This article was downloaded by:
On: *15 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Journal of Experimental Nanoscience
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t716100757

Preparation and investigation on properties of lysozyme chemically bonded to single-walled carbon nanotubes

Zahra Borzooeian[a]; Afsaneh Safavi[b]; Mohammad Hossain Sheikhi[c]; Mahmood Aminlari[a]; Mohammad Mahdi Doroodmand[b]
[a] Department of Biochemistry, School of Veterinary Medicine, Shiraz University, Shiraz 71345, Iran [b] Department of Chemistry, College of Science, Shiraz University, Shiraz 71454, Iran [c] Nanotechnology Research Institute, Shiraz University, Shiraz, Iran

Online publication date: 17 December 2010

To cite this Article Borzooeian, Zahra , Safavi, Afsaneh , Hossain Sheikhi, Mohammad , Aminlari, Mahmood and Mahdi Doroodmand, Mohammad(2010) 'Preparation and investigation on properties of lysozyme chemically bonded to single-walled carbon nanotubes', Journal of Experimental Nanoscience, 5: 6, 536 — 547
To link to this Article: DOI: 10.1080/17458081003699122
URL: http://dx.doi.org/10.1080/17458081003699122

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Experimental Nanoscience
Vol. 5, No. 6, December 2010, 536–547

Taylor & Francis
Taylor & Francis Group

Preparation and investigation on properties of lysozyme chemically bonded to single-walled carbon nanotubes

Zahra Borzooeian[a]*, Afsaneh Safavi[b], Mohammad Hossain Sheikhi[c], Mahmood Aminlari[a] and Mohammad Mahdi Doroodmand[b]

[a]*Department of Biochemistry, School of Veterinary Medicine, Shiraz University, Shiraz 71345, Iran;* [b]*Department of Chemistry, College of Science, Shiraz University, Shiraz 71454, Iran;* [c]*Nanotechnology Research Institute, Shiraz University, Shiraz, Iran*

(*Received 3 November 2009; final version received 13 February 2010*)

Downloaded At: 11:10 15 January 2011

The purpose of this research is the preparation of a lysozyme-single-walled carbon nanotube (SWCNT) conjugate and investigation of the effect of conjugation on the structure and function of lysozyme. At first, SWCNTs were functionalised by oxygen and lysozyme was coupled to the SWCNTs by carbodiimide method. The high chemical stability of conjugation was purposed for the SWCNTs conjugated enzyme as approved by Fourier transform infrared (FT-IR) spectroscopy, transmission electron microscopy (TEM) images, agarose gel electrophoresis and X-ray diffraction (XRD) patterns. The degree of conjugation was determined by thermogravimetric analysis (TGA) process. The results showed no significant differences between the XRD patterns of the native lysozyme and conjugated lysozyme–SWCNTs and these indicated excellent capacity of the SWCNTs to conjugated enzyme. The gel electrophoresis studies and TEM image confirmed the covalent attachment of the enzyme to the functionalised SWCNTs. These results revealed that the enzyme retain a high fraction of their native structure and activity upon attachment to SWCNTs. Therefore, this conjugated protein represents novel preparations that make it an attractive choice as a natural antimicrobial agent.

Keywords: lysozyme; single-walled carbon nanotubes; enzyme conjugation

1. Introduction

During recent decades, the bioapplications of carbon nanostructures have been predicted and explored ever since the discovery of one-dimensional carbon allotropes. Among carbon nanostructures, carbon nanotubes (CNTs) with unique properties have been considered as an interesting nanomaterial due to their potential use in a variety of biological and biomedical systems and nanoscale devices. Their fantastic properties have caused the CNTs to be applicable in different branches of science including their use as nanobiocatalysts, a vehicle for enzyme encapsulation [1], DNA transfection [2–4], drug delivery [5–7] and biosensors [8–10].

*Corresponding author. Email: zborzooeian50@gmail.com; borzooian@shirazu.ac.ir

ISSN 1745–8080 print/ISSN 1745–8099 online
© 2010 Taylor & Francis
DOI: 10.1080/17458081003699122
http://www.informaworld.com

Recently, rapid developments in the application of CNTs as biosensor and bioreactor systems have been activated. The performance of these nanoscale biosensors and bioreactor devices has been driven via immobilisation of biological materials such as proteins and enzymes either in the hollow cavity or on the surface of CNTs [11,12].

However, a lot of important bioapplications of enzymes conjugated with CNT bundles rely on the interfacing effects of enzymes with the CNT matrix, the stability of the native structure and also the biological activity of enzymes, immobilised on the nanoscale supports are considered as serious challenge for the supported proteins and enzymes [13–23]. To solve this problem, it is necessary to control the biological activity of enzymes and also, large amounts of enzyme molecules are needed to be strongly coupled to the CNT bundles.

