Mini-Review

Correspondence
Gavin H. Thomas
ght2@york.ac.uk

Sialic acid utilization by bacterial pathogens
Emmanuele Severi,1 Derek W. Hood2 and Gavin H. Thomas1

1Department of Biology (Area 10), University of York, PO Box 373, York YO10 5YW, UK
2Molecular Infectious Diseases Group, University of Oxford Department of Paediatrics, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS, UK

Sialic acid occupies the terminal position within glycan molecules on the surfaces of many vertebrate cells, where it functions in diverse cellular processes such as intercellular adhesion and cell signalling. Pathogenic bacteria have evolved to use this molecule beneficially in at least two different ways: they can coat themselves in sialic acid, providing resistance to components of the host’s innate immune response, or they can use it as a nutrient. Sialic acid itself is either synthesized de novo by these bacteria or scavenged directly from the host. In this mini-review we will summarize recent findings relating to sialic acid transport, modification of sialic acid by O-acetylation, and the mechanisms of sialic acid-mediated complement resistance.

Introduction
The surfaces of many cells, both prokaryotic and eukaryotic, are decorated with glycoconjugates that play important roles in a wide range of biological processes, including cell–cell and small molecule–cell recognition. Sialic acid is a generic term to indicate a wide family of related nine-carbon sugar acids that feature prominently at terminal positions of many eukaryotic surface-exposed glycoconjugates, where they confer important properties upon the resulting cell surface. It is therefore perhaps not surprising that many pathogenic bacteria have also evolved to decorate their cell surfaces with sialic acid, which results in important phenotypes regarding their ability to resist the host’s innate immune response and also their ability to interact specifically with different host-cell surfaces. The most abundant and best-studied sialic acid is N-acetyll neuraminic acid (Neu5Ac, Fig. 1), although there are numerous naturally occurring variations including substitutions at carbon 5, or covalent modifications of the sugar’s hydroxyls (for a more general overview of the diversity of sialic acid forms in nature see Angata & Varki, 2002). Most experimental work on bacterial sialic acid metabolism relates to Neu5Ac, therefore the term ‘sialic acid’ has been used in the literature to mean this particular chemical primarily. For the sake of simplicity we will not attempt to distinguish between these two terms; however, bacteria may encounter other sialic acid forms in their environment and process them through the same pathways as used for Neu5Ac metabolism. This review will summarize recent literature describing how pathogenic bacteria use sialic acid in their lifestyles and will draw primarily from a limited number of well-studied pathogens, including Escherichia coli K1, Haemophilus influenzae, Pasteurella multocida, Neisseria spp., Campylobacter jejuni and Streptococcus agalactiae. These bacteria use sialic acid for a variety of different purposes that play important roles in their ability to colonize, persist and cause disease in mammalian species.

Although bacterial sialic acid metabolism is not restricted to bacterial pathogens, this topic will not be covered by this review, which will rather focus on the role of sialic acid in pathogenesis [for a complete review of bacterial sialic acid metabolism, readers are directed to the excellent article by Eric Vimr and colleagues (Vimr et al., 2004)]. The mini-review will consider recent discoveries in three areas related to pathogenic bacteria and sialic acid, namely (i) how bacteria acquire sialic acid, either by scavenging from the environment or by de novo biosynthesis, (ii) how bacteria incorporate this compound into their cell-surface features, and (iii) how these sialic acid-bearing surfaces modify the interaction of the bacterium with its host.

Preparing the supply line – sialic acid acquisition and biosynthesis
Bacteria have two primary routes to obtain sialic acid: de novo biosynthesis or acquisition from the environment (Fig. 1) (Vimr & Lichtensteiger, 2002; Vimr et al., 2004). The first route is used by a number of bacteria including E. coli K1, Neisseria meningitidis and C. jejuni (Vimr et al., 2004). The metabolite UDP-GlcNAc is the precursor for sialic acid biosynthesis (Fig. 1; abbreviations for the names of metabolites are reported in the legend) and is produced by most cells, as it is used in cell wall biosynthesis; the combined actions of the NeuC and NeuB proteins convert this to Neu5Ac via ManNAc (Fig. 1).

The second source of sialic acid is the environment, which for the pathogens mentioned in this review is the mammalian host. Many pathogens secrete a sialidase that
Fig. 1. Overview of the major pathways for sialic acid utilization in bacterial pathogens. This figure summarizes the major pathways and mechanisms discussed in this review (see text for a description of each pathway) and is not meant to represent all known pathways for prokaryotic sialic acid utilization. Sialic acid is shown as Neu5Ac (inset), as most, if not all, experimental research has been carried out with this particular form, and as Neu5Ac is the most abundant form of sialic acid in nature. The site of interaction of factor H (fH) on the gonococcal cell surface is deliberately indicated in an ambiguous manner (black arrow) as it is still unclear whether the sialylated LPS is an actual binding site for fH. The monomeric O-acetylated Neu5Ac produced by E. coli NeuD is believed to enter the normal PSA biosynthetic pathway via NeuA/NeuS (Steenbergen et al., 2006); hence the omission of the activated intermediate from the figure. The light-green discontinuous arrow represents the pathways (mostly not defined) by which the LPS is exposed on the outer membrane. The asterisk indicates O-acetylation of the disialylated LPS of C. jejuni; the reaction is catalysed by the product of the gene orf11, which is defined as a sialic acid O-acetyltransferase (SOAT) (Houliston et al., 2006). The figure takes into account that the modified sialylated LPS of C. jejuni is exposed on the cell surface (Houliston et al., 2006); however, as the role of LPS sialylation in C. jejuni is not clearly established, the interaction indicated between LPS and C3 must be taken to refer solely to H. influenzae. **Abbreviations:** IM, inner membrane; OM, outer membrane; Neu5Ac, N-acetylneuraminic or sialic acid; ManNAc, N-acetylmannosamine; GlcNAc, N-acetylgucosamine; GlcN, glucosamine; Fru, fructose; PSA, polysialic acid; PEP, phosphoenolpyruvate; Lst, Neisseria LPS sialyltransferase; NanC, E. coli Neu5Ac-specific porin; Kps, E. coli PSA capsule export system; SatABCD, H. ducreyi Neu5Ac ABC (ATP-binding cassette) transporter; SiaPQM, H. influenzae/P. multocida Neu5Ac TRAP (tripartite ATP-independent periplasmic) transporter; NanT, E. coli Neu5Ac MFS (major facilitator superfamily) transporter; SatA and SatB, respectively H. influenzae and E. coli CMP-Neu5Ac synthetases (NeuA also possesses deacetylase activity; Steenbergen et al., 2006); Lic3A, Lic3B: H. influenzae sialyltransferases; SOAT, C. jejuni Neu5Ac O-acetyltransferase; NeuC, E. coli UDP-GlcNAc 2-epimerase; NeuB, E. coli Neu5Ac synthase; NeuS, E. coli polysialyltransferase; NeuO, E. coli PSA O-acetyltransferase; NeuD, E. coli Neu5Ac O-acetyltransferase; NanA, Neu5Ac aldolase; NanK, ManNAc kinase; NanE, ManNAc-6P epimerase; NagB, GlcNAc-6P deacetylase; NagA, GlcN-6P deaminase (the catabolic pathway is present in several bacteria: Vimr et al., 2004).
releases sialic acid from a diverse range of host sialoglycoconjugates (Fig. 1) (Corfield, 1992); however, other sialic acid-utilizing bacteria, such as the respiratory pathogen *H. influenzae*, lack genes for a sialidase yet are reliant on host-derived sialic acid (Bouchet et al., 2003). Presumably free sialic acid is made available to such pathogens by other, sