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Mini Review

Periplasmic glucans isolated from Proteobacteria

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Periplasmic glucans (PGs) are general constituents in the periplasmic space of Proteobacteria. PGs from bacterial strains are found in larger amounts during growth on medium with low osmolarity and thus are often been specified as osmoregulated periplasmic glucans (OPGs). Furthermore, they appear to play crucial roles in pathogenesis and symbiosis. PGs have been classified into four families based on the structural features of their backbones, and they can be modified by a variety of non-sugar substituents. It has also recently been confirmed that novel PGs with various degrees of polymerization (DPs) and/or different substituents are produced under different growth conditions among Proteobacteria. In addition to their biological functions as regulators of low osmolarity, PGs have a variety of physico-chemical properties due to their inherent three-dimensional structures, hydrogen-bonding and complex-forming abilities. Thus, much attention has recently been focused on their physico-chemical applications. In this review, we provide an updated classification of PGs, as well as a description of the occurrences of novel PGs with substituents under various bacterial growth environments, the genes involved in PG biosynthesis and the various physico-chemical properties of PGs. [BMB reports 2009; 42(12): 769-775]

INTRODUCTION

Periplasmic glucans (PGs), which are produced by many Proteobacteria, are important intrinsic components of the Gram-negative bacterial envelope that are especially important for host-bacteria interactions. PGs have glucopyranosyl residues as the only carbohydrates in their backbones, in which the glucose molecules are linked with β-glycosidic bonds (1). It has been reported that PG synthesis is generally greater in cells cultured on medium with low osmolarities. PGs are composed of 5 to 40 glucosyl residues per molecule and show distinct structural diversity in their glucose backbones depending on the species they are produced by (Table 1).

The first PGs identified were cyclic β-glucans, which were discovered in 1942 in the extracellular media of *Agrobacterium tumefaciens* cultures (2). Since then, several Gram-negative bacteria including *Sinorhizobium, Mesorhizobium* and *Brucella* species have been shown to produce cyclic β-glucans with similar structures. After the discovery of cyclic β-glucans, a class of PGs known as membrane-derived oligosaccharides (MDOs) was discovered in *Escherichia coli* in 1973. That

Table 1. Classification of PGs, bonds and biosynthesis-associated genes produced by Proteobacteria

Family	Bacterial species	[a]DP	Linkages	Substituents	Genes involved in PG biosynthesis
I	*E. coli*	5-12	β-1,2 β-1,6	[b]PG, [c]PEA, [d]Suc	*opgG, opgH, opgB, opgC, opgD*
	P. syringae	6-28	β-1,2 β-1,6	Suc, [e]Ac	*opgG, opgH*
	P. aeruginosa	6-10	β-1,2 β-1,6	Suc	*opgG, opgH*
	E. chrysanthemi	5-12	β-1,2 β-1,6	Suc, Ac	*opgG, opgH*
II	*S. meliloti*	17-40	β-1,2	PG, Suc, [f]MeMal	*ndvB, cgmB*
	M. huakuii	17-28	β-1,2	None	[h]ND
	A. tumefaciens	17-25	β-1,2	PG	*chvB*
	B. abortus	17-25	β-1,2	Suc	*cgs, cgm*
III	*B. japonicum*	10-13	β-1,3 β-1,6	[g]PC	*ndvB, ndvC*
	A. brasilense	12-13	β-1,3 β-1,4 β-1,6 α-1,3	Suc	ND
	A. caulinodans	10-13	β-1,3 β-1,6	None	ND
IV	*X. campestris*	16	β-1,2 α-1,6	PG	*opgH$_{Xcv}$, opgB*
	R. solanacearum	13	β-1,2 α-1,6	Ac	*opgG, opgH*
	R. sphaeroides	18	β-1,2 α-1,6	Suc, Ac	*opgG, opgI, opgH, opgC*

[a]DP: degree of polymerization, [b]PG: phosphoglycerol group, [c]PEA: phosphoethanolamine group, [d]Suc: succinyl group, [e]Ac: acetyl group, [f]MeMAL: methylmalonyl group, [g]PC: phosphocholine group, [h]ND: not detected.