Both physical and chemical absorption processes have been reported for binding the enzymes on the nanoscale supports. Both covalent [15,16,24,25] and noncovalent bonds [13,14,18–22] have been reported for the attachment of enzymes to the CNT matrix. Despite considerable progresses in the preparation of CNT–protein conjugates, few studies have yielded detailed information on the structure and function of enzymes attached to CNTs [18,19].

Among different forms of enzymes, lysozyme (EC 3.2.1.17, mucopeptide, *N*-acetyl muramic hydrolase) is considered a natural antimicrobial protein that is of widespread distribution in avian eggs, mammalian milk, tears and other secretions, insects and fish [26]. The conjugate of SWCNTs with chicken egg white lysozyme was found to be soluble in aqueous solutions [27]). Silica nanotubes have been used as enzyme immobilisation carriers. The immobilisation profiles are described by the adsorption of lysozyme molecules from an aqueous solution onto the hydrophilic silica surface [28].

In this research, lysozyme is selected as a representative enzyme to study its chemical attachment to the SWCNTs. The structure and catalytic activities of the attached lysozyme are studied by FT-IR spectroscopy, TEM images, agarose gel electrophoresis, XRD patterns and TGA analysis to further evaluate the performance of SWCNTs as micro-reactors for enzyme immobilisation.

Downloaded At: 11:10 15 January 2011

2. Experimental

2.1. *Materials*

Lyophilised chicken egg white lysozyme (EC 3.2.1.17) were purchased from Inovatech Inc., (Abbotsford, BC, Canada). *Micrococcus lysodeikticus* cells were provided by Sigma (St. Louis, MO, USA) as salt-free, dry powders and were used without further purification. Highly purified activated SWCNTs were synthesised by the chemical vapour deposition (CVD) process. Ferrocene was prepared by Merck, Darmstadt, Germany and MES [2-(*N*-morpholino)ethanesulfonic acid] buffer, *N*-ethyl-*N*′-(3-(dimethylamino)propyl) carbodiimide hydrochloride (EDC) and all other chemicals were purchased from Sigma and used as received.

2.2. *Synthesis of SWCNTs*

Highly purified (99%) aligned activated SWCNTs with a 50 mM internal diameter were synthesised by a CVD instrumentation system at a temperature of around 1300°C in argon

as an inert atmosphere using acetylene gas (Pars Balloon, Iran) as a source of carbon and ferrocene as a source of catalyst. The raw SWCNTs were then purified, end-opened and finally activated by oxygen. The activated SWCNT bundles were then used as an appropriate support for the modification with lysozyme.

2.3. *Enzyme attachment onto SWCNTs*

Conjugated lysozyme–SWCNTs solution was prepared by carbodiimide method [27] with modifications in some steps. To attach the enzyme onto the SWCNTs, about 1.0 mg of oxygen-treated SWCNTs were dispersed into 1.0 ml of 50 mM of MES [2-(*N*-morpholino) ethanesulfonic acid] buffer solution at a pH of 6.2. Equal volumes of *N*-hydroxy succinimide (NHS) in MES buffer (400 mM) was then added to the mixture. Afterwards, the mixture was sonicated (MSE Ultrasonic Disintegrators, 150 W,England) for around 30 min, along with the addition of 20 mM *N*-ethyl-*N*′-(3-(dimethyl amino)propyl) carbodiimide hydrochloride (EDC) to start the coupling of NHS to the carboxylic functional groups on the oxidised SWCNTs. After stirring at 200 rpm for 30 min, the resulting mixture was centrifuged at 7000 rpm for 15 min.

The suspension was then removed followed by the addition of MES buffer to the remaining precipitate. The centrifuge steps were repeated three times to remove excess EDC and NHS. The enzyme solution (10 mg/ml, 10 mM phosphate buffer, pH 8) was added to the rinsed nanotubes and sonicated for ca. 1 min to redisperse the SWCNTs. The mixture was then shaken at room temperature in an orbital shaker at 200 rpm during the conjugation of the enzyme with SWCNTs.

The SWCNT–enzyme suspension was then centrifuged and washed three times with triply distilled water and once with 1% (v/v) Tween-20 to completely remove all nonspecifically adsorbed enzyme. Control nanotube–enzyme conjugates were prepared using the same procedure in the absence of EDC and NHS.

Downloaded At: 11:10 15 January 2011

2.4. *Characterisations of nanotube–enzyme*

The morphology of lysozyme supported on SWCNT bundles was compared with that of pure SWCNTs using TEM (Philips, CM10, 100 kV). For this purpose, the water suspension of 6% enzyme on SWCNTs was first sonicated for ca. 5 min to prepare a homogenous suspension. Then, one drop of the mixture was placed on a formvar-covered copper grid and the solvent was allowed to evaporate in air, at room temperature, using a 100 W tungsten lamp and was observed through the TEM. The patterned XRD was performed using XRD (D8, IIIV, Advance, Bruker, AXS) at $\lambda = 0.1542$ nm. The amount of bound lysozyme was determined by measuring the unbound protein content in the supernatant after the binding process by using a spectrophotometer (ULTEROSPEC PLUS, Pharmacia LKB Biotechnology, Sweden) (scan demo, 200–500 nm). The chemical binding stability of lysozyme onto the SWCNTs was characterised using a FT-IR spectrometer (8300 Shimatzu). A lab-made TG analyser was also used to evaluate the binding efficiency.

