Periplasmic Proteins: Signalling, Kinetics, Thermodynamics and Expression

Neeraj Kharor¹* J. S. Dhanda²

¹ Department of Biochemistry, Kurukshetra University, Kurukshetra, Haryana, India
² Department of Biochemistry, Kurukshetra University, Kurukshetra, Haryana, India

Abstract – Periplasmic proteins are an important class among the proteins. These proteins are important in signalling and transport of specific signals and the molecules. Hence, this property of periplasmic proteins can be exploited for the expression and the production of the different proteins. This review elaborates the extraction, binding, folding, signalling, role as transport proteins, kinetic and the thermodynamic properties of periplasmic proteins.

Keyword: Periplasmic Proteins, Signalling, Kinetics, Expression

INTRODUCTION

Proteins found in periplasm of the organisms with cell wall are the periplasmic proteins. The periplasmic proteins are not found in the organisms without cell wall. Ecotin protein, NapH protein, NapG, MdoG proteins etc. are some of the periplasmic proteins which are found in Ecoli, pseudomaonas, chlamydomonas etc. The periplasm is a space bordered by two selective permeable barriers, i.e. biological membranes, which are the inner membrane (i.e. cytoplasmic membrane) and the outer membrane in Gram-negative bacteria. Strictly speaking, there is no periplasmic space in Gram-positive bacteria because there is only one biological membrane, the cytoplasmic membrane, but a region termed inner wall zone (IWZ) has been observed between the cytoplasmic membrane and the mature cell wall. The periplasm may constitute up to 40% of the total cell volume in Gram-negative species, whereas the IWZ is drastically smaller in Gram-positive species. Composition of the IWZ has not been as yet clearly determined.

Periplasmic proteins are present as the integral proteins or they can be found as the embedded in the cell wall. These proteins help in transport purpose from the outside to inside of the cell or from inside to outside of the cell. Disulfide bonds are formed to fold the proteins in the periplasm of the cell. In the periplasm, disulfide bond (Dsb) formation is a necessary part of the protein folding pathway of many cell envelope proteins. Here, oxidation of dithiols is catalysed rapidly and in a rather unspecific way by DsbA and DsbB. Further periplasmic thiol: disulfide oxido reductases are known, which are responsible for the transfer of reducing power towards the cell envelope and for reduction and isomerisation of randomly formed disulfide bonds. These proteins are responsible for the uptake of minerals from the surroundings such as nickel etc. which are responsible for the synthesis of the products such as the enzymes. Periplasmic proteins act as the active transporters which are dependent upon the utilization of ATP. The periplasmic proteins can be extracted from the periplasm of the cell by using the different methods such as by chloroform extraction, osmotic shock etc.

Identification and Binding:

The periplasmic proteins can be identified by different methods used by different researchers. Wroblewski et.al, 1990 developed a method for the identification of bacterial periplasmic glycine betaine-binding proteins (GB-BP). Antibodies were elicited in rabbits against periplasmic protein obtained by cold osmotic shock from the Gram-negative eubacterium Rhizobium meliloti. When analysed by crossed immune electrophoresis (CIE), the periplasmic proteins gave rise to 20 distinct immune precipitates corresponding to the same number of bands in polyacrylamide gel electrophoresis (PAGE) under non-denaturing conditions and in SDS-PAGE. The periplasmic glycine betaine-binding protein (GB-BP) was identified by autoradiography after affinity labelling with [¹³C] glycine betaine in PAGE and in CIE gels. The binding proved to be quite specific to glycine betaine, since the GB-BP was not labelled by choline (a metabolic precursor or glycine betaine
in *Escherichia coli* and *Rhizobium melliloti*) and 15 distinct l-amino acids, including l-proline which, like glycine betaine is also an osmoprotectant. Affinity labelling of the GB-BP with [14C] glycine betaine after protein separation by PAGE or CIE is a simple and sensitive technique permitting the GB-BP to the unambiguously detected and identified in samples of complex protein mixtures containing down to 2μg of GB-BP in PAGE and only 0.2 μg in CIE