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study confirmed that the turnover of membrane phospholipids in *E. coli* is closely related to MDO biosynthesis in their periplasmic space (3, 4). Furthermore, regulation of the biosynthesis of periplasmic cyclic β-glucans in *A. tumefaciens* has been shown to be similar to the osmotic regulation of MDO biosynthesis in *E. coli*, suggesting a general role for periplasmic oligosaccharides in the osmotic adaptation of Gram-negative bacteria (5). PGs can also be modified by several non-sugar substituents depending on the species or growth environment. However, the nature of the biological relationships between PGs and their substituents remains unclear.

Although a review on PGs in Proteobacteria was reported early in 2000 (6), no updated information is available regarding the occurrence of novel PGs, the genes involved in PG biosynthesis, and the various physico-chemical properties of PGs. Thus, an updated review is needed so that the functions of novel PGs at the molecular level and their chemical properties can be understood.

Classification of PGs

Proteobacteria subdivided into α, β and γ types, which produce PGs with varying structures. Generally, PGs are classified into four families based on their respective glucose backbones (Table 1), and these backbones also show structural diversity depending on the bacterial strain by which they are produced.

The PGs of Family I are heterogeneous in size, varying from 5 to 28 glucosyl residues per molecule. Their linear backbone structures consist of β-1,2-linked glucosyl residues with branches of glucosyl units linked by β-1,6 bonds. The PGs produced by *E. coli* are representative of Family I and have degrees of polymerization (DPs) of 5-12. PGs from *E. chrysanthemi* have the same number of DPs as those from *E. coli*. In addition, it was recently reported that *Pseudomonas syringae* (7) and *P. aeruginosa* (8) produce PGs with 6-28 and 6-10 DPs, respectively.

Family II PGs consist of cyclic glucan backbones linked by β-1,2-glycosidic bonds. The cyclic glucans are heterogeneous in size, ranging from 17 to 40 glucosyl residues (9, 10). Bacterial strains such as the *Agrobacterium*, *Sinorhizobium, Mesorhizobium* and *Brucella* species produce periplasmic cyclic β-glucans belonging to Family II (9, 11, 12).

PGs belonging to Family III are cyclic glucans linked by β-(1,3) and β-(1,6) glycosidic bonds with DPs that range from 10 to 13 glucose units that are produced by *Bradyrhizobium* spp. Similarly, *Azorhizobium caulinodans* produce β-(1,3) and β-(1,6) glucans containing 10 to 13 glucose residues (13). *Azospirillum brasilence* has also been found to produce PGs belonging to Family III with 12-13 DPs; however, these PGs consist of three cyclic glucans linked by β-(1,4) and α-(1,3) bonds as well as β-(1,3) and β-(1,6) (14,15). Based on the presence of glycosidic bonds that strictly control ring size, the PGs of Family III have few similarities with those of Family II.

The PGs of Family IV consist of cyclic glucans linked by β-(1,2) bonds containing a single α-(1,6) glycosidic linkage. The structural characteristics of Family IV PGs differ from those of Family II or III. *Xanthomonas* species (16-18, 19, 20), *Ralstonia solanacearum* (17, 21, 22) and *Rhodobacter sphaeroides* (23) produce PGs with 16, 13 and 18 DPs, respectively.

Substituents on PGs and the occurrence of novel PGs

PGs can be substituted with non-sugar moieties, such as phosphoglycerol, phosphoethanolamine, phosphocholine, acetyl, succinyl and methylmalonyl (Table 1). Among these substituents, phosphoglycerol, phosphoethanolamine and phosphocholine residues originate from periplasmic membrane phospholipids, while acetyl, succinyl and methylmalonyl residues originate from metabolic intermediates in cellular systems (1, 9). These substituents are modified independently of the backbone structures of the PGs. The degree of substitution (DS) of the PGs varies among bacterial strains, but some species often contain no negative-charged substituents as in the case of cyclic β-(1,2)-glucans produced by *Sinorhizobium* sp. strain GRH2, *Rhizobium trifoli*, *Rhizobium phaseoli* and certain strains of *S. leguminosarum* that synthesize Family II PGs (9, 24, 25).

The growth stage of bacterial strains has also been shown to affect the DS of PGs. For example, several studies have indicated that the conversion of neutral to anionic cyclic β-(1,2)-glucans in *S. meliloti* 1021 is much more rapid and extensive in exponentially growing cultures than in cultures in the stationary phase (26). Thus, depending on the bacterial strain and growth conditions, PGs can be non-substituted or substituted with neutral or anionic substituents (Table 1).

