Print ISSN: 2321-6379
Online ISSN: 2321-595X
DOI: 10.17354/ijss/2018/1

Review Article

Bacterial Serine/Threonine/Tyrosine Phosphoproteomics: A Current Status and Their Role in Diverse Biological Processes

Asha Mishra

Department of Zoology, HRPG College, Khalilabad, Sant Kabir Nagar, Uttar Pradesh, India

Abstract

Protein phosphorylation is the most common post-translational modification in proteins. The majority of amino acids modified in a protein are serine, threonine, and tyrosine. It is estimated that 30–60% of proteins in eukaryotes is phosphorylated and the role of these modifications is well studied. The protein phosphorylation is much less abundant in prokaryotes. The role of protein phosphorylation in prokaryotes is recently being realized. Identification of all the phosphoproteins present in a bacterial cell by high-resolution mass spectrometry and using gel-free protein digestion approaches has revolutionized the field. This review is mainly focused on the role of bacterial phosphoproteome and pathogenicity. A number of biochemical pathways such as glycolysis, translation, response to stress, sugar transport, and most importantly virulence are also regulated by protein phosphorylation.

Key words: Bacterial phosphoproteomics, Mass spectrometry, Serine, Threonine, Tyrosine phosphorylation

INTRODUCTION

Serine, threonine, and tyrosine (Ser/Thr/Tyr) residues are the most common amino acids in a protein that is subjected to phosphorylation. In eukaryotes, this most common protein post-translational modification plays a significant role in a large number of biochemical pathways including signal transduction, regulation of protein function, glycolysis, and protein-protein interaction. Until recently, the protein phosphorylation was thought to be limited to eukaryotes. The importance of protein phosphorylation in bacteria is being realized now. Phosphorylated proteins play an important role in the regulation of almost all physiological processes in prokaryotes. These processes include several key steps in the host infection such as adhesion to the host, triggering and regulation of pathogenic functions, as well as biochemical warfare.

Access this article online

www.ijss-sn.com

Month of Submission : 10-2018
Month of Peer Review : 11-2018
Month of Acceptance : 12-2018
Month of Publishing : 12-2018

In prokaryotes, it is believed that about 1–5% proteins are phosphorylated as compared to the eukaryotes which have about 30–60% phosphorylated proteins. Hence, to identify the phosphoproteins in a bacterium, the phosphopeptides must be enriched from non-phosphopeptides. Several methods for phosphopeptide enrichment have been developed, including immobilized metal affinity chromatography, strong cation exchange chromatography, and enrichment by antibodies specific to phosphorylated peptides. The recent developments in the high-resolution mass spectrometry and methods to enrich the phosphopeptides led to the discovery of phosphoproteome in a number of prokaryotes.

In one of the earlier studies, Macek *et al.* identified the Ser/Thr/Tyr phosphoproteome of model bacterium *Escherichia coli*.[1] It was the first prokaryote where the large-scale phosphoproteome was identified using phosphopeptides enrichment and high-resolution mass spectrometry. This publication resulted in a significant interest in bacterial phosphoproteomics and soon a number of other studies were published. Soufi *et al.* identified the phosphoproteome of *Lactococcus lactis* and Misra *et al.* identified the phosphoproteome of *Listeria monocytogenes* by phosphopeptide enrichment and "gel-free"

Corresponding Author: Dr. Asha Mishra, Department of Zoology, HRPG College, Siddhartha University, Khalilabad, Uttar Pradesh, India. E-mail: asha.hrpg@gmail.com

high-resolution mass spectrometry.[2,3] Most recently, the tyrosine phosphoproteome of Gram-negative *Shigella flexneri* was determined by Standish *et al*. These studies clearly established that Ser/Thr/Tyr phosphorylation plays a very important role in different aspects of bacterial life including virulence. The list containing some of the bacterial species where the phosphoproteome has been identified by phosphopeptide enrichment and high-resolution mass spectrometry is presented in Table 1.

