

Application of one-dimensional nanostructures in biomedical sciences

Eunice Mercado Feliciano
Catedrática Auxiliar - Departamento de Química y Física
UPR-Ponce

Abstract

This article explores the application of carbon nanotubes in biomedical sciences. In order to explore the nanotubes properties for the biomedical sciences field, single-wall and multi-wall carbon nanotubes were functionalized with an amino group. The functionalization makes possible the study of the tubes' interaction with the myoglobin protein in aqueous solution. Obtained UV/Vis measurements suggest protein-nanotubes interactions. These results suggest a possible site of adsorption, deposition on a surface, on the nanotubes walls.

Keywords: carbon nanotubes, myoglobin, functionalization, interaction, adsorption

Resumen

Este artículo investiga la aplicación de nanotubos de carbono en las ciencias biomédicas. Nanotubos de carbono de pared simple y pared múltiple fueron funcionalizados con un grupo amino. La funcionalización hace posible el estudio de la interacción de los tubos con la proteína mioglobina en solución acuosa. Medidas obtenidas de UV/Vis sugieren interacciones proteína-nanotubos. Estos resultados sugieren un posible sitio de adsorción, deposición sobre una superficie, sobre las paredes del tubo.

Palabras clave: nanotubos de carbono, mioglobina, funcionalización, interacción, adsorción

Proteins are molecules that can adopt different structural arrangements. A protein's three-dimensional structure is tied to its biological function. The present article focuses on the study of the interaction of the myoglobin protein with single-wall carbon nanotubes (SWCNT) and multi-wall carbon nanotubes (MWCNT) in aqueous solution. To achieve the protein-nanotubes interaction, the carbon nanotubes' surface modification was necessary. Obtained results suggest a protein-nanotubes interaction. Our interest in specifically this protein detection is focused in the contribution for the identification of the rhabdomyolysis disease.

Myoglobin contains a single polypeptide chain and is relatively small (16,700 daltons) [1, 2]. It is an oxygen-binding protein that is found in muscle cells. Myoglobin was the first protein to have its structure determined by X-ray crystallography [3]. Myoglobin is a globular protein. Globular proteins are usually very soluble in aqueous solutions which fold into compact units that are roughly spherical in shape. Myoglobin's tertiary structure consists of 8 α -helices which fold to make the compact globular protein. The folding occurs in such a manner that almost all of the hydrophilic groups are on the outside of the protein, facing the aqueous environment. The hydrophobic groups are almost all inside

the protein and the hydrophobic effect plays a large role in maintaining the stability of the folded protein [4]. Figure 1 shows a schematic representation of the myoglobin's active site, the heme group, in aqueous media. This group is responsible for the oxygen-binding properties of myoglobin. The myoglobin protein surrounds the heme group, protecting the oxygen from release until the tissue needs it. As is shown in the representation, four nitrogen atoms are coordinated with the central Fe (II) atom. The Fe atom is coordinated on top by the proximal side chain of histidine and should be

coordinated on the bottom with a diatomic oxygen molecule. In water, a free ferrous heme group can bind oxygen, but it only does so for a brief moment [5, 6]. The reason is that O₂ very rapidly oxidizes the Fe (II) to Fe (III), which cannot bind oxygen. This leaves a sixth ligation position, on the side of the heme plane opposite to the histidine. In aqueous solution, water molecules interact in this position. The heme iron is the binding site for ligands. The binding occurs at the distal (from the histidine) side of the heme, the position occupied by the water molecule in Figure 1.

