SYNTHESIS OF BIO-INORGANIC NANOHYBRIDS MATERIAL (BINHs) OF DEOXYRIBONUCLEIC ACID ENCAPSULATED INTO LAYERED DOUBLE HYDROXIDE

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OF DEOXYRIBONUCLEIC ACID ENCAPSULATED INTO LAYERED
DOUBLE HYDROXIDE

By

ISWAN BUDY HJ. SUYUB

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science

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Dedicated to Emak and Ayah
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Chairman : Professor Datin Paduka Khatijah Mohd. Yusoff, PhD
Faculty : Biotechnology and Biomolecular Sciences

Deoxyribonucleic acid (DNA) from salmon sperm was encapsulated into the inorganic Zn/Al-layered double hydroxides (LDHs) interlamellar for the formation of bio-inorganic nanohybrids materials (BINHs) by self-assembly method. Intercalation of DNA into Zn/Al-layered double hydroxides inorganic interlamellar host was carried out at different Zn to Al molar ratios, Rs and various concentrations of DNA. This work is meant to synthesize BINHs as drug or gene carrier. Among the numerous DNA delivery approaches developed so far, viral methods are well known and can be extremely efficient as its were used in the first human gene therapy test, but the safety especially the toxicity and immunogenecity of viral vectors is unknown. The physicochemical properties of the as-synthesized LDH and the resulting BINHs nanocomposite were studied using powder x-ray diffraction (PXRD) analysis. Expansion of basal spacing to about 21.2 Å (for R=3 with highest concentration of DNA which is 100 mg/ml) and 21.9 Å (for R=1 with lowest concentration of DNA
which is 44.4 mg/ml) in the resulting nanomaterials was observed compared to their respective LDHs precursor with basal spacing of 9.0 Å and 8.9 Å, respectively. This shows that the expansion is due to spatial orientation of the DNA into the LDH inorganic interlamellar and FTIR spectra further confirmed that the DNA was intercalated into the host-matrix of the LDHs. Polymerase Chain Reaction (PCR) analysis verified that DNA within LDH interlayer still sustained its integrity because amplification of certain region of DNA can still be carried out.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBENTUKAN BAHAN NANOHIBRID BIO-INORGANIK (BINHs) BAGI ASID DEOKSIRIBONUKLEIK TERSELIT DALAM LAPISAN BERGANDA HIDROKSIDA

Oleh

ISWAN BUDY HJ. SUYUB

November 2008

Pengerusi : Professor Datin Paduka Khatijah Mohd. Yusoff, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Asid deoksiribonukleik (DNA) daripada sperma salmon telah diinterkalasikan ke dalam lapisan inorganik Zn/Al hidroksida berlapis ganda (LDH) bagi pembentukan bahan nanohibrid bio-inorganik (BINHs) dengan menggunakan cara pembentukan-sendiri. Interkalasi DNA ke dalam ruang antara lapisan inorganik Zn/Al hidroksida berlapis ganda telah dilakukan pada nisbah molar Zn terhadap Al (Rs) yang berbeza, dan pada pelbagai kepekatan DNA. Kajian ini dilakukan bertujuan mensintesiskan BINHs sebagai pembawa gen atau dadah. Antara kebanyakan pendekatan dalam pembangunan pembawa DNA yang telah dilakukan, kaedah viral lebih dikenali dan dianggap paling efisien oleh kerana ia telah digunakan dalam ujian terapi gen yang pertama, namun keselamatan terutamanya dari segi ketoksidan dan immunogenesiti
vector viral masih tidak diketahui. Ciri-ciri kimia-fizik LDH dan nanokomposit BINHs yang terhasil telah dikaji menggunakan analisis pembeluan sinaran x-ray serbuk. Pengembangan ruang antara dua lapisan asas kira-kira sebanyak 21.2 Å (bagi R=3 dengan kepekatan DNA yang tertinggi iaitu 100 mg/ml) dan 21.9 Å (bagi R=1 dengan kepekatan DNA yang terendah iaitu 44.44 mg/ml) pada bahan nano yang terhasil dibandingkan perbezaannya dengan bahan awal LDH masing-masing yang berketebalan 9.0 Å dan 8.9 Å. Ini menunjukkan pengembangan ketebalan berpunca daripada orientasi DNA di dalam lapisan inorganik LDH dan analisis spektrum FTIR sekaligus membuktikan bahawa DNA telah diinterkalasikan ke dalam ruang matrik LDH. Analisis Tindakbalas Berantai Polimeres (PCR) menunjukkan bahawa DNA di dalam ruang antara lapisan LDH masih mengekalkan integritinya yang mana amplifikasi bahagian tertentu DNA masih boleh dihasilkan.
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Although not everyone individually named here for their contribution towards this study, I humbly extends my appreciation to all of you and prays the very best of life for all of you.
I certify that an Examination Committee has met on 29th of August 2008 to conduct the final examination of Iswan Budy Hj. Suyub on his Master of Science entitled “Synthesis of Bio-Inorganic Nanohybrids Materials (BINHs) Containing Deoxyribonucleic Acid (DNA) Encapsulated in Layered Double Hydroxide” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the relevant degree.