2.5. *Lysozyme activity*

Lysozyme activity was assayed by the lysis of *M. lysodeikticus* cell walls consistent with the method of Imoto and Yagishita [29]. Nine milligrams of dried *M. lysodeikticus* cell walls

were dissolved in 25 ml of 0.1 M potassium phosphate buffer (pH 7.0) and diluted to a final volume of 30 ml with the same buffer. Lysozyme or conjugated lysozyme at a concentration of 1 mg of protein/ml was dissolved in cold distilled water. The cell wall suspension (2.9 ml) was poured into a cuvette and incubated at 25°C for 4–5 min to achieve temperature equilibration. The enzyme solution (0.1 ml) was added to the cuvette, and the change in absorbance at 450 nm was then recorded. One unit of activity of lysozyme is defined as the decrease in the absorbance at 450 nm of 0.001/min at pH 7.0 and 25°C using *M. lysodeikticus* cells as a substrate.

3. Results and discussion

3.1. *Binding efficiency*

To have an enzyme with maximum activity, supporting the enzyme on nanomaterials seems to be effective. Maintaining the native structure and function of bound lysozyme is an important aspect of its immobilisation and it has a strong influence on the reproducibility of antimicrobial and industrial applications of lysozyme. In this research, to increase the chemical attachment of the enzyme to the SWCNTs, several parameters were optimised and conjugated processes were achieved with a 1 mg enzyme to 1 mg SWCNTs ratio and the remaining enzyme activity was about 87.5%. The spectroscopic results of the unbound protein assay in the supernatant after the binding process showed that almost all the enzyme was attached to SWCNTs (Figure 1(a) and (b)). As shown in Figure 1(b), after loading the enzyme to SWCNTs, no peak was observed at 280 nm, and this shows that all of the lysozyme was bound when added to the same amount of SWCNTs.

Downloaded At: 11:10 15 January 2011

3.2. *Enzyme activity*

The enzyme was attached onto the surface of oxygen-oxidised SWCNTs using carbodiimide activation of the nanotube-bound carboxylic acid groups. Standard kinetic assays suggested that lysozyme retained a high percentage of its native solution activity, 87% (Figure 2). The control sample (lysozyme exposed to oxidised SWCNTs in the absence of EDC and NHS) showed less than 3% of the activity of the covalently attached SWCNT–lysozyme conjugate, suggesting that the least nonspecific adsorption of lysozyme onto oxidised SWCNTs had occurred.

In addition, in this study, the conjugation process of lysozyme on 4% and 6% functionalised SWCNTs was studied. The complete binding of lysozyme with the activated SWCNTs was also approved by using thermograms shown in Figure 3. The TG analysis shows the percent of the conjugated lysozyme as well as the activation percent.

3.3. *Agarose gel electrophoresis, TEM and XRD studies*

Before electrophoresis, samples were washed several times with a phosphate buffer (10 mM, pH 8) to remove any physically adsorbed enzyme. The results of agarose gel electrophoresis of native lysozyme and conjugated lysozyme–SWCNTs are shown in Figure 4. Significant brightness of the lane 5, conjugated lysozyme–SWCNTs, in comparison with that of the control is considered as good evidence for the covalent

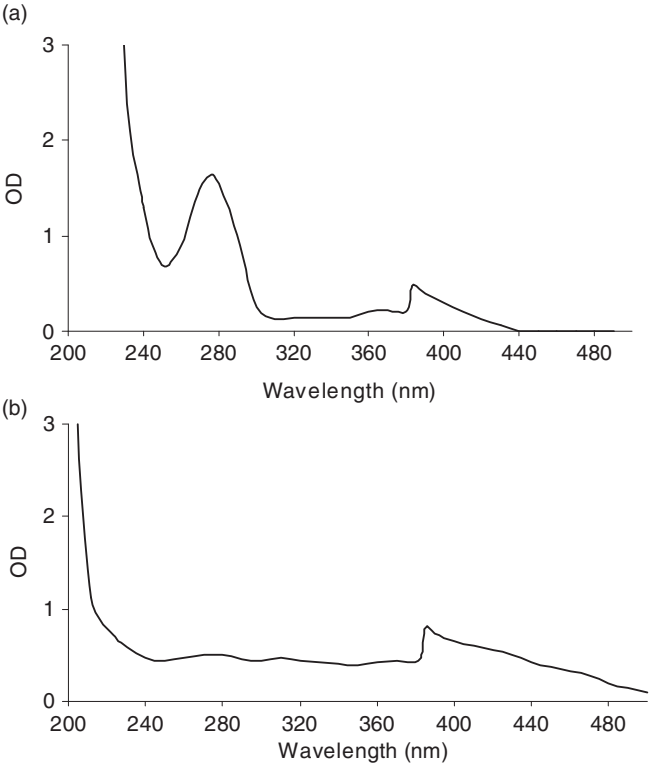

Figure 1. Scan demo spectra of (a) native enzyme solution and (b) supernatant of conjugated lysozyme–SWCNTs solution.

