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DESIGN AND SYNTHESIS OF NEW 1-ALKYL-3-BUTYLIMIDAZOLIUM BROMIDE IONIC LIQUIDS AS MEDIA FOR DNA SOLVATION

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By

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DESIGN AND SYNTHESIS OF NEW 1-ALKYL-3-BUTYLIMIDAZOLIUM BROMIDE IONIC LIQUIDS AS MEDIA FOR DNA SOLVATION

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Chair: Mohd Basyaruddin Abdul Rahman, PhD

Faculty: Faculty of Science

The influence of ionic liquids (ILs) on the structural properties of DNA was revealed by experimental and molecular dynamics (MD) simulation. In the first part of experimental section, six new 1-alkyl-3-butylimidazolium bromide ILs ([$C_n$bim][Br] where *n* = 2, 4, 6, 8, 10 and 12) were successfully synthesized. All of the ILs was obtained using simple alkylation reaction of 1-butylimidazole with various bromoalkanes, which gave high yield above 85%. Their physico-chemical properties, including the spectroscopic characteristics have been comprehensively studied. Three of these ILs (C_2, C_4, C_6) exist in liquid form while the others appear as semi solid at room temperature. Proton and carbon NMR and CHN elemental analysis were carried out to identify the molecular structure and purity of ILs produced. The thermal stability studied using TGA indicated that these new ILs were stable up to 270°C. As expected, the viscosity of three liquid salts hugely increased from 199 mPa·s ([C_2bim][Br]) to 1180 mPa·s ([C_6bim][Br]), while the density slightly decreased with increasing length of alkyl chains.

The properties of Calf thymus DNA in hydrated ILs were studied using spectroscopic analysis. The strong interactions between the P-O bond of DNA phosphate groups and the $[C_nbim]^+$ lead to compact DNA conformation, which excludes the intercalation of ethidium with DNA. Although the DNA stability is mainly due to the electrostatic attraction between DNA and ILs' cation, hydrophobic

interactions between hydrocarbon chains of $[C_nbim]^+$ and DNA bases also provided a major driving force for the binding of ILs to DNA. The effect of ILs concentration at 25°C shows that the DNA maintains its B-conformation in all solution of hydrated ILs despite the high concentration up to 75% (w/w). During heating process, hydrated ILs are observed to stabilize DNA helical structure up to 56°C ± 1.0°C, almost 11°C higher than DNA in water. The DNA melting temperature is found gradually increases with increasing length of alkyl chain from 56°C ± 1.0°C (in $[C_2bim][Br]$) to 58°C ± 1.0°C in the presence of $[C_6bim][Br]$.

In the first part of MD simulation, the force fields (FFs) parameter for these three liquid ILs ($[C_nbim][Br]$ where n = 2, 4 and 6) was validated based on experimental evidences. The modified collision parameter (σ) to 0.369 nm for the anion shows the simulation data obtained were in agreement with experimental density and viscosity with the percentage error below ± 2.0% and ± 10.0%, respectively. The validated FFs were then applied for simulation of DNA in these ILs. The MD data offers clear evidence that the DNA maintains its B-conformation in all $[C_4bim]Br$ systems (25, 50 and 75% w/w). The hydration layer around the DNA phosphate group was the main factor in determining DNA stabilization. Stronger hydration shells in 25% $[C_4bim][Br]$ in water (w/w) reduced the binding ability of ILs' cations to the DNA phosphate groups. The computed energy shows that the electrostatic energy between $[C_4bim]^+$–$[PO_4]^-$ (-46.55 ± 4.75 kcal mol^{-1}) is lower than water–$[PO_4]^-$(-12.78 ± 2.12 kcal mol^{-1}). Effect of temperature revealed that ILs was able to retain DNA native conformation at high temperature up to 373.15 K in the presence of 75% $[C_4bim]Br$. All the simulations findings were in agreement with experimental evidences. The prediction solvation free energy of nucleic acids bases performed in last part of MD simulation revealed that the nucleic acid bases were better solvated in ILs rather than in aqueous solution.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