PHOSPHORYLATED PROTEINS IN VIRULENCE

The most important role played by phosphoproteins is in the pathogenesis of the bacterium. A number of proteins already known to play an important role in host infection and bacterial survival have been reported to be phosphorylated. Examples include superoxide dismutase, catalase, PrfA, and WcaJ proteins.[4-7] Arguably, the tyrosine phosphorylation plays a more important role in virulence than serine/threonine phosphorylation in pathogenic bacteria. Understanding the full role of the phosphoproteins in bacterial pathogenesis is important in discovering novel ways to block the infection by pathogenic bacteria and treat the infection after symptoms develop. The discovery of phosphoproteome of many pathogenic bacteria has opened new ways to achieve this goal.[8-11]

PHOSPHORYLATED PROTEINS IN PEP:SUGAR TRANSFERASE SYSTEM

Bacteria use PEP:sugar transport system also known as PTS system to transport sugars and sugar derivatives. In addition to this, they are involved in a large number of cellular processes. A central protein of this system, Hpr is reported to be phosphorylated on five different sites in *L. monocytogenes*. In *L. lactis*, the fructose-specific EIIABC is phosphorylated on two different serine residues. Mannose-specific EIIAB is serine phosphorylated in *E. coli* and threonine phosphorylated in *S. pneumoniae*. Several other components of PTS system are reported to be phosphorylated on serine or threonine including EIIA, EIIB, and EIIBCA.

Table 1: Ser/Thr/Tyr phosphoproteome of bacteria

Bacterium name	Number of phosphosites	Number of phosphoproteins	Year
Escherichia coli	81	79	2008
Bacillus subtilis	78	78	2007
Lactococcus lactis	73	63	2008
Streptococcus pneumoniae	163	84	2010
Listeria monocytogenes	143	112	2011
Helicobacter pylori	126	67	2011
Mycobacterium tuberculosis	500	301	2010
Klebsiella pneumoniae	93	81	2009
Acinetobacter baumannii	91	77	2014
Clostridium acetobutylicum	52	44	2012

PHOSPHORYLATED PROTEINS OF SUGAR METABOLISM

A large number of proteins of sugar metabolism, like glycolysis, have been identified to be phosphorylated.[12,13] In *L. monocytogenes*, the majority of glycolytic including fructose-1, 6-bisphosphate aldolase, glyceraldehyde 3-phosphate, pyruvate kinase, and enolase are phosphorylated on either serine or threonine amino acids. The site of phosphorylation on these enzymes appears to be conserved. For example, in *L. monocytogenes*, the S211 is phosphorylated, and in *L. lactis*, serine 216 is phosphorylated. Both of these sites are conserved as seen by aligning the protein sequences of these two enzymes. Likewise, glyceraldehyde-3-phosphate dehydrogenase is phosphorylated on threonine 211 in *L. monocytogenes* and threonine 212 in *L. lactis*. Phosphorylation on some of the glycolytic proteins seems to be limited in some bacterial species only. For example, triosephosphate isomerase is phosphorylated only in *Bacillus subtilis*.[14] The widespread conservation of phosphorylated sites on the enzymes of sugar metabolism suggests an important role of phosphorylation in the activity of these enzymes.

PHOSPHORYLATED PROTEINS IN RESPONSE TO STRESS

Bacteria live in an environment where they are subjected to continuous exposure to chemicals and conditions that are stressful. They need a mechanism to survive adverse conditions.[15,16] A large number of proteins have been identified in bacteria that help bacteria in overcoming challenges faced by bacteria. Phosphoproteomics studies identified a number of these proteins to be phosphorylated. DPS family proteins demonstrate potential to bind iron and have been implicated in protecting DNA in bacterial stress conditions. It has been reported to be phosphorylated and phosphorylation might play an important role in its function. Heat shock protein DnaK protects the bacterial DNA in thermal stress. It has been reported to be phosphorylated in *E. coli*, *L. monocytogenes*, and *Streptococcus pneumoniae*.

CONCLUSION

Improvements in phosphopeptide enrichment and mass spectrometry methods for phosphopeptide identification methods allowed the identification of phosphoproteome

of a number of bacterial species including pathogenic bacteria. Phosphoproteins representing almost all the bacterial processes have been identified. The sites of phosphorylation are also mostly conserved. This indicates that phosphorylated proteins play an important role in bacteria including virulence.[17,18]

