UNIVERSITI PUTRA MALAYSIA

MOLECULAR INSIGHTS ON THE EFFECTS OF ANIONS TOWARDS HYDROLASES IN [BMIM]-BASED IONIC LIQUIDS

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FS 2014 68

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By

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Thesis Submitted to School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

August 2014

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfillment of the requirement for the degree of Doctor of Philosophy

MOLECULAR INSIGHTS ON THE EFFECTS OF ANIONS TOWARDS HYDROLASES IN [BMIM]-BASED IONIC LIQUIDS

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August 2014

Chair: Professor Mohd Basyaruddin Abdul Rahman, PhD

Faculty: Science

The use of non-aqueous solvents in biocatalysis has shown improvements in enzyme performance. A new type of non-aqueous solvent has attracted a lot of interests in biocatalysis, called the Room Temperature Ionic Liquid (RTIL). A lot of biocatalysis experiments have showed that RTILs can further increase the reaction rates and yields when used instead of conventional organic solvents. However, since there are many RTIL combinations available, selecting a specific RTIL for use in biocatalysis have proven to be quite challenging. A detailed understanding on the effects that different RTIL combination imposed on enzymes is therefore important. Here, the behavior of enzymes in RTILs was characterized by their effects when different RTIL anions were used. A series of molecular-level investigations were conducted using molecular dynamics (MD) and stochastic dynamics (SD) simulations in order to gain more information on the structural and dynamics properties of enzymes in RTILs. Four hydrolases, consisted of α-Chymotrypsin, thermolysin, *Candida Antarctica* Lipase B (CALB) and *Candida rugosa* Lipase (CRL) were studied. These hydrolases were solvated in aqueous and five, 1-butyl-3-methylimidazolium ([BMIM])-based RTILs with different anions such as hexafluorophosphate ($[PF_6]^-$), tetrafluoroborate ($[BF_4]^-$), chloride ($[Cl]^-$), trifluoromethanesulfonate ($[TfO]^-$) and bis-trifluoromethylsulfonylimide ($[Tf_2N]^-$). The effects of water molecules in the systems were studied at 5%, 10%, 15%, 20% and 50% of water, based on the weight/weight percentages of the protein mass (w/w protein). All RTIL solvent models produced a liquid ordering at room temperature and an average density that was close to experimental data with a percentage error of below than 5%.

The structural stability of all hydrolases studied showed a dependency towards the water content, in which the minimum atomic displacements were observed around 10 to 20% of water. Around this water percentage region, $[TfO]^-$ anion rendered the most stable conformation for α-Chymotrypsin, CALB and CRL. The smallest $[Cl]^-$ anion was found to produce the least stable conformations compared to other RTILs studied. In the case of thermolysin, the order of structural stability between the RTIL anions at 15% of water was $[PF_6]^- > [TfO]^- \sim [Tf_2N]^- > [Cl]^- \sim [BF_4]^-$ which was different from other hydrolases studied. Further investigations revealed that in [BMIM][PF_6], thermolysin showed better structural stability than in aqueous, even when simulated at 90 °C. The effect of changing the RTIL anions towards the enzyme flexibility was only clearly visible at higher water content (20% and 50% w/w protein), especially for $[PF_6]^-$and $[Tf_2N]^-$ anions. The analysis on local flexibility showed that only the surface of the protein was affected. For the lipases, the local flexibility was found significantly reduced in certain regions which were highly flexible in aqueous solution, particularly for the lid of the CRL. MD simulations revealed a structured ordering of RTIL anions around the enzymes while the water molecules were found localized at certain region of the protein surface. Hydrophobic anions such as $[PF_6]^-$ covered more areas and were more organized at low water content while $[Cl]^-$anion behave otherwise. Meanwhile, a number of water molecules were stripped off from the surface of α-Chymotrypsin, CALB and CRL. RTILs with $[PF_6]^-$ and $[TfO]^-$ anions retained more water on the surface as compared to $[BF_4]^-$ and $[Cl]^-$ anions, consistently for the three hydrolases. $[Tf_2N]^-$ anion was found stripping the most number of water for the case ofα-Chymotrypsin and CALB while the least was found for CRL.