REKABENTUK DAN SINTESIS CECAIR IONIK BAHARU 1-ALKIL-3-BUTILIMIDAZOLIUM BROMIDA SEBAGAI MEDIA UNTUK SOLVASI DNA

Oleh

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Pengaruh cecair ionik (ILs) pada sifat-sifat struktur DNA telah didedahkan oleh experimen dan simulasi molekul dinamik (MD). Dalam bahagian pertama seksyen experiment, enam 1-alkil-3-butilimidazolium bromida ILs baharu ([$C_n$bim][Br] di mana n = 2, 4, 6, 8, 10 dan 12) telah berjaya disintesis. Kesemua ILs diperolehi menggunakan tindak balas pengalkilan mudah 1-butilimidazol dengan pelbagai bromoalkana, yang memberi hasil tinggi di atas 85%. Sifat-sifat fiziko-kimia termasuk ciri-ciri spektroskopi telah dikaji secara menyeluruh. Tiga daripada ILs ini (C_2, C_4, C_6) wujud dalam bentuk cecair manakala yang lain muncul sebagai separuh pepejal pada suhu bilik. Proton dan karbon NMR dan analisis elemen CHN dilakukan untuk mengenal pasti struktur molekul dan ketulenan ILs yang dihasilkan. Kestabilan terma yang telah dikaji menggunakan TGA menunjukkan bahawa ILs baharu ini stabil sehingga suhu 270°C. Seperti yang dijangka, kelikatan tiga garam cecair meningkat mendadak daripada 199 mPa·s ([C_2bim][Br]) kepada 1180 mPa·s ([C_6bim][Br]), manakala data ketumpatan sedikit menurun dengan peningkatan rantai alkil.

Sifat-sifat DNA daripada Calf thymus dalam ILs terhidrat telah dikaji menggunakan analisis specktroskopi. Interaksi kuat antara ikatan P-O kumpulan fosfat DNA dan $[C_n bim]^+$ membawa kepada bentuk DNA yang padat, yang mana menyingkirkan interkalasi etidium dengan DNA. Walaupun kestabilan DNA terutamanya adalah

disebabkan oleh tarikan elektrostatik antara DNA dan kation ILs, interaksi hidropobik antara rantaian hidrokarbon $[C_nbim]^+$ dan bes DNA juga memberikan daya pendorong utama untuk pengikatan ILs kepada DNA. Kesan kepekatan ILs pada suhu 25°C menunjukkan bahawa DNA mengekalkan konformasi-B dalam semua larutan ILs terhidrat meskipun dalam kepekatan yang tinggi sehingga 75% (w/w). Semasa proses pemanasan, larutan ILs terhidrat diperhatikan menstabilkan struktur helix DNA sehingga suhu 56°C ± 1.0°C, hampir 11°C lebih tinggi daripada DNA di dalam air. Suhu lebur DNA didapati beransur-ansur meningkat dengan peningkatan panjang rantaian alkil daripada 56°C ± 1.0°C (di dalam $[C_2bim][Br]$) kepada 58°C ± 1.0°C dalam kehadiran $[C_6bim][Br]$.

Dalam bahagian pertama simulasi MD, parameter medan daya (FFs) untuk tiga cecair ILs ($[C_nbim][Br]$ di mana n = 2, 4 dan 6) telah disahkan berdasarkan bukti-bukti eksperimen. Parameter perlanggaran (σ) untuk anion yang telah diubahsuai kepada 0.369 nm menunjukkan bahawa data simulasi yang diperolehi didapati bersetuju dengan data experimen ketumpatan dan kelikatan dengan peratus ralat masing-masing di bawah ±2.0% and ±10.0%. FFs yang telah disahkan kemudiannya digunakan untuk simulasi DNA dalam ILs ini. MD data menunjukkan bukti yang jelas bahawa DNA mengekalkan konformasi-B di dalam semua sistem $[C_4bim]Br$ (25%, 50% dan 75% w/w). Lapisan penghidratan sekitar kumpulan fosfat DNA adalah faktor utama dalam menentukan kestabilan DNA. Lapisan penghidratan lebih kuat dalam 25% $[C_4bim][Br]$ (w/w) di dalam air telah mengurangkan keupayaan pengikatan kation ILs kepada kumpulan fosfat DNA. Tenaga yang dikira menunjukkan bahawa tenaga elektrostatik antara $[C_4bim]^+$–$[PO_4]^-$ (-46.55 ± 4.75 kcal mol^{-1}) adalah lebih rendah berbanding air–$[PO_4]^-$ (-12.78 ± 2.12 kcal mol^{-1}). Kesan suhu mendedahkan bahawa ILs telah berupaya mengekalkan konformasi asal DNA pada suhu tinggi sehingga 373.15 K dalam kehadiran 75% $[C_4bim][Br]$. Semua penemuan simulasi didapati bersetuju dengan bukti-bukti eksperimen. Ramalan tenaga bebas pensolvatan bes nukleik acid yang dilakukan dalam bahagian akhir simulasi MD mendedahkan bahawa bes nukleik acid lebih mudah terlarut di dalam ILs berbanding di dalam larutan akues.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

