Effects of Tris Buffer pH Values on the Adsorption of Single-Stranded DNA onto Carbon Nanotubes

Haniyeh Pourtaghi Khorsand[1], Farrokh Karimi[2]*
[1] Department of biotechnology, faculty of science, university of Maragheh, Maragheh, Iran
[2] Department of biotechnology, faculty of science, university of Maragheh, Maragheh, Iran
*karimifm@maragheh.ac.ir

ABSTRACT

Carbon nanotubes (CNTs) have attracted considerable attention as nanomaterials for the development of biosensing and molecular diagnostic platforms because of their distinctive carbon-based structure and surface properties. The interaction of nucleic acids with CNT surfaces is influenced by environmental factors, including buffer composition and pH. Tris buffer is widely used in DNA-related applications; however, changes in its pH can modify the chemical environment and consequently affect DNA–nanomaterial interactions. Therefore, this study investigated the effect of Tris buffer pH on the fluorescence response of a single-stranded DNA (ssDNA)–CNT system to identify an appropriate pH for subsequent experiments. ROX-labeled ssDNA was incubated with CNTs in Tris buffer at different pH values, and changes in fluorescence intensity were evaluated as an indicator of DNA–CNT interactions and fluorescence quenching resulting from the proximity of the fluorophore to the CNT surface. In addition, scanning electron microscopy (SEM) was used to examine morphological differences in CNTs before and after interaction with ssDNA. The presence of CNTs resulted in a substantial reduction in ssDNA fluorescence intensity, consistent with the interaction of ssDNA with the CNT surface and the associated fluorescence quenching. Increasing the pH of the Tris buffer resulted in a progressive increase in fluorescence intensity. The fluorescence intensities at pH 4, 5, 6, 7, and 8 were approximately 65, 145, 190, 230, and 355. Among the conditions evaluated, pH 8 produced the highest fluorescence intensity. SEM analysis also revealed observable morphological differences in CNTs following interaction with ssDNA. Overall, these findings demonstrate that Tris buffer pH influences the fluorescence response of the ssDNA–CNT system. Within the investigated pH range, higher pH values were associated with greater fluorescence intensity, indicating that buffer pH is an important experimental parameter to consider when selecting conditions and optimizing CNT-based DNA nanobiosensing platforms.

Keywords: Carbon nanotubes; single-stranded DNA; Tris buffer; pH; nanosensor

1. *INTRODUCTION*

Carbon nanotubes (CNTs) have attracted considerable attention as promising nanomaterials for the development of biosensors and molecular diagnostic platforms. This interest stems from their distinctive structural characteristics, high specific surface area, favorable electrical and optical properties, and ability to interact noncovalently with biomolecules [1]. The graphitic surface of CNTs can interact with a variety of biomolecules, including nucleic acids, leading to the formation of CNT-DNA complexes. In particular, single-stranded DNA (ssDNA) can interact with CNT surfaces through π–π interactions between the aromatic bases of nucleotides and the graphitic lattice, in addition to van der Waals forces and electrostatic interactions [2]. Previous studies have shown that the adsorption and interaction of ssDNA with CNT surfaces are influenced by the physicochemical properties of DNA, the structural characteristics of the nanotubes, and the surrounding environmental conditions [3]. A key property of CNTs in fluorescence-based systems is their ability to quench the fluorescence of fluorophores located in close proximity to their surfaces. When a fluorophore-labeled DNA molecule interacts with a CNT surface and the fluorophore approaches the nanotube sufficiently closely, its fluorescence intensity can be substantially reduced.