Recent studies have indicated that the occurrence of novel PGs depends on the bacteria and their environments. Until 2008, PGs of *P. syringae* belonging to Family I were known to be highly branched linear glucans ranging from 6 to 13 glucosyl residues without any substituents. However, it has recently been reported that novel anionic PGs with one succinyl residue at the C-6 position of the glucose unit as well as neutral PGs including large-sized glucans with up to 28 DPs are produced by *P. syringae* pv. syringae. (7) (Fig. 1). Interestingly, it has also been demonstrated that the synthesis of large PGs from *P. syringae* pv. syringae is not subject to strict size controls (7). One acetylated PG with 6-22 DPs and two acetylated PGs with 7-17 DPs were also newly detected in a culture medium of *P. syringae* (27). *P. aeruginosa* in Family I have also been shown to synthesize novel PGs with 6-10 DPs in which the glucans have been substituted by succinyl residues (8).

Among the Family II PGs, cyclic β-(1,2)-glucans from *B. abortus* are modified with succinyl residues that confer an anionic character to the PGs. In addition, the backbone of the PGs is substituted at the C-6 positions with an average of two succinyl resides per molecule. Moreover, the succinyl substituents of cyclic β-(1,2)-glucans in *B. abortus* are known to be necessary for hypo-osmotic adaptation (28).

In Family IV, novel PGs substituted with non-sugar residues

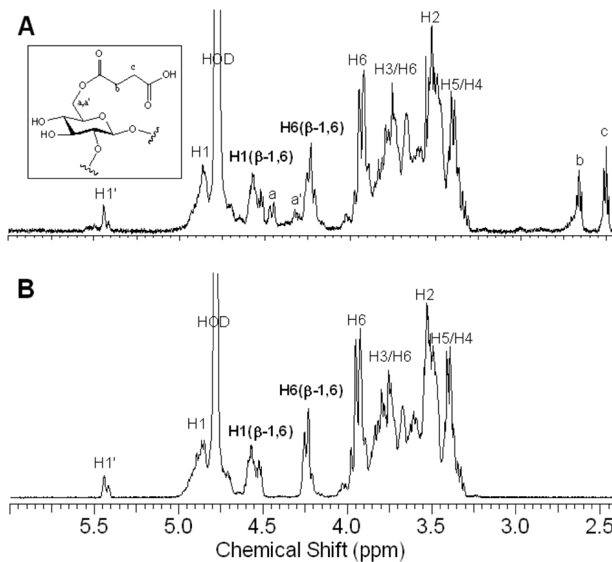

Fig. 1. ^{1}H NMR spectra of (A) succinylated PGs and (B) neutral PGs produced by *P. syringae* pv. syringae. The inset of (A) indicates a proposed structure of one succinylated PG, while a, a′, b and c indicate protons directly linked to carbons. Reprinted with permission from Ref. (7).

have recently been found in *X. campestris, R. solanacearum* and *R. sphaeroides*. Among these strains, the free-living photosynthetic bacterium *R. sphaeroides* is known to produce a cyclic glucan with 18 DPs in which the PG is modified with succinyl residues of 1-7 at the C-6 positions of the glucosyl unit (23). The plant pathogen, *X. campestris*, has been shown to produce a novel anionic PG in which neutral α-cyclosophorohexadecaose (α-C16) was substituted with one or two phosphoglycerol substituents at the C-6 positions of the glucose residues, which differs from the PGs produced by *X. oryzae* and *X. phaseoli* (16, 20). The plant pathogen, *R. solanacearum*, also produces a novel cyclic PG known as acetylated α-cyclosophorotridecaose (acetylated α-C13) (21).

Although the occurrence of novel PGs substituted with a variety of non-glycosyl residues has been reported, any biological correlation between the structure of the PGs and the nature of the substituents remains unclear.

Genes involved in PG biosynthesis

It is well known that the *opgG* and *opgH* genes involved in the biosynthesis of OPGs are the most widely distributed Proteobacterial genes (Table 1). In *E. coli*, four genes are known to be involved in the biosynthesis of Family I PGs. Among these, two genes forming an *opgGH* locus play a role in the synthesis of the linear glucan backbones (29), whereas the *opgB* (30, 31) and *opgC* genes (32) are involved in substitution of the PG by phosphoglycerol and succinyl residues, respectively. However, the gene associated with PG modification by phosphoenthanolamine residues has yet to be identified. The *opgD* gene that controls the size of PGs produced by *E. coli* has also been identified (33). In *E. coli*, membrane-bound phosphoglycerol transferase I and periplasmic phosphoglycerol transferase II are involved in modifying phosphoglycerol residue (34-36); however, it has recently been demonstrated that periplasmic phosphoglycerol transferase II is a soluble form of membrane-bound phosphoglycerol transferase I, indicating that the two enzymes are encoded by the *opgB* gene (37).

