, belongs to the carotenoid family. The study of DNA interactions with various drugs is very important for estimating the mechanism of interaction and developing new drugs. The purpose of this study is to investigate the binding affinity of Ax to double strand (ds) DNA evaluated by using a fluorescence spectroscopy, surface plasmon resonance (SPR) and docking approaches. The fluorescence results shown that Ax can quench the intensity amount of DNA fluorescence via a static quenching way. In the SPR method, DNA molecules were attached on a gold sensor surface. Using different amounts of ds DNA, the kinetic values $K_D$, $K_A$, and $K_a$ were calculated. The obtained finding confirm that the binding of Ax to DNA has an exothermic and spontaneous mechanism. Also, thermodynamic studies were carried out using fluorescence analysis at four different temperatures, and the resulted negative data for $\Delta H$ and $\Delta S$ displayed that the main binding strength in the interaction of Ax to DNA was hydrogen bonding. Molecular docking results confirmed that the side chains of Ax interact specifically with base pairs and the DNA backbone.

Highlights

- The binding strength of astaxanthin (Ax) to double strands (ds) DNA was investigated.
- The fluorescence results shown that Ax can quench the DNA.
- The obtained negative values for $\Delta H$ and $\Delta S$ indicated that the binding of Ax to DNA was spontaneous process.
- Major binding force involved in intercalation of AX to DNA was hydrogen bonding.
- Molecular docking results confirmed that the side chains of Ax interact with DNA.

1. Introduction

The use of food additives is at least challengeable due to their vital and direct role of them in health problems. Natural source materials have been applied to provide color in foods, make-ups and drugs. Some of the most common ones are carotenoids, chlorophyll, anthocyanin, and turmeric[1]. Hydrocarbon carotenoids like $\beta$-carotene and oxygen-replaced carotenoids (also known as "xanthophylls," like astaxanthin) make up the family of carotenoids with a deep red, yellow, or orange color. The carotenoid astaxanthin (Ax), potent herbal antioxidant, known as 3,30-dihydroxy-$\beta$, $\beta$-carotene-4,40-dione, has enormous profitable usages in the food and medicinal sectors and it has been widely used in the food [2, 3]. Ax, also has been introduced as a new food in food supplements at maximum amounts of 8 mg/day [4]. The benefits of eating a diet heavy in fruits and vegetables and consuming a lot of carotenoids are frequently attributed to the antioxidant properties of carotenoids [5–7]. A associated class of more than 600 chemicals, excluding stereo- and geometric-isomers, are known as carotenoids and some of them have strong antioxidant properties [8]. The presence of hydroxyl (OH) and keto (C@O) moieties on each ionone ring explains some of its distinguishing characteristics, including the ability to be esterified, higher antioxidant activity, and a more polar nature than other carotenoids [9].
Deoxyribonucleic acid (DNA) is one of the most important biological macromolecules which can control all changes in cells. Replication and transcription are the two main processes in cells and are required to cell proliferation and cell survival. These processes are started when regulatory proteins (as a signal) bind to specific regions of the DNA structure [10–12]. So, if a small molecule mimics the binding specificity of the regulatory proteins (by activation or inhibition of DNA function) it will be an effective therapeutic agent [13]. Many drugs and therapeutic agents such as anticancer, antiviral and antibiotics interact with DNA to control cell functions by interfering with replication or regulating gene expression [14]. It will be useful to explore the interaction mechanism between DNA and various small molecules for understanding the action mechanism and developing new agents [15]. The interaction investigation between biomolecules and DNA has an overall interest and significance due to develop of different DNA-targeted molecules. Drugs and small molecules can bind to DNA via the following models: covalent binding to DNA bases, minor groove or major groove binding, intercalation between DNA base pairs and finally using the electrostatic interaction [16]. Fluorescence spectroscopy is one of the techniques which used to measure the binding affinity, kinetic and thermodynamic parameters of drug interaction to DNA [17, 18]. Spectroscopic methods have proven to be very suitable to characterized binding thermodynamics of ligands biomolecules like DNA and albumin. In addition, surface plasmon resonance (SPR) based biosensors which related to label-free optical methods, recently used for small molecule and DNA interactions [19, 20]. In this method, any refractive index shifts which is proportional to the mass of analyte molecules bound near the sensor surface was recorded in real time form [21, 22].

