UNIVERSITI PUTRA MALAYSIA

REDUCTIVE ALKYLATION OF CANDIDA RUGOSA LIPASE:
STRUCTURAL APPROACHES

BIMO ARIO TEJO

FBSB 2004 3
REDUCTIVE ALKYLATION OF CANDIDA RUGOSA LIPASE: STRUCTURAL APPROACHES

BIMO ARIO TEJO

DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

2004
REDUCTIVE ALKYLATION OF CANDIDA RUGOSA LIPASE: STRUCTURAL APPROACHES

by

BIMO ARIO TEJO

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

September 2004
Reductive Alkylation of *Candida rugosa* Lipase: Structural Approaches

By

Bimo Ario Tejo

September 2004

Chairman: Professor Abu Bakar Salleh, Ph.D.

Faculty: Biotechnology and Biomolecular Sciences

The properties of alkylated lipase are successfully explored through experimental and molecular modelling methods. Alkylation was done using aldehydes with different degree of modification to represent different levels of hydrophobicity which is important for enzymes to work in nonaqueous environment.

Far ultraviolet circular dichroism (CD) spectroscopy of the lipase in aqueous solvent shows that increasing the degree of modification from 49% to 86% resulted in loss in secondary structure which is attributed to the enzyme unfolding. The secondary structure elements of the CD spectra of native and modified lipase were analysed using the CDPro software and the K2D program. Both methods yield the same results in that the ratio of α-helical
structure is lost. This result explains why alkylated lipases have much lower activity in an aqueous environment.

Molecular modelling simulations were performed to study the structural and dynamical changes of the lipase upon different levels of modification. Simulations were run for 1 ns (300 K) with five different initial velocities to obtain better conformational sampling. Two solvent systems were used: TIP3P water model and carbon tetrachloride (CCl4) solvent model in periodic boundary condition (PBC). Generally, lipases simulated in water are less deviated in term of root mean square deviations (rmsd) compared to lipases simulated in CCl4.

Lid movements are essential for lipase function, both in water and water-lipid environments. Analyses of lid dynamics were done using time-correlation function and second-order Legendre polynomial function. Lipase in water and CCl4 shows different properties of dynamics. Without alkylation, the time correlation function of lipase in water shows one slow exponential decay with a correlation time of \( \tau = 92.8 \) ps. In contrast, for simulations in CCl4 the lid has a more complex dynamics. Exponential fit of open CRL in CCl4 results in two different \( \tau \) values: a fast motion \( \tau_1 = 5.6 \) ps and a slow motion \( \tau_2 = 163.8 \) ps.
Upon alkylation, different levels of modification show different properties of lid motions. In CCl₄, lid region is highly stabilised upon 95% alkylation with slow motion mode of $\tau_1 = 4.1$ ps and $\tau_2 = 577.8$ ps. Slow motion effect of lid region is also observed at 63% with $\tau_1 = 2.9$ ps and $\tau_2 = 209.2$ ps and 43% modification with $\tau_1 = 3.4$ ps and $\tau_2 = 117.9$ ps. In water, 43% and 95% modification show similar motion with unmodified lipase, with one slow exponential decay of $\tau = 142.8$ ps and 133.6 ps, respectively. However, 63% modification shows more complex dynamics with different $\tau$ values which mimics the dynamics properties in CCl₄.

A novel lid-locking mechanism which stabilises the opening form of lid region has been observed during simulations of unmodified CRL in CCl₄, i.e. a salt bridge between Lys85 and Asp284. This salt bridge is highly stabilised on unmodified lipase with a distance of 3.3 Å compared with lipase simulated in water with a distance of 15.25 Å. Alkylation at 43% causes the salt bridge to be deformed in CCl₄ with a distance of 6.03 Å; however, 63% modification stabilises the salt bridge with a distance of 3.88 and 95% modification shows the most stabilising effect with a distance of 3.19 Å.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGALILAN REDUKSI LIPASE CANDIDA RUGOSA:
PENDEKATAN STRUKTUR

Oleh

BIMO ARIO TEJO

September 2004

Pengerusi : Profesor Abu Bakar Salleh, Ph.D

Fakulti : Bioteknologi dan Sains Biomolekul

Sifat-sifat lipase teralkil telah berjaya dikaji dengan kaedah uji kaji dan dinamik molekul. Pengalkilan dilakukan dengan aldehida pada beberapa peringkat pengubahsuaian yang berbeza untuk menggambarkan perbezaan hidrofobisiti yang penting bagi enzim untuk bekerja dalam persekitaran tanpa air.