The solvation thermodynamics of amino acid side chain analogues in water and five [BMIM]-based RTILs was investigated using SD simulations. The solvation free energy was calculated using Bennett's Acceptance Ratio method. Results from the simulations in water were in agreement with published experimental and simulation data. RTILs showed better solvation capabilities when compared with water. Non-polar analogues produced lower solvation free energy in hydrophobic anions such as $[PF_6]^-$ and $[Tf_2N]^-$ while the polar ones showed better solvation in hydrophilic anions such as $[BF_4]^-$, $[Cl]^-$ and $[TfO]^-$. The solvation properties in [BMIM][Cl] also explained why the enzymes experienced more conformational distortions in this RTIL at low water content. Overall, computer simulations were able to explain several effects of RTIL anions on the structure and dynamics of enzymes at molecular level. The structural stability and flexibility of the enzymes were found affected by the water content, more than the types of the RTIL anions studied. MD simulation results were correlated with experimental reports. It was found that the behavior of anions and water at the protein surface played a major role towards the properties of enzymes in RTILs. The results also suggested that the surface properties of the biocatalyst and the physicochemical properties of the substrate should be taken into consideration when choosing a particular RTIL as the solvent system.

Abstrak tesis yang dikemukakan kepada Senat of Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENDEKATAN MOLEKULAR BERKENAAN KESAN ANION TERHADAP HIDROLASE DI DALAM CECAIR IONIK BERASASKAN [BMIM]

Oleh

MUHAMMAD ALIF BIN MOHAMMAD LATIF

Ogos 2014

Pengerusi: Profesor Mohd Basyaruddin Abdul Rahman, PhD

Faculti: Sains

Penggunaan larutan bukan akues di dalam biokatalisis telah menunjukkan peningkatan prestasi enzim. Sejenis larutan bukan akues telah berjaya menarik perhatian di dalam bidang biokatalisis, bernama Cecair Ionik Suhu Bilik (RTIL). Terdapat banyak laporan eksperimen yang menunjukkan bahawa RTIL mampu menambah lagi kadar reaksi dan hasil apabila digunakan sebagai pelarut bukan akues menggantikan organik konvensional. Walaubagaimanapun, terdapat banyak kombinasi RTIL yang boleh digunakan, maka untuk memilih RTIL yang spesifik untuk biokatalisis adalah agak sukar. Pemahaman yang terperinci tentang kesan yang berbeza apabila RTIL yang berbeza digunakan ke atas enzim adalh amat penting. Di sini, perilaku enzim di dalam RTIL dicirikan oleh kesan mereka apabila menggunakan RTIL anion yang berbeza. Satu siri siasatan di tahap molekul telah dijalankan menggunakan simulasi dinamik molekul (MD) dan dinamik stokastik (SD) untuk mendapatkan maklumat lanjut mengenai sifat-sifat dinamik dan struktur enzim di dalam RTIL. Empat hidrolase, terdiri daripada α-Chymotrypsin, thermolysin, *Candida antartica* Lipase B (CALB) dan *Candida rugosa* Lipase (CRL) telah dikaji. Hidrolase-hidrolase ini telah dilarutkan di dalam akues dan di dalam lima RTILs berasaskan kation 1-butil-3-methylimidazolium ([BMIM]) dengan anion yang berbeza seperti hexafluorofosfat ($[PF_6]^-$), tetrafluoroborat ($[BF_4]^-$), klorida ($[Cl]^-$), trifluorometanaesulfonat ($[TfO]^-$) dan bis-trifluorometilsulfonilimida ($[Tf_2N]^-$). Kesan molekul air di dalam setiap sistem dikaji pada 5%, 10%, 15%, 20% dan 50% air, berdasarkan peratusan berat/berat jisim protein (w/w protein). Kesemua model larutan RTIL menghasilkan aturan cecair pada suhu bilik dan kepadatan purata yang dekat dengan data eksperimen dengan ralat peratusan kurang daripada 5%. Kestabilan struktur semua hidrolase yang dikaji menunjukkan pergantungan kearah kandungan air, dan anjakan atom minimum diperhatikan pada kira-kira 10 hingga 20% air.