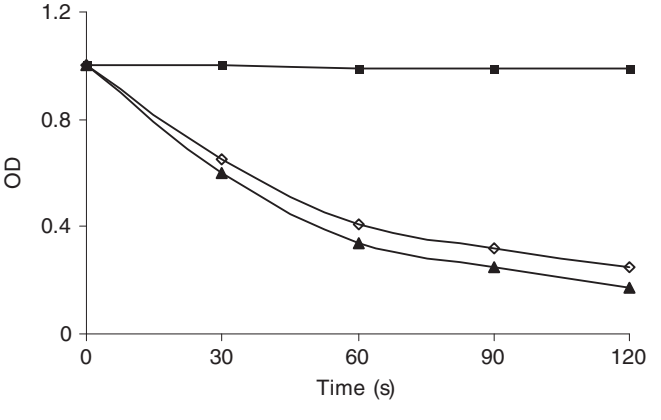

Figure 2. Comparison of native lysozyme (▲), conjugated lysozyme–SWCNTs (◇) and control solution activity (■).

Downloaded At: 11:10 15 January 2011

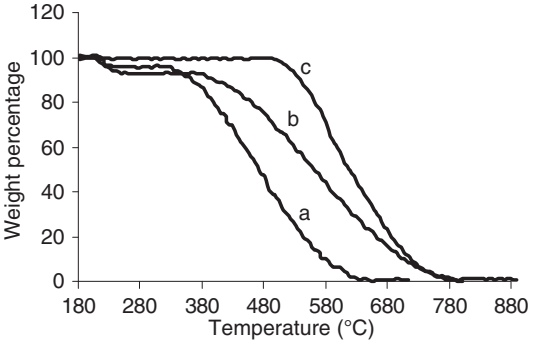

Figure 3. TGA measuring of (a) 4% functionalised SWCNTs, (b) 6% functionalised SWCNTs and (c) highly purified aligned SWCNTs.

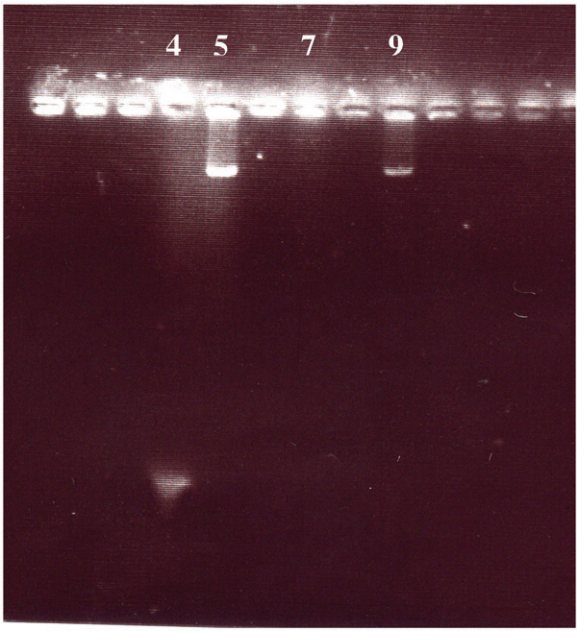

Figure 4. Agarose gel electrophoresis. Lane 4: native enzyme; lane 5: conjugated lysozyme–SWCNTs; lane 7: SWCNTs; lane 9: control.

bond between the lysozyme and SWCNTs. The weak brightness of control also indicates the slight adsorption of the enzyme to SWCNTs.

The covalent bond in conjugated lysozyme–SWCNTs was confirmed with XRD patterned and TEM images. According to the XRD patterns, the strong peaks of the purified SWCNTs and conjugated lysozyme correspond to the (0 0 2), (1 0 0) and (1 0 1) planes of carbon [30].