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

ISWAN BUDY HJ. SUYUB

Date:
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<td>Å</td>
<td>Angstrom</td>
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<tr>
<td>BINH</td>
<td>Bio-Inorganic Nanohybrid</td>
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<tr>
<td>°C</td>
<td>Celsius</td>
</tr>
<tr>
<td>CHO</td>
<td>Chinese Hamster Ovarian</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>CHNS</td>
<td>Carbon, Hydrogen, Nitrogen, Sulphur</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle’s Medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>FCS</td>
<td>Foetal Calf Serum</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
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<tr>
<td>mg</td>
<td>Milligram</td>
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<tr>
<td>g</td>
<td>Gram</td>
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<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>Inhibition Concentration at 50% Viability</td>
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<td>ICP-AES</td>
<td>Inductive Couple Plasma – Atomic Emission Spectroscopy</td>
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<tr>
<td>LDH</td>
<td>Layered Double Hydroxide</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>M²⁺/M²⁺</td>
<td>Divalent metal cation</td>
</tr>
<tr>
<td>M³⁺/M³⁺</td>
<td>Trivalent metal cation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>nm</td>
<td>nanometer</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>PXRD</td>
<td>Powder X-Ray Diffraction</td>
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CHAPTER 1

INTRODUCTION

1.1 Bio-inorganic nanohybrids material (BINHs)

Bio-inorganic chemistry is becoming a specialized area that combines biological part and inorganic chemistry part to develop new hybrid materials. Research in bio-inorganic materials has advanced significantly with interests in the formation of new materials based on nanotechnological tools. This new technology known as ‘nanobiotechnology’ has seen the development of improved materials called bio-inorganic nanohybrid materials (BINHs). The ultimate goal is the production of BINHs as the active material at a nano-scale with atomic precision.

Nanobiotechnology which includes “molecular nanotechnology” is a subset of nanotechnology (Drexler, 1992). It involves atomic level engineering and manufacturing using biological models for guidance and requires human design for construction at nanoscale level. It is also closely related to biotechnology but adds the ability to design and modify more details at the atomic level of the objects created (Goodsell, 2004).

Eventhough nanobiotechnology has many different kinds of focus areas, they all share a central concept which is the ability to design molecular machinery to atomic specifications. Today, individual bionanomachines are being designed and created to perform specific nanoscale tasks, such as the targeting of cancer cells (Oh et al., 2006), solution of a computational task (Kassner et al., 2005),
antifungal drug therapy of amphotericin B (Kadimi et al., 2007), sensitive gas sensor detection for air quality monitoring systems (Rickerby and Morrison, 2007), edible and biodegradable nanocomposite films for food packaging applications (Sorrentino et al., 2007). As nanobiotechnology matures, researchers will be able to redesign the biomolecular machinery of the cell to perform large scale tasks for the benefit of human health and technology. Macroscopic structures will be built to atomic precision with existing biomolecular assemblers or by using biological models for assembly.

The construction of BINH materials such as layered double hydroxides (LDHs) intercalated with DNA provides a new way of gene therapy (Kwak et al., 2001). These hybrids provide a shield transportation for oligonucleotide antisense sequence to inhibit the growth of cancer cell by blocking the translation of cancer cell mRNA.

LDH can be described as layered compounds having the general formula of $[\text{M}^{2+}_{1-x}\text{M}^{3+}_x(\text{OH})_2][\text{A}^{m-}_{x/m}]\cdot n\text{H}_2\text{O}$. Partial substitutions of divalent cations (M$^{2+}$) by trivalent cations (M$^{3+}$) generate positive charges in the layer, which will be compensated by the counter anion ($\text{A}^{m-}$) and water molecules (Vaccari, 1999). One of the most important properties of LDHs is that it has the flexibility to incorporate various anions into the interlayer region. This unique property of LDH has attracted considerable attention in line to create a new compound, which is called ‘nanocomposites’ through intercalation process. Intercalation is the insertion of a guest species in the interlayer region of a layered solid with preservation of the layered structure. Intercalation of various anions into the
interlayer of LDH has led to production of useful and multifunctional material such as plant growth regulator (Hussein et al., 2002), pH stabilizer (Vatier et al., 1994), optimizer for medicine effectiveness (Zhang et al., 2006), water treatment (Mohmel et al., 2002), dyeing process for textile industry (Hussein et al., 2004b), supplementary for cement in construction industry (Raki et al., 2004), eliminate allergy in cosmetic products (Perioli et al., 2006) and cancer therapy (Hoyo, 2007).