$[AlCl_4]^-$	Tetrachloroaluminate
$[BF_4]^-$	Tetrafluoroborate
$[C_{10}mim][Cl]$	1-decyl-3-methylimidazolium chloride
$[C_{10}mim][PF_6]$	1-decyl-3-methylimidazolium hexafluorophosphate
$[C_1mim][Cl]$	1,3-dimethylimidazolium chloride
$[C_2mim][AlCl_4]$	1-ethyl-3-methylimidazolium tetrachloroaluminate
$[C_2mim][BF_4]$	1-ethyl-3-methylimidazolium tetrafluoroborate
$[C_2mim][Cl]$	1-ethyl-3-methylimidazolium chloride
$[C_2mim][N(SO_2CF_3)_2]$	1-ethyl-3-methylimidazolium bis(trifluoromethanesulfonate)imide
$[C_2mim][PF_6]$	1-ethyl-3-methylimidazolium hexafluorophosphate
$[C_2mim]^+$	1-ethyl-3-methylimidazolium
$[C_4mim][BF_4]$	1-butyl-3-methylimidazolium tetrafluoroborate
$[C_4mim][Cl]$	1-butyl-3-methylimidazolium chloride
$[C_4mim][PF_6]$	1-butyl-3-methylimidazolium hexafluorophosphate
$[C_4mim]^+$	1-butyl-3-methylimidazolium
$[C_6mim][PF_6]$	1-hexyl-3-methylimidazolium hexafluorophosphate
$[CF_3O_3S]^-$	Trifluoromethanesulfonate
$[C_nbim][Br]$	1-alkyl-3-butylimidazolium bromide

$[C_n mim][BF_4]$	1-alkyl-3-methylimidazolium tetrafluoroborate
$[C_n mim][PF_6]$	1-alkyl-3-methylimidazolium hexafluorophosphate
$[C_n mim]^+$	1-alkyl-3-methylimidazolium
$[E]^+$	Ethidium
$[EtNH_3][NO_3]$	Ethylammonium nitrate
$[Ms_2N]^-$	Bis(methanesulfonyl)amide
$[N(SO_2CF_3)_2]^-$	Bis(trifluoromethanesulfonate)imide
$[NO_3]^-$	Nitrate
$[PF_6]^-$,	Hexafluorophosphate
$[SO_4]^{2-}$	Sulfate
$[TF]^-$	Trifluoromethylsulfonate
^{31}P NMR	^{31}Phosphorus NMR
A_{260}	Absorbance at wavelength 260 nm
AA	All-atom
AM1 / SM2	Austin Model 1 / Semiempirical quantum chemical solvation Model 2
AM1-MST	Austin Model 1 / Miertus–Scrocco–Tomasi
AMBER	Assisted Model Building with Energy Refinement
A-T	Adenine-Thymine
B3LYP	Becke's Three-parameter Hybrid Functional
BAR	Bennet Acceptance Ratio
bp	Base pairs

Br	Bromine
CD	Circular dichroism
C-G	Cytosine-Guanine
CH_2	Methylene
CH_3	Methyl
CHARM	Chemistry at HARvard Molecular Mechanics
CHN	Carbon, Hydrogen, Nitrogen
COM	Centre-of-mass
$_d$-$CDCl_3$	Deuterated chloroform
DESO	Diethylsulfoxide
DFT	Density Functional Theory
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DPSO	Dipropylsulfoxide
dsDNA	Double-stranded DNA
EB	Ethidium bromide
ESP	Electrostatic potential
FDPB	Finite Difference Poisson-Boltzmann
FEP	Free Energy Perturbation
FFs	Force Fields
FRET	Fluorescence resonance energy transfer
FT-IR	Fourier transform-Infrared
GROMOS	GROningen Molecular Simulation