REFERENCES

1. Sun X, Ge F, Xiao CL, Yin XF, Ge R, Zhang LH, *et al.* Phosphoproteomic analysis reveals the multiple roles of phosphorylation in pathogenic bacterium *Streptococcus pneumoniae*. J Proteome Res 2010;9:275-82.
2. Soufi B, Gnad F, Jensen PR, Petranovic D, Mann M, Mijakovic I, *et al.* The ser/Thr/Tyr phosphoproteome of *Lactococcus lactis* IL1403 reveals multiply phosphorylated proteins. Proteomics 2008;8:3486-93.
3. Misra SK, Milohanic E, Aké F, Mijakovic I, Deutscher J, Monnet V, *et al.* Analysis of the serine/threonine/tyrosine phosphoproteome of the pathogenic bacterium *Listeria monocytogenes* reveals phosphorylated proteins related to virulence. Proteomics 2011;11:4155-65.
4. Lin MH, Hsu TL, Lin SY, Pan YJ, Jan JT, Wang JT, *et al.* Phosphoproteomics of *Klebsiella pneumoniae* NTUH-K2044 reveals a tight link between tyrosine phosphorylation and virulence. Mol Cell Proteomics 2009;8:2613-23.
5. Ravichandran A, Sugiyama N, Tomita M, Swarup S, Ishihama Y. Ser/Thr/Tyr phosphoproteome analysis of pathogenic and non-pathogenic pseudomonas species. Proteomics 2009;9:2764-75.
6. Prisic S, Dankwa S, Schwartz D, Chou MF, Locasale JW, Kang CM, *et al.* Extensive phosphorylation with overlapping specificity by *Mycobacterium tuberculosis* serine/threonine protein kinases. Proc Natl Acad Sci U S A 2010;107:7521-6.
7. Macek B, Gnad F, Soufi B, Kumar C, Olsen JV, Mijakovic I, *et al.* Phosphoproteome analysis of *E. coli* reveals evolutionary conservation of bacterial ser/Thr/Tyr phosphorylation. Mol Cell Proteomics 2008;7:299-307.
8. Misra SK, Bhakuni V. Unique holoenzyme dimers of the tetrameric enzyme *Escherichia coli* methylenetetrahydrofolate reductase: Characterization of structural features associated with modulation of the enzyme's function. Biochemistry 2003;42:3921-8.
9. Schmidl SR, Gronau K, Pietack N, Hecker M, Becher D, Stülke J, *et al.* The phosphoproteome of the minimal bacterium *Mycoplasma pneumoniae*: Analysis of the complete known ser/Thr kinome suggests the existence of novel kinases. Mol Cell Proteomics 2010;9:1228-42.
10. Parker JL, Jones AM, Serazetdinova L, Saalbach G, Bibb MJ, Naldrett MJ, *et al.* Analysis of the phosphoproteome of the multicellular bacterium *Streptomyces coelicolor* A3(2) by protein/peptide fractionation, phosphopeptide enrichment and high-accuracy mass spectrometry. Proteomics 2010;10:2486-97.
11. Misra SK, Moussan Désirée Aké F, Wu Z, Milohanic E, Cao TN, Cossart P, *et al.* Quantitative proteome analyses identify prfA-responsive proteins and phosphoproteins in *Listeria monocytogenes*. J Proteome Res 2014;13:6046-57.
12. Zheng J, Liu L, Liu B, Jin Q. Phosphoproteomic analysis of *Bacillus* calmette-guérin using gel-based and gel-free approaches. J Proteomics 2015;126:189-99.
13. Schmalstig AA, Benoit SL, Misra SK, Sharp JS, Maier RJ. A non-catalytic antioxidant role for *Helicobacter pylori* urease. J Bacteriol 2018: JB.00124-18.
14. Macek B, Mijakovic I, Olsen JV, Gnad F, Kumar C, Jensen PR, *et al.* The serine/threonine/tyrosine phosphoproteome of the model bacterium *Bacillus subtilis*. Mol Cell Proteomics 2007;6:697-707.
15. Bai X, Ji Z. Phosphoproteomic investigation of a solvent producing bacterium *Clostridium acetobutylicum*. Appl Microbiol Biotechnol 2012;95:201-11.
16. Yang MK, Qiao ZX, Zhang WY, Xiong Q, Zhang J, Li T, *et al.* Global phosphoproteomic analysis reveals diverse functions of serine/threonine/tyrosine phosphorylation in the model cyanobacterium *Synechococcus* sp. Strain PCC 7002. J Proteome Res 2013;12:1909-23.
17. Soares NC, Spät P, Méndez JA, Nakedi K, Aranda J, Bou G, *et al.* Ser/Thr/Tyr phosphoproteome characterization of *Acinetobacter baumannii*: Comparison between a reference strain and a highly invasive multidrug-resistant clinical isolate. J Proteomics 2014;102:113-24.
18. Ge R, Sun X, Xiao C, Yin X, Shan W, Chen Z, *et al.* Phosphoproteome analysis of the pathogenic bacterium helicobacter pylori reveals over-representation of tyrosine phosphorylation and multiply phosphorylated proteins. Proteomics 2011;11:1449-61.

How to cite this article: Mishra A. Bacterial Serine/Threonine/Tyrosine Phosphoproteomics: A Current Status and Their Role in Diverse Biological Processes. Int J Sci Stud 2018;6(9):96-98.

Source of Support: Nil, **Conflicts of Interest:** None declared.