

The Comparison of Optical Properties of Acridine Orange when Interact with Hybrids of Single-Walled Carbon Nanotubes and Single-Stranded DNA or Double-Stranded DNA

Ying Tan, Kazuo Umemura*

Department of Physics, Graduate School of Science, Tokyo University of Science, Japan

*Corresponding author

Kazuo Umemura, Department of Physics, Graduate School of Science, Tokyo University of Science, Japan, Tel: 81-3-52288228; E-mail: meicun2006@163.com

Submitted: 24 Dec 2017; Accepted: 05 Jan 2018; Published: 04 Feb 2018

Abstract

The optical properties (visible-ultraviolet emission and fluorescence) of acridine orange (AO) were compared and investigated to try to discuss the AO adsorption mechanisms when AO interacted with four aqueous dispersions, for example, single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), hybrids of ssDNA and single-walled carbon nanotubes (SWNT-ssDNA), and hybrids of dsDNA and single-walled carbon nanotubes (SWNT-dsDNA). Molecule structure deformation and energy transfer maybe existed when AO attached to DNA chains or emerged into SWNT/DNA suspensions. DsDNA seemingly brought about an obvious structure deformation to AO; delocalized π -bond of AO/SWNT perhaps produce a little absorbance red-shift effect to AO, because of lower electronic transition energy for π -electron or n-electron; the energy transferred from DNA to AO, or from AO to SWNT. Most of AO molecules "prioritized" DNA chains, rather than SWNT surface; although AO molecules adsorbed to DNA chain and SWNT surface at the same time.

Keywords: Single-Walled Carbon Nanotubes (SWNT), Single-Stranded DNA, Double-Stranded DNA, Acridine Orange (AO)

Introduction

A uniform suspension of carbon nanotubes (CNTs), especial single-walled carbon nanotubes (SWNTs), is essential for its all fundamental and industrial applications. Researchers conducted many studies to disperse SWNT using various assisting agents, which include DNA short oligo, proteins, surfactants, synthetic polymers [1-4]. Single-walled carbon nanotubes (SWNT) dispersed by single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) were first presented in 2003 by and, respectively. It is known that the emission from fluorescent dyes is quenched when the dyes are adsorbed onto CNT surfaces [5-7]. AO is a nucleic acid selective fluorescent cationic dye useful for cell cycle determination. Although possessing an inherent emission of 520-526 nm, when bound to dsDNA, an emission maximum at 526-533 nm (green) with an excitation maximum at 494-500 nm could be observed; while when it associates with ssDNA or RNA, the excitation maximum shifts to 420-460 nm (blue) and the emission maximum shifts to 630-650 nm (red).

In our previous studies, AO was used as intercalating dyes to react with two kinds of SWNT-DNA hybrids (SWNT-ssDNA and SWNT-dsDNA), the quenching phenomenon and fluorescent results of AO were collected for the hybridization behaviors. In this work, in order to further discuss the AO adsorption mechanism onto the surface

of SWNT-DNA, we compared and researched the photo-physical properties (not only fluorescence but also UV emission) of AO when mixed with four aqueous dispersions, for example, single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), hybrids of ssDNA and single-walled carbon nanotubes (SWNT-ssDNA), and hybrids of dsDNA and single-walled carbon nanotubes (SWNT-dsDNA). The fundamental information was necessary for the AO detection and quantification in aqueous solution, also useful for the behavior of DNA molecules on CNT surfaces, and potential nanosensor applications.

Materials and Methods

Single-walled carbon nanotubes (SWNT) produced by the high-pressure carbon monoxide (HiPco) method were purchased from Raymor Nanotech (Canada) as purified powders.