Jay et.al, 1989 compared the four methods for extracting the periplasmic proteins from 7 species of bacteria which were earlier reported by other researchers. Overall, chloroform (CHL) extracts contained higher quantities of periplasmic proteins from three of seven bacteria than either cold osmotic shock (COS) or slow-and rapid-freeze/thaw (SF/T, RF/T) methods. CHL extracts contained equally higher levels for the six periplasmic enzymes tested. Considerably more protein was extracted by all four methods from two *Pseudomonas* spp. than from two *Acinetobacter* spp., and the RF/T method extracted considerably more from *Acinetobacter* than the other methods

Periplasmic binding proteins scavenging or sense diverse nutrients. In the bacterial environment they usually couple to transporters or chemotaxis receptors on the inner bacterial membrane. The periplasmic binding protein HmuT from *Yersinia pestis* (YpHmuT) is a component of the heme uptake locus hmu and delivers bound heme to the inner-membrane-localized, ATP-binding cassette (ABC) transporter HmuUV for translocation into the cytoplasm. The mechanism of this process, heme transport across the inner membrane of pathogenic bacteria, is currently insufficiently understood at the molecular level

Woo JS et.al, 2010 described the crystal structures of the substrate-free and heme-bound states of YpHmuT, revealing two lobes with a central binding cleft. Superposition of the apo and holo states reveals a minor tilting motion of the lobes surrounding concomitant with heme binding. Unexpectedly, YpHmuT binds two stacked hemes in a central binding cleft that is larger than those of the homologous periplasmic heme-binding proteins ShuT and PhuT, both of which bind only one heme. The hemes bound to YpHmuT are coordinated via a tyrosine side chain that contacts the Fe atom of one heme and a histidine that contacts the Fe atom of the other heme. The coordinating histidine is only conserved in a subset of periplasmic heme binding proteins suggesting that its presence predicts the ability to bind two heme molecules simultaneously. The structural data are supported by spectroscopic binding studies performed in solution, where up to two hemes can bind to YpHmuT. Isothermal titration calorimetry suggests that the two hemes are bound in discrete, sequential steps and with dissociation constants (K_D) of ~ 0.29 and ~ 29 nM, which is similar to the affinities observed in other bacterial substrate binding proteins. Their findings suggested that the cognate ABC transporter HmuUV may simultaneously translocate two hemes per reaction cycle.

Park SH et.al, 2012 performed structural analysis and serological test of arginine periplasmic binding protein 2 from *Chlamydia pneumoniae*. The ‘art’ genes encode specific arginine uptake proteins, and are repressed by the repressible promoters of ArgR, affecting transcription of artJ. Cpb0502, the arginine-binding periplasmic protein 2 precursor from *Chlamydia pneumoniae* TW-183 strains, is responsible for arginine transport. Here they reported Cpb0502 as a specific immunogenic antigen against *C. pneumoniae* as it was detected only in human infection sera of *C. pneumoniae* but not in *Legionella pneumophila* and *Mycoplasma pneumoniae* infection sera, showing high specificity and sensitivity by micro immune fluorescence assay (MIF), western blot and ELISA analysis. And also the crystal structure of Cpb0502 was determined to be a dimer at 2.07 Å, revealing a similar backbone structure to a histidine kinase receptor, HK29S. They suggested that Cpb0502 is a candidate immunogenic antigen for better diagnosis of *C. pneumoniae*.

Periplasmic binding proteins of a new family particularly well represented in *Bordetella pertussis* have been called Bug receptors. One *B. pertussis* Bug protein is part of a tripartite tricarboxylate transporter while the functions of the other 77 are unknown. Villeret et.al, 2006 reported the first structure of a Bug receptor, BugD. It adopts the characteristic Venus flytrap motif observed in other periplasmic binding proteins, with two globular domains bisected by a deep cleft. BugD displays a closed conformation resulting from the fortuitous capture of a ligand, identified from the electron density as an aspartate. The structure reveals a distinctive α carboxylate-binding motif, involving two water molecules that bridge the carboxylate oxygen atoms to the protein. Both water molecules are hydrogen bonded to a common carbonyl group from Ala14, and each forms a hydrogen bond with one carboxylate oxygen atom of the ligand. Additional hydrogen bonds are found between the ligand α carboxylate oxygen atoms and protein backbone amide groups and with a threonine hydroxyl group. This specific ligand-binding motif is highly conserved in Bug proteins, indicating that they may all be receptors of amino acids or other carboxylated solutes, with a similar binding mode. The present structure thus unveils the bases of ligand binding in this large family of periplasmic binding proteins, several hundred members of which have been identified in various bacterial species.