Until now, there has been no study on temperature dependence of DNA and small molecules interaction. In this study, not only the binding affinity of Ax to double strand (ds) DNA was investigated by spectral and SPR methods but also the thermodynamic values at four different temperatures was calculated. For this purpose, the quench of DNA intensity was investigated by fluorescence spectroscopy at different temperatures. Also, in the SPR method, the biotinylated DNA molecules layers were attached on a streptavidin coated gold sensor surface for evaluation of the kinetic values $K_D$, $K_A$, and $K_a$. Finally in silico molecular docking analyses was applied for comparison of obtained results from in vitro spectral and sensing measurements.

2. Materials and methods

2.1. Materials

Ax was purchased from sigma. The biotin-terminated ds DNA solution (OD: 4) with sequences of $3'$ CGCGAATTCGCG $5'$ were obtained from takapouzist Company (Iran). To determine the purity of DNA, the absorbance ratio of the DNA solution was measured at A260/A280 and the obtained results showed great purity of the sample (A260/A280 = 1.78). In general, A260/A280 ratios greater than 1.7 are frequently indicative of pure DNA samples. Also, Astaxanthin (Ax) (C40H52O4) $\geq$ 97%, ammonia (NH$_4$OH, 30%), glycine-HCl, and mercaptoethanol (MCH) were purchased from Sigma-Aldrich (Steinheim, Germany). The streptavidin coated gold chip was prepared from Bionavis Company (Tampere-region,
Finland). To prepare the stock solution of Ax, an adequate amount of its powder was dissolved in distilled water, and the prepared stock solution was then diluted in a series of dilutions with varying concentrations.

2.2. Fluorescence measurements

All fluorescence spectra of the samples were measured using a Cytation™ 5 (bioteck) spectrophotometer attached with a thermostated cell holder and a black 96-well plate. The prepared samples were excited at 280 nm, and then the emission curves were recorded from 390 to 470 nm. Ax showed a maximum emission intensity at 416 nm ($\lambda_{em} = 416$ nm) by exciting at 280 nm ($\lambda_{ex} = 280$ nm). To study of the Ax/DNA interaction, a fixed amount of Ax was added to various DNA solutions and vortexed slowly for 20 min.

2.3. SPR measurements

To obtain a stable baseline in the sensorogram, the streptavidin-coated gold chip surface was washed with NaCl (2.0 M) and NaOH (0.1 M) for 3 min. The SPR instrument was then cleaned with PBS as a running buffer, and the biotinylated probes were immobilized on the sensor surface by the avidin-biotin interaction during a 30 min injection. The biotinylated-DNA was then immobilized by injecting a 100 M DNA solution into channel 1 for 5 min and acetate buffer (running buffer) into channel 2 (reference channel). Mercaptohexanol (MCH) (1 mM) was used to block non-specific interacting places. All injection steps were done real-time at a flow rate of 30 ml/min during 3 minutes and also different concentrations of Ax solution (20–100 M) were injected into both flow cells. The DNA was immobilized only in the test flow channel, and the other one without any immobilized DNA was used as a reference channel. Before investigating the interaction of Ax with DNA, the DNA-modified gold surface was washed using a one-min injection of glycine-HCl (10 mM). The Ax solution was introduced into reference and test channels. The kinetic parameters were calculated using Trace Drawer TM for SPR Navi™ and SPR Navi™ Data viewer software, which was fitted based on a one-to-one interaction model.

2.4. Molecular docking

For the computational study of the interaction of Ax with the DNA macromolecule in its most stable form, the method of molecular docking simulation was used. The molecular modeling method allows for predicting the most suitable conformation and the most favorable orientation of the ligand on biomacromolecules. On the other hand, molecular docking results provide the main pieces of information about the macromolecular ligand system that is subjected to the docking process, which are the binding value of free energy ($\Delta G$), suitable binding site in the macromolecule structure, and different forms of non-covalent interaction. In this study, the three dimensional structures of DNA and Ax were taken from the RCSB protein data bank (http://www.pdb.org) and the PubChem website (PubChem CID: 5281224), respectively, and used in the simulation process. The DNA taken (PDB ID: 2GVR) has 1.65 Å resolution of the self-complementary dodecamer 5′-d(CGCGAATTCGCG)-3′. The heteroatoms or water molecules in the crystallographic structure of DNA macromolecules were removed from the PDB file in preparation for use
in the docking simulation. In this work, free and widely used docking software, Auto Dock Tools 1.5.7 is used [23].