Downloaded At: 11:10 15 January 2011

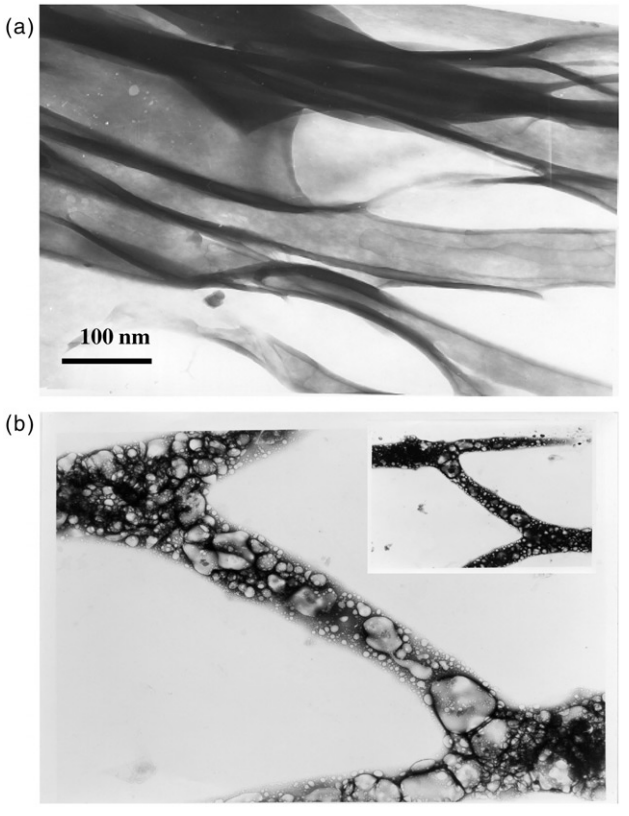

Figure 5. TEM images of carbon nanotubes (a) without and (b) with bound lysozyme. Lysozyme/SWCNTs = 1 mg/1 mg (w/w); magnification: 45,000.

Downloaded At: 11:10 15 January 2011

The typical micrographs for SWCNTs with and without bound lysozyme are shown in TEM images (Figure 5). It is clear that the SWCNTs are essentially very smooth and Y shaped with a mean diameter of 50 nm. After binding the lysozyme, as shown in Figure 4(b), an agglomeration of enzyme is seen on the surface of SWCNTs and also in the holes of SWCNTs.

3.4. *Mechanism of binding or FT-IR analyses*

Figure 7 shows the FT-IR spectra recorded for native lysozyme (curve (a)), the SWCNTs (curve (b)) and lysozyme-immobilised on SWCNTs (curve (c)). The amide linkages between amino acid residues in polypeptides and protein provides the well known fingerprints from the FT-IR spectrum, exhibiting the character of those substances [31]. The positions of the amide type I and II bands in the FT-IR spectra of proteins are sensitive indicators of the conformational changes in the proteins secondary structure [32]) and have been used in studies to investigate the immobilised enzyme molecules. The FT-IR spectra of the pure SWCNTs functionalised with carboxylic acid and also that of pure

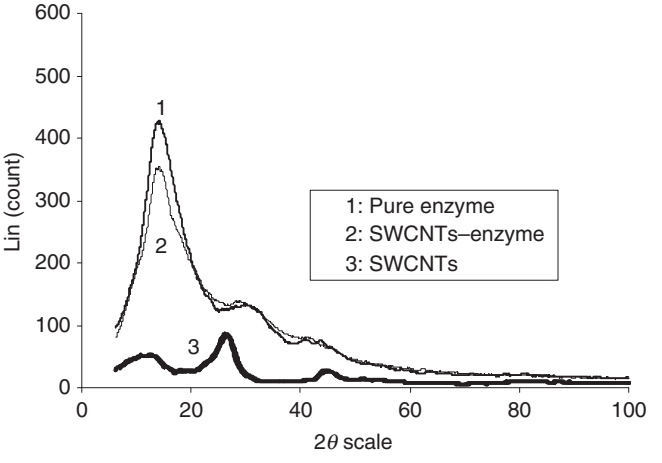

Figure 6. XRD patterns of (1) naked lysozyme, (2) conjugated lysozyme–SWCNTs and (3) SWCNTs without bound lysozyme.

Downloaded At: 11:10 15 January 2011

lysozyme are shown in Figure 7(a) and (b). As shown in Figure 7(b), the peak at around 1616 cm^{-1} is related to the carboxyl group in SWCNTs. The absence of this peak in the case of conjugated enzyme–SWCNT reveals the participation of a carboxyl group in lysozyme–SWCNT chemical bonding (Figure 7(c)).

A broad, strong NH_3 stretching band in the 3100–2600 cm^{-1} region is characteristic for amino acids. The absence of these extended and their peaks in Figure 7(c) reveals a decrease of amine groups in the conjugated enzyme and it confirms the incorporation of the amine groups in the formation of amide bond type II.

Multiple combination and overtone bands extend the absorption to about 2000 cm^{-1}. This overtone region usually contains a prominent band near 2222–2000 cm^{-1} assigned to a combination of asymmetrical NH_3^+ bending vibrations and the torsional oscillation of the NH_3^+ group [33]. The 2000 cm^{-1} band is absent if the nitrogen atom of the amino acid is substituted. The significant decrease in the transmittance intensity in Figure 7(c) indicates the decrease of the amino group in the conjugated enzyme.