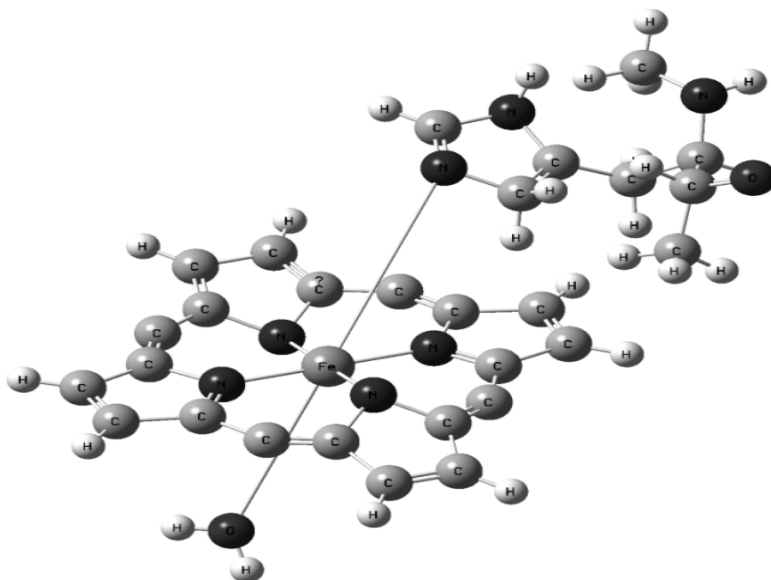


Figure 1: Schematic representation of the heme group

Actually, the carbon nanotubes modification has been the subject of numerous studies. Because of their unique mechanical, physical, and chemical properties, carbon nanotubes have great potential applications in many fields including molecular electronics, medical chemistry, and biomedical engineering [7-10]. Carbon nanotubes can be functionalized to achieve improved properties and functions such

as biomedical capabilities [11, 12]. Functionalized nanotubes have potential applications in the sensing of several diseases. Cai et al. reported that carbon nanotubes coated with a thin layer of protein-recognizing polymer form a biosensor capable of detecting minute amounts of proteins, which could provide a crucial new diagnostic tool for the detection of a range of illnesses [13]. They also reported that the carbon

nanotubes biosensors are capable of detecting human ferritin, the primary iron-storing protein of cells, and E7 oncoprotein derived from human papillomavirus, among other diseases. Also, further tests showed the sensor could discriminate between varieties of the protein that take different shapes. Z. Liu et al. reported that protein-conjugated-nanotubes can cross cellular membranes and enter into the cell's body [14]. In this scenario, they can be filled with nucleic acids and be used as a delivery system for therapy genes or drugs.

Myoglobin is a component of heart and skeletal muscles. When muscle is damaged, the myoglobin in muscle cells is released into the bloodstream [15]. The kidneys help remove myoglobin out of the body. In large amounts, myoglobin can damage the kidneys. Possible complications of the disease include acute tubular necrosis and acute renal failure. Rhabdomyolysis associated with high levels of the protein in the bloodstream. In this condition, the muscle fibers breakdown, resulting in the release of the fiber contents, myoglobin, into the bloodstream.

To make a positive detection of this disease, it is necessary to carry out various analyses. These include: the verification of the levels of the creatinine phosphokinase enzyme, the myoglobin-serum, the potassium test, and urinalysis and myoglobine urine among others. Some of these tests must be repeated for several days to obtain reliable results. In addition, the patient must monitor the consumption of medications and food during the study, since the results could be affected.

A research conducted by Grover et al. suggests that the accuracy and clinical utility of the assay used for the detection and diagnosis of rhabdomyolysis in patients has a poor and clinically inadequate sensitivity [16]. They conducted an analysis of the relationship between creatine kinase, serum-myoglobin, the urine qualitative assay for myoglobin and the semi-quantitative assay for urine pigments in patients evaluated for rhabdomyolysis. They found that that the negative test was not a result of the absence of the disease.

In order of the achieve myoglobin-nanotubes interaction, the functionalization of the nanotubes with an amino group was necessary. The nanotubes' functionalization makes possible the study of their interaction with the protein in aqueous solution. Obtained UV/Vis measurements suggest a protein-nanotubes interaction. The results also suggest the possible site of the protein adsorption on the nanotubes walls.

The myoglobin protein used in our experiments was from equine skeletal muscle, salt free and lyophilized. The initial protein concentration in the solutions analyzed by UV/Vis was 7.55×10^{-6} M in the SWCNT-NH₂ solution and 1.32×10^{-5} M in the MWCNT-NH₂ solution. Distilled water was used as the solvent solution.

The nanotubes functionalization was achieved following the procedure explained by B. Pan et al. [17]. The samples modified with carboxylic acid groups are represented as SWCNT-COOH and MWCNT-COOH, and the samples modified with amino groups are

represented as SWCNT-NH₂ and MWCNT-NH₂. J. Zhang et al. reported that the carbon nanotubes modified via the procedure used in our experiments contain some amine groups on the surface [18].