IL	Ionic liquid (singular)
ILs	Ionic liquids (plural)
kbp	Kilo base pairs
KCl	Potassium chloride
LINCS	Linear Constraint Solver
LJ	Lennard-Jones
MC	Monte Carlo
MD	Molecular dynamics
mdeg	Milidegree
MSD	Mean square displacement
MST	Miertus–Scrocco–Tomasi
NaCl	Sodium chloride
NDB	Nucleic Acids Database
NEMD	Non-equilibrium molecular dynamics
NMR	Nuclear Magnetic Resonance
NPT	Isobaric-isothermal ensemble
NVT	Canonical ensemble
OPLS	Optimized Potential for Liquid Simulation
PBC	Periodic boundary condition
PCR	Polymerase chain reaction
PDB	Protein Data Bank
PME	Particle Mesh-Ewald
QM	Quantum mechanics
QM/MM	Quantum mechanics / Molecular mechanics

RDF	Radial distribution function
RES-COM	Residue-centre of mass
RESP	Restrained electrostatic potential
RMSD	Root mean square deviation
RMSF	Root mean square fluctuation
RTILs	Room temperature ionic liquids
S1	Vibrational level 1
SCRF	Self consistent reaction field
SDF	Spatial distribution function
SF_6	Sulfur hexafluoride
SFE	Solvation free energy
So	Ground state
ssDNA	Single-stranded DNA
TGA	Thermogravimetric Analysis
TI	Thermodynamics Integration
T_m	Melting temperature
T_{onset}	Onset temperature
UA	United-atom
w/w	Weight over weight
ΔG_{solv}	Absolute solvation free energy

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Since the discovery of DNA over half a century ago, doing research on DNA has become a subject of intense interest. In many aspects of nucleic acid metabolism, the stability of double helical DNA structure is extremely important and plays a main role especially in biomedical applications. The specificity of hybridization is at the core of many molecular biology techniques including the DNA sequencing, polymerase chain reaction (PCR), microarray technology as well as an essential material in the development of advanced molecular devices (Krishnan and Simmel, 2011; Kutzler and Weiner, 2008; Jobling and Gill, 2004).

For many years, the solution environment strongly influences the stability of DNA. Both aqueous and organic solvents are widely used as an extraction media or molecular solvent for DNA solvation. The stability of biological structure of DNA mainly depends on the water molecules. Particularly, the conformational and stability of DNA are controlled by the interactions between DNA and nearby water molecules (Westhof, 1988; Saenger, 1987; Texter, 1978). Since there is a close connection between DNA structure and their biological function, understanding the water-DNA relationship is significantly important. Other than water, the ambient environment such as different buffer conditions (pH, types of buffer solutions), concentrations of molecules, higher salt concentrations or even different type of non-aqueous solvents may all affect the stability of DNA conformation (Bonner and Klibanov, 2000). Previous studies of DNA in non-aqueous solutions have revealed that most organic solvents such as methanol, phenols, chloroform and DMSO, whether neat or in a mixture with water, all spontaneously denature DNA.

The dry storage of DNA, utilizing the basic concept of anhydrobiosis or “life without water” is an alternative to old-style DNA storage (Bonnet *et al.,* 2009). The development of other non-aqueous media which can stabilize and maintain native DNA structure for a long period especially at ambient temperature is increasing. Recently, huge attention is directed to the development of specific solvent for DNA. Ionic liquids (ILs) which is one of the non-aqueous ionic solvent have attracted many attentions due to their interesting

properties. The usage of ILs is attractive as they are almost non-toxic, having good solubility and high conductivity (Sun *et al.,* 2008a; He *et al.,* 2006; Nishimura *et al.,* 2005; Qin and Li, 2003). They offer unique opportunities as alternatives to aqueous and non-aqueous solvents in DNA biotechnology.