Single stranded DNA with thymine 30 mers (ssDNA, or T30) and double stranded DNA (dsDNA, deoxyribonucleic acid sodium salt from salmon testes) were purchased from Life Technologies Japan, Ltd., Tokyo, Japan and Sigma-Aldrich Co., MO, USA, respectively. Acridine orange (1 mg/mL H₂O), was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Buffer solution

10mM Tris (hydroxymethylaminomethane-HCl (Tris) (pH=8.0)

Hybrids of ssDNA and SWNT (SWNT-ssDNA, or SWNT-T30)

0.6 mL of ssDNA solution of deionized water (1 mg/mL) and 0.3 mg of SWNT powder were mixed and sonicated using a probe-type sonicator in an ice-water bath for 90 min (VCX 130, Sonics & Materials, Inc., CT, USA) with a 2-mm diameter tip at 3 W [8,9]. The hybrid suspension was centrifuged at 12,500 g for 3 h at 8 h (high speed refrigerated micro centrifuge, MX-150, TOMY, Japan). After centrifugation, the upper 75% of the volume was stored as the SWNT-ssDNA suspension. The relative concentration of SWNT-ssDNA and SWNT-dsDNA origin suspension was estimated based on their largest absorbance at 730 nm, as 0.124 and 0.073, respectively. The concentration of dsDNA in both dsDNA origin solution and prepared SWNT- dsDNA could be calculated as 800 and 1200 $\mu\text{g/mL}$, based on the DNA absorbance at 270 nm.

AO-H₂O solution

Purchased Acridine orange (AO) solution (1 mg/mL H₂O) was 10-fold diluted with deionized water to prepared 0.1 mg/mL (0.33mM) AO-H₂O solution.

Vis-absorption (UV) measurement

The UV spectra of abs. intensity upon concentration curve of AO, AO/DNA and AO/SWNT-DNA were recorded by JASCO V-630 spectrophotometer. All of mixture solutions were diluted with the buffer solution up to 1 mL to prepare the absorbance samples.

Fluorescence measurement

The fluorescence spectra of AO, AO/DNA and AO/SWNT-DNA were recorded for different concentration by JASCO FP-6500 spectrophotometer. All of mixture solutions were diluted with the buffer solution up to 2 mL to prepare the fluorescence samples.

Results and Discussions

Depending on our previous work, when quenched by SWNT-DNA (both ssDNA and dsDNA), AO seemed showed unique performance than other common dyes. In order to obtain detailed information, we designed four adsorb situations for AO, with single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), hybrids of ssDNA and single-walled carbon nanotubes (SWNT-ssDNA), and hybrids of dsDNA and single-walled carbon nanotubes (SWNT-dsDNA), respectively. Figure 1 showed the absorbance spectra of AO, AO/DNA, AO/SWNT-DNA solutions, with a fixed content of AO and increasing amount of DNA or SWNT-DNA. Moreover, the concentration of both ssDNA (a) and dsDNA (b) was adjusted to the same (800 $\mu\text{g/mL}$), the dsDNA content of SWNT- dsDNA solution (d) was also adjusted to the same value as used in (b) (800 $\mu\text{g/mL}$); the SWNT-ssDNA solution (c) was 3.6-diluted to make sure the similar SWNT concentration in both SWNT-ssDNA (c) and SWNT-dsDNA (d). Figure 2 illustrated the fluorescent property of AO in the AO/ssDNA, AO/dsDNA, AO/SWNT-ssDNA and AO/SWNT-dsDNA systems, all of the utilized DNAs was adjusted to the same concentration; all the used SWNT-DNA solutions were also shared the same SWNT relative concentration. The summary of optical performance summary of AO, AO/DNA and AO/SWNT-DNA was listed in Table 1.

As a typical SWNT spans a wide absorption range of 500-900 nm, resonant energy transfer is allowed to occur because of the absorption wavelengths overlapping the photoluminescence spectra of various fluorophores [10]. This is a type of energy transfer when a donor photon is not radiated but is reabsorbed by an acceptor [11]. CNTs

were regarded as electron acceptors due to their electron-deficiency property based on molecular orbital theory and experimental results, thus, upon photoexcitation, single excited fluorophore would transfer an electron to a SWNT, resulting in single-electron oxidation of fluorophore and single-electron reduction of SWNT [12]. Relative researches were also carried out to deduce the occurrence of a photoinduced charge-separation state upon electrochemical results and the electron-transfer process from redox potentials [7,13,14].