This fluorescence-quenching property has enabled CNTs to serve as functional components of fluorescence-based sensing platforms. In these systems, adsorption of DNA onto the CNT surface can bring the fluorophore into close proximity to the nanotube, resulting in fluorescence quenching. Conversely, changes in the DNA-CNT interaction may alter the extent of quenching and consequently modify the fluorescence signal. Thus, fluorescence intensity can serve as an indirect optical readout of changes in DNA-CNT interactions and fluorophore proximity to the CNT surface [4]. The choice of fluorophore is an important consideration when monitoring DNA-CNT interactions. ROX is a rhodamine-family fluorophore that can be covalently attached to oligonucleotides and exhibits fluorescence emission in the red region of the visible spectrum. When conjugated to ssDNA, changes in ROX fluorescence intensity can be used as an optical readout to investigate the interaction of DNA with CNT surfaces. Under controlled experimental conditions, a reduction in ROX fluorescence in the presence of CNTs may indicate close association of the fluorophore with the nanotube surface and the resulting fluorescence quenching. Accordingly, variations in the ROX fluorescence signal can be used to monitor changes in the interaction between ssDNA and CNTs under different environmental conditions [5]. The interaction between DNA and CNTs is strongly influenced by the surrounding chemical environment. DNA is a polyanion with a negatively charged phosphate backbone, and its charge distribution, conformation, and interactions with nanomaterial surfaces can vary as a function of solution conditions. Among the environmental factors that influence DNA-CNT interactions, pH and ionic strength are particularly important. Changes in pH can modify the protonation state of chemical species and alter the electrostatic environment of the solution, thereby affecting electrostatic and other intermolecular interactions between DNA and the CNT surface [6,7]. Ionic conditions can likewise affect DNA conformation and its organization near CNT surfaces. Consequently, changes in the chemical environment may shift the equilibrium between free DNA and surface-associated DNA, thereby influencing the extent of fluorophore quenching and the resulting fluorescence signal [8]. Tris buffer is widely used in molecular biology and biochemical research, particularly in applications involving DNA and other nucleic acids. It provides a controlled buffering environment and is routinely used in nucleic acid-related experiments. Variations in Tris buffer pH can modify the chemical environment surrounding both DNA and the CNT surface and may consequently influence their interaction [9,10]. Systematic evaluation of Tris buffer pH is therefore important for identifying conditions that provide a stable and reproducible fluorescence response in DNA-CNT systems. This is particularly relevant when CNTs and DNA are used as functional components of fluorescence-based diagnostic and biosensing platforms. In fluorescence-based DNA-CNT systems, the magnitude of fluorescence quenching may depend on the physicochemical properties of the surrounding environment. However, changes in fluorescence intensity may not exclusively reflect alterations in DNA-CNT interactions; they may also arise from changes in fluorophore behavior or DNA conformation. Therefore, systematic evaluation of pH is necessary to identify experimental conditions that provide a reproducible fluorescence response and facilitate control of the DNA-CNT interaction [11]. Fluorescence measurements consequently offer a practical approach for assessing changes in the response of DNA-CNT systems under different environmental conditions. Accordingly, the present study aimed to evaluate the effect of different Tris buffer pH values on the fluorescence response of a ROX-labeled ssDNA/CNT system and to identify an appropriate pH condition for subsequent experiments. The relationship between buffer pH and fluorescence intensity was examined to determine conditions suitable for controlling and optimizing the response of the ssDNA-CNT system. Establishing an appropriate buffering condition may contribute to the development of stable and reproducible experimental protocols for DNA- and CNT-based fluorescence sensing and molecular diagnostic applications.

MATERIALS AND METHODS

Evaluation of the Interaction between ROX-Labeled ssDNA and CNTs

The interaction between single-stranded DNA (ssDNA) and carbon nanotubes (CNTs) was initially evaluated using ROX-labeled ssDNA in the absence and presence of CNTs. CNTs were added to the ssDNA solution at a final concentration of 20 µg/mL. Following incubation for a

predetermined period, the fluorescence intensity of each sample was measured. Fluorescence emission spectra were recorded over the wavelength range of approximately 585–685 nm. The fluorescence response of ROX-labeled ssDNA in the presence of CNTs was compared with that of the corresponding ssDNA control in the absence of CNTs. A decrease in ROX fluorescence intensity upon addition of CNTs was interpreted as evidence of an interaction between ssDNA and the CNT surface. This decrease is consistent with fluorescence quenching caused by the close proximity of the ROX fluorophore to the graphitic surface of CNTs. Because CNTs are highly efficient fluorescence quenchers, adsorption or association of ssDNA with the CNT surface can bring the fluorophore into closer proximity to the nanotube, thereby increasing fluorescence quenching. Accordingly, changes in the ROX fluorescence signal were used as an indirect measure of the interaction between ssDNA and CNTs.