Before performing molecular docking simulation, only polar hydrogen atoms, the setting of gasteigers, and the application of Kollman charges were added to DNA. To determine the proper binding site of Ax with the highest affinity on the stable form of the DNA macromolecule, blind molecular docking was done. In this technique, the proper ligand binding site was identified by scanning the entire surface of the DNA macromolecule. The estimated grid box sizes at grid focus in x × y × z bearings were settled at 25 × 31 × 47 Å for DNA and the grid spacing value was determined at 1 Å. 100 runs were considered for molecular docking of DNA with Ax. Default values for Auto Dock Tools parameters were considered, and the Lamarckian genetic algorithm mode was set on molecular docking simulation [24, 25].

3. Results

3.1. Fluorescence measurements

Fluorescence spectroscopy is a popular tool for investigating drug-DNA interactions. The fluorescence spectra is the most useful method for detection of functional groups types present in biomolecules [26]. Fluorescence quenching experiments reveal additional information regarding ligand location and mode of binding to DNA. It has been reported that Ax can display solid fluorescence spectra because of its carbonyl agents with extremely conjugated double-bonds. With excitation at 280 nm, the Ax releases emission spectra in the wavelength range of 420–430 nm. In this work, the possible effects of DNA on the fluorescence intensity of Ax were investigated using fluorescence method. The fluorescence data analysis revealed that adding DNA to Ax solution quenched its emission spectra without causing a significant change in spectrum position (Fig. 1, 2). However, increasing the DNA concentration caused a remarkable shift in the emission maxima to 681 nm, and the quenching of AX emission was related to the DNA concentration.

Based on their temperature response, the quenching approaches were categorized as static (complex creation), dynamic (collisional processes), or a combination both of static and dynamic [27–29]. The fluorescence quenching constant ($K_{sv}$) values were evaluated using following equation (Eq. 1) [30, 31] and the calculated values are shown in Table 1.

$$\frac{F_0}{F} = 1 + K_{sv}[DNA]$$

(1)

Where $F_0$ and F denote the emission intensity of Ax without and with DNA, [DNA] represent the complete amount of DNA (as a quencher) and $K_{sv}$ is the quenching constant of Stern–Volmer. The curve of $F_0/F$ against [DNA] was plotted at four different temperatures and $K_{sv}$ data were obtained from the slope of the regression curve and presented in Table 1. It can be seen from Fig. 1,2, the slope of the plots reduced
with temperature growth. This findings suggest that the $K_{sv}$ values were inversely correlated with temperature and the fluorescence quenching of Ax-DNA occurred through a static model [32, 33].

Hydrogen bonds, hydrophobic forces, van der Waals interactions, and electrostatic interactions all play important roles in ligand-receptor binding. The thermodynamic parameters were evaluated using the slope and intercept of the plot of $\ln K_D$ against $1/T$ and Van't Hoff's equation (Eq. 2) and used to define the type of the forces between Ax and DNA (Table 1).

$$\log K_d = -\frac{\Delta H}{2.30RT} + \frac{\Delta S}{2.30R}$$  \hspace{1cm} (3)

Where $K_A$, $T$ and $R$ correspond to the interaction constant, the temperature, and universal the gas constant [34]. In general, negative data of $\Delta H$ and $\Delta S$ characterize hydrogen bonding and van der Waals forces [35]. As a result, Ax can bound to DNA through primarily hydrogen bond and van der Waals formation. Planar heterocyclic small molecules in the structure of AX act as intercalators which cause a significant $p$-electron overlap [36]. Intercalators molecules stack vertical to the DNA building without making covalent bonds or forming hydrogen bonds between DNA bases [37]. It seems that the hydroxyl or carbonyl groups in the AX structure which defines as the hydrogen bond acceptors or donors led to the AX intercalation in DNA structure.