Also, weak asymmetric NH_3^+ bending bands are near 1660–1610 cm^{-1} and fairly strong symmetric bending bands are near 1550–1485 cm^{-1} [33].

The decrease of intensity of peak 2200 and 1661 in Figure 7(c) indicates the absence of amine groups in the conjugated enzyme.

On the other hand, primary amides and secondary amides display a band in the region of 1650–1515 cm^{-1} primarily due to NH_2 or NH bending: the amide type II band. This adsorption involves coupling between N–H bending and other fundamental vibrations and requires a trans configuration [33].

Out-of-plane NH wagging in amides is responsible for a broadband of medium intensity in the 800–666 cm^{-1} region. Surprisingly, an increase of these peaks related to only the type II amide was displayed in accordance with the FT-IR spectrum of the lysozyme-functionalised SWCNTs, as shown in Figure 7(a) and (c).

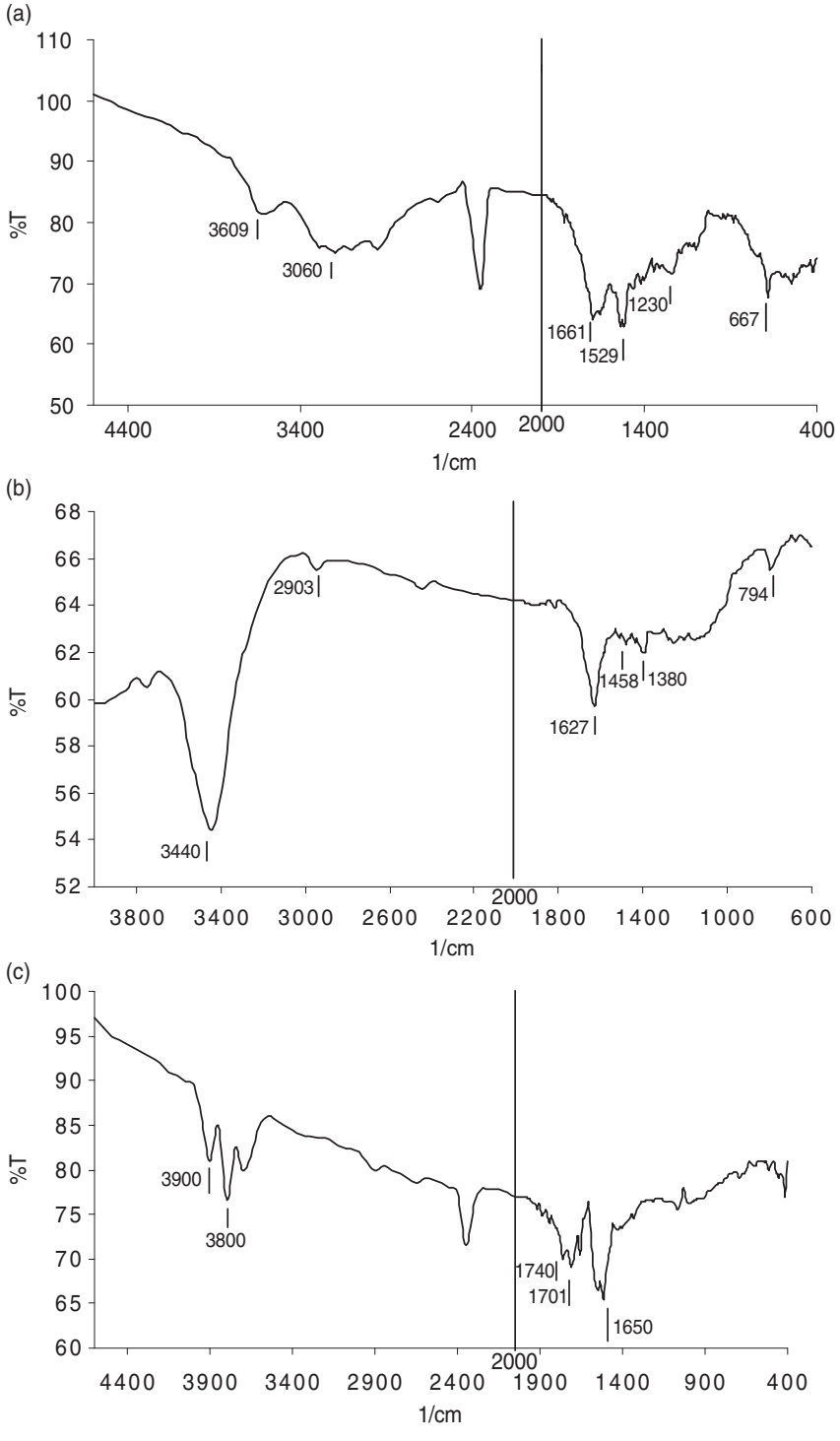

Figure 7. FT-IR spectra of (a) native enzyme, (b) SWCNTs and (c) attached lysozyme–SWCNTs.