SEM Characterization

Scanning electron microscopy (SEM) was used to characterize the morphology of CNTs before and after interaction with DNA. The untreated CNT sample and the DNA-treated CNT sample were designated as CNT and CNT-DNA, respectively. SEM images were compared to assess differences in nanotube morphology, aggregation state, and overall structural organization. Particular attention was given to changes in the apparent arrangement and aggregation of CNTs following interaction with DNA.

Effect of pH on the DNA-CNT Interaction

The effect of Tris buffer pH on the fluorescence response of the DNA-CNT system was evaluated at pH values of 4, 5, 6, 7, and 8. At each pH, ROX-labeled ssDNA was incubated with CNTs in Tris buffer adjusted to the corresponding pH. After completion of the experimental procedure, the fluorescence intensity of each sample was measured under identical conditions. The fluorescence intensity obtained at each pH was compared across the tested pH range to determine the effect of buffer pH on the response of the DNA-CNT system. The observed changes in fluorescence intensity were used to characterize the pH dependence of the system and to identify the Tris buffer pH providing the most suitable fluorescence response for subsequent experiments.

RESULTS AND DISCUSSION

Fluorescence Quenching of ROX-Labeled ssDNA by CNTs

To evaluate the interaction between single-stranded DNA (ssDNA) and carbon nanotubes (CNTs), the fluorescence behavior of ROX-labeled ssDNA was examined in the absence and presence of CNTs. As shown in Figure 1, ROX-labeled ssDNA in the absence of CNTs exhibited a distinct fluorescence emission peak at approximately 600 nm, with a maximum intensity of approximately 190. In the presence of CNTs at a final concentration of 20 μg/mL, the fluorescence intensity decreased markedly, and the ROX emission signal was reduced to near-background levels. The pronounced fluorescence quenching observed following CNT addition is consistent with an interaction between ssDNA and the graphitic surface of CNTs. This effect may be associated with the close proximity of the ROX fluorophore to the CNT surface following adsorption or association of ssDNA with the nanotubes. Localization of ROX near the graphitic surface can facilitate nonradiative deactivation of the excited fluorophore, thereby resulting in substantial attenuation of its fluorescence signal [12]. Overall, the substantial decrease in ROX fluorescence intensity after CNT addition demonstrates the effective quenching capability of CNTs toward the fluorophore attached to ssDNA. This fluorescence response provides a basis for further investigation of CNT-mediated fluorescence modulation in ssDNA-based sensing systems.

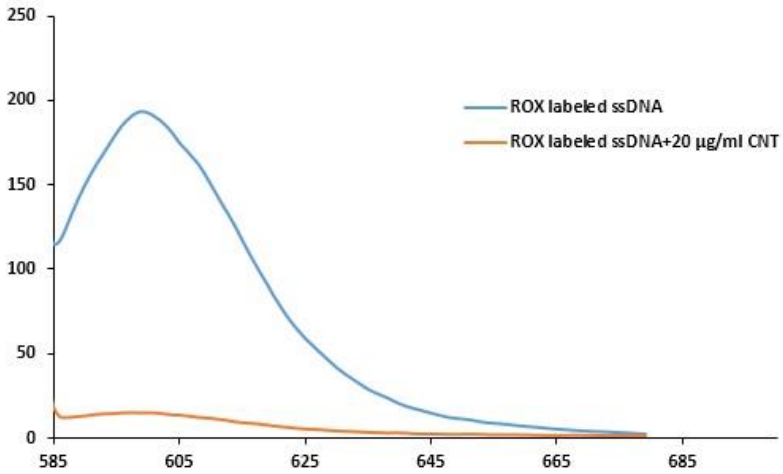


Fig. 1. *Fluorescence emission spectrum of ROX-labeled ssDNA in the absence and presence of carbon nanotubes (CNTs). The fluorescence emission of ROX-labeled ssDNA was recorded over an approximate wavelength range of 585–685 nm. ROX-labeled ssDNA exhibited a maximum emission at approximately 600 nm, whereas the addition of CNTs at a final concentration of 20 µg/mL resulted in substantial fluorescence quenching. The observed reduction in fluorescence intensity is consistent with close association of ssDNA with the CNT surface and the resulting proximity of the ROX fluorophore to the graphitic surface.*