Form Table 1 we can see, with the content of DNA or SWNT-DNA increased, both absorbance and fluorescence properties of AO showed a peak shift phenomenon, more or less; moreover, variation of intensity could also be observed. In the case of AO/ssDNA, during the ssDNA added, the maximum absorbance of AO did not change, only absorbance intensity decreased (Figure 1 (a)); the fluorescent peaks and intensities were nearly the same (Figure 2 (a)). Maybe it could be suggested that AO molecules just attached to ssDNA chains simply and some energy transfer from DNA to AO depending on the decreased absorbance intensity. While in the AO/dsDNA situation with more dsDNA added, the absorbance red-shift (from 490 to 499 nm, Figure 1 (b)) perhaps illustrated somehow AO structure deformation in AO/dsDNA hybrids, and the energy transfer to AO from dsDNA caused the declined absorbance intensity; slight blue-shift (from 532 to 530 nm, Figure 2 (b)) fluorescence and increased emission intensity indicated that AO gained much more energy in AO/dsDNA hybrids than AO lonely.

Theoretically, introducing the chromophores could result in the red-shift of absorbance wavelength because of molecular structural changes/deformations in some degree; while here AO is just regarded as a kind of chromophore group as its conjugated structure. Here, considering the conformations of ssDNA and dsDNA, we thought AO molecule just loosely attached to ssDNA chains, while intercalated closely into dsDNA chains. Thus, it was assumed that AO/dsDNA complex showed an obvious red-shift absorbance compared with AO/ssDNA complex, in virtue of a chromophore introduction into dsDNA chains.

As far as AO/SWNT-ssDNA system, it was thought that AO molecules attached to DNA chains and SWNT surface at the same time. With the SWNT-ssDNA increased, the absorbance of AO showed a little red-shift of 4 nm (from 490 to 494 nm, Figure 1 (c)), illustrated that maybe lower electronic transition energy for electrons in AO occurred when AO attached onto SWNT surface; while the absorbance intensity first decreased then increased, perhaps could be due to the energy transfer from ssDNA to AO, and from AO to SWNT, respectively. The fluorescent intensity declined constantly but there was rarely fluorescence peak-shift (Figure 2 (c)), showing a typical quenching phenomenon of AO by SWNT-ssDNA. Complex optical properties of AO took place in AO/SWNT-dsDNA suspensions. As shown in Figure 1 (d), with SWNT-dsDNA added, the absorbance performance of AO could be described as superposition effect of AO/dsDNA (Figure 1 (b)) and AO/SWNT-ssDNA system (Figure 1 (c)). The fluorescence properties revealed a similar tendency. As we adjusted the same dsDNA concentration (800 $\mu\text{g/mL}$) of dsDNA aqueous solution as that of SWNT-dsDNA aqueous suspension, maybe it could be supposed that AO molecules adsorbed to DNA chain and SWNT surface at the same time, because the absorbance red-shift dramatically in AO/SWNT-dsDNA system (Figure 1 (d), from 490 to 504 nm); however, the fluorescent intensity trends of AO/SWNT-dsDNA maybe demonstrated that most of AO

molecules “prioritized” DNA chains, rather than SWNT surface. In the case of AO/SWNT-dsDNA hybrids, perhaps besides the red-shift owing to the structural deformation of AO/dsDNA complex when AO intercalated into dsDNA chains, another type of red-shift emerging in delocalized π -bond of AO/SWNT complex was seemingly explicable since both AO and SWNT possess conjugated structures. It is well known that absorbance redshift effect could be realized due to lower electronic transition energy of π -electron or n-electron in the delocalized conjugated situation, although here the conjugated effect existed between AO and SWNT was not so strong.

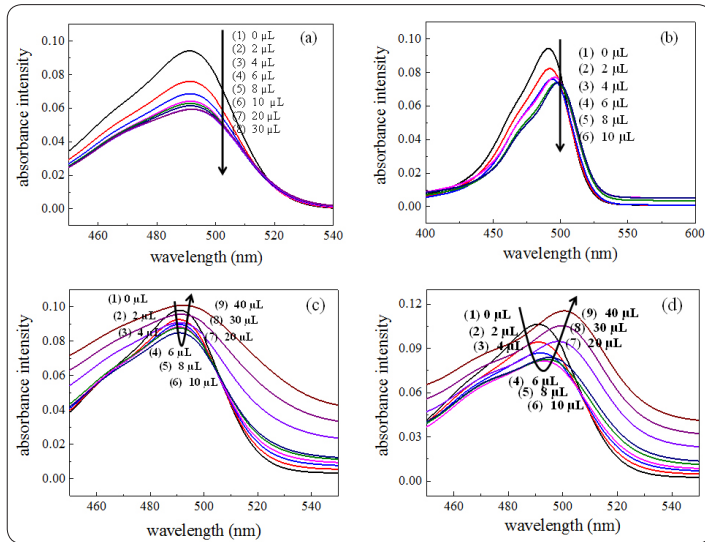


Figure 1: Absorbance spectra about AO origin solution. a) AO(10 μ L)/ssDNA(0, 2, 4, 6, 8, 10, 20, 30 μ L); b) AO(10 μ L)/dsDNA(0, 2, 4, 6, 8, 10 μ L); c) AO(10 μ L)/SWNT-ssDNA(0, 2, 4, 6, 8, 10, 20, 30 μ L); d) AO(10 μ L)/SWNT-dsDNA(0, 2, 4, 6, 8, 10, 20, 30 μ L). The concentration of both ssDNA (used in (a)) and dsDNA (used in (b)) was adjusted to the same (800 μ g/mL), the ds-DNA content of SWNT-dsDNA solution (used in (d)) was also adjusted to the same

same value as used in (b) (800 μ g/mL); the SWNT-ssDNA solution (used in (c)) was 3.6-diluted to make sure the similar SWCNT concentration in both SWNT-ssDNA (used in (c)) and SWNT-dsDNA (used in (d)). All the mixtures were diluted to 1 mL. The origin concentration of AO- H_2O solution was 0.33 mM.

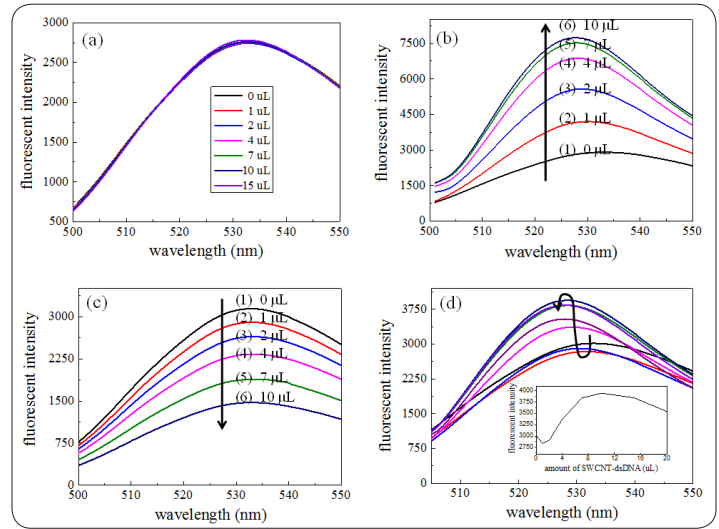


Figure 2: Fluorescent spectra about AO mixture solutions with exciting of 490 nm. a) AO(2 μ L)/ssDNA(0, 1, 2, 4, 7, 10, 15 μ L); b) AO(2 μ L)/dsDNA(0, 1, 2, 4, 7, 10 μ L); c) AO(2 μ L)/SWNT-ssDNA(0, 1, 2, 4, 7, 10 μ L); d) AO(10 μ L)/SWNT-dsDNA(0, 1, 2, 4, 7, 10 μ L). The concentration of both ssDNA (used in (a)) and dsDNA (used in (b)) was adjusted to the same (800 μ g/mL), the dsDNA content of SWNT-dsDNA solution (used in (d)) was also adjusted to the same value as used in (b) (800 μ g/mL); the SWNT-ssDNA solution (used in (c)) was 3.6-diluted to make sure the similar SWCNT concentration in both SWNT-ssDNA (used in (c)) and SWNT-dsDNA (used in (d)). All the mixtures were diluted to 2 mL. The origin concentration of AO- H_2O solution was 0.33 mM.

Table 1: Optical performance summary of AO in AO/DNA and AO/SWNT-DNA systems, with fixed content AO and increasing amount of DNA or SWNT-DNA.

	peak shift		intensity	
	absorbance	fluorescence	absorbance	fluorescence
AO + ssDNA	---	---	↘	---
AO + dsDNA	red-shift (9 nm)	slight blue-shift (3 nm)	↘	↗
AO + SWNT-ssDNA	slight red-shift (4 nm)	---	↘ ↗	↘ ↗
AO + SWNT-dsDNA	red-shift (14 nm)	blue-shift (6 nm)	↘ ↗	↘ ↗ ↘

--- : no change ↘ : decrease ↗ : increase ↘ ↗ : first decrease, then increase

Conclusion

Two kinds of changes of AO, molecule structure deformation and energy transfer, were supposed based on the absorbance and fluorescence performances of AO, when attached to DNA chains or emerged into SWNT/DNA suspensions. In the condition of existence of dsDNA (dsDNA only or SWNT-dsDNA), the photo-property AO would be varied obviously. Regarded as a kind of chromophore group, AO lead to an obvious red-shift absorbance of AO/dsDNA complex compared with AO/ssDNA complex, as AO intercalated closely into dsDNA chains and caused a slight structural deformation. While SWNT could bring about a little absorbance red-shift effect to AO, because of lower electronic transition energy for π -electron or n-electron in delocalized π -bond of AO/SWNT

complex since both AO and SWNT possess conjugated structures. Depending on the optical intensity, two energy transfer phenomena existed, both from DNT to AO and from AO to SWNT. Most of AO molecules “prioritized” DNA chains, rather than SWNT surface; although AO molecules adsorbed to DNA chain and SWNT surface at the same time.

Acknowledgement

This work was supported by a Grant-in-Aid for Scientific Research (26400436) of the Japan Society for the Promotion of Science (JSPS).

References

1. M Zheng, A Jagota, ED Semke, BA Diner, RS McLean, et al. (2003) *Nat Mater* 2: 338.
2. BD Holt, KN Dahl, MF Islam (2011) *small* 7: 2348.
3. V Georgakilas, K Kordatos, M Prato, DM Guldi, M Holzinger, et al. *J Am Chem Soc* 124: 760.
4. R Haggemueller, SS Rahatekar, JA Fagan, J Chun, ML Becker, et al. (2008) *Langmuir* 24: 5070.
5. M Zheng, A Jagota, MS Strano, AP Santos, P Barone, et al. (2003) *Science* 302: 1545.
6. N Nakashima, S Okuzono, H Murakami, T Nakai, K Yoshikawa, et al. (2003) *Chem Lett* 32: 456.
7. R Chitta, ASD Sandanayaka, AL Schumacher, L D'Souza, Y Araki, et al. (2007) *J Phys Chem C* 111: 6947.
8. S Ichimura, M Zama, H Fujita (1971) *Biochem Biophys Acta* 240: 485.
9. D Nii, T Hayashida, K Umemura (2013) *Colloids Surf B* 106: 234.
10. RB Bachilo, MS Strano, C Kittrell, RH Hauge, RE Smalley, et al. (2002) *Science* 298: 2361.
11. A Ahmad, K Kern, K Balasubramanian (2009) *Chem Phys Chem* 10: 905.
12. PW Fowler, A Ceulemans (1995) *J Phys Chem* 99: 508.
13. XW Tang, S Bansaruntip, E Yenilmes, YI Chang, Q Wang (2006) *Nano Lett* 6: 1632.
14. JP Casey, SM Bachilo, RB Weisman (2008) *J Mater Chem* 18: 1510.

Copyright: ©2018 Kazuo Umemura. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.