Downloaded At: 11:10 15 January 2011

4. Conclusions

Activated SWCNTs characterise exciting materials for the preparation of enzyme conjugates with high active-enzyme loadings per unit weight of the material. They exhibit unique behaviours that distinguish them from traditional immobilisation systems. High active loading of lysozyme onto the SWCNTs enabled characterisation using spectrophotometric, electrophoretic, XRD and FT-IR studies. This confirmed that a variety of lysozyme retained a high fraction of their native activity and structure when attached to SWCNTs.

Indeed, to our knowledge, this is the first XRD, electrophoresis, FT-IR and TGA analysis of protein structure and function by covalent attachment onto SWCNTs. The availability of these nanomodified enzyme constructs opens up an entire exciting research direction in the field of nanoenzymology, finally, aiming to target and to alter the enzyme behaviour at the molecular level.

Acknowledgements

The authors thank Dr K. Javidnia for the guidance in FT-IR and M. Tavana and M. Masoodian for their assistance in this study. This research was supported by grant numbers 88-GR-VT-11 and 88-GR-ENG-29 from Shiraz University Research Council.

Downloaded At: 11:10 15 January 2011

References

[1] T.M.S. Chang and S. Prakash, *Procedures for engineered microorganisms*, Mol. Biotechnol. 17 (2001), pp. 246–260.

[2] C. Kneuer, M. Sameti, U. Bakowsky, T. Schiestel, H. Schirra, H. Schmidt, and C.-M. Lehr, *A nanoviral DNA delivery system based on surface modified silica-nanoparticles can efficiently transfect cells in vitro*, Bioconj. Chem. 11 (2000), pp. 926–932.

[3] J. Radler, I. Koltover, T. Salditt, and C.R. Safinya, *Structure of DNA–cationic liposome complexes: DNA intercalation in multilamellar membranes in distinct intermedical packing regimes*, Science 275 (1997), pp. 810–814.

[4] I. Koltover, T. Salditt, J.O. Radler, and C.R. Safinya, *An inverted hexagonal phase of cationic liposome–DNA complexes related to DNA release and delivery*, Science 281 (1998), pp. 78–81.

[5] K.E. Ulrich, S.M. Cannizzaro, R.S. Langer, and K.M. Shakeshelf, *Polymeric systems for controlled drug release*, Chem. Rev. 99 (1999), pp. 3181–3198.

[6] K.E. Lee, B.K. Kim, and S.H. Yuk, *Biodegradable polymeric nanospheres formed by temperature-including phase transition in a mixture of poly(lactide-co-glycolide) and poly(ethylene oxide)poly (propylene oxide).poly(ethylene oxide) triblock copolymer*, Biomacromolecules 3 (2002), pp. 1115–1119.

[7] N. Murthy, Y.X. Thng, S. Schuck, M.C. Xu, and J.M.J. Frechet, *A novel strategy for encapsulation and release of proteins, hydrogels and microgels with acid-labile acetal cross-linkers*, J. Am. Chem. Soc. 124 (2002), pp. 12398–12399.

[8] Y.W.C. Cao, R.C. Jin, and C.A. Mirkin, *Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection*, Science 297 (2002), pp. 1536–1540.

[9] L.M. Demers, D.S. Ginger, S.-J. Park, Z. Li, S.-W. Chung, and C.A. Mirkin, *Direct patterning of modified oligoncleotides on metals and insulators by dip-pen nanolithography*, Science 296 (2002), pp. 1836–1838.

[10] S.J. Park, T.A. Taton, and C.A. Mikin, *Array-based electrical detection with nanoparticles probes*, Science 295 (2002), pp. 1503–1506.