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$K_{sv}$ ($\mu$M$^{-1}$)</th>
<th>$R^2$</th>
<th>$\Delta H$ (kJ mol$^{-1}$)</th>
<th>$\Delta S$ (J mol$^{-1}$ K$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>304</td>
<td>0.00446</td>
<td>0.97</td>
<td>-35.667</td>
<td>-19.47 J mol$^{-1}$ K$^{-1}$</td>
</tr>
<tr>
<td>307</td>
<td>0.00405</td>
<td>0.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>310</td>
<td>0.003432</td>
<td>0.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>313</td>
<td>0.003075</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 3.2. SPR measurements

The SPR measurements were done by a Double-channel bionavis attached with a cuvette. A streptavidin coated gold chip was used for DNA probe immobilization. The SPR millidegree (m) angle change was detected in an air state and the change in millidegrees in the SPR curve reveals the amount of DNA immobilized. Every 120-m angle changes is corresponded to 1 ng/mm$^2$. SPR data analysis was used to obtain kinetic parameters of Ax/DNA complex including of the affinity (KD), association (ka) and dissociation (kd) constants. To decrease the mass transport influence, all solutions were injected at a low concentration, and the binding test flow rate was set at a higher rate of 30 µl/min.
Figure 3A shows the schematic illustration of Ax interaction with DNA on gold sensor surface in SPR method. In Fig. 3B the related SPR sensogram for biotinylated DNA immobilization on streptavidin coated gold chip was shown. As shown in this figure, near 0.03 RU of DNA was attached on gold sensor surface. Due to the addition of thickness to the gold surface when the gold sensor chip surface was altered by a biomolecules, the resonance peaks shifted to upper incident angles and widened.

The DNA-drug interaction tests were done out by injecting of different concentrations of Ax (1-2.5 nM) on the chip surface during 3 min. After the sensor signal stabilized, the SPR angle change was monitored to investigate the binding of Ax to DNA modified sensor. SPR curves at various Ax concentrations were set to a global 1:1 Langmuir-based association/dissociation form to ascertain the binding kinetics. Figure 4 represents SPR sensorgrams of the Ax-DNA interactions and the reference subtraction.

The computed data of the kinetic values $k_a$, $k_d$, and $K_D$ were $1.96 \times 10^2 \text{ 1/M s}$, $2.73 \times 10^{-2} \text{ 1/s}$ and $6.89 \times 10^{-5} \text{ M}$ respectively. A small amount of $K_D$ displays a more affinity of two molecules to each other [29, 38].

### 3.3. Molecular docking results

The lowest binding free energy value calculated in the docking molecular simulation for the interaction of Ax with DNA is -9.95 kcal mol$^{-1}$. According to the conformers obtained from molecular simulation, it can be concluded that the type of interaction of the considered compound with DNA is groove-like manner (Fig. 5A). The nucleotides participating in the hydrogen bond between Ax and DNA that have been identified after examining all docking runs are, deoxyguanosine (DG) 10, DG12, DG14, deoxythymidine (DT) 19, and the phosphodiester backbone of DNA. The formation of a hydrogen bond between nucleotides is created with the oxygen of the Ax carbonyl group, and the deoxynucleotides' hydrogens. Also, the constructed DNA backbone's H-bonds are established between the hydrogen of the Ax hydroxyl group and the phosphodiester's oxygen. Figure 5B shows a two-dimensional illustration of interactions between the DNA molecule and Ax. The hydrogen link between the nucleotide and the carbonyl and hydroxyl group of Ax is shown as a green dashed line. The three-dimensional representation of the formed hydrogen bond is visible in Fig. 5C. Due to the absence of an aromatic ring in the structure of Ax, the possibility of interaction of $\pi-\pi$ stacking is weak. Therefore, in the analysis of all conformers of the DNA-Ax system, $\pi-\pi$ stacking was not observed. In addition, nucleotides, DT8, deoxycytidine (DC)9, DG10, DC11, DG12, DC15, DG16, deoxyadenosine (DA)17, DT18, DT19, and DT20 have hydrophobic interactions. Due to the long chain of non-polar carbon-carbon in the structure of the Ax, the interaction of the mentioned compound with a large number of nucleotides is hydrophobic. On the other hand, the presence of a small number of groups capable of forming hydrogen bonds on the chemical structure, as was also observed in the analysis of the docking outputs, leads to the weak interaction.

