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Fluorescence enhancement of glutaraldehyde functionalized polyaniline nanofibers in the presence of aromatic amino acids

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ABSTRACT

Polyaniline nanofibers (PNFs) synthesized by dilute polymerization method have been surface functionalized with glutaraldehyde at their N-terminals in Phosphate Buffered Saline (PBS) at $P^H = 7.4$ in order to achieve improved interaction of surface functionalized polyaniline nanofibers (SF-PNFs) with aromatic amino acids—Tyrosine, Tryptophan and Phenylalanine through incorporation of aldehyde (−CHO) and hydroxyl (−OH) functionalities. HRTEM reveals nanofibers of average diameter of 35.66 nm. FESEM depicts interconnected networks of nanofibers of polyaniline (PAni). UV–visible absorption and Fluorescence spectroscopy indicate that the PNFs and SF-PNFs are in emeraldine base (EB) form. FT-IR, ^{1}H NMR spectroscopy suggests covalent interactions of SF-PNFs with aromatic amino acids and possible reaction mechanisms have been proposed based on these results. Remarkable enhancement in fluorescence signals of SF-PNFs in the presence of aromatic amino acids has been observed and the apparent binding constant (K_A) and the number of binding sites (n) have been calculated using fluorescence enhancement equation. The K_A value is found to be highest for SF-PNFs + Tyrosine and n is two for all the polymer amino acid complexes, which are in agreement with the FT-IR and ^{1}H NMR results. Fluorescence resonance energy transfer (FRET) efficiency has been found to be highest for SF-PNFs + Tyrosine giving maximum fluorescence enhancement. The study of interaction mechanisms by means of an extremely sensitive technique like fluorescence using SF-PNFs as a substrate may provide a promising analytical tool for detection and monitoring any biochemical reactions involving these three aromatic amino acids.

1. Introduction

The study of π-conjugated conducting polymers (CPs) have attracted much interest in the past two decades because they display the physical and chemical properties of organic polymers and the electrical characteristics of metals due to the formation of non linear defects such as solitons, polarons and bipolarons by the process of doping during polymerization [1]. Polyaniline due to its unique properties such as ease of synthesis, low cost, environmental stability, excellent redox stability, high conductivity and biocompatibility has found tremendous applications in various fields like supercapacitors [2], electrochromic displays [3], sensors [4], actuators [5], electrorhelogical materials [6], tissue engineering [7,8] and controlled release of drugs [9].

Over the past decades, the advent of nanotechnology has prompted the integration of one-dimensional (1D) nanostructures as transducers, which offered ample benefits for using 1D CPs as novel functional materials for rapid, inexpensive and reliable biosensors for massive parallel sequences analysis of biological activities. Increased surface to volume ratio and facilitation of the electron transport through the bulk of 1D nanomaterials cause a small perturbation by adsorbed charged chemical/biological molecules on their surface. It significantly affects the charge distribution within the bulk of 1D nanomaterial, enhancing the sensitivity and detection limits [10]. In spite of the great potential of 1D conducting polymers as functional materials for advanced technology in detection and monitoring, the device fabrication as well as their scalability and reproducibility in a cost effective manner are still challenging.

Amino acids are one of the most important classes of biomolecules to be investigated which are the essential components of life processes. The detection of amino acids is of importance as they are essential substances in protein metabolism as well as in pharmaceutical and food products. It is noteworthy that amino acids function as the building blocks of proteins, as biosynthetic precursors of many biologically relevant small molecules, and as metabolic fuel [11,12]. Recent development in the field of amino acid sensing have focused on the use of fluorescent and colorimetric methods for the enantioselective discrimination of amino acids by means of indicator displacement assays (IDAs), metal complexes coordination and specific reactions between probes and amino acids [12–15]. Though several similar techniques were reported earlier, none of those were intended to study the interaction mechanisms between substrate and the amino acids.

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Generally, the surface of conducting polymers is considered to be inert in nature. Surface functionalization is a technique to make a completely or partially inert surface more active towards a specific event to recognize. It can be achieved by turning the hydrophobic surface of polyaniline to hydrophilic through incorporation of polar chemical groups like aldehyde (CHO), hydroxyl (OH), carboxylic (COOH) and amino ($-NH_2$) groups. Here, we have reported large scale production of nanofibers of polyaniline by dilute polymerization method in a cost effective manner as described by N. R. Chiou et al. [16]. We have further functionalized the surface of synthesized polyaniline nanofibers by glutaraldehyde through incorporation of aldehyde and hydroxyl groups as glutaraldehyde has a higher reactivity towards amine group [17]. Remarkable enhancement in fluorescence has been observed after surface functionalization of PNFs. Though photoluminescence of aniline has been found much earlier, photoluminescence of polyaniline has been reported more elaborately by Shimano et al. Normally, the photoluminescence of PAni is quenched due to adjacent quinoid units as reported by Shimano et al. [18]. We have described here fluorescence enhancement phenomenon of our parent material after exposure to aromatic amino acids—Tyrosine, Tryptophan and Phenylalanine through a simple chemical modification technique. It lacks use of several dyes as in IDAs and more interestingly, SF-PNFs act as a fluorophore. The fluorescence of PAni has not been widely reported. Moreover, it has not been used to study the molecular interactions with amino acids till date. The present embodiment deals with a new functionalization technique of polyaniline nanofibers and uses its fluorescence activity to monitor interaction mechanisms with aromatic amino acids.