Spektroskop “circular dichroism” sinar ultraungu jauh menunjukkan bahawa kenaikan peringkat pengubahsuaian dari 43% kepada 86% menyebabkan kehilangan struktur kedua yang berhubung kait dengan struktur enzim yang lebih terbuka. Unsur-unsur struktur kedua spektrum CD bagi lipase semula jadi dan terubah suai telah dianalisis menggunakan program CDPro dan K2D. Kedua kaedah itu menghasilkan keputusan yang sama bahawa nisbah struktur α-helix telah menurun. Keputusan ini memberi
penjelasan mengapa enzim lipase terubah suai memiliki aktiviti rendah di persekitaran yang mengandungi air.

Penyerupaan model dinamik molekul telah dijalankan untuk mengkaji struktur dan dinamik enzim lipase pada peringkat pengubahsuaian yang berbeza. Penyerupaan model telah dijalankan selama 1 ns (300 K) dengan lima kelajuan awal yang berbeza untuk mendapatkan contoh-contoh struktur molekul yang lebih baik. Dua sistem pelarut telah digunakan: model molekul air TIP3P dan model pelarut karbon tetraklorida (CCl₄) dalam keadaan berkala bersempadan (PBC). Secara amnya, lipase yang menjalani penyerupaan dalam pelarut air adalah kurang menyimpang dari segi “root mean square deviation” (rmsd) berbanding dengan lipase yang menjalani penyerupaan dalam pelarut CCl₄.

Pergerakan kawasan penutup adalah penting untuk fungsi lipase samada di dalam air atau dalam persekitaran yang mengandungi air-lipid. Analisis dinamik kawasan penutup telah dilakukan dengan menggunakan fungsi pertalian masa dan fungsi polinomial Legendre urutan kedua. Lipase dalam air dan CCl₄ menunjukkan sifat-sifat dinamik yang berbeza. Tanpa pengalkilan, fungsi pertalian masa lipase dalam air menunjukkan satu persebutan eksponen yang lambat dengan pertalian masa τ = 92,8 ps. Sebaliknya, kawasan penutup menunjukkan dinamik yang lebih rumit ketika
dilakukan penyerupaan dalam pelarut CCl₄. Kepadanan eksponen CRL terbuka dalam pelarut CCl₄ menunjukkan dua pertalian masa yang berbeza: satu pergerakan cepat \( \tau_1 = 5.6 \) ps dan satu pergerakan lambat \( \tau_2 = 163.8 \) ps.

Dengan pengalkilan, beberapa peringkat ubah suai yang berbeza menunjukkan sifat-sifat dinamik kawasan penutup yang berbeza. Dalam CCl₄, kawasan penutup sangat stabil pada pengalkilan 95% dengan pergerakan lambat \( \tau_1 = 4.1 \) ps dan \( \tau_2 = 577.8 \) ps. Pergerakan lambat kawasan penutup juga dilihat pada pengalkilan 63% dengan \( \tau_1 = 2.9 \) ps dan \( \tau_2 = 209.2 \) ps dan pada pengalkilan 43% dengan \( \tau_1 = 3.4 \) ps dan \( \tau_2 = 117.9 \) ps. Dalam air, pengubahsuaian 43% dan 95% menunjukkan pergerakan yang serupa untuk lipase semula jadi, dengan satu pertalian masa yang lambat, masing-masing 142.8 ps dan 133.6 ps. Tetapi, pengubahsuaian 63% menunjukkan dinamik yang lebih rumit dengan dua nilai \( \tau \) berbeza yang menyerupai sifat-sifat dinamiknya dalam CCl₄.

