

# Table of contents

<b>SUMMARY .....</b>	<b>1</b>
<b>ACKNOWLEDGMENT.....</b>	<b>2</b>
<b>TABLE OF CONTENTS.....</b>	<b>4</b>
<b>ABBREVIATIONS .....</b>	<b>7</b>
<b>INTRODUCTION .....</b>	<b>8</b>
<b>A.1 THE ROLES OF PROTEOLYTIC ENZYMES IN LIFE AND DISEASES .....</b>	<b>9</b>
<b>A.2 LEXICON ABOUT PROTEOLYTIC ENZYMES .....</b>	<b>10</b>
<i>A.2.1 MEROPS classification.....</i>	<i>10</i>
<i>A.2.2 Nomenclature of peptidase active sites .....</i>	<i>11</i>
<b>A.3 THE PROTEOLYTIC COMPLEXES IN PROKARYOTES .....</b>	<b>11</b>
<i>A.3.1 Proteasome 20S.....</i>	<i>12</i>
<i>A.3.2 HslUV .....</i>	<i>13</i>
<i>A.3.3 Anbu and BPH .....</i>	<i>13</i>
<i>A.3.4 ClpP.....</i>	<i>14</i>
<i>A.3.5 Lon .....</i>	<i>15</i>
<i>A.3.6 FtsH.....</i>	<i>16</i>
<i>A.3.7 HtrA.....</i>	<i>16</i>
<b>A.4 PROTEASOME-ASSOCIATED PEPTIDASES IN PROKARYOTES .....</b>	<b>17</b>
<i>A.4.1 Peptidases A, B, D, N, E, P, and Q.....</i>	<i>18</i>
<i>A.4.2 Hsp31 peptidases.....</i>	<i>20</i>
<i>A.4.3 Acylpeptide hydrolases and prolyl oligopeptidases.....</i>	<i>20</i>
<i>A.4.4 Tricorn peptidases.....</i>	<i>21</i>
<i>A.4.5 TET aminopeptidases.....</i>	<i>22</i>
<b>A.5 M42 AMINOPEPTIDASES .....</b>	<b>23</b>
<i>A.5.1 M42 family.....</i>	<i>23</i>
A.5.1.1 MH clan .....	23
A.5.1.2 M42 family phylogeny.....	24
<i>A.5.2 Activity of M42 aminopeptidases.....</i>	<i>25</i>

A.5.2.1 Substrate specificity .....	25
A.5.2.2 Proposed catalytic mechanism .....	26
A.5.2.3 Metal ion cofactor .....	27
A.5.2.4 Does an M42 aminopeptidase moonlight? .....	28
A.5.3 Structure of M42 aminopeptidases.....	30
A.5.3.1 Subunit structure .....	30
A.5.3.2 Quaternary structure .....	32
A.5.3.3 Dodecamer assembly.....	33
A.5.3.4 Miscellaneous quaternary structures .....	34
A.5.4 Physiological function .....	35
A.5.4.1 Proteasome link .....	35
A.5.4.2 Heterocomplexes .....	36
A.5.4.3 Cellular localization .....	36
<b>OBJECTIVES.....</b>	<b>38</b>
<b>RESULTS AND DISCUSSION.....</b>	<b>40</b>
<b>B.1 HOW METAL COFACTORS DRIVE DIMER-DODECAMER TRANSITION OF THE M42 AMINOPEPTIDASE</b>	
<b>TmPEP1050 OF <i>THERMOTOGA MARITIMA</i> .....</b>	<b>41</b>
B.1.1 Background.....	41
B.1.2 Methodology.....	42
B.1.3 Article summary and discussion.....	42
B.1.4 The article with supplementary data .....	45
B.1.5 Unpublished related results .....	67
<b>B.2 X-RAY CRYSTALLOGRAPHY TO STUDY OLIGOMERIC STATE TRANSITION OF THE <i>THERMOTOGA MARITIMA</i> M42</b>	
<b>AMINOPEPTIDASE TmPEP1050.....</b>	<b>68</b>
B.2.1 Background.....	68
B.2.2 Methodology.....	69
B.2.3 Article summary and discussion.....	69
B.2.4 The article .....	71
<b>B.3 M42 AMINOPEPTIDASE CATALYTIC SITE: THE STRUCTURAL AND FUNCTIONAL ROLE OF A STRICTLY CONSERVED</b>	
<b>ASPARTATE RESIDUE .....</b>	<b>83</b>
B.3.1 Background.....	83
B.3.2 Methodology.....	83
B.3.3 Article summary and discussion.....	84

<i>B.3.4 The article with supplementary data</i> .....	86
<b>B.4 CHARACTERIZATION OF TmPEP1048 AND TmPEP1049</b> .....	<b>98</b>
<i>B.4.1 Background</i> .....	98
<i>B.4.2 Methodology</i> .....	98
<i>B.4.3 Results and discussion</i> .....	98
<b>B.5 CHARACTERIZATION OF M42 AMINOPEPTIDASES FROM <i>E. COLI</i></b> .....	<b>99</b>
<i>B.5.1 Background</i> .....	99
<i>B.5.2 Methodology</i> .....	100
<i>B.5.3 Results and discussion</i> .....	101
<b>CONCLUSIONS</b> .....	<b>104</b>
<b>APPENDICES</b> .....	<b>110</b>
<b>C.1 MATERIAL AND METHODS</b> .....	<b>111</b>
<i>C.1.1 Cloning and mutagenesis</i> .....	111
<i>C.1.2 Production and purification of recombinant enzymes</i> .....	111
<i>C.1.3 Activity assays with aminoacyl-pNA</i> .....	112
<i>C.1.4 Activity assays with peptides</i> .....	113
<i>C.1.5 Cobalt binding assays</i> .....	113
<i>C.1.6 Thermal shift assay</i> .....	114
<i>C.1.7 Native mass spectrometry</i> .....	114
<i>C.1.8 Crystallization</i> .....	114
<i>C.1.9 Structure determination and analysis</i> .....	115
<i>C.1.10 Gene deletion in <i>E. coli</i></i> .....	115
<i>C.1.11 FrvX-SgcX-YpdE heterocomplexes detection</i> .....	115
<b>REFERENCES</b> .....	<b>117</b>