[11] S.C. Tsang, J.J. Davis, M.L.H. Green, H.A.O. Hill, Y.C. Leung, and P.J. Sadler, *The immobilisation of small proteins in carbon nanotubes: High resolution transmission electron microscopy and catalytic activity*, J. Chem. Soc. Chem. Commun. (1995), pp. 1803–1804, 2579.
[12] J.J. Davis, M.L.H. Green, H.A.O. Hill, Y.C. Leung, P.J. Sadler, J. Sloan, A.V. Xavier, and S.C. Tsang, *The immobilisation of proteins in carbon nanotubes*, Inorg. Chim. Acta 272 (1997), pp. 261–266.
[13] P.W. Barone, S. Baik, D.A. Heller, and M.S. Strano, *Near-infrared optical sensors based on single-walled carbon nanotubes*, Nat. Mater. 4 (2005), pp. 86–92.
[14] R.J. Chen, S. Bangsaruntip, K.A. Drouvalakis, N.W.S. Kam, M. Shim, Y. Li, W. Kim, P.J. Utz, and H. Dai, *Noncovalent functionalization of carbon nanotubes for highly specific electronic biosensors*, Proc. Natl. Acad. Sci. USA 100 (2003), pp. 4984–4989.
[15] K. Besteman, J.O. Lee, F.G.M. Wiertz, H.A. Heering, and C. Dekker, *Enzyme-coated carbon nanotubes as single-molecule biosensors*, Nano Lett. 3 (2003), pp. 727–730.
[16] N.W.S. Kam, T.S. Jessop, P.A. Wender, and H. Dai, *Nanotube molecular transporters: Internalization of carbon nanotube–protein conjugates into mammalian cells*, J. Am. Chem. Soc. 126 (2004), pp. 6850–6851.
[17] P. Asuri, S.S. Karajanagi, R.S. Kane, and J.S. Dordick, *Polymer–nanotube–enzyme composites as active antifouling films*, Small 3 (2007), pp. 50–53.
[18] S.S. Karajanagi, A.A. Vertegel, R.S. Kane, and J.S. Dordick, *Structure and function of enzymes adsorbed onto single-walled carbon nanotubes*, Langmuir 20 (2004), pp. 11594–11599.
[19] P. Asuri, S.S. Karajanagi, H. Yang, T.-J. Yim, R.S. Kane, and J.S. Dordick, *Increasing protein stability through control of the nanoscale environment*, Langmuir 22 (2006), pp. 5833–5836.
[20] B.F. Erlanger, B.-X. Chen, M. Zhu, and L. Brus, *Binding of an anti fullerene IgG monoclonal antibody to single wall carbon nanotubes*, Nano Lett. 1 (2001), pp. 465–467.
[21] M. Shim, N.W.S. Kam, R.J. Chen, Y. Li, and H. Dai, *Functionalization of carbon nanotubes for biocompatibility and biomolecular recognition*, Nano Lett. 2 (2002), pp. 285–288.
[22] M. Panhius, C. Salvador-Morales, E. Franklin, G. Chambers, A. Fonseca, J.N. Nagy, W.J. Blau, and A.I. Minett, *Characterization of an interaction between functionalized carbon nanotubes and an enzyme*, J. Nanosci. Nanotechnol. 3 (2003), pp. 209–213.
[23] P. Asuri, S.S. Karajanagi, J.S. Dordick, and R.S. Kane, *Directed assembly of carbon nanotubes at liquid–liquid interfaces: Nanoscale conveyors for interfacial biocatalysis*, J. Am. Chem. Soc. 128 (2006), pp. 1046–1047.
[24] D. Pantarotto, J.-P. Briand, M. Prato, and A. Bianco, *Structure and function of enzymes adsorbed onto single-walled carbon nanotubes*, Chem. Commun. 16 (2004), pp. 11594–11598.
[25] P. Asuri, S.S. Karajanagi, E. Sellito, D.-Y. Kim, R.S. Kane, and J.S. Dordick, *Water-soluble carbon nanotube–enzyme conjugates as functional biocatalytic formulations*, Biotechnol. Bioeng. 95 (2006), pp. 804–811.
[26] P.M. Davidson, *Food Microbiology: Fundamentals and Frontier*, 2nd ed., Chapter 29, ASM Press, Washington, DC, USA, 2001.
[27] P. Asuri, S.S. Bale, R.C. Pangule, D.A. Shah, R.S. Kane, and J.S. Dordick, *Structure, function, and stability of enzymes covalently attached to single-walled carbon nanotubes*, Langmuir 23 (2007), pp. 12318–12321.
[28] H.-M. Ding, L. Shao, R.-J. Liu, Q.-G Xiao, and J.-F. Chen, *Silica nanotubes for lysozyme immobilization*, J. Colloid Interface Sci. 290 (2005), pp. 102–106.
[29] T. Imoto and K.A. Yagishita, *Simple activity measurement of lysozyme*, Agric. Biol. Chem. 35 (1971), pp. 1154–1156.
[30] X.P. Gao, Y. Zhang, X. Chen, G.L. Pan, J. Yan, F. Wu, H.T. Yuan, and D.Y. Song, *Carbon nanotubes filled with metallic nanowires*, Carbon 42 (2004), pp. 47–52.

Downloaded At: 11:10 15 January 2011

[31] A. Dong, P. Huang, and W.S. Caughey, *Redox-dependent changes in β-extended chain and turn structures of cytochrome-c in water solution determined by 2nd derivative amide I infrared spectra*, Biochemistry 31 (1992), pp. 182–189.
[32] A. Gole, G. Thakar, and M. Sastry, *Protein diffusion into thermally evaporated lipid films: Role of protein charge/mass ratio*, Colloids Surf. B 28 (2003), pp. 209–214.
[33] R.M. Silveratein, G.C. Bassler, and T.C. Morrill, *Spectrometric Identification of Organic Compounds*, 3rd ed., John Wiley & Sons, New York, USA, 1974.

Downloaded At: 11:10 15 January 2011