

**Characterization of IphP from *Nostoc commune* UTEX 584  
and a Dual-Specificity Protein Phosphatase from *Anabaena* PCC  
7120**

by

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A dissertation submitted to the faculty of Virginia Polytechnic Institute & State  
University in partial fulfillment of the requirements for the degree of

**Doctor of Philosophy  
in  
Biochemistry**

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September 17, 1997  
Blacksburg, Virginia

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(Abstract)

Protein phosphorylation is utilized universally as a mechanism of signal transduction. However, the use of tyrosine phosphorylation by bacteria has been a matter of dispute. Conventional wisdom dictated that "prokaryotic phosphorylation" was typified by phosphorylation of histidine and aspartate residues of proteins, while "eukaryotic phosphorylation" was characterized by modification of serine, threonine, or tyrosine residues. Increasing numbers of reports have emerged challenging the traditional view of "prokaryotic" and "eukaryotic" phosphorylation. One of the strongest links unifying prokaryotic and eukaryotic protein phosphorylation to date is IphP, a genomically-encoded dual-specificity protein phosphatase from the cyanobacterium *Nostoc commune* UTEX 584 bearing the active-site signature sequence of eukaryotic tyrosine-specific and dual-specificity protein phosphatases.

The catalytic properties and substrate specificity of IphP were examined in detail. The enzyme was able to discriminate among a variety of exogenous peptides and proteins. Kinetic studies revealed that IphP favors protein / peptide substrates over low molecular weight compounds.

Heparin effected IphP activity in a substrate-dependent manner. Enzyme activity toward casein (P-Ser) and MAP kinase (P-Thr/P-Tyr) was stimulated in the presence of the polyanion, whereas activity was inhibited by heparin toward other protein substrates. Both stimulation and inhibition by heparin were dose-dependent. The ability to stimulate IphP activity toward select substrates was attributed to the ability of heparin recruit the enzyme and substrates to the same microenvironment.

To facilitate future genetic studies to examining the role of tyrosine phosphorylation in cyanobacteria, we searched for evidence of protein tyrosine phosphorylation in *Anabaena* PCC 7120. In a collaborative effort with the laboratory of Dr. Potts, tyrosine phosphorylated proteins were identified in *Anabaena* utilizing several approaches, including comparative labelling with  $[\alpha]$ - vs  $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$ , phosphoamino acid analysis, and selective hydrolysis with a tyrosine specific protein phosphatase. Together, these data unequivocally demonstrate the presence of tyrosine-phosphorylated proteins in *Anabaena* PCC 7120.

Extracts of *Anabaena* PCC 7120 were examined for protein tyrosine phosphatase. An apparent PTP activity was detected, partially purified, and characterized. The protein phosphatase, ~38kDa by SDS-PAGE and sucrose density gradient centrifugation that displayed dual-specificity protein phosphatase (DSP) activity *in vitro*. The enzyme was localized to the periplasm and was thus assigned the title PAD, for Periplasmic *Anabaena*

DSP. Periplasmic phosphoproteins of ~120 and 55 kDa that had been radiolabelled *in vitro* were dephosphorylated by partially purified PAD. PAD activity varied *in vivo* ~5-fold in a rhythmic, seemingly diurnal manner. These and other proteins were also labelled *in vivo* and the degree of radiolabel incorporated into the periplasmic proteins varied inversely with PAD activity.

## ACKNOWLEDGEMENTS

I would like to thank all of those people who played a crucial role in my success here at Virginia Tech., especially the members of my committee: Dr. Brenda Shirley, Dr. Timothy Larson, Dr. Thomas Sitz, Dr. Malcolm Potts, and Dr. Peter Kennelly. I would especially like to thank my advisor for allowing me the freedom to pursue my own ideas and passing on to me his tried-and-true philosophies about the "doing of science" -- May we always tell a good story.

