

## **TABLE OF CONTENTS**

Chapters	Title	Page No.
Chapter 1	GENERAL INTRODUCTION	1-33
1.1.	PROTEINS: The sophisticated masters of cell	2
1.2.	PROTEIN ARCHITECTURE	2
1.2.1.	The amino acids as the building blocks of proteins	2
1.2.1.1.	Nonpolar aliphatic	3
1.2.1.2.	Non-polar aromatic	3
1.2.1.3.	Polar uncharged	3
1.2.1.4.	Polar charged amino acids	3 4
1.2.2.	Primary, secondary, tertiary structures and quaternary organization	4
1.2.3.	Primary structure of a protein or polypeptide chain	5
1.2.4.	Secondary structures	6
1.2.4.1.	α-helix	6
1.2.4.2.	β-sheets	7
1.2.4.3	Turns	7
1.2.4.4.	β-bulges	8
1.2.5.	Tertiary structure	8
1.2.6.	Quaternary organization	9 a
1.3.	PROTEIN FOLDING AND STABILITY	9
1.3.1.	From polypeptide to a functional native conformation	9
1.4.	MODELS PROPOSED TO EXPLAIN PROTEIN FOLDING	10
1.4.1.	Small proteins as model systems to understand the mechanism of protein folding	12
1.4.2.	Ubiquitin: The lethal tag	12
1.4.3.	Ubiquitin: The conserved gene	13
1.4.4.	The structure of ubiquitin	13
1.4.5.	Ubiquitin stability	15

1.4.6.	Folding of ubiquitin	17
1.4.6.1.	N-terminal β-bulge	17
1.4.6.2.	C-terminal β-bulge	18
1.5.	UBIQUITIN IN THE CELL	19
1.5.1.	THE Ubiquitin gene family	19
1.5.2.	Protein degradation and the ubiquitinproteasome pathway	20
1.5.3.	The proteolytic machinery: The ubiquitin proteasome system	23
1.5.4.	Protein half life and substrate recognition	25
1.5.4.1.	The N-end rule	25
1.5.4.2.	Primary Destabilizing Residues (N-d <sup>p</sup> )	26
1.5.4.3.	Secondary Destabilizing Residues (N-d <sup>s</sup> )	26
1.5.4.4.	Tertiary Destabilizing Residues (N-d <sup>t</sup> )	26
1.5.4.5.	Stabilizing Residues	26
1.5.5.	The role of ubiquitin proteasome system in biological function and its pathogenesis	27
1.6.	RATIONALE FOR SELECTING YEAST UBIQUITIN AND YEAST AS A MODEL SYSTEM	29
<b>1.7.</b>	THE ROLE OF CONSERVED RESIDUES IN THE PARALLEL G1 β-BULGE IN THE DETERMINATION OF STRUCTURE-FUNCTION RELATIONSHIPS OF UBIQUITIN	29
Chapter 2	CONSTRUCTION, EXPRESSION, PURIFICATION OF UbE64G AND ITS STRUCTURAL ANALYSIS ·	34-52
2.1.	INTRODUCTION	35
2.2.	MATERIALS AND METHODS	37
2.2.1.	Plasmid Constructs	37
2.2.2.	Construction and expression of UbE64G - pKK 223-3	39
2.2.3.	Purification of UbWt, UBF45W and UBE64G proteins	40
2.2.4.	Circular dichroism and fluorescence spectroscopy	41
2.3.	RESULTS	42
2.3.1.	Expression and purification of UbWt and UbF45W	42
2.3.2.	Construction, expression and purification of UbE64G from	42

	рКК 223-3	
2.3.3.	Sequence analysis of UbE64G gene in pKK223-3	43
2.3.4.	Far and near UV CD spectra of the three variants of ubiquitin: UbWt, UbF45W and UbE64G	44
2.3.5.	Fluorescence spectrum of the three forms of ubiquitin UbWt, UbF45W and UbE64G	46
2.3.6.	UbE64G shows greater content of hydrated hydrophobic residues.	47
2.3.7.	Thermal denaturation/ renaturation profiles of UbE64Gand UbF45W are identical.	49
2.3.8.	Guanidine hydrochloride denaturation of UbE64G and UbF45W	49
2.4.	DISCUSSION	52
		1.9979999999999999999999999999999999999
Chapter 3	CONSTRUCTION, EXPRESSION, PURIFICATION OF UbS65D AND ITS STRUCTURAL ANALYSIS	53-66
3.1.	INTRODUCTION	54
3.2.	MATERIALS AND METHODS	56
3.2.1.	Construction and expression of UBS65D-pKK 223-3	56
3.2.2.	Purification of UbS65D protein	58
3.2.3.	Circular dichroism and fluorescence Spectroscopy	58
3.3.	RESULTS	59
3.3.1.	Construction, expression and purification of UbS65D from pKK 223-3	59
3.3.2.	Sequence analysis of UbS65D gene in pKK223-3	60
3.3.3.	Far and near UV CD spectra of ubiquitin variants UbF45W and UbS65D	60
3.3.4.	Fluorescence spectrum of UbF45W and UbS65D	62
3.3.5.	ANS binding studies with UbS65D and UbF45W	63
3.3.6.	Thermal denaturation/ renaturation profiles of UbS65D and UbF45W	64
	Guanidine hydrochloride denaturation of UbS65D and	65
	UbF45W	

