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

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Abstract

The study of molecule-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 intercalation and minor-grove binding. In the present study, we present a novel approach based on the two-photon absorption (2PA) cross-sections of chromophores to differentiate the intercalation and minor-grove binding interactions. The investigations are carried out using three cyanine derivatives Dicyanomethylene (DTI), 3,3-Dicyanomethylene triquinacene (DTDCI) and 3,3-Dicyanomethylene triquinacene 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-grove than intercalate. The results show that the 2PA cross-sections of DTI bound to salmon-sperm DNA are unchanged or slightly decreased with increased DNA concentration. On the other hand, the 2PA cross-sections of DTDCI have increased by 3-4 fold with increase in DNA concentration. The results show that DTI binds to DNA via intercalation while DTDCI binds with DNA via minor-grove. 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 investigate if 2PA cross-sections can be used as markers to differentiate between intercalation and minor-grove binding interactions of ligands and DNA.

Introduction-Motivation

Molecule-DNA interactions

Interactions of small molecules with DNA push the DNA base pairs from one another and induce structural changes in the DNA and increase the helix length.

Why to study chromophore-DNA interactions?

These are important for several pharmaceutical applications

Chemotherapy - Doxorubicin and adriamycin
Antibiotics
Prevents DNA replication

Popular DNA-binding experiments

a. Molecular “Switching” – Luminescence based detection
b. Viscometry

Why is 3D imaging of DNA binding interaction is tough with these techniques. Novel approaches and molecules needed for this purpose.

Our approach is to use two-photon absorption (2PA) cross-sections of chromophores as markers to study chromophore-DNA interactions

Investigated Molecules and Techniques

2.7- Diarylthiacyanine iodide (DTI)
3.3-Dicyanomethylene triquinacene iodide (DTDCI)
Salmon sperm DNA

Interaction of DTI with DNA

Figure 1. Molecular structures of the investigated chromophores

Figure 2. Optical absorption spectra of DTI with increasing DNA concentrations

Figure 3. Two-photon excited fluorescence spectrum of DTI with increasing DNA concentrations. Observe the enhancement of fluorescence intensity suggesting DNA binding

Figure 4. Circular dichroism spectrum of DTI at different DNA concentrations.

Figure 5. 2PA cross-section spectra of DTI at different DNA concentrations.

Interaction of DTDCI with DNA

Figure 6. The circular dichroism of DTI 0 DNA and dye after bound to DNA. It can be observed that the dye’s absorption also polarized with DNA suggesting effective DNA binding

Figure 7. Optical absorption spectra of DTDCI with increasing DNA concentrations

Figure 8. One photon fluorescence spectra of DTDCI at different DNA concentrations

Figure 9. Two-photon excited fluorescence spectrum of DTDCI with increasing DNA concentrations

Figure 10. 2PA cross-section spectrum of DTDCI with increasing DNA concentrations

Figure 11. Optical absorption spectra of DTDCI with increasing DNA concentrations

Figure 12. One photon fluorescence spectra of DTDCI with increasing DNA concentrations

Figure 13. Two-photon excited fluorescence spectra of DTDCI with increasing DNA concentrations

Figure 14. Relative 2PA cross-sections of DTDCI with increasing DNA concentrations

Fluorescence lifetimes

Figure 15. Fluorescence decay traces of DTI at different DNA concentrations

Figure 16. Fluorescence lifetime changes for DTDCI at different DNA concentrations

Mechanism

The 2PA cross-section of DTI is unchanged with DNA even though it binds to DNA suggesting that it binds with DNA via intercalation.

While the 2PA of DTI and DTDCI enhanced by 4-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 DTI is unchanged with DNA suggesting that it binds with DNA via intercalation.

The 2PA cross-sections of DTI 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