The behavior of DNA structure in ILs is of both practical and fundamental interest. Structural studies using crystallography and NMR provide tremendous amounts of information with the determination of three-dimensional pictures of various complexes. From a historical point of view, the first structure of DNA solved by single crystal x-ray analysis was a tetramer reported by Viswamitra *et al.* (1978). Until now, there are many ligand/compound-DNA structures deposited to the Nucleic Acids Database (NDB) and also Protein Data Bank (PDB) (Berman *et al.,* 2000). However, these techniques only provide static pictures and often it is desirable to follow the progression of molecules as a function of time. Additionally, not all structures are possible to obtain via these methods due to technical issues. Molecular dynamics (MD) simulation technique provides another way to look at the structures and interactions and complements the experimental evidences nicely.

This study focused on understanding of how the ILs influence the dynamics and structural stability of DNA from both experimental and computational point of views. The results of our work may provide more insight into the studied system, allowing a better understanding of the IL-DNA binding and expanding the overall capabilities and applications of ILs in biological and biomedical applications. This study is also vital for future development of specific solvent especially for DNA and RNA solutes.

1.2 Problem Statements

The problems in current DNA technology are related to the use of aqueous and conventional organic solvents as a media for DNA solvation. Although DNA is considered stable in aqueous solution, it is susceptible to slow hydrolytic reaction such as deamination and depurination, which caused serious damage to DNA helical structure (Lukin and de los Santos, 2006). Furthermore, aqueous solution is not able to stabilize DNA helical structure over a long period especially at room temperature (several days up to 1 month) (Vijayaraghavan *et al.,* 2010a) due to the degradation by contaminating nucleases (Sasaki *et al.,* 2007) and inherent chemical instability.

Moreover, DNA in various organic solvents such as DMSO, DMF, formamide, methanol or pyridine is found to have lost its native structure, undergo strand separation or formation of toroid-like conformations (Ke *et al.,* 2010; Hammouda and Worcester, 2006; Montesi *et al.,* 2004; Bonner and Klibanov, 2000). Even worse, addition of ethanol to an aqueous solution induces drastic changes in the duplex structure, forcing a B- to A-DNA transition (Herskovits and Harrington, 1972). Traditional extractions using chloroform/phenol (Muller *et al.,* 1983) can also cause denaturation of DNA during the extraction process. More importantly, the contamination of extracted DNA by organic solvents is unavoidable and creates vital problems for the biological investigations as the traditional organic solvents are known to be toxic to bioprocesses (Matsumoto *et al.,* 2004; Albarino and Romanowski, 1994). Physical factors such as ionic strength, pH and temperature can also disturb the helical structure and cause denaturation (Cheng and Pettitt, 1992; Lindahl and Nyberg, 1972). Therefore, the development of potential molecular solvent is aimed to overcome these limitations and its application especially in the DNA biotechnology.

Therefore, there is a great need to introduce other solvent for DNA such as ILs. Based on their remarkable properties, ILs have proved to be preferred solvents to replace traditional organic solvents and aqueous solution in many types of reactions. Over the last few years, several authors have reported the use of ILs in extraction and separation/purification of traces species of interest from complex matrixes including metal and organic compounds as well as amino acids (Han and Armstrong, 2007). ILs also have been used for gene delivery vectors, capillary electrophoresis and DNA isolation (Zhang *et al.,* 2009b; Wang *et al.,* 2007a; Qin and Li, 2003). It has been reported that ILs are able to extract DNA without any contamination from proteins and metal specifies during the extraction process (Wang *et al.,* 2007a). This finding provides an alternate approach for the measurement of DNA in ILs as well as for the separation/purification of trace amounts of DNA in real-world biological matrices.

1.3 Research Objectives

This research was embarked with the main goal to show that ionic liquids (ILs) have good properties as molecular solvent for DNA. Hence, the experimental and computational studies were performed to fulfill the objectives as below:

i) Experimental part

1. To design and synthesize new alkylimidazolium-based ILs.
2. To characterize the physico-chemical properties of the synthesized ILs.
3. To elucidate the IL-DNA interaction using biophysical characterizations.

ii) Computer modeling via MD simulation

4. To study the properties and behavior of ILs and IL-DNA at molecular level.
5. To determine the solvation free energy of nucleic acid bases in ILs.

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