Chapter 4	CONSTRUCTION, EXPRESSION, PURIFICATION OF UbQ2N AND ITS STRUCTURAL ANALYSIS	67-78
4.1.	INTRODUCTION	68
4.2.	MATERIALS AND METHODS	69
4.2.1.	Construction and expression of UbQ2N-pKK 223-3	69
4.2.2.	Purification of UbQ2N protein	69
4.2.3.	Circular dichroism and fluorescence spectroscopy	69
4.3.	RESULTS	71
4.3.1.	Construction, expression and purification of UbQ2N from pKK 223-3	71
4.3.2.	Sequence analysis of UbQ2N gene in pKK223-3	72
4.3.3.	Far and near UV CD spectra of ubiquitin variants UbF45W and UbQ2N	72
4.3.4.	Fluorescence spectrum of UbF45W and UbQ2N	74
4.3.5.	Comparison of the content of hydrated hydrophobic residues in UbF45W and UbQ2N	75
4.3.6.	Thermal denaturation/ renaturation profiles of UbQ2N and UbF45W	76
4.3.7.	Gaundine hydrochloride denaturation of UbQ2N and UbF45W	77
4.4.	DISCUSSION	78
		4101100.001101000.0000.0000
Chapter 5	FUNCTIONAL ASSESSMENT OF THREE SITE DIRECTED MUTANTS OF UBIQUITIN IN Saccharomyces cerevisiae	79-81
5.1.	INTRODUCTION	80
5.1.1.	Ubiquitin and its role in biological system	80
<b>5.1.2.</b>	Differential ubiquitination	81
5.1.3.	Specific degradation signals on substrate protein for ubiquitination	81
5.1.4.	The N-end rule	82
5.1.5.	The ubiquitin N-terminal fusions of $\beta$ -galactosidase as an assay system for ubiquitin function	82
	system for uniquitin function	

5.2.	MATERIALS AND METHODS	85
5.2.1.	Yeast strains, media, and plasmids	85
5.2.2.	Plasmid construction (yeast expression vector)	85
5.2.3.	Growth Effects	86
5.2.4.	Complementation assay	87
5.2.5.	Heat sensitive test	87
5.2.6.	UV-C sensitivity test	87
5.2.7.	Antibiotic sensitivity test	87
5.2.8.	N-end rule as degradation signals	88
5.2.9.	Western blot analysis	88
5.2.10.	Plan of work	89
5.2.11.	Bacterial strains and media	89
5.2.12.	Sequence Analysis	90
5.3.	RESULTS	91
5.3.1.	Construction yeast expression vector carrying UbF45W and UbE64G gene in pUB175 and YEp96 vectors	91
5.3.1.1.	PCR amplification of ubiquitin genes generated from pKK223-3	91
5.3.1.2.	Construction of pUB175-UbF45W	92
5.3.1.3.	Construction of pUB175-UbE64G	92
5.3.1.4.	Construction of YEp96-UbF45W	93
5.3.1.5.	Construction of YEp96-UbE64G	94
5.3.2.	Construction yeast expression vector carrying UbS65D mutation in YEp96 vector	95
5.3.3.	Construction yeast expression vector carrying UbQ2N mutation in YEp96 vector	95
5.3.4.	Effects of mutants gene expression on growth of S. cerevisiae.	98
5.3.5.	Complementation of Stress hypersensitive phenotype SUB60 cells	99
5.3.5.1.	UV-C Sensitivity Complementation	99
5.3.5.2.	Heat Stress Complementation	99
5.3.6.	N-end rule as degradation signal in Saccharomyces cerevisiae	101

.

5.4.	DISCUSSION	105
Chapter 6	IN VITRO EVOLUTION OF UBIQUITIN	107-130
6.1.	INTRODUCTION	108
6.1.1.	Ubiquitin: The Conserved protein	108
6.1.2.	Random Mutagenesis	109
6.2	MATERIALS AND METHODS	111
6.2.1.	Yeast strains, media and plasmids	111
6.2.2.	Plasmid construction (yeast expression vector)	111
6.2.3.	Error prone PCR	112
6.2.4.	Mutant screening	112
6.2.5.	Heat sensitive phenotype	113
6.2.6.	Plan of work	113
6.2.7.	Functional analysis using antibiotic sensitivity test, efficiency of degradation based on N-end residue and Western blot analysis for polynucleotide chain formation	114
6.2.8.	Sequence Analysis	114
6.2.9.	Bacterial strains and media	114
6.2.10.	Construction of Bacterial expression vector	114
6.2.11.	Gene expression and purification	114
6.3.	RESULTS	115
6.3.1.	Plasmids construct YEp96/UbWt (yeast expression vector)	115
6.3.2.	Amplicons generated under various error prone conditions	115
6.3.3.	Screening of error prone mutants for loss of stress hypersensitive phenotype (Heat Stress) complementation	116
6.3.4.	Isolation of a dosage dependent lethal mutant of ubiquitin (UbEP42) in Saccharomyces cerevisiae	119
6.3.5.	Sequence analysis of UbEP42 gene	120
6.3.6.	Stress-hypersensitive Phenotype complementation	121
6.3.7.	Heat Stress Complementation	121
6.3.8.	Antibiotic Sensitivity test	122
6.3.9.	N-end rule as degradation signals in Saccharomyces cerevisiae	123

6.3.10.	UbEP42 polyubiquitination of substrate protein in <i>Sacchromyces cervisiae</i>	125
6.3.11.	Construction, expression and purification of UbEP42 gene in bacterial expression system pKK 223-3	126
6.4.	DISCUSSION	128
		a di kanangan kanang
1.1.7 <b>- 1.1.7</b>	SUMMARY	131-134
	·	ан байлаган нуулуу туулуу түүнүү түүнө
	BIBLIOGRAPHY	135-151
-		
	PUBLICATIONS AND PRESENTATIONS	**************************************

•