Two-photon Absorption Spectroscopy to Differentiate Chromophore-DNA Binding Interactions

Saad H. Alotaibi, b, Kelly M Usakoski aMohammad Hatshan and aGuda Ramakrishna
a Department of Chemistry, Western Michigan University, Kalamazoo MI 49008 , United States
b Chemistry Department, Kalamazoo College, Kalamazoo, MI 49006-3295, United States

Abstract
The study of molecular-DNA binding interactions are vital as they are the principal components behind drug action, transcription and replication. Optical spectroscopic techniques are often used to study these interactions however, the existing techniques cannot differentiate different binding motifs of interaction and minor-groove binding. In the present study, we present a novel approach based on the two-photon absorption (2PA) cross-sections of chromophores to differentiate the interaction and minor-groove binding interactions. The investigations are carried out using three cyanine derivatives: Diethylthiacarbocyanine iodide (DTIC) and 3,3′-Diethylthiadiazocarbocyanine iodide (DTDCI). The chromophores were chosen as they are similar series of dye molecules but with different carbon chain lengths. As the chain length increases, the chromophores start to bind to minor-groove that intercalates. The results show that the 2PA cross-sections of DTIC bound to salmon-sperm DNA are unchanged or slightly decreased with increase in DNA concentration. On the other hand, the 2PA cross-sections of DTDCI have increased by 5-fold with increase in DNA concentration. The results show that DTIC binds to DNA via intercalation while DTDCI binds via DNA via minor-groove. The results are rationalized based on the DNA backbone’s electric field. Additional results of fluorescence lifetimes, anisotropy and circular dichroism will be presented.

Objectives of the study

To monitor the 2PA cross-sections of chromophores when they are bound to DNA. The hypothesis is that the local electric field environment in DNA can enhance the 2PA cross-sections of chromophores with the way the chromophore’s dipoles are aligned with the electric field of phosphate backbone.

To investigate if 2PA cross-sections can be used as markers to differentiate between intercalation and minor-groove binding interactions of ligands and DNA.

Introduction-Motivation
Molecule-DNA interactions
Interactions of small molecules with DNA push the DNA base pairs apart from one another and induce structural changes in the DNA and increase the helix length.

Why to study chromosome-DNA interactions?
These are important for several pharmaceutical applications
Chemotherapy - Doxorubicin and adriamycin
Antibiotics Prevents DNA replication
Popular DNA-binding experiments
a. Molecular “Swiching”- Luminiscence based detection
b. Victermy
- Thermal denaturation
d. Photobleaching
3D-imaging of DNA binding interaction is budge with these techniques. Novel approaches and molecules needed for this purpose.

Investigated Molecules and Techniques

Interaction of DTIC with DNA

DTIC (Diethylthiazocarbocyanine iodide) is a popular panel for DNA binding studies. A variety of observations can be made using 2PA absorption spectroscopy and two-photon excited fluorescence technique to measure 2PA cross-sections.

Figure 1. Molecular structures of the investigated chromophores

Interaction of DTDCI with DNA

DTDCI (3,3′-Diethylthiaadiazocarbocyanine iodide) has been studied extensively for its binding interactions with DNA. The 2PA cross-sections of DTDCI were found to be increased by 5-fold with increase in DNA concentration.

Fluorescence lifetimes

DTIC and DTDCI exhibit fluorescence lifetimes that are characteristic of DNA binding interactions. The lifetimes are determined using time-resolved fluorescence techniques.

Mechanism

The 2PA cross-section of DTIC is increased with DNA even though it binds to DNA through an intercalation mode.
While the 2PA of DTIC and DTDCI enhanced with 5-fold indicating that they bind with DNA via minor-groove where the dipole is parallel to E field.

Conclusions

All the investigated chromophores bound strongly with DNA with apparent changes in fluorescence intensity and absorption spectral variations and CD.
The 2PA cross-section of DTIC increased with DNA indicating that it binds with DNA via intercalation.
The 2PA cross-sections of DTIC and DTDCI have increased by more than 4-fold suggesting that it binds with DNA via minor-groove.
The results show that 2PA spectroscopy can be used to differentiate different modes of DNA-chromophore binding.

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References