Di rantau peratusan air ini, anion $[TfO]^-$memberikan bentuk yang paling stabil untuk α-Chymotrypsin, CALB dan CRL. Anion yang paling kecil $[Cl]^-$ didapati menghasilkan bentuk yang paling kurang stabil berbanding dengan RTIL lain yang dikaji. Di dalam kes thermolysin, susunan kestabilan struktur antara anion RTIL pada 15% air adalah $[PF_6]^- > [TfO]^- \sim [Tf_2N]^- > [Cl]^- \sim [BF_4]^-$ yang mana, berbeza dari hidrolases lain yang dikaji. Siasatan lanjut mendedahkan bahawa dalam [BMIM][PF_6], thermolysin menunjukkan kestabilan struktur yang lebih baik daripada di dalam akueus, walaupun simulasi dijalankan pada suhu 90 °C. Kesan daripada mengubah anion RTIL terhadap fleksibiliti enzim hanya jelas kelihatan pada kandungan air yang tinggi (20% dan 50% w/w protein), terutamanya untuk anion $[PF_6]^-$ dan $[Tf_2N]^-$. Analisa ke atas fleksibiliti setempat menunjukkan hanya permukaan protein yang terjejas. Bagi lipase, fleksibiliti didapati berkurangan di kawasan-kawasan tertentu yang amat fleksibel dalam larutan akues, terutamanya bagi bahagian penutup struktur CRL. Simulasi MD turut mendedahkan yang anion RTIL mempunyai aturan berstruktur di sekitar enzim manakala molekul air ditemui secara setempat di kawasan tertentu pada permukaan protein. Anion hidrofobik seperti $[PF_6]^-$ melindungi lebih banyak kawasan dan lebih teratur pada kandungan air yang rendah manakala anion $[Cl]^-$ berkelakuan sebaliknya. Sementara itu, beberapa molekul air telah dilucutkan dari permukaan α-Chymotrypsin, CALB dan CRL.

RTIL dengan anion $[PF_6]^-$ dan $[TfO]^-$ mengekalkan lebih banyak air di permukaan berbanding dengan anion $[BF_4]^-$ dan $[Cl]^-$, secara konsisten untuk tiga hidrolase itu. Anion $[Tf_2N]^-$ pula didapati melucutkan paling banyak air untuk kes α-Chymotrypsin dan CALB manakala sebaliknya berlaku untuk CRL. Termodinamik pensolvatan bagi analog-analog rantaian sisi asid amino di dalam air dan lima RTIL berasaskan [BMIM] telah dikaji dengan menggunakan simulasi SD. Tenaga bebas pensolvatan telah dikira menggunakan kaedah Penerimaan Nisbah Bennett. Keputusan dari simulasi di dalam air didapati bersetuju dengan data eksperimen dan simulasi yang telah diterbitkan. RTIL menunjukkan keupayaan pensolvatan yang lebih baik berbanding dengan air. Analog tak berkutub menghasilkan tenaga bebas pensolvatan yang lebih rendah di dalam anion hidrofobik seperti $[PF_6]^-$ dan $[Tf_2N]^-$manakala analog berkutub menunjukkan pensolvatan yang lebih baik di dalam anion hidrofilik seperti $[BF_4]^-$, $[Cl]^-$dan $[TfO]^-$. Sifat-sifat pensolvatan di dalam [BMIM][Cl] juga menjelaskan mengapa enzim mengalami gangguan struktur yang lebih didalam RTIL ini pada kandungan air yang rendah. Secara keseluruhan, simulasi komputer dapat menjelaskan berbagai kesan anion RTIL kepada struktur dan dinamik enzim di peringkat molekul. Kestabilan struktur dan fleksibiliti enzim didapati dipengaruhi oleh kandungan air, lebih daripada jenis anion RTIL yang dikaji. Keputusan simulasi MD didapati berkait rapat dengan laporan eksperimen. Juga, didapati bahawa kelakuan anion dan air di permukaan protein memainkan peranan utama kearah sifat-sifat enzim dalam RTIL. Hasil kajian juga menunjukkan bahawa sifat-sifat permukaan biomangkin dan sifat-sifat fizikokimia substrat perlu diambil kira apabila memilih RTIL tertentu sebagai sistem pelarut.

ACKNOWLEDGEMENT

Individual thanks to Allah (SWT), the Creator and Nabi Muhammad (PBUH), thus I can complete my thesis dissertation as fulfillment of requirements for the degree of Doctor of Philosophy at Universiti Putra Malaysia.

Firstly, I would like to take this opportunity to express my huge appreciation towards my main supervisor, Prof. Dr. Mohd Basyaruddin Abdul Rahman, for giving me countless opportunities and for his endless support throughout my candidature. I am deeply grateful to have such great person to be my guide. My acknowledgement also goes to my co-supervisors with a special tribute to Dr. Nuno Miguel Da Silva Micaêlo for a wonderful and enlightening experience in Braga, Portugal. My sincere gratitude also goes towards my colleagues, students at Macromolecular Simulation Laboratory and at the faculty along with all members of Enzyme and Microbial Technology Research Centre, UPM. I also acknowledge the National Science Fellowship MOSTI for the financial support.