Specific recognition by a Periplasmic Binding Protein Involved in Pectin Catabolism. The process of pectin depolymerization by pectate lyases and
glycoside hydrolases produced by pectinolytic organisms, particularly the phytopathogens from the genus Erwinia, is reasonably well understood. Indeed each extracellular and intracellular catabolic stage has been identified using either genetic, bioinformatics or biochemical approaches. Nevertheless, the molecular details of many of these stages remain unknown. In particular, the mechanism and ligand binding profiles for the transport of pectin degradation products between cellular compartments remain entirely uninvestigated. Boraston et.al, 2007 presented the structure of TogB, a 45.7 kDa periplasmic binding protein from Yersinia enterocolitica. This protein is a component of the TogMNAB ABC transporter involved in the periplasmic transport of oligogalacturonides. In addition to the unliganded complex (at 2.2 Å), they have also determined the structures of TogB in complex with digalacturonic acid (at 2.2 Å), trigalacturonic acid (at 1.8 Å) and 4, 5-unsaturated digalacturonic acid (at 2.3 Å). The molecular determinants of oligogalacturonide binding include a novel salt-bridge between the non-reducing sugar uronate groups, selectivity for the unsaturated ligand, and the overall sugar configuration. Complementing this are UV difference and isothermal titration calorimetry experiments that highlight the thermodynamic basis of ligand specificity. The ligand binding profiles of the TogMNAB transporter complex nicely complement pectate lyase-mediated pectin degradation, which is a significant component of pectin depolymerization reactions.

Folding:

Periplasmic proteins have the different folding pattern. A large number of groups are present which are helpful in the folding of these proteins in the periplasm. Disulfide bond formation is part of the folding pathway for many periplasmic and outer membrane proteins that contain structural disulfide bonds. Meyer et.al, 2000 identified in Escherichia coli, a broad variety of periplasmic protein thiol: disulfide oxidoreductases in which disulfide bond formation is part of their folding pathway. These periplasmic proteins contain the conserved C-X-X-C motif in their active site. Most of them have a domain that displays the thioredoxin-like fold. The function of these proteins is either the oxidative formation of disulfides, which is often necessary for folding and stability of secondary proteins, the reduction of non-native disulfides, or the isomerisation of disulfide bonds in proteins, especially when wrong disulfide bonds were formed.

Proteins often require specific helper proteins, chaperones, to assist with their correct folding and to protect them from denaturation and aggregation. The cell envelope of Gram-negative bacteria provides a particularly challenging environment for chaperones to function in as it lacks readily available energy sources such as adenosine 5' triphosphate (ATP) to power reaction cycles. Periplasmic chaperones have therefore evolved specialized mechanisms to carry out their functions without the input of external energy and in many cases to transduce energy provided by protein folding or ATP hydrolysis at the inner membrane.

Structural and biochemical studies have in recent years begun to elucidate the specific functions of many important periplasmic chaperones and how these functions are carried out. This includes not only specific carrier chaperones, such as those involved in the biosynthesis of adhesive fimbriae in pathogenic bacteria, but also more general pathways including the periplasmic transport of outer membrane proteins and the extra cytoplasmic stress responses. This study by Waksman et.al, 2009 aimed to provide an overview of protein chaperones so far identified in the periplasm and how structural biology has assisted with the elucidation of their functions.