The *P. aeruginosa* genome contains two ORFs, PA5077 and PA5078, which are similar to the *opgH* and *opgG* of *P. syringae*, respectively. In addition, *P. aeruginosa* contains the PA 1163 ORF, which is similar to *ndvB* of *S. meliloti*. A recent study demonstrated that the *opgGH* locus of *P. aeruginosa* PA14 is involved in the synthesis of PG and that the transcription of the *opgGH* is repressed by high osmolarity (8). In *P. syringae* (38) and *E. chrysanthemi* (39), the *opgGH* locus was reported in 1988 and 2001, respectively, but no genes involved in the substitution of the PGs in these organisms have been identified to date.

It is well known that the *ndvB* gene in *S. meliloti* and the *chvB* gene in *A. tumefaciens* encode a large-molecular-mass cytoplasmic membrane protein involved in the biosynthesis of the neutral cyclic β-1,2-glucan backbone from UDP-glucose (40-42). Additionally, the *cgmB* gene encodes a soluble protein required for phosphoglycerol modification of the PG (43). Conversely, the identity of the genes involved with the PG substitution by succinyl residues in *S. meliloti* and *A. tumefaciens* remains unclear. The *ndvB* genes are involved in the synthesis of Family II and III PGs in *Sinorhizobium* and *Bradyrhizobium*, but the ndvB proteins produced by these organisms do not show any significant sequence similarity, indicating non-homology between the two $_{n}$dvBs. The gene locus involved in the synthesis of cyclic β-(1,3) and β-(1,6)-glucan in *Bradyrhizobium* spp. is composed of at least two genes, *ndvB* and *ndvC*. It was found that no PGs were synthesized when *ndvB* was inactivated, but that PGs containing β-(1,3) linkages alone were produced when *ndvC* was inactivated, indicating that *ndvC* may be involved in the formation of β-(1,6)-linkages (44).

In the case of *B. abortus*, an open reading frame (BAB1_1781) homologous to *R. sphaeroides* glucans succinyltransferase (opgC) has been recently identified as the *cgm* gene, which encodes the enzyme responsible for succinyl modification of the PGs (28). The *opgGIH* and *opgC* genes in *R. sphaeroides* have been shown to form an operon that controls the backbone synthesis and succinylation of the PGs, respectively (45).

A genetic locus, $opgH_{Xcv}$, in *X. campestris* pv. *vesicatoria* that is translationally homologous to *hrpM* of *P. syringae* and *opgH* of *E. chrysanthemi* has been identified through cloning and molecular analysis (46). Furthermore, in *R. solanacearum*, an *opgH* (*mdoH*) gene that encodes the PG biosynthesis of glucosyltransferase H and the *opgG* (*mdoG*) gene which is closely

related to the biosynthesis gene of the PGs from *P. aeruginosa* have been shown to be involved in periplasmic cyclic glucan synthesis (47, 48). Recently, the presence of the *opgB* gene in *X. campestris* pv. campestris, which is similar to the *mdoB* (*opgB*) gene that encodes phosphoglyceroltransferase I in *E. coli*, was confirmed (16).

Although similarities exist between *opgG* genes produced by different organisms such as *R. solanacearum* and *P. aeruginosa*, not all genes show similar functions between bacterial strains. For example, no significant similarity has been observed between Cgm and opgC, which contain two enzymes that catalyze the substitution of the succinyl residues on PGs from *B. abortus* and *E. coli*, respectively. This fact suggests that different PG-substituting enzymes that catalyze very similar biochemical reactions such as Cgm and *opgC* can evolve independently.