Last but not least, I would like to thank my beloved family and friends for their help and support throughout my candidature. Thank you for always being there for me.

APPROVAL

I certify that a Thesis Examination Committee has met on 19th August 2014 to conduct the final examination of Muhammad Alif bin Mohammad Latif on his thesis entitled "Molecular Insights on the Effects of Anions towards Hydrolases In [BMIM]-based Ionic Liquids" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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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

ρ	density
°C	degree Celsius
$AlCl_3$	aluminiumtrichloride
AMBER	Assisted Model Building with Energy Refinement
a_w	water activity
BAR	Bennett's Acceptance Ratio
BF_4	tetrafluoroborate
BMIM	1-butyl-3-methylimidazolium
BPTI	bovine pancreatic trypsin inhibitor
C153	Coumarin 153
Ca	calcium
CALB	*Candida antarctica*lipase B
CCl_4	carbon tetrachloride
CD	circular dichroism
CHARMM	Chemistry Harvard Macromolecular Mechanics
Cl	chloride
CRL	*Candida rugosa* lipase
Cα	carbon-alpha
ee	enantiomeric excess
EMIM	1-ethyl-3-methylimidazolium
E^N_T	Reichardt's Dye Polarity
FEP	Free Energy Perturbation
fs	femto-seconds
FT-IR	Fourier-transformed infrared
GROMACS	Groningen Machine for Chemical Simulations
H-bond	hydrogen bond
IL	ionic liquid
IUPAC	International Union of Pure and Applied Chemistry
K	Kelvin
kg/m^3	kilogram per meter cube
kJ/mol	kilo Joule per mole
LINCS	Linear Constraints Solver
LJ	Lennard-Jones
MD	molecular dynamics
MM	molecular mechanics
MTBE	methyl tert-butyl ether
nm	nano-meter
NMR	Nuclear Magnetic Resonance

NO_3	nitrate
NPT	Number, Pressure, Temperature
ns	nano-seconds
NVT	Number, Volume, Temperature
OPLS	Optimized Potential for Liquid Simulations
OPLS-AA	Optimized Potential for Liquid Simulations – All Atom
PBC	periodic boundary condition
PCL	*Pseudomonas cepacia* Lipase
PF_6	hexafluorophosphate
PME	Particle-Mesh Ewald
ps	pico-seconds
QM	quantum mechanics
QMMM	quantum mechanics molecular mechanics
QSPR	quantitative structure property relationship
RB	Ryckaert-Bellemans
RDF	radial distribution function
R_g	radius of gyration
RMSD	root mean square deviation
RMSF	root mean square fluctuation
RTIL	room temperature ionic liquid
SASA	solvent accessible surface area
SbF_6	hexafluoroantimonate
Tf_2N	bis-trifluoromethylsulfonylimide
TfO	trifluoromethanesulfonate
TI	Thermodynamics Integration
w/w	weight per weight
Zn	zinc
ΔG	Gibb's free energy difference

CHAPTER 1

INTRODUCTION

Biotechnology can be generally defined as the application of living organisms for producing useful products. For many years, biotechnology has been applied in agricultural, food productions and medicine. A sub-specialty of biotechnology such as industrial biotechnology, also known as "white biotechnology" is one of the highly progressing fields in biotechnology applications. In an era where production of chemicals on industrial scale is in need of sustainable processes, chemical synthesis of organic compounds have benefited most from the use of natural catalysts such as enzymes. The process where organic compounds were transformed chemically using enzymes is called biocatalysis (Anthonsen, 2000). In organic synthesis, enzymes have shown a remarkable performance as a biocatalyst. Unlike the traditional chemical and metal-catalyzed reactions, biocatalysis is more environmental-friendly and is a sustainable process while producing excellent yields (Wohlgemuth, 2010). Even though this technology has been vastly applied by academicians and industrial companies worldwide, the future of biocatalysis field promises much more. The developments in enzyme engineering has allowed synthetic biocatalysts to emerge with the potential to be better than the natural ones (Coelho *et al.*, 2013; Narayan and Sherman, 2013). Besides enzyme modification such as immobilization, substantial efforts have been made to re-engineer the natural biocatalysts. Screening of enzyme variants, directed evolutions and rational designs were applied in order to produce mutants that can expand their functionality in biocatalysis (Zhang *et al.*, 2003; Kazlauskas, 2005; Wahab *et al.*, 2012).