Figure 2 illustrates the dependence of the absorption spectra of myoglobin on time in the presence of (a) SWCNT-COOH and (b) MWCNT-COOH. The insert in each figure shows the maximum of the absorption band centered at 409 nm. These UV/Vis spectra are similar to the myoglobin spectrum in aqueous solution. Previous works demonstrates that the most intense absorption band results from the

interaction of the iron center with the protein body. This band is sensitive to changes in the coordination of the heme group. As the protein unfolds (or is denatured), the intensity of this band decreases. When the protein is denatured, the signal disappears, due to the loss of the heme group from the protein matrix. The signal at 502 nm is a Q band and the one at 630 nm corresponds to a charge transfer band. It is a less intense signal due to a ligand-to-metal charge transfer. These controlled experiments do not exhibit any decrease in the intensity of the absorption band at 409 nm in function of time, suggesting no protein-nanotubes interactions

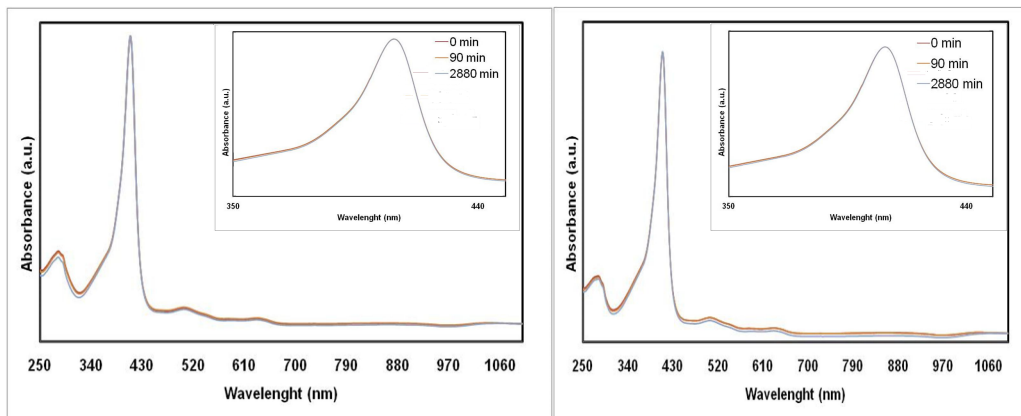


Figure 2: UV/Vis spectra showing the dependence of the myoglobin absorption on time in presence of (a) SWCNT-COOH and (b) MWCNT-COOH

Figure 3 shows the dependence of the absorption spectra of myoglobin on time in presence of water soluble (a) SWCNT-NH₂ and (b) MWCNT-NH₂. As shown in the insert of the figures, the intensity of the band centered at 409 nm decreases with time in the presence of the amine modified nanotubes. The radical decrease of the myoglobin concentration in solution in presence of

SWCNT-NH₂ in comparison with the MWCNT-NH₂, suggest a more effective adsorption of the protein by the SWCNT-NH₂. This decrease in the absorption band should not be attributed to the protein denaturalization, since it is not observed in presence of SWCNT-COOH and MWCNT-COOH; experiments carried out under the same conditions.