Signalling:

A new type of solute importer has been identified recently in various bacterial genera and called the tripartite tricarboxylate transporter (TTT). TTTs consist of two cytoplasmic membrane proteins and a periplasmic solute-binding protein. In the whooping cough agent Bordetella pertussis, a TTT system that has been called BctCBA mediates the uptake of citrate, with BctA and BctB being the membrane components and BctC, the periplasmic protein. Dubuisson et.al, 2005 described that the expression of the bctCBA operon is induced by the presence of citrate in the milieu. The signalling cascade involves both BctC and the signal transduction two-component system BctDE, encoded by an operon adjacent to bctCBA. Furthermore, two-hybrid analyses and affinity chromatography experiments indicated that citrate-ligated BctC interacts with the periplasmic domain of the sensor protein, BctE. Thus, BctC is part of the signalling cascade leading to up regulation of the transporter operon in the presence of its solute, a new function for periplasmic binding proteins of TT transporters.

As Transport proteins

Until recently, extra cytoplasmic solute receptor (ESR)-dependent uptake systems were invariably found to possess a conserved ATP-binding protein (the ATP-binding cassette protein or ABC protein), which couples ATP hydrolysis to the translocation of the solute across the cytoplasmic membrane. While it is clear that this class of ABC transporter is ubiquitous in prokaryotes, it is now firmly established that other, unrelated types of membrane transport systems exist which also have ESR components. These systems have been designated tripartite ATP- independent periplasmic (TRAP) transporters, and they form a distinct class
of ESR-dependent secondary transporters where the driving force for solute accumulation is an electrochemical ion gradient and not ATP hydrolysis. According to Kelly et al., 2001 the best characterised TRAP transporter at the functional and molecular level is the high-affinity C4-dicarboxylate transport (Dct) system from *Rhodobacter capsulatus*. This consists of three proteins; an ESR (DctP) and small (DctQ) and large (DctM) integral membrane proteins. The characteristics of this system are discussed in detail. Homologues of the *R. capsulatus* DctPQM proteins are present in a diverse range of prokaryotes, both bacteria and archaea, but not in eukaryotes. The deduced structures and possible functions of these homologous systems are described. In addition to the DctP family, other types of ESRs can be associated with TRAP transporters.

A conserved family of immunogenic extra cytoplasmic proteins is shown to be invariably associated with TRAP systems that contain a large DctQM fusion protein. All of the currently known archaeal systems are of this type. It is concluded that TRAP transporters are a widespread and ancient type of solute uptake system that transport a potentially diverse range of solutes and most likely evolved by the addition of auxiliary proteins to a single secondary transporter.

Bacterial periplasmic transport systems are complex permeases composed of a soluble substrate-binding receptor and a membrane-bound complex containing 2–4 proteins. Recent developments have clearly demonstrated that these permeases are energized by the hydrolysis of ATP. Several in vitro systems have allowed a detailed study of the essential parameters functioning in these permeases. According to Ames et al., 1990 several of the component proteins have been shown to interact with each other and the actual substrate for the transport process has been shown to be the liganded soluble receptor. The affinity of this substrate for the membrane complex is approximately 10 μM. The involvement of ATP in energy coupling is mediated by one of the proteins in the membrane complex. For each specific permease, this protein is a member of a family of conserved proteins which bind ATP. The similarity between the members of this family is high and extends itself beyond the consensus motifs for ATP binding.

Interestingly, over the last few years, several eukaryotic membrane-bound proteins have been discovered which bear a high level of homology to the family of the conserved components of bacterial periplasmic permeases. Most of these proteins are known to, or can be inferred to participate in a transport process, such as in the case of the multidrug resistance protein (MDR), the *STE6* gene product of yeast, and possibly the cystic fibrosis protein. This homology suggests a similarity in the mechanism of action and possibly a common evolutionary origin. This exciting development will stimulate progress in both the prokaryotic and eukaryotic areas of research by the use of overlapping procedures and model building. Ames et al., proposed that this universal class of permeases be called ‘Traffic ATPase’s’ to distinguish them from other types of transport systems, and to highlight their involvement in the transport of a vast variety of substrate in either direction relative to the cell interior and their use of ATP as energy source.

