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" phosphorlyation. 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, wheras 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 [α]- vs [γ-^{32}P]-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 rhthymic, seemingly diurnal manner. These and other proteins were also labelled *in vivo* and the degree of radiolabel incorporated into the periplasmic proteins varied inversly 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 contageous 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"

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BSA	bovine serum albumin
CAPS	3-(cyclohexylamino)-1-propane sulfonic acid
casein (^{32}P-Ser)	[^{32}P]phosphoseryl casein
casein (^{32}P-Tyr)	[^{32}P]phosphotyrosyl casein
CP	cellulose phosphate
CPM	counts per minute
DEAE	diethylaminoethyl
DSK	Dual-Specificity Protein Kinase
DSP	dual-specificity phosphatase
DTT	dithiothreotol
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylenedioxydiethylenedinitrolo tetraacetic acid
ERK	extracellular-regulated protein kinase
g	acceleration due to gravity
GST	glutathione-S-transferase
HMB	*p*-hydroxymercuribenzoate
HMPSA	*p*-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 *Anabaena* DSP
PAGE	polyacrylamide gel electrophoresis
PBP	phycobiliprotein
PCC	Pasteur Culture Collection
pH	log H^+ concentration
P_i	inorganic phosphate
PKA	protein kinase A
PKC	protein kinase C
PMSF	phenylmethylsulfonyl fluoride
pNPP	p-nitrophenyl phosphate
PS-I	photosystem I
PS-II	photosystem II

PTK	protein tyrosine kinase
PTP	protein tyrosine phosphatase
PVDF	polyvinylidene difluoride
RCML (^{32}P-Ser)	[^{32}P]phosphoseryl RCM-lysozyme
RCML	reduced, carboxymethylated, and maleylated lysozyme
RCML (^{32}P-Tyr)	[^{32}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