Mekanisma baru tentang penguncian kawasan penutup yang menstabilkan bentuk terbuka lipase telah dilihat dalam penyerupaan lipase semula jadi dalam CCl₄, iaitu satu jambatan garam Lys85 dengan Asp284. Jambatan garam ini sangat stabil pada lipase semula jadi dalam CCl₄ dengan jarak 3.3 Å berbanding lipase dalam air dengan jarak 15.25 Å. Pengalkilan 43% menyebabkan jambatan garam menjadi cacat dalam CCl₄ dengan jarak 6.03 Å.
Å; tetapi pengubahsuaian 63% menstabilkan jambatan garam tersebut dengan jarak 3.88 Å dan pengubahsuaian 95% menunjukkan kesan penstabilan terbaik dengan jarak 3.19 Å.
ACKNOWLEDGEMENTS

I must express heartfelt thanks to my supervisor, Professor Abu Bakar Salleh for scientific challenge he has given me. His encouragement has been a source of my enthusiasm to explore this novel research area in the country. I have learnt so many things from him, not only as a researcher, but also about life. Thanks, Prof!

I am also grateful to my co-supervisors: Professor Mahiran Basri, Associate Professor Dr. Raja Noor Zaliha Raja Abd. Rahman, and Associate Professor Dr. Basyaruddin Abd. Rahman for their invaluable guidance, comments, and constant support during the period of this study. My deep appreciation is also extended to Associate Professor Dr. Che Nyonya Abd. Razak who was my co-supervisor; I felt such a loss when she decided to retire in the middle of my study.

For the scientists around the world who helped me to complete this challenging job, I would like to thank all of them. I have met them either physically and virtually through our communications by e-mails. Some of them came to Malaysia: Professor Teruna Siahaan (Kansas University), Dr. Mitsuru Haruki (Osaka University), and Dr. Farid Khan (Cambridge University). Thank you for coming! Some of them have assisted me through
e-mails: Professor Romas Kazlauskas (McGill University), Professor Karl Hult (Royal Swedish Institute of Technology), and Professor Sergio Emanuel Galembeck (Universidade de Sao Paulo). Nice to talk to you, guys!

I also would like to thank Dr. Juergen Pleiss for accepting and allowing me to work in his laboratory in Stuttgart. I have experienced working with one of the most advanced computing facilities in Germany during my stay there. Beside scientific matters, I have also enjoyed a good friendship with my German colleagues: Dr. Erik Henke, Dr. Sandra Barth, Dr. Peter Oelschlaeger, Markus Fischer, Florian Barth, Fabian Boes, and Alexander Steudle. Danke schön!

I also would like to thank Professor Rahmah Mohamed and Firdaus Raih for allowing me to work in their laboratory in Bangi. Special thanks to Associate Professor Dr. Sharifuddin Zain for introducing me to computational chemistry and a friendship we have built.

I must also express my gratitude to all my lab mates in Malaysia, especially Kak Shidah, Thanges, Kak Su, Laith, Leow, Ain, Ina, Rofandi, Ferrol, Aiman, Shah, Mohammad, Daim, Ghani, Shukuri, and Azreen for their sincere friendship, support, and their help. It was nice to spend my life in the lab with them.
I would like to acknowledge the Malaysian Government for financial support through the GRA scholarship and to German Ministry of Education and Research for financial support during my research visits to Stuttgart.

I must express my heartfelt thank to Azira for her patience and moral support I much needed during writing process of this thesis. I appreciate her understanding for many evenings and weekends that we could not spend them together while I was working. As a scientist with swingy mood, I am indeed very much lucky to have someone with extra patience and understanding like her.

Finally, my deepest appreciation goes to my family: Bapak, Ibu, Mas Koko, Mbak Devita, Mbak Ita, Mas Kukuh, and Dik Galuh for being so supporting, for their love and encouragement. Thank you, Bapak…thank you, Ibu…I dedicate this work to both of you.
I certify that an Examination Committee met on 9th September 2004 to conduct the final examination of Bimo Ario Tejo on his Doctor of Philosophy thesis entitled "Reductive Alkylation of Candida rugosa Lipase: Structural Approaches" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree.