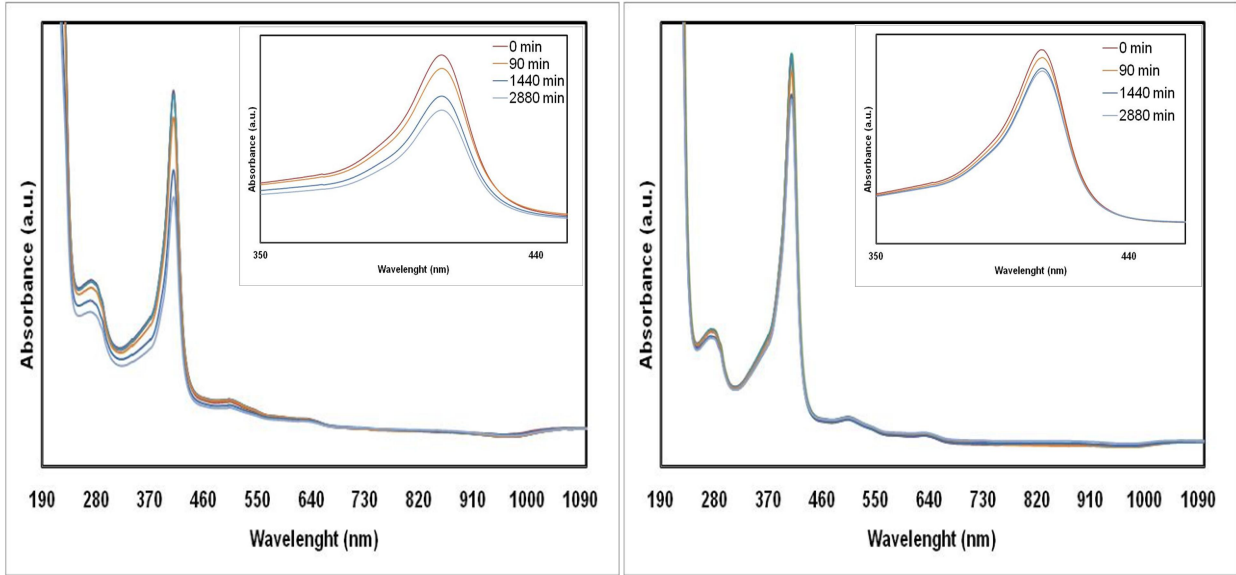


Figure 3: UV/Vis spectra showing the dependence of the myoglobin absorption on time in presence of water soluble (a) SWCNT-NH₂ and (b) MWCNT-NH₂.

Figure 4 illustrate the tendency in the increment of the experimental amount of protein adsorbed on the (a) SWCNT-NH₂ and (b) MWCNT-NH₂ surfaces in function of time. These results confirmed that the SWCNT-NH₂ adsorbs a larger amount of protein than the MWCNT-NH₂ at the same time limit. As is stated in the literature, most

protein-surface combinations result in adsorption (i.e. sticking at the interface). The protein adsorption on a surface could be described using mathematical models. Equation 1 describes the Kisiuk adsorption isotherm, used to obtain the coverage of the carbon nanotubes surface with the protein [19].

$$[\text{Protein}/\text{SWCNT}-\text{NH}_2] = \frac{1 - e^{-r(1+K)t}}{1 + Ke^{-r(1+K)t}} \quad \text{Equation 1}$$

This equation is employed in order to make an approximation in the tendency of the protein adsorption on the nanotubes' surface. In the equation, [Protein/SWCNT-NH₂] corresponds to the fractional coverage of the SWCNT-NH₂ surface, r is used to represent the impact of diffusion on monolayer formation according to Kisiuk, t represents the surface immersion time

and K is a sticking coefficient. This equation was employed in determining the same information for the MWCNT-NH₂. Figure 4 also shows the protein coverage of the amine modified nanotubes' surface as a function of time. The correlation of the experimental and modeled curves suggests that the model describes the tendency of the protein adsorption on the nanotubes' surface.

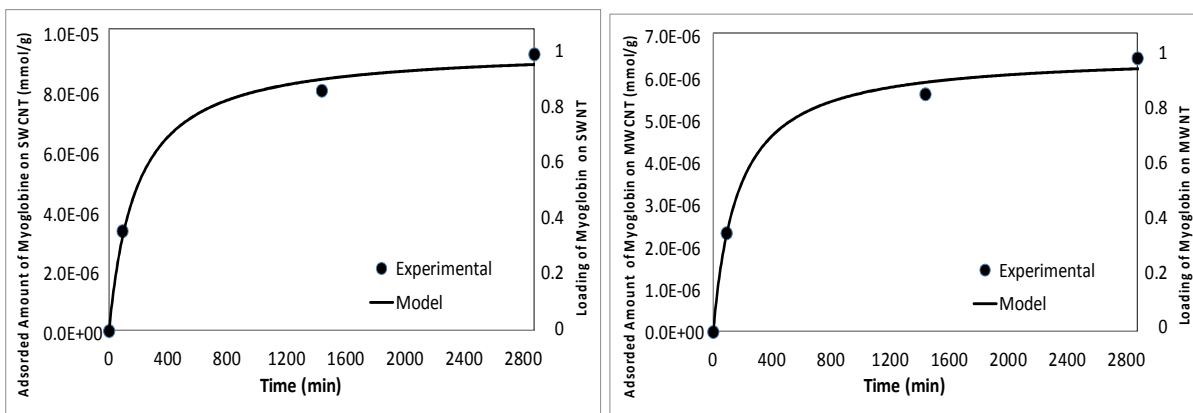


Figure 4: Dependence of the myoglobin absorption on time; (a) — modeled and ··· experimental adsorbed protein amount on SWCNT-NH₂ and (b) — modeled and ··· experimental adsorbed protein amount on MWCNT-NH₂.