ATP-binding cassette superfamily of periplasmic metal transporters are known to be vital for maintaining ion homeostasis in several pathogenic and non-pathogenic bacteria. Amit Sharma et al., 2007 determined the crystal structure of the high-affinity zinc transporter ZnuA from *Escherichia coli* at 1.8 Å resolution. This structure represents the first native (non-recombinant) protein structure of a periplasmic metal binding protein. ZnuA reveals numerous conformational features, which occur either in Zn\(^{2+}\) or in Mn\(^{2+}\) transporters, and presents a unique conformational state. A comprehensive comparison of ZnuA with other periplasmic ligand binding protein structures suggests vital mechanistic differences between bound and release states of metal transporters. The key new attributes in ZnuA include a C-domain disulfide bond, an extra α-helix proximal to the highly charged metal chelating mobile loop region, alternate conformations of secondary shell stabilizing residues at the metal binding site, and domain movements potentially controlled by salt bridges. Based on in-depth structural analyses of five metal binding transporters, they presented a mechanistic model termed as “partial domain slippage” for binding and release of Zn\(^{2+}\).

**Kinetics and thermodynamics:**

Krupka et al., 1992 investigated the kinetics of transport system dependent on periplasmic proteins. Rate equations were derived for a transport model involving a water-soluble binding protein outside the plasma membrane. On addition of the substrate, the conformation of the binding protein changes, the complex then combines with the membrane carrier, transferring the substrate to the carrier site. The free binding protein leaves and the carrier shift inward, releasing the substrate inside the cell. Exit follows the reverse path. The predicted behaviour is as follows. (i) Uptake does not necessarily conform to Michaelis-Menten kinetics. (ii) In both the energized and de-energized states, the maximum rate of exit is far lower than that of entry; the asymmetry is determined by the conformational change in the binding protein, which is independent of the energy state of the system. (iii) Exchange transport is inhibited by external substrate and is extremely slow; consequently counter-transport is not expected. (iv) The half-saturation constant in uptake can differ from the dissociation constant of the binding protein. (v) The maximum rate of uptake depends on the intrinsic substrate affinity of the membrane carrier relative...
to that of the binding protein. (vi) The maximum rate of uptake and the substrate half-saturation constant depend on the concentration of the binding protein.

Tripartite ATP-independent periplasmic transporters (TRAP-Ts) are bacterial transport systems that have been implicated in the import of small molecules into the cytoplasm. A newly discovered subfamily of TRAP-Ts [tetratricopeptide repeat-protein associated TRAP transporters (TPATs)] has four components. Three are common to both TRAP-Ts and TPATs: the P component, a ligand-binding protein, and a transmembrane symporter apparatus comprising the M and Q components (M and Q are sometimes fused to form a single polypeptide). TPATs are distinguished from TRAP-Ts by the presence of a unique protein called the “T component”. In Treponema pallidum, this protein (TatT) is a water-soluble trimer whose protomers are each perforated by a pore. Its respective P component (TatP_R) interacts with the TatT in vitro and in vivo. Norgard et al. 2012 further characterized this interaction. Co-crystal structures of two complexes between the two proteins confirm that up to three monomers of TatP_R can bind to the TatT trimer. A putative ligand-binding cleft of TatP_R aligns with the pore of TatT, strongly suggesting ligand transfer between T and P_R. They used a combination of site-directed mutagenesis and analytical ultracentrifugation to derive thermodynamic parameters for the interactions. These observations confirm that the observed crystallographic interface is recapitulated in solution. These results prompt a hypothesis of the molecular mechanism(s) of hydrophobic ligand transport by the TPATs.