Members of the Examination Committee are as follows:

NOR ARIPIN SHAMAAN, Ph.D.
Associate Professor
Universiti Putra Malaysia
(Chairman)

SUHAIMI NAPIS, Ph.D.
Associate Professor
Universiti Putra Malaysia
(Member)

ZULKARNAIN ZAINAL, Ph.D.
Associate Professor
Universiti Putra Malaysia
(Member)

JEREMY C. SMITH, Ph.D.
Professor
University of Heidelberg
(Independent Examiner)

GULAM RUSUL RAHMAT ALI, Ph.D.
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:
This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

ABU BAKAR SALLEH, Ph.D.
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

MAHIRAN BASRI, Ph.D.
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

RAJA NOOR ZALIHA RAJA ABD. RAHMAN, Ph.D.
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

MOHD. BASYARUDDIN ABD. RAHMAN, Ph.D.
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

__________________
AINI IDERIS, Ph.D.
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

xiii
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.

_________________
BIMO ARIO TEJO

Date:
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>ix</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>xii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xx</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxiii</td>
</tr>
</tbody>
</table>

CHAPTER

I  INTRODUCTION

II  LITERATURE REVIEW

  Protein Structure: Conformational Properties
    Introduction                                              6
    Determination of Protein Conformation                      7
  Protein Dynamics: Molecular Simulations
    Introduction                                              11
    Force Fields                                              12
    Time Scale of Simulation                                 18
    Solvent Model                                             20
  Protein Dynamics and Catalytic Functions
    Protein Dynamics in Enzymatic Catalysis                     23
    The Effect of Mutations on Protein Dynamics                26
  Enzymes in Organic Solvents
    Introduction                                              29
    Protein Structure and Dynamics in Organic Solvents         31
    Intramolecular Interactions                                34
  Chemical Modification of Enzymes
    Introduction                                              40
    Reductive Alkylation of Amino Groups                       42
    Effect of Reductive Alkylation on Candida rugosa Lipase    44
Detection and Localisation of Modification Site 46
Properties and Structure of *Candida rugosa* Lipase
  Molecular Properties 48
  Catalytic Properties 51
  Structural Properties 51
  Influence of CRL Structure on the Catalytic Properties 52

### III MATERIALS AND METHODS

#### Chemicals
55

#### Computer Hardwares and Softwares
57

#### Methods
58

**Purification of *Candida rugosa* Lipase**
- Preparation of Crude Extract
- Anion Exchanger Chromatography on DEAE-Sephacel
- Gel Filtration Chromatography on Sephadex G-100
- Determination of Lipolysis Activity
- Determination of Protein Concentration
- SDS-PAGE Analysis
- Determination of N-terminal Sequence

**Protein Modification**
- Modification with Aldehyde
- Determination of Percent Modification

**Determination of Protein Conformation**
- Circular Dichroism Spectroscopy
- Analysis of Circular Dichroism Spectra

**Computational Modelling**
- Preparation of Initial Structure
- Charge Assignment of Alkylated Lysine
- Solvent Accessible Surface Area Analysis
- Parameterisation of Carbon Tetrachloride Solvent Box
- Molecular Dynamics Simulations
- Atomic Fluctuation Analysis
- Time-correlation Analysis
- Analysis of Scalar Movements
IV RESULTS
Experimental Studies
- Purification of Lipase
- Determination of N-terminal Sequence
- Modification of Lipase
- Effect of Modifiers on the Lipase Conformation

Computational Modelling
- Preparation of the Systems
  - Determination of Protonation State of the Protein
  - Preparation of Modified Lysine
  - Determination of Alkylation Site
  - Parameterisation of Carbon Tetrachloride Solvent Box
- Dynamics of Unmodified Lipase in Water and Carbon Tetrachloride
  - Equilibration
  - Atomic Fluctuations
  - Time-correlation Analysis of Lid Movements
  - The Role of Lys85 and Lys75
- Dynamics of Modified Lipase in Water and Carbon Tetrachloride
  - Equilibration
  - Atomic Fluctuations
  - Time-correlation Analysis of Lid Movements
  - The Role of Lys85 and Lys75

V DISCUSSION
Experimental Studies
- Effect of Alkylation on the Protein Structure

Molecular Modelling Study
- Preparation of the System
  - Determination of Protonation State of the Protein
  - Preparation of Modified Lysine
  - Importance of Counterions
- Dynamics of Unmodified Lipase in Water and Carbon Tetrachloride
  - Structure
  - General Insight of Flexibility
  - Lid Dynamics
- Dynamics of Modified Lipase in Water and Carbon Tetrachloride
  - Structure
  - General Insight of Flexibility
  - Lid Dynamics
Remote Effect of Modification 191