EDS measurements were performed with the purpose of characterizing the product of the protein-nanotubes interactions. The results, weight percent (*Wt %*) and atomic percent (*At%*), are summarized in Table 1. These measurements reflect strong evidence for the protein adsorption by the amine functionalized carbon nanotubes. The amount of iron (Fe) in the dry deposits of SWCNT-NH₂ and MWCNT-NH₂ in comparison with the amount in SWCNT-COOH and MWCNT-COOH, suggests the

myoglobin interaction with the amine group in the nanotubes' surface. The amount present in the carboxylic acid modified nanotubes is unimportant in comparison with the quantity existing in the amine modified nanotubes. It could be graphically observed in Figure 5. Although the EDS results show a larger amount of iron in the MWCNT-NH₂ dry deposits than in the SWCNT-NH₂, contrasting the stated before, it should be acknowledged that EDS is a localized surface analysis.

Table 1: EDS quantitative results

Element	CK		NK		OK		FeK	
	<i>Wt %</i>	<i>At %</i>	<i>Wt %</i>	<i>At %</i>	<i>Wt %</i>	<i>At %</i>	<i>Wt %</i>	<i>At %</i>
SWCNT-NH ₂	51.24	59.79	3.41	3.41	40.67	35.63	4.68	1.17
MWCNT-NH ₂	62.03	78.98	3.39	3.71	11.48	10.98	23.10	6.33
SWCNT-COOH	70.72	76.01	3.63	3.35	25.53	20.60	0.11	0.02
MWCNT-COOH	70.72	76.02	3.63	3.35	25.53	20.60	0.11	0.02

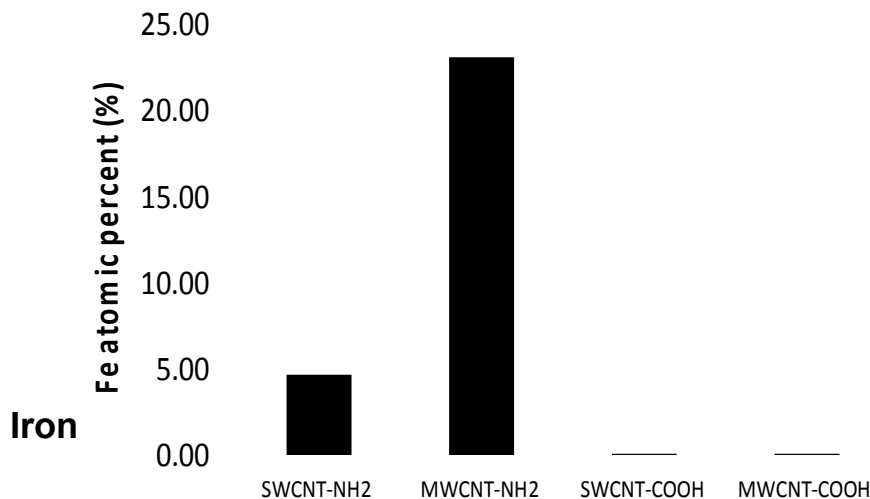


Figure 5: Iron atomic percent present on dry deposits of modified carbon nanotubes-proteinsolutions.

Our results suggest that the bonded water molecule to the iron center in the myoglobin's active site was replaced by the amine group in the functionalized carbon nanotubes. The evidence in reduction of the myoglobin concentration in presence of SWCNT-NH₂ and MWCNT-NH₂ observed in the UV/Vis spectra, and the presence of a significant amount of iron in the dry deposits of the amine modified carbon nanotubes, suggest a possible detection of the protein in aqueous solution.

A clear interaction between the myoglobin protein and the amine modified carbon nanotubes was suggested due to a decrease with time in the intensity of the band

centered at 409 nm. The nanotubes modified with the carboxylic acid group, do not exhibit any decrease in the intensity of the absorption band at 409 nm in function of time, suggesting no protein-nanotubes interactions. The radical decrease of the myoglobin concentration in solution in presence of SWCNT-NH₂ suggests a more effective protein adsorption than the MWCNT-NH₂. Evidence in reduction of the myoglobin concentration in presence of SWCNT-NH₂ and MWCNT-NH₂, and the presence of a significant amount of iron in the dry deposits of the amine modified carbon nanotubes, suggest a possible detection of the protein in aqueous solution.

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