Expression of periplasmic proteins:

It is well recognised that phosphate limitation in Escherichia coli causes enhanced synthesis of a variety of proteins involved in maximising the uptake and utilisation of the available phosphate. In contrast to this situation, Payne et al. 1992 reported that these same conditions repress synthesis of the periplasmic binding proteins for both the oligopeptide (Opp) and dipeptide permeases (Dpp), and of certain other periplasmic proteins. Regulation in the former case is mediated by the Pho regulon; genes controlled by this mechanism lack efficient −35 promoter regions, and instead, an activator protein, PhoB, binds to a specific ‘Pho box’ sequence, ten bases upstream from a −10 promoter, thereby facilitating binding of RNA polymerase and leading to enhanced transcription. In the latter case, putative Pho boxes can be identified in the promoter regions of opp and dpp (and of other binding proteins), but in these genes they overlap the RNA polymerase binding sites of good promoters. We speculate that this different Pho box location may allow PhoB to act as a repressor of transcription of these genes. The promoter region for the sigma factor, ω32, (RpoH) also contains a putative Pho box, implying that it may be involved in the enhanced synthesis and secretion of proteins required under phosphate limitation.

Act as an Acid-responsive Chaperone

HdeA, a periplasmic protein found to be required for E. coli cells to survive in acidic environments, works to prevent the aggregation of proteins at extremely low pH values. Experimental observations include the following: (a) HdeA exhibits chaperone-like activity, i.e. being able to bind to the denatured substrate proteins, exclusively at extremely low pH values and releases them at neutral pH; (b) HdeA possesses a globally disordered conformation at extremely low pH values but an ordered conformation at neutral pH; and (c) HdeA exposes hydrophobic surfaces that appear to be involved in binding denatured substrate proteins at extremely low pH values. It is proposed that HdeA might have evolved to protect E. coli cells for survival in the extremely low pH environment that bacteria typically encounter in mammalian stomachs. In particular, HdeA appears to be able to act as an acid-responsive molecular chaperone that prevents protein aggregation in the periplasmic space (that is exposed to the outside environment because of the porous nature of the outer membrane) at such extremely low pH values. The physiological role of HdeA during the passage of the enterobacteria through the stomach may be visualized. Upon the entrance of the enterobacteria from a neutral environment into an acidic stomach, whose pH is typically maintained in the range of 1–3 by the secreted HCl, the structure of the HdeA protein is immediately transformed from an ordered conformation into a disordered one, which allows it to effectively bind to the denatured substrate proteins and thereby prevent their aggregation. When the enterobacteria reach the small intestine, where the pH is typically around 7, the proteins bound to HdeA are released and may eventually refold into their native conformation with or without aid or they may be degraded. The decrease of pH to values lower than 3, conformational disordering somehow transforms HdeA into an “amphipathic molecule” having a hydrophobic tail (consisting of the two hydrophobic fragments) that is involved in substrate binding and a positively charged hydrophilic head (consisting of the two Lys-rich terminals) that enhances the solubility of HdeA-substrate complexes via the hydration of the charges. Such a postulated “amphipathic molecule” would act as an effective molecular chaperone in preventing the aggregation of substrate proteins.

Periplasmic proteins of Escherichia coli are highly resistant to aggregation

Periplasmic proteins of Gram-negative bacteria like Escherichia coli are subjected to immediate effect of environmental fluctuation that may unfold proteins, due to the permeability of the outer membrane to small molecules. They are thus
supposedly protected by certain molecular chaperones. Nevertheless, no homologues of typical molecular chaperones have so far been found in periplasm, and the recently reported chaperone activities of periplasmic protein disulfide isomerase (PDI) and peptidyl prolyl isomerase (PPI) seem to be too weak to satisfy such assumed needs. In an attempt to reveal whether periplasmic proteins exhibit certain unusual properties, Chang et al. 2004 discovered that such proteins as a whole are highly resistant to aggregation under a wide variety of denaturing conditions. Furthermore, in an effort to unveil the nature behind this phenomenon they purified and examined four prominent periplasmic proteins. The results demonstrate that these proteins unfold at rather mild denaturing conditions and expose hydrophobic surfaces during such unfolding process, but hardly form complexes with a typical molecular chaperone. Based on these observations, it was proposed that the periplasmic proteins have been evolved to resist the formation of aggregates when subjected to various denaturing conditions and molecular chaperones may thus not be needed in periplasm.