VI CONCLUSION AND RECOMMENDATION
 Conclusion 195
 Recommendation 196

REFERENCES 198
APPENDICES 215
BIODATA OF THE AUTHOR 349
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Summary of the rms deviation, radius of gyration, SASA, hydrophobic exposed SASA, and number of hydrogen bonds from the simulations of γ-chymotrypsin in three different solvation environments</td>
</tr>
<tr>
<td>2</td>
<td>Amino acid composition of lipase A and B in mol%</td>
</tr>
<tr>
<td>3</td>
<td>Amino terminal sequences of CRLs A and B</td>
</tr>
<tr>
<td>4</td>
<td>Force field parameters and partial charges of CCl₄</td>
</tr>
<tr>
<td>5</td>
<td>Purification Table of lipase A and B from <em>Candida rugosa</em></td>
</tr>
<tr>
<td>6</td>
<td>Secondary structure ratio of modified and unmodified lipases calculated using different methods</td>
</tr>
<tr>
<td>7</td>
<td>List of atoms and residues affected of open CRL upon transfer from water to CCl₄</td>
</tr>
<tr>
<td>8</td>
<td>List of atoms and residues affected of closed CRL in water and CCl₄</td>
</tr>
<tr>
<td>9</td>
<td>List of atoms and residues affected upon 43% alkylation in water</td>
</tr>
<tr>
<td>10</td>
<td>List of atoms and residues affected upon 63% alkylation in water</td>
</tr>
<tr>
<td>11</td>
<td>List of atoms and residues affected upon 95% alkylation in water</td>
</tr>
<tr>
<td>12</td>
<td>List of atoms and residues affected upon 43% alkylation in CCl₄</td>
</tr>
<tr>
<td>13</td>
<td>List of atoms and residues affected upon 63% alkylation in CCl₄</td>
</tr>
<tr>
<td>14</td>
<td>List of atoms and residues affected upon 95% alkylation in CCl₄</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Schematic representation of the four contributions to a molecular mechanics force field</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>Residue-residue based map of correlated motions</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>Correlations between correlated motions of DHFR/DHF/NADPH and the location of debilitating mutants</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>Atom types of the CCl₄ molecule</td>
<td>64</td>
</tr>
<tr>
<td>5</td>
<td>Chromatography profile of CRL on DEAE-Sephacel</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>Chromatography profile of CRL on Sephadex G-100</td>
<td>72</td>
</tr>
<tr>
<td>7</td>
<td>Chromatography profiles of N-terminal amino acids sequence of Candida rugosa lipase B</td>
<td>74</td>
</tr>
<tr>
<td>8</td>
<td>CD spectra of unmodified lipase, 49% lysines modified, 86% lysines modified, and buffer</td>
<td>76</td>
</tr>
<tr>
<td>9</td>
<td>Chemical structure of alkylated lysine</td>
<td>81</td>
</tr>
<tr>
<td>10</td>
<td>Stereopicture of open CRL with positions of alkylated Lys at 43% degree of modification are marked red</td>
<td>84</td>
</tr>
<tr>
<td>11</td>
<td>Stereopicture of open CRL with positions of alkylated Lys at 63% degree of modification are marked red</td>
<td>85</td>
</tr>
<tr>
<td>12</td>
<td>Stereopicture of open CRL with positions of alkylated Lys at 95% degree of modification are marked red</td>
<td>86</td>
</tr>
<tr>
<td>13</td>
<td>Density fluctuation of CCl₄ solvent box over 50 ps of equilibration</td>
<td>87</td>
</tr>
<tr>
<td>14</td>
<td>One-dimensional averaged root mean square deviations of open CRL in water and CCl₄</td>
<td>89</td>
</tr>
<tr>
<td>15</td>
<td>One-dimensional averaged root mean square deviations of closed CRL in water and CCl₄</td>
<td>90</td>
</tr>
</tbody>
</table>
16 Superimposition of open and closed conformers of \textit{C. rugosa} lipase