In addition to the members of my committee, I would like to thank Dr. Neihaus for the use of his 12-liter fermentor (which will most likely be green forever), Dr. Story for teaching me via E-mail from Germany how to take pictures of my cultures with the CCD camera, Dr. John Cundiff for helping me keep life in perspective (btw, doesn't Carla look lovely today?) and Dr. Hess and Dr. Bevan for nominating me for Full Membership into Sigma Xi.

I am extremely grateful to the many members of the Kennelly lab with whom I was fortunate enough to work: Jie, Keith, Christina, Charmaine, Barb, Ronda, Tom, Ken, Danielle, and Liang. I especially thank Jie Leng for all the encouragement he provided during my early years in the lab and for the many insightful lunch conversations at "Wendy" and "Hardee", Ken Bischoff for his help interpreting results and designing experiments, and Liang Shi for his contagious enthusiasm for cyanobacteria!

Many others outside of Virginia Tech contributed to my general well-being throughout graduate school. I would like to thank my best friend Stuart Whitaker for the hours of stimulating conversation we shared and for teaching me to appreciate a good fire. I thank my parents, Larry and Neoma, and my sister, Angela Teter, for their constant love and support throughout the years. And finally, my deepest appreciation goes to my wife, Carla, who's taught me more about life than any biochemistry text ever could. I love you.

J'aime Dieu, ma femme, ma famille, et mon métier. J'espère que je serai toujours satisfait avec ces cadeaux et que je n'ai jamais envie de plus.

*"Thanks be to God in all things"*

## TABLE OF CONTENTS

<b>ACKNOWLEDGMENTS</b> .....	iv
<b>LIST OF FIGURES</b> .....	viii
<b>LIST OF TABLES</b> .....	x
<b>LIST OF ABBREVIATIONS</b> .....	xi
<b>CHAPTER 1: INTRODUCTION</b> .....	1
Protein Phosphorylation.....	1
Protein Phosphorylation in the Bacteria.....	5
The Cyanobacteria.....	14
Protein Phosphorylation in the Cyanobacteria.....	17
Thesis Objectives.....	25
<b>CHAPTER 2: MATERIALS AND METHODS</b> .....	27
Materials.....	27
Procedures.....	27
SDS-PAGE.....	27
IEF / 2D-PAGE.....	27
Electroblotting to PVDF Membranes.....	28
Media Formulations.....	28
Growth of Cyanobacteria.....	29
Preparation of Cyanobacterial Extracts.....	29
Expression and Isolation of IphP.....	30
Expression and Purification of p56 <sup>lyn</sup> kinase.....	30
Expression and Purification of MAP kinase.....	31
Preparation of <sup>32</sup> P-phosphotyrosyl-RCM-lysozyme.....	31
Preparation of <sup>32</sup> P-phosphotyrosyl-casein.....	31
Preparation of <sup>32</sup> P-phosphoserine-casein.....	32
Preparation of <sup>32</sup> P-phosphoserine-RCM-lysozyme.....	32
Phosphatase assays.....	32
Radiolabelling Cyanobacterial Phosphoproteins.....	34
Phosphoamino Acid Analysis.....	34
Molybdc Acid Extraction.....	36
Sucrose Density Gradient Ultracentrifugation.....	36
Heparin-agarose Affinity Chromatography.....	37