Novel physico-chemical properties of PGs

PGs in Proteobacteria have been studied in a variety of physicochemical applications as well as with regard to their biological functions. In early studies with PGs, sinorhizobial cyclic β-(1,2) glucans belonging to Family II were used as solubility enhancers for poorly soluble compounds due to their cyclic structure and high solubility in water (49, 50). Furthermore, it has been suggested that sinorhizobial cyclic β-glucans can form non-covalent complexes with some hydrophobic plant flavonoids (51-53). Actually, the complex-forming abilities of the sinorhizobial cyclic β-glucans may be important during plant infection in terms of biological function. Additional studies have indicated that sinorhizobial cyclic β-(1,2)-glucans have the potential for use as commercial complexation agents for a variety of poorly soluble molecules (54-59). Recent studies have also shown that cyclic β-(1,2)-glucans act as chiral selectors for various enantiomers (60-66), as catalytic carbohydrates for methanolysis (67-69), and as morphology-directing agents for the synthesis of selenium nanowires (70). These characteristic functions of PGs may be due to inherent three-dimensional structures, hydrogen-bonding and the complex-forming abilities of the cyclic glucans in aqueous solutions. In the case of Family II sinorhizobial cyclic β-(1,2)-glucans, the PGs have a high degree of molecular flexibility, as demonstrated by molecular dynamics simulation studies (71). These findings suggest that the molecular flexibility of PGs gives rise to preferential interactions of the PGs with target molecules such as 5(*4H*)-oxazolones or phospholipids, which demonstrates that the PGs function as catalytic carbohydrates in specific reactions (Fig. 2).

Based on the unusual features of the PGs in Family II, the PGs of Families I, III and IV have been exploited. It was recently reported that the Family I PGs produced by *P. syringae*, as well as linear β-1,2 glucans and Family III PGs produced by *B. japonicum* and cyclic β-(1→3),(1→6)-glucans act as chiral selectors in enantioseparation (72, 73). The Family IV PGs produced by *X. oryzae* and α-cyclosophorohexadecaose (α-C16) have also been shown to act as catalytic carbohydrates in the methanolysis of some benzoates (19) and the Strecker reaction of some imine compounds (74).

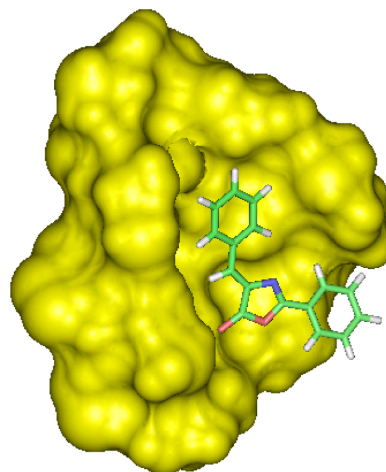

Fig. 2. Energy-minimized structure showing a preferential binding mode with a target molecule, 4-benzylidene 2-phenyloxazolone, induced by one of the Family II PGs, sinorhizobial cyclic β-(1,2)-glucan. The cyclic β-(1,2)-glucan is colored yellow and the target molecule is partially colored green.

Interestingly, it has been reported that the α-1,6 glycosidic linkage in cyclic glucans of Family IV can reduce structural constraints existing in those glucans, as demonstrated by nuclear magnetic resonance (NMR) spectroscopy (75, 76) and molecular dynamics simulations (11, 18, 77, 78). Thus, the physiological functions of these types of PG might be closely related to their intrinsic conformation.

Relationship between PGs and pathogenesis

The critical role of PGs in pathogenesis has been shown for many human, animal and plant pathogens (1). Mutants defective in PG biosynthesis display highly pleiotropic phenotypes such as hyperproduction of exopolysaccharides, motility defects and hypersensitivity to antibiotics, indicating that PGs are essential biomolecules for normal organization of the cell envelope.

Conclusions and perspectives

PGs have long been considered to be general constituents of the envelope of Proteobacteria. These compounds appear to play essential roles in interactions with specific eukaryotic hosts. Occurrences of PGs with distinct structural diversities among Proteobacteria have been reported continually. Structural variations occur at levels such as the glucan backbone organization of PGs and include the presence or absence

of non-sugar substituents. Moreover, it is expected that novel PGs with various DPs and different substituents can be produced under different growth conditions and by different bacterial strains. Because it is assumed that the inherent physiological functions of PGs are closely related to their structural conformations, there has been considerable interest in determining their structural features. PGs have also been extensively exploited for many novel applications based on their ability to act as solubility enhancers, chiral selectors, catalysts and morphology-directing agents. These properties may be attributed to their inherent three-dimensional structures, hydrogen-bonding and complex-forming abilities. More importantly, recent studies of the physico-chemical properties of PGs strongly suggest that they play special roles in cellular systems beyond osmoregulation.

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