17 One-dimensional averaged root mean square deviations of protein core of open CRL in water and CCl$_4$

18 One-dimensional averaged root mean square deviations of protein core of closed CRL in water and CCl$_4$

19 One-dimensional rmsd of a 1000 ps CRL simulation in CCl$_4$; two-dimensional rmsd plot of the same simulation

20 B-factors plot of open CRL simulated in water and CCl$_4$

21 Correlation plot of open CRL between B-factors values simulated in water and in CCl$_4$

22 B-factors plot of closed CRL simulated in water and CCl$_4$

23 Correlation plot of closed CRL between B-factors values simulated in water and in CCl$_4$

24 Time-correlation functions graph of open CRL simulated in water and in CCl$_4$

25 Time-correlation functions graph of closed CRL simulated in water and in CCl$_4$

26 Stereopicture of positions of Lys75, Lys85, Asp284, and Asn292 in water and CCl$_4$

27 Distance plot of a salt bridge between Lys85 and Asp284 in water and in CCl$_4$ (a). Distance plot of a hydrogen bond between Lys75 and Asn292 in water and in CCl$_4$ (b)

28 One-dimensional rmsd plot of 1000 ps simulation of 43\% alkylated CRL in water (a) and CCl$_4$ (b), 63\% alkylated CRL in water (c) and CCl$_4$ (d), and 95\% alkylated CRL in water (e) and CCl$_4$ (f)

29 Simulated and crystallographic B-factors of unmodified (a), 43\% alkylated CRL (b), 63\% alkylated CRL (c), and 95\% alkylated CRL (d) in water
30 Correlation plot of simulated B-factors between unmodified CRL with 43% alkylated CRL in water 129

31 Correlation plot of simulated B-factors between unmodified CRL with 63% alkylated CRL in water 130

32 Correlation plot of simulated B-factors between unmodified CRL with 95% alkylation in water 131

33 Simulated and crystallographic B-factors of unmodified (a), 43% alkylated CRL (b), 63% alkylated CRL (c), and 95% alkylated CRL (d) in CCl$_4$ 143

34 Correlation plot of simulated B-factors between unmodified CRL with 43% alkylated CRL in CCl$_4$ 147

35 Correlation plot of simulated B-factors between unmodified CRL with 63% alkylated CRL in CCl$_4$. 148

36 Correlation plot of simulated B-factors between unmodified CRL with 95% alkylated CRL in CCl$_4$ 149

37 Time correlation functions of 43% alkylated CRL (a), 63% alkylated CRL (b), and 95% alkylated CRL (c) in water 160

38 Time correlation functions of 43% alkylated CRL (a), 63% alkylated CRL (b), and 95% alkylated CRL (c) in CCl$_4$ 162

39 Distance plot of a salt bridge between Lys85 and Asp284 in water for different degree of alkylation (a). Distance plot of a hydrogen bond between Lys75 and Asn292 for different degree of alkylation (b) 165

40 Distance plot of a salt bridge between Lys85 and Asp284 in CCl$_4$ for different degree of alkylation (a). Distance plot of a hydrogen bond between Lys75 and Asn292 in CCl$_4$ for different degree of alkylation (b) 166

41 Superimposition of snapshots taken from trajectories of simulated CRL in water and CCl$_4$ 178

42 Orientation of acyl chain containing phenoxyl group around the hypothetical binding pocket, Phe344, Phe345, Phe296, and Leu267 181
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPTI</td>
<td>bovine pancreatic trypsin inhibitor</td>
</tr>
<tr>
<td>CD</td>
<td>circular dichroism</td>
</tr>
<tr>
<td>CRL</td>
<td><em>Candida rugosa</em> lipase</td>
</tr>
<tr>
<td>DHFR</td>
<td>dihydrofolate reductase</td>
</tr>
<tr>
<td>GCL</td>
<td><em>Geothricum candidum</em> lipase</td>
</tr>
<tr>
<td>HF</td>
<td>Hartree-Fock</td>
</tr>
<tr>
<td>MD</td>
<td>molecular dynamics</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>PDB</td>
<td>Protein Data Bank</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>PME</td>
<td>Particle Mesh Ewald</td>
</tr>
<tr>
<td>RESP</td>
<td>restrained electrostatic potential</td>
</tr>
<tr>
<td>rmsd</td>
<td>root mean square deviation(s)</td>
</tr>
<tr>
<td>SASA</td>
<td>solvent accessible surface area</td>
</tr>
<tr>
<td>TNBS</td>
<td>trinitrobenzenesulfonate</td>
</tr>
</tbody>
</table>