On the other hand, advancements in peptide synthesis have encouraged the use of peptidomimetics in order to design smaller biocatalysts with similar functions as the natural enzymes (Fernandez *et al.*, 1995). One of the crucial parts in a biocatalytic reaction is the reaction media. A solvent could affect both the enzymes and substrates in a biocatalysis process. Thus, the selection of a solvent would crucially depend on its effects toward the enzyme and substrates that are involved in the targeted reaction. The use of non-aqueous systems especially organic solvents in biocatalysis has shown increased reaction rates and higher conversions or yields (Klibanov, 2001). Non-aqueous solvents can be characterized by their physical and physicochemical properties such as boiling point, volatility, polarity, hydrophobicity, and viscosity to name a few. In the new millennium era, a new class of non-aqueous solvents have emerged as an exciting media for biocatalysis, called the Ionic Liquids (IL)s. Like the classical molten salts such as sodium chloride, an IL is composed entirely of ions (Davis and Fox, 2003). To differentiate ILs from the classical molten salts, the ones which are "low melting" or exist in liquid state at a temperature of below 100 °C can be called Room Temperature Ionic Liquids (RTIL)s (Welton, 1999).

Enzymatic reactions carried out in the presence of RTILs have been reported to produce more yield (increased enzyme activity) as compared to conventional organic solvents (Eckstein *et al.*, 2002; Lozano *et al.*, 2003; Maruyama *et al.*, 2004; Noritomi *et al.*, 2009). A growing interests in RTILs have seen numerous researches conducted in them, involving many types of reactions, enzymes and co-solvents (Sheldon *et al.*, 2002; Yang and Pan, 2005). In particular, RTILs have shown a remarkable influence toward the productivity of hydrolases such as lipases and proteases in the various organic reactions. It is widely known that there are many factors affecting the performance of enzymes in biocatalysis reactions. One of the major influences is the stability of the protein conformation. Structural changes to the protein conformation, particularly at the active site, can affect enzyme's catalytic capability. Selectivity is also very important in order to get the better yield for the desired product. The flexibility of protein conformation played a major role in determining the selectivity of an enzyme that is used in a biocatalytic reaction (Broos, 2002). Controlling the enzyme flexibility is therefore an important characteristic of a good solvent. One of the major advantages of using organic solvents in biocatalysis is that they stabilize the enzyme conformation (Ogino and Ishikawa, 2001). Furthermore, in conventional organic solvents, the enzyme flexibility can be controlled by the water concentration in the system (Kurkal *et al.*, 2005).

What makes organic solvents like RTILs fascinating is that they can be composed of cations and anions with different physicochemical properties. For example, [BMIM][BF_4] consists of a hydrophobic cation in 1-butyl-3-methylimidazolium ([BMIM]$^+$) and a hydrophilic tetrafluoroborate ([BF_4]$^-$) anion. Theoretically, this RTIL can provide two distinctive characteristics toward the enzyme and substrates involved when used as the reaction media. Therefore, the physicochemical properties of RTIL cations and anions can have a huge influence on the solvation properties of the solute molecules. In a system which contains an enzyme, water and RTILs, the enzyme's structure and dynamics properties can be affected by the interactions between RTILs and water, particularly at the enzyme's surface. These interactions however, are extremely difficult to be observed experimentally. From many attempts, researchers have been trying to explain the mechanisms of RTILs interactions in chemical reactions (Zhao, 2010). Most of the published reports include the effect of RTILs on activity and stability of enzymes, but the interactions between RTILs, enzymes and water have been sparsely investigated. Only a small part of these researches were focusing on the structural and dynamics behavior of enzymes and RTILs in such system (Bourissou *et al.*, 2000; Raza *et al.*, 2001; Micaelo *et al.*, 2005; Logotheti *et al.*, 2009; Klähn *et al.*, 2011).