**Involvement of a periplasmic protein kinase in DNA strand break repair**

The involvement of signal transduction in the repair of radiation-induced damage to DNA has been known in eukaryotes but remains understudied in bacteria. the periplasmic lipoprotein (YfgL) with protein kinase activity transducing a signal for DNA strand break repair in Escherichia coli. Purified YfgL protein showed physical as well as functional interaction with pyrroloquinoline-quinone in solution and the protein kinase activity of YfgL was strongly stimulated in the presence of pyrroloquinoline-quinone. Transgenic E. coli cells producing Deinococcus radiodurans pyrroloquinoline-quinone synthase showed nearly four log cycle improvement in UVC dark survival and 10-fold increases in gamma radiation resistance as compared with untransformed cells. Pyrroloquinoline-quinone enhanced the UV resistance of E. Coli through the YfgL protein and required the active recombination repair proteins. The yfgL mutant showed higher sensitivity to UVC, mitomycin C and gamma radiation as compared with wild-type cells and showed a strong impairment in homologous DNA recombination. The mutant expressing an active YfgL in trans recovered the lost phenotypes to nearly wild-type levels. The results strongly suggest that the periplasmic phosphorolipoprotein kinase YfgL plays an important role in radiation-induced DNA strand break repair and homologous recombination in E. coli.

**Effect of copper ions**

Novo et al, 2003 studied the effects of 200 mM copper ions on the synthesis of membrane and periplasmic proteins were investigated in iron-grown cells of Acidithiobacillus ferrooxidans (At. ferrooxidans). Total membrane protein profiles of cells grown in the absence of copper ions (unadapted cells) and in the presence of copper ions (copper-adapted cells) were compared by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Crude preparations of outer membrane and periplasmic proteins were analysed by SDS-PAGE. The synthesis of proteins was diminished or increased in the presence of copper ions. Low molecular weight proteins (<14 kDa) were significantly repressed by copper. These proteins are probably acidic proteins located in the outer membrane. An over-expression of a periplasmic protein of about 17 kDa was detected in the copper-adapted cells and was assumed to be rusticyanin, a 16.5-kDa periplasmic copper protein present in At. ferrooxidans cells and involved in the electron-transport chain of the iron oxidation pathway. To our knowledge, this is the first report of a possible involvement of the rusticyanin and outer membrane proteins in the mechanism of copper resistance in At. ferrooxidans.

**Act as chelators**

*Escherichia coli* require nickel for the synthesis of [NiFe] hydrogenases under anaerobic growth conditions. Nickel import depends on the specific ABC-transporter NikABCDE encoded by the *nik* operon, which deletion causes the complete abolition of hydrogenase activity. Christine Cavazza et al. 2011 have previously postulated that the periplasmic binding protein NikA binds a natural metallophore containing three carboxylate functions that coordinate a Ni (II) ion, the fourth ligand being His416, the only direct metal-protein contact, completing a square-planar coordination for the metal. In this work, they studied the physiologal consequences of mutating His416 into isoleucine which cannot bind the metal. The crystal structure of the H416I mutant showed no electron density corresponding to a metal-chelator complex. In agreement with the role suggested previously by His416 in Ni complexation by the wild-type NikA its mutation provoked (1) the absence of the metal ion in the NikA crystal structure, (2) a low concentration of intracellular nickel and (3) decreased hydrogenase activity in the mutant. All these observations confirm the central role that plays His416, the only protein ligand to nickel, in the transport of this ion by NikA.

**Periplasmic proteins as enzymes**

Mo- and W-enzymes are widely distributed in biology as they can be found in all domains of life. They perform key roles in several metabolic pathways catalyzing important reactions of the biogeochemical cycles of the more abundant elements of the earth. These reactions are usually redox processes involving the transfer of an atom from the substrate to the metal ion or vice versa. The Mo or W reactivity and specificity toward a

---

**Neeraj Kharor**1,2 J. S. Dhanda2

---

1. Department of Chemistry, University of Delhi, Delhi, India
2. CSIR-Indian Institute of Chemical Biology, Kolkata, India