### 4. Conclusion
Due to having the direct interactions of molecules and DNA, in the present study, we have attempted to probe the interaction of Ax with the dsDNA by a spectral, biosensor-based SPR and docking approaches. Studying the interaction between DNA and Ax show that, Ax non-covalently bind to DNA via hydrogen bonding. The Ax caused the quenching of the fluorescence emission intensity in DNA sequences. The $\Delta G$, the best parameter for the estimation of macromolecule stability, was determined by spectral and docking methods. Our results showed a decrease in the $\Delta G$, indicating DNA destabilization due to its interaction with the Ax ligands. The fluorescence results shown that Ax can quench the intensity amount of DNA fluorescence via a static quenching way. The resulted value for affinity, $\Delta H$ and $\Delta S$ was obtained $6.89 \times 10^{-5}$ M, -35.667 kJ mol$^{-1}$ and -19.47 J mol$^{-1}$ K$^{-1}$ respectively. The obtained finding confirm that the binding of Ax to DNA has an exothermic and spontaneous mechanism. Also, thermodynamic studies were carried out using fluorescence analysis at four different temperatures, and the resulted negative data for $\Delta H$ and $\Delta S$ displayed that the main binding strength in the interaction of Ax to DNA was hydrogen bonding. Molecular docking results confirmed that the side chains of Ax interact specifically with base pairs and the DNA backbone. It should be noted, these affinities have a significant effect on life phenomena at the molecular ranges.

Declarations

Ethical Approval

This declaration is not applicable.

Competing interests

The authors declare no conflict of interest.

Authors' contributions

Farideh Ranjbary: Methodology, Formal analysis, Docking analyses, Writing - Review & Editing

Farzaneh fathi : Supervision, Methodology, Validation, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization

Somaiyeh Maleki: Methodology, Investigation

Funding

The authors are grateful for financial support (grant no: IR.ARUMS.REC.1400.228) from Ardabil University of Medical Sciences.

Availability of data and materials

Data will be made available on request.
Acknowledgements

The authors are grateful for financial support from Ardabil University of Medical Sciences.

References


**Figures**

![Figure 1](image)

Figure 1

Fluorescence emission spectra of fixed concentration of Ax (0.55 µM) without and with various amounts of DNA solution (0, 14, 28, 43, 57 and 71 µM) at temperature of 31° C and 34° C and the related plot of
curve of $F_0/F$ against [DNA]. $\lambda_{\text{ex}}=280$ nm, $\lambda_{\text{em}}=390-470$ nm.

Figure 2

Fluorescence emission spectra of fixed concentration of Ax (0.55 µM) without and with various amounts of DNA solution (0, 14, 28, 43, 57 and 71 µM) at temperature of 37° C and 40° C and the related plot of curve of $F_0/F$ against [DNA]. $\lambda_{\text{ex}}=280$ nm, $\lambda_{\text{em}}=390-470$ nm.
Figure 3

(A) Schematic image of Ax interaction with DNA on gold sensor surface in SPR method and (B) SPR sensogram for biotinylated DNA immobilization on streptavidin coated gold chip.
Figure 4

The SPR curve of interaction between DNA and various concentrations of Ax (0.5-2.5 nM)
Figure 5

A) The lowest binding free energy conformer based on molecular docking simulations binding between astaxanthin and DNA. The interaction of the compound with DNA is groove mode. B) Illustration of the interaction between Ax and DNA nucleotides in two dimensions. Hydrophobic interactions with red spoked arcs and H-bonding with green dashed lines are shown. Calculated distances from DG14 to
binding oxygen of Ax carbonyl group are presented. C) 3D representation of the interaction between Ax and DNA. Green spheres presented hydrogen binding.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- [GraphicalAbstract.png](#)