<b>CHAPTER 3: SUBSTRATE SPECIFICITY &amp; CATALYTIC PROPERTIES OF IHP</b> .....	38
Objectives.....	38
Rationale.....	38
Relative IphP Activity toward Potential Protein / Peptide	
Substrates.....	38
IphP displays selectivity among potential peptide and protein substrates.....	38
IphP favors protein/peptide substrates vs low molecular weight organophosphates <i>in vitro</i> .....	39
IphP Dephosphorylates P-Tyr of MAPK faster than P-Thr.....	39
Heparin Affects IphP Activity in a Substrate-Specific Manner... ..	39
Heparin lowers the apparent $K_M$ of IphP toward MAPK.....	40
MAPK (P-Thr/P-Tyr), Casein (P-Ser), and IphP associate with heparin.....	40
Proteins with high pI values block enhancement by heparin.....	41
Membrane lipids do not mimic heparin enhancement.....	41
 <b>CHAPTER 4: DETECTION OF TYROSINE-PHOSPHORYLATED PROTEINS IN ANABAENA PCC 7120</b> .....	52
Objectives.....	52
Rationale.....	52
Phosphorylation vs Nucleotidylation.....	52
Phosphoamino Acid Analysis of Radiolabelled Phosphoproteins.....	53
Dephosphorylation of Radiolabelled Phosphoproteins by PTP1B.....	53
 <b>CHAPTER 5: PARTIAL PURIFICATION AND CHARACTERIZATION OF A SOLUBLE 38kDa DSP</b> .....	57
Objectives.....	57
Rationale.....	57
Detection of PTP Activities Extracts of <i>Anabaena</i> PCC 7120.....	57
Partial Purification of a Soluble PTP.....	57
Is the Enzyme in the DE52 Fraction a PTP?.....	58

<i>In Vitro</i> Characterization of the Soluble PTP.....	59
Subcellular Localization of the DSP.....	60
PAD Activity <i>In Vivo</i> is Rhythmic.....	61
Dephosphorylation of a Periplasmic Phosphoprotein by PAD.....	62
<b>CHAPTER 6: DISCUSSION</b> .....	85
Specificity and Catalytic Properties of IphP.....	85
Detection of Phosphotyrosyl-phosphoproteins in <i>Anabaena</i> PCC 7120.....	86
Characterization of a DSP from <i>Anabaena</i> PCC 7120.....	87
Conclusions.....	89
<b>VITA</b> .....	90

## List of Figures

Figure 1.1	Modification of a protein by phosphorylation.....	2
Figure 1.2	A schematic diagram of a typical cyanobacterial vegetative cell.....	16
Figure 1.3	The photosynthetic apparatus of a typical cyanobacterium...	19
Figure 2.1	Depiction of a TLE plate for phosphoamino acid analysis....	35
Figure 3.1	IphP dephosphorylates P-Tyr from MAPK (P-Thr/P-Tyr) faster than P-Thr.....	47
Figure 3.2	Enhancement of IphP activity toward casein (P-Ser) and MAPK (P-Thr/P-Tyr) by heparin is concentration-dependent.....	48
Figure 3.3	Associative model for the enhancement of IphP activity toward MAPK (P-Thr/P-Tyr) by heparin.....	49
Figure 3.4	Sucrose gradient ultracentrifugation of heparin-protein complexes.....	50
Figure 3.5	Proteins with high pI values block heparin enhancement.....	51
Figure 4.1	Cyanobacterial proteins are phosphorylated by endogenous protein kinases.....	54
Figure 4.2	Phosphoamino acid analyses of cyanobacterial phosphoproteins.....	55
Figure 4.3	PTP1B, IphP, and alkaline phosphatase liberate <sup>32</sup> P from radiolabelled <i>Anabaena</i> phosphoproteins.....	56
Figure 5.1	PTP activities in <i>Anabaena</i> PCC 7120.....	68
Figure 5.2	DEAE chromatography of the soluble fraction.....	69
Figure 5.3	Cellulose phosphate chromatography of the DE52 fraction...	70

Figure 5.4	Molybdic acid extraction of inorganic [ <sup>32</sup> P]phosphate.....	71
Figure 5.5	In-gel assay of soluble, DE52, and CP fractions.....	72
Figure 5.6	Native molecular weight determination of the soluble PTP...	73
Figure 5.7	Catalytic activity of the soluble phosphatase as a function of pH.....	74
Figure 5.8	Determination of the isoelectric point of the 38kDa PTP.....	75
Figure 5.9	RCML (P-Tyr) and RCML (P-Ser) are both dephos- phorylated by the DE52 fraction.....	76
Figure 5.10	The effect of known phosphatase inhibitors on the soluble DSP.....	77
Figure 5.11	Assay of isocitrate dehydrogenase activity in subcellular fractions of <i>Anabaena</i> .....	78
Figure 5.12	Total PTP activities in subcellular fractions of <i>Anabaena</i> .....	79
Figure 5.13	In-gel assay of subcellular fractions of <i>Anabaena</i> .....	80
Figure 5.14	PAD activity <i>in vivo</i> is rhythmic.....	81
Figure 5.15	Rhythmic PAD activity is independent of light cycle.....	82
Figure 5.16	Soluble fraction and periplasmic fraction phosphoproteins are dephosphorylated by PAD.....	83
Figure 5.17	Incorporation of <sup>32</sup> P into periplasmic phosphoproteins <i>in vivo</i> varies inversely with PAD activity.....	84