---

**Periplasmic Proteins: Signalling, Kinetics, Thermodynamics and Expression**

---

1 www.ignited.in

---

143
substrate is determined by the polypeptide chain of the enzyme, which tunes the chemical properties of the metal ion. Two enzymes sharing almost identical active sites but catalyzing very different reactions are periplasmic nitrate reductase and formate dehydrogenase from bacteria. They represent a good example of how key changes in the amino acid sequence tune the properties of an enzyme. In order to analyze the chemistry of Mo and W in these enzymes, structural, kinetic and spectroscopic data were reviewed by Moura et al. 2013, along with the role of these enzymes in cell metabolism. In addition, the features that govern selectivity of metal uptake into the cell and Mo/W-cofactor biosynthesis were revised.

Development of ELISA for Diagnosis of Mycoplasma pulmonis Infection in Rodents.

Mycoplasma pulmonis is one of the most prevalent bacterial pathogens that infects laboratory mice and rats. To develop a M. pulmonis-specific antigen for serological diagnosis, we cloned the cDNA of P46-like lipoprotein (P46L), an M. pulmonis putative periplasmic protein. P46L is a homolog of P46, an M. hyopneumoniae antigen. We produced recombinant P46L fused to glutathione S-transferase (GST) in Escherichia coli. Immunoblot analysis revealed that sera from Mycoplasma-infected mice and rats contained anti-P46L antibodies. We developed an ELISA using the recombinant P46L-GST protein as an antigen. Thirteen of the 14 samples from rats naturally infected with M. pulmonis were determined to be positive according to the commercial ELISA (MONILISA Myco) and positive by our ELISA. Furthermore, 18/19 samples from mice experimentally infected with M. pulmonis were positive using our P46L-GST ELISA. In contrast, only 8/19 samples from infected mice were positive by the commercial ELISA. Our results indicate that P46L-GST was an appropriate antigen for developing a serological test to determine M. pulmonis infection in laboratory mice and rats.

As biosensor

The members of the superfamily of *Escherichia coli* periplasmic binding proteins have a high affinity for a wide variety of small molecule ligands. This offers a range of specificities for which to design sensing systems utilizing the proteins natural affinities for many analyte of interest. Genetically engineered periplasmic glucose receptors could be used as biomolecular recognition elements on gold nanoparticles (AuNPs). This would allow to develop a sensitive and reagentless electrochemical glucose biosensor. The receptors were immobilized on AuNPs by a direct sulfur–gold bond through a cysteine residue that was engineered in position 1 on the protein sequence. The study of the attachment of genetically engineered and wild-type proteins binding to the AuNPs was first carried out by Andreescu et al., 2008 in colloidal gold solutions. These constructs were studied and characterized by UV–Vis spectroscopy, transmission electron microscopy, particle size distribution, and zeta potential. They showed that the genetically engineered cysteine is important for the immobilization of the protein to the AuNPs. Fabrication of the novel electrochemical biosensor for the detection of glucose used these receptor-coated AuNPs. The sensor showed selective detection of glucose in the micro molar concentration range, with a detection limit of 0.18 μM.

CONCLUSION

The diversity of biological function, ligand binding, conformational changes and structural adaptability of the periplasmic binding protein superfamily could be used in many ways. As the periplasmic proteins play an important role in the transport process and in the uptake of the elements. This property of the periplasmic proteins can be exploited for the transport of the small molecules from the outside to cytoplasm. They also help in detecting nutrients in the environment, provide protection from harmful compounds. So these properties of the periplasmic proteins could be exploited. Periplasmic proteins are also helpful in the signal transduction. The expression of specific periplasmic proteins can be increased in the periplasm which is of human interest. By the use of the genetic engineering involving the insertion of the gene of interest Periplasmic binding protein based biosensor could also be developed.

REFERENCES


Escherichia coli"Biochimie, Volume 70, Issue 6, Pages 727–733.


**Corresponding Author**

**Neeraj Kharor**

Department of Biochemistry, Kurukshetra University, Kurukshetra, Haryana, India

E-Mail – nkharor@gmail.com