In order to understand the behavior of the BINH nanocomposites, which have been synthesized, various physico-chemical properties have to be studied. Standard techniques such as x-ray diffraction and infrared spectra are used to confirm the insertion of the DNA into the molecules. From the x-ray patterns, insertion is considered successful if an expansion of the basal spacing occurred and data from the infrared spectrum indicating the presence of functional groups from the host and the DNA will confirm the intercalation process. Techniques of Inductive Couple Plasma – Atomic Emission Spectroscopy (ICP-AES) and Carbon, Hydrogen, Nitrogen, Sulphur (CHNS) are used to check on the elemental contents of the sample. Thermal stability of the nanocomposite can be tested by using the technique of Thermal Gravimetry Analysis – Derivatives Thermal Gravimetry (TGA-DTG). Intercalation of DNA into the interlayer of LDH will exhibit some modification to the morphology of the sample and this can be examined by Scanning Electron Microscope (SEM) technique. Because DNA is a biomolecule material, some biomolecular tools such as agarose gel electrophoresis and Polymerase Chain Reaction (PCR) procedures were also used to further confirm the intercalated substance is still the intact DNA. These BINHs
were examined for their cell cytotoxicity by using methylthiazolyldiphenyl-tetrazolium (MTT) assay to determine their safe use in the human being.

1.2 Statement of Problems

The problem arise when DNA delivery becoming an important tool in biomedical sciences. DNA delivery is a powerful and popular technique in elucidating gene regulation and function. Among the numerous DNA delivery approaches developed so far, viral methods are well known and can be extremely efficient as its were used in the first human gene therapy test, but the safety including the toxicity and immunogenicity of viral vectors is unknown (Luten et al., 2008). As a result, nonviral approaches to DNA delivery are becoming popular.

The search and enhancement for alternate nonviral synthetic approaches to DNA delivery such as lipids and polymers, as well as mechanical and electrical methods has been in progress. Unfortunately, synthetic DNA delivery systems are usually inefficient and toxic (Chowdhury et al., 2004). Barriers along DNA delivery pathway help us understand the fundamental mechanisms of DNA delivery, and thus point out new directions in developing novel synthetic DNA delivery systems that are safer and more efficient.

The barriers along the DNA delivery pathway lie on three major levels:

1. Extracellular (DNA condensation and complexation)
2. Intracellular (endocytosis)
3. Nuclear (nuclear entry and targeting)

On the extracellular level, negatively charged DNA molecules must first be condensed usually via complexing with cationic reagents into particles small enough to be taken up by cells. On the intracellular level, major route of DNA entry which is endocytosis determines DNA lifetime within cytoplasm. Formation of endosome and fusion with lysosomes after DNA entry will expose DNA to extreme degradable enzymes that pose serious challenges to DNA integrity. On the nuclear level, DNA that has survived then dissociate from the condensed complexes, localize the nucleus, and enter through nuclear pores. Only DNA that survives all these barriers has the potential to be functional (Xu et al., 2006; Park et al., 2008).

One way to solve the problem mentioned above is to create an appropriate system, which able to deliver DNA more efficiently and slowly release the DNA to specific targets. There has been development of bio-inorganic nanohybrid (BINHs) systems that can allow safe and controlled release of various bioagents for safe genes and drug delivery. Among a variety of inorganic materials, layered double hydroxides (LDHs) offer great potential as an inorganic matrix for this purpose.

A distinguish achievement made by Choy et al. (2000) show a great example that comply the requirement needed to counter the barrier that DNA will face in cell delivery process. In their study, an antisense oligonucleotide molecule that was intercalated in the LDHs was able to enter leukemia cells to become potential
gene therapy. Treatment of leukemia cells with LDH-encased As-myc oligonucleotides was found out to disrupt a gene in the cells thus inhibit leukemia cell growth by 65%.

Another accomplishment of LDH in contributing towards biomedical science was made by Xia et al. (2008) by intercalating antihypertensive drugs (Enalpril, Lisinopril, Captopril and Ramipril) into Zn/Al-LDH. Better thermal property and stability of intercalated drugs against acid attack indicate potential applicability of LDHs as supports for the preparation of sustained-release formulations of antihypertensive drugs.

Chan et al. (2008) developed an innovative method of rapid disease diagnosis through fluidic system by extracting DNA molecules using media that contain immobilized inorganic LDHs on the polycarbonate (PC) substrate. LDHs as DNA extractor was designed and formulated with immobilization in the polymeric tunnels to capture and release DNA molecules more efficiently from diseased blood through fluidic solution for applying as bio-chip. These methods also enhance the extraction efficiency of DNA molecules from extreme low concentration solution.

In terms of improving BINHs efficiency, good formulations are designed to reduce excessive utilization of DNA to produce high good crystallinity BINHs with a minimum amount of DNA. In this study, attempts were made to study the formation of BINH nanocomposites, which is the hybrid material containing layered double hydroxides (LDH) being inserted with DNA. Therefore, this work