The study of variation of fluorescence activity of fluorescence active molecules is an extremely sensitive optical technique for real time monitoring of their interactions with analytes [12,19,20]. Fluorescence active molecules used as chemosensors have been explored extensively in diverse fields such as biology [19–21], medical analysis [12,20,22] and environment monitoring [12,20,23]. In the present work, we have reported the fluorescence enhancement of SF-PNFs after treatment with Tyrosine, Tryptophan and Phenylalanine based on fluorescence resonance energy transfer (FRET) effect between SF-PNFs and the three aforementioned amino acids. The interaction mechanisms have been extensively investigated by FT-IR and ^{1}H NMR techniques and correlated with the results obtained from analysis of fluorescence enhancement effect.

2. Experimental details

2.1. Materials

Aniline (p.a. Merck) was distilled under reduced pressure before use. Ammonium peroxydisulfate (p.a. Merck) and Hydrochloric acid (Rankem) were used without further purification. Deionized water (12 MΩ cm) used for the synthesis was obtained from a Milli-Q system. 25% solution of Glutaraldehyde (p.a. Merck) was diluted to 1% using Milli-Q water. All other chemicals and reagents were of analytical grade and used as received.

2.2. Synthesis of polyaniline nanofibers

Polyaniline nanofibers were synthesized using the dilute polymerization method described by Chiou et al. [16]. A solution of 1 M HCl (dopant acid) was prepared and the monomer aniline was dissolved in a small portion of that solution. Ammonium peroxydisulfate (oxidizing agent) was dissolved in the remaining portion of the dopant acid solution. The initial concentration of aniline in the reaction mixture was kept at 8 mM and the molar ratio of the monomer to the oxidant was maintained at 2:1. The monomer solution was then carefully transferred to the solution of APS. The reaction was allowed to take place in a magnetic stirrer at a very slow stirring rate at room temperature for about 24 h till the whole mixture became dark green. The whole mixture was then filtered and washed with deionized water and methanol for several times. Colloidal solution of PNFs in 0.1 M PBS at $p^H = 7.4$ at a concentration of 0.125 mg/ml has been used throughout the further experimentation.

2.3. Surface functionalization of polyaniline nanofibers

Surface functionalization of the PAni nanofibers was accomplished by treating the purified PAni nanofibers with 1% Glutaraldehyde solution for a period of 24 h. Subsequently, SF-PNFs were washed with deionized water to remove the excess Glutaraldehyde. The different molecular forms of glutaraldehyde in aqueous solution enable it to cross-link two materials having active amine groups [17]. Thus, it was expected that the SF-PNFs would be highly reactive towards other chemical species having active amine groups such as in amino acids.

2.4. Fluorescence enhancement effect

When biomolecules co-exist with some fluorophore or ligand in a solution, the obvious change in fluorescence intensity can be observed, which can be used to study the binding event of biomolecules with the fluorophore [24–28]. In our case, surface functionalized polyaniline nanofibers act as a fluorophore and the change in fluorescence intensity of it in the presence of aromatic amino acids, has been utilized to determine the apparent binding constant (K_A) and the number of binding sites (n) of these complexes. A biomolecule (P) binds with a fluorophore or ligand (M) with 'n' number of the same and separating binding sites according to the following reaction:

$$P + nM \leftrightarrow M_nP \tag{1}$$

Accordingly we can obtain the fluorescence enhancement Eqs. (27–29) will be as follows:

$$\frac{[M_t]}{[M_b]} = \frac{1}{K_A(n[P_t]-[M_b])} + 1 \tag{2}$$

$$M_b = M_t \times \frac{F - F_D - F_A}{F_b - F_A} \tag{3}$$

$$\frac{1}{F - F_D - F_A} = \frac{1}{F_b - F_A}\left(1 + \frac{1}{K_A(n[P_t]-[M_b])}\right) \tag{4}$$

where $[M_t]$ is the total concentration of the fluorophore, which is fixed throughout the experiment, $[M_b]$ is the concentration of the fluorophore bound to the biomolecule, $[P_t]$ is the total concentration of biomolecule, the concentration of resultant M_nP can be expressed as $[M_n]/n$, and $[M] = [M_t]–[M_b]$, $[P] = [P_t][M_b]/n$; F is the fluorescence intensity of the fluorophore in the presence of biomolecule, F_D is the fluorescence intensity of free biomolecule, F_A is the fluorescence intensity of the free fluorophore and F_b is the maximum fluorescence intensity of the fluorophore after the biomolecule is added to it. According to Eq. (4), the double reciprocal curve of $[F - F_D - F_A]^{-1}$ against $n[P_t] - [M_b]^{-1}$ is plotted varying 'n' from 1 to 3. The curve is a straight line and the slope of the best fitted double reciprocal curve gives the apparent dissociation constant ($K_D = 1/K_A$) and the corresponding value of 'n' is the number of binding sites of the fluorophore-biomolecule complex.

2.5. Analytical techniques

The structural, morphological and conformational characterizations of PNFs and SF-PNFs were carried out using different characterization techniques. Transmission electron microscopy was accomplished using a JEOL JEM 200 CX transmission electron microscope (TEM). Morphological characterization was performed using Field emission scanning electron microscopy (FESEM) model Sigma 300 from Zeiss. FTIR