## List of Tables

Table 1.1	Bacterial phosphotyrosyl-phosphoproteins.....	10
Table 3.1	Relative activity of IphP toward peptide and protein substrates.....	42
Table 3.2	Kinetic parameters of IphP toward selected substrates.....	43
Table 3.3	The effect of various polyanions of IphP activity.....	44
Table 3.4	Affinity of IphP and substrates toward heparin-agarose.....	45
Table 3.5	Effect of membrane & sheath components of IphP activity...	46
Table 5.1	Purification of the soluble PTP.....	64
Table 5.2	Low molecular organophosphate hydrolase activity in the DE52 fraction.....	65
Table 5.3	The effect of monovalent, divalent, or trivalent metal chlorides on the soluble PTP activity.....	66
Table 5.4	The effect of key metabolites on PAD activity <i>in vitro</i> .....	67

## List of Abbreviations

BSA	bovine serum albumin
CAPS	3-(cyclohexylamino)-1-propane sulfonic acid
casein ( <sup>32</sup> P-Ser)	[ <sup>32</sup> P]phosphoserine
casein ( <sup>32</sup> P-Tyr)	[ <sup>32</sup> P]phosphotyrosyl casein
CP	cellulose phosphate
CPM	counts per minute
DEAE	diethylaminoethyl
DSK	Dual-Specificity Protein Kinase
DSP	dual-specificity phosphatase
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylenedioxydiethylenedinitrolo tetraacetic acid
ERK	extracellular-regulated protein kinase
<i>g</i>	acceleration due to gravity
GST	glutathione-S-transferase
HMB	<i>p</i> -hydroxymercuribenzoate
HMPSA	<i>p</i> -hydroxymercuriphenyl sulfuric acid
IAA	iodoacetic acid
IEF	isoelectric focusing
IPTG	isopropylthio-β-D-galactoside
MAPK	mitogen-activated protein kinase
MBP	myelin basic protein
NEM	N-ethylmaleimide
P-Ser or PS	phosphoserine
P-Thr or PT	phosphothreonine
P-Tyr or PY	phosphotyrosine
PAA	phosphoamino acid analysis
PAD	Periplasmic <i>Anabaena</i> DSP
PAGE	polyacrylamide gel electrophoresis
PBP	phycobiliprotein
PCC	Pasteur Culture Collection
pH	log H <sup>+</sup> concentration
P <sub>i</sub>	inorganic phosphate
PKA	protein kinase A
PKC	protein kinase C
PMSF	phenylmethylsulfonyl fluoride
pNPP	<i>p</i> -nitrophenyl phosphate
PS-I	photosystem I
PS-II	photosystem II

PTK	protein tyrosine kinase
PTP	protein tyrosine phosphatase
PVDF	polyvinylidene difluoride
RCML ( <sup>32</sup> P-Ser)	[ <sup>32</sup> P]phosphoserine RCM-lysozyme
RCML	reduced, carboxymethylated, and maleylated lysozyme
RCML ( <sup>32</sup> P-Tyr)	[ <sup>32</sup> P]phosphotyrosyl RCM-lysozyme
SDS	sodium dodecyl sulfate
ssDNA	single-stranded DNA
TCA	trichloroacetic acid
TLE	thin-layer electrophoresis
Tris	tris (hydroxymethyl) aminomethane
UTEX	University of Texas Culture Collection
YINAS	peptide ENDYINASL