1.1 Problem Identification

Due to the complexity of RTILs, some may work well with a certain enzyme but not with others. These have been highlighted by several reports in recent years (Kaar *et al*., 2003; Park and Kazlauskas, 2003; Klähn *et al*., 2011). This phenomenon is related to the fact that the cation-anion combination can affect the performance of RTILs as the reaction media. Since a vast number of cation-anion combinations of RTILs have been introduced and studied in recent years, finding the best RTIL for a particular enzyme or a certain reaction is time, and resource-consuming. Many experimental works have been carried out to determine the effect of different cation-anion combinations toward the performance of biocatalysts such as hydrolases (Irimescu and Kato, 2004; Paljevac *et al.*, 2006; Lee *et al.*, 2008; Hernández-Ferńandez *et al.*, 2009; Zhao, 2010). However, the focus usually tends to go toward activity, reaction rate and yield but not onto the enzyme properties. Therefore, to relate the findings with structural and dynamics properties of enzymes such as stability, flexibility, surface interactions and solvation is a tricky task. To really understand how enzymes react with RTILs and how RTILs affect the enzyme performance as a whole, it is essential to look into the structural and dynamics properties in the presence of these solvents. Understanding of RTILs solvent effect in more detail could provide imperative support when the study focuses on the interactions between enzymes, water and cation/anion at enzyme's surface. This will ensure that before a particular RTIL is chosen to work with an enzyme, one will have an idea what criteria each must have in order to work well.

This can be predicted by using computational approach, such as molecular modeling and simulations, where the behavior of enzymes during solvation with RTILs can be predicted at molecular level. As one of the popular computer simulation methods, molecular dynamics (MD) has been proven as an excellent tool to distinguish the structural properties of biomolecules in aqueous and organic media (Lousa *et al.*, 2013). In MD, understanding of enzyme behavior in RTILs can be increased by mimicking the interactions between the enzyme and RTIL components in great atomic details. By combining reported experimental findings available and computer simulation studies, the relationship between enzyme activity and its molecular properties in RTILs can be further explained. It is believed that the effects of using different RTIL anions toward hydrolases' properties at molecular level can be revealed by the use of computer simulation techniques such as MD. From the analyses performed, a certain order can be established between anions, in relation toward the structural and dynamics properties of all hydrolases studied. The hypothesis is that the order is dependent on the physicochemical properties of the five anions and should coincide with experimental evidences. Meanwhile, thermodynamics characterizations from the free energy calculations can be used to predict the solvation properties of enzymes and substrates in different RTILs.

1.2 Objectives

The main goal is to utilize molecular dynamics technique in order to investigate the structural and dynamics properties of different hydrolases such as α-Chymotrypsin, thermolysin, *Candida antarctica* Lipase B and *Candida rugosa* lipase when solvated in BMIM-based RTILs, composed of different anions such as $[PF_6]^-$, $[BF_4]^-$, $[Cl]^-$, $[TfO]^-$ and $[Tf_2N]^-$. Therefore, these objectives will be pursued:

1. To determine the effect of RTILs toward the structural stability and flexibility of hydrolases

2. To observe the effect of water concentration on enzyme properties in RTILs

3. To characterize enzyme:water:RTIL interactions on the protein surface and correlate with enzyme's structure and dynamics properties

4. To estimate the solvation free energy of small molecules in RTILs

The main focus of this project is on the effects when different RTIL anions are used. MD simulations was used to predict the behavior of several hydrolases in five [BMIM]-based RTILs. The five anions chosen for this project are consisted of four fluorine-based anions, commonly reported to increase enzyme activity. They were hexafluorophosphate ($[PF_6]^-$), tetrafluoroborate ($[BF_4]^-$), trifluoromethanesulfonate ($[TfO]^-$) and bis-trifluoromethylsulfonylimide ($[Tf_2N]^-$). Chloride ($[Cl]^-$) anion was also selected due to its physical and physicochemical properties for comparison purposes. In the next chapter, the literatures related to the project will be discussed. The theoretical background and methodologies such as algorithms, parameters and analysis tools that were used during the project will be presented in the third chapter. In chapter four, the results from the simulations will be presented, correlated with experimental evidences and discussed. In order to verify that the models used can produce similar properties as determined experimentally, molecular modeling and simulations studies on selected RTILs were performed and reported. After validations, these models were used to investigate the effects of using different RTIL anions toward the structure and dynamics of an α-Chymotrypsin at different hydration level. Due to the success of modeling the α-Chymotrypsin's behavior in RTILs, a similar approach was applied for thermolysin, which is currently under-utilized in RTILs. The structural stability and flexibility of both proteases were compared. Lipases behavior in RTILs with different anions was compared between *Candida antarctica* Lipase B (CALB) and *Candida rugosa* Lipase (CRL). Lastly, the solvation thermodynamics of neutral amino acid side chain analogues in different RTIL anions were predicted and the results were discussed in relation to the solvation dynamics of enzymes and substrates in RTILs. In the last chapter, the summary of all findings will be provided. The structural stability and dynamics of all enzymes were summarized and a general trend on the effects of changing RTIL anions was elucidated. This was followed by the general conclusions that obtained by this project and recommendations for future works.

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