Morphological Changes Associated with DNA-CNT Interaction

Following the pronounced change in fluorescence intensity, scanning electron microscopy (SEM) was performed to examine morphological features associated with the interaction between DNA and carbon nanotubes (CNTs). As shown in Figure 2, the CNT sample exhibited distinct nanotubular structures with a pronounced tendency toward aggregation. In contrast, the CNT–DNA sample displayed noticeable differences in the aggregation pattern and apparent surface morphology of the CNT structures following incubation with DNA. These morphological differences may reflect changes in the intermolecular interactions between adjacent nanotubes upon association with DNA. The presence of DNA at or near the CNT surface may modify interactions between neighboring nanotubes, thereby affecting their aggregation behavior [13]. Overall, the SEM observations were consistent with the fluorescence results presented in Figure 1. Both analyses revealed detectable changes following incubation of DNA with CNTs, providing complementary evidence supporting an interaction between the DNA and CNTs.

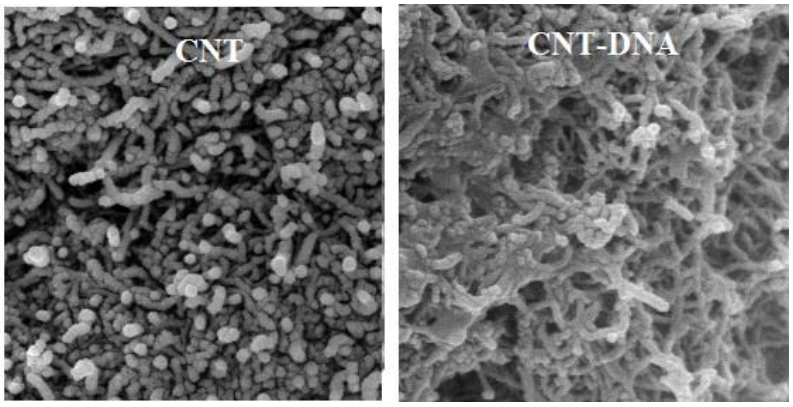


Fig.2. *Scanning electron microscopy (SEM) images of carbon nanotubes before and after interaction with single-stranded DNA. Representative SEM images of CNTs before interaction with DNA (CNT) and following incubation with single-stranded DNA (CNT–DNA) are presented. Comparison of the images shows differences in the aggregation pattern and apparent surface morphology of the CNT structures following incubation with DNA.*

Effect of pH on the Fluorescence Response of the DNA-CNT System

Following the initial assessment of the interaction between DNA and CNTs, the effect of Tris buffer pH on the fluorescence response of the DNA–CNT system was investigated to identify suitable conditions for subsequent experiments. As shown in Figure 3, fluorescence intensity increased progressively with increasing buffer pH from 4 to 8. The fluorescence intensities at pH 4, 5, 6, 7, and 8 were approximately 65, 145, 190, 230, and 355. Thus, the lowest fluorescence intensity was observed at pH 4, whereas the highest intensity was recorded at pH 8. The pH-dependent increase in fluorescence intensity indicates that the fluorescence response of the DNA–CNT system is sensitive to the pH of the surrounding buffer under the experimental conditions examined. Because CNTs can efficiently quench fluorophores in close proximity to their graphitic surfaces, variations in fluorescence intensity may reflect changes in the extent of fluorophore–CNT interactions and, consequently, fluorescence quenching. In addition, changes in pH may alter the physicochemical environment of the system, potentially affecting the interaction between DNA and the CNT surface and the spatial arrangement of the ROX fluorophore. At pH 4, the markedly low fluorescence intensity suggests a relatively high apparent degree of fluorescence quenching. In contrast, fluorescence intensity increased progressively with increasing pH, reaching approximately 355 at pH 8. Under the conditions investigated, higher Tris buffer pH was therefore associated with increased fluorescence intensity and reduced apparent fluorescence quenching of the DNA–CNT system. These results demonstrate a clear dependence of the fluorescence response on buffer pH and provide a basis for selecting an appropriate pH for subsequent experiments involving the DNA–CNT fluorescence sensing system.

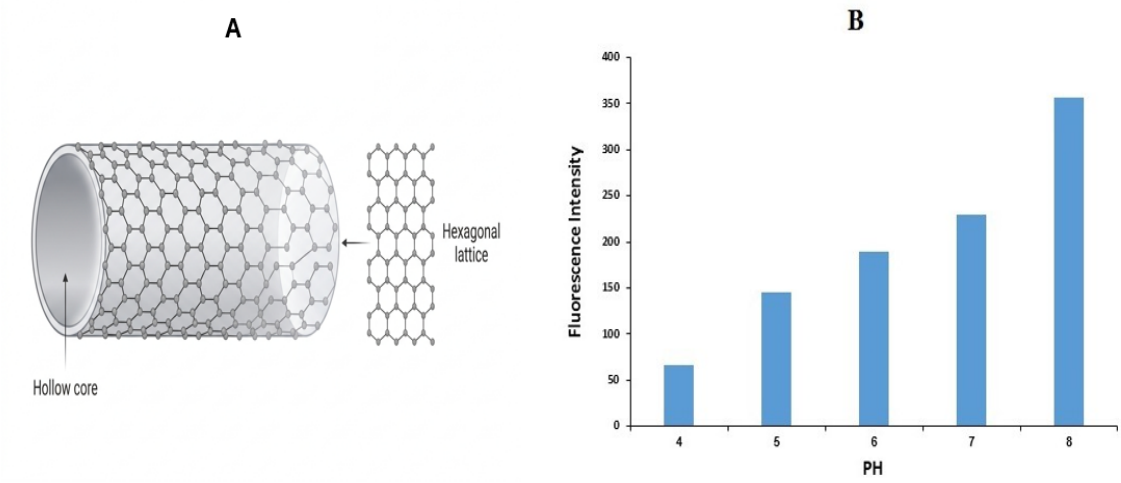


Fig. 3. *Effect of Tris buffer pH on the fluorescence response of the ssDNA–CNT system. (A) Schematic representation of the hollow cylindrical structure and hexagonal carbon network of a carbon nanotube. (B) Fluorescence intensity of ROX-labeled single-stranded DNA (ssDNA) in the presence of CNTs at pH 4, 5, 6, 7, and 8. Fluorescence intensity increased progressively with increasing pH, from approximately 65 at pH 4 to 355 at pH 8.*

CONCLUSION

In this study, the interaction between ROX-labeled single-stranded DNA (ssDNA) and carbon nanotubes (CNTs) was investigated, together with the effect of Tris buffer pH on the fluorescence response of the DNA–CNT system. Fluorescence measurements demonstrated a substantial decrease in ROX fluorescence intensity in the presence of CNTs. This reduction is consistent with an interaction between ssDNA and the CNT

surface that brings the fluorophore into close proximity to the carbon nanomaterial, thereby promoting fluorescence quenching. SEM analysis further demonstrated changes in the morphology and aggregation pattern of CNTs following interaction with DNA. These observations were consistent with the fluorescence results and indicated detectable structural changes in the CNT-containing system after DNA exposure. The effect of Tris buffer pH on the fluorescence response was subsequently evaluated to identify suitable conditions for further experiments. Within the investigated pH range of 4–8, fluorescence intensity increased from approximately 65 to 355 as the pH increased. Thus, higher pH values were associated with increased fluorescence intensity and a lower apparent degree of fluorescence quenching. Collectively, these findings demonstrate that CNTs substantially attenuate ROX fluorescence in the ssDNA–CNT system and that Tris buffer pH is an important experimental parameter affecting the observed fluorescence response. Systematic evaluation of pH conditions can therefore assist in establishing appropriate experimental conditions for subsequent investigations and in optimizing DNA–CNT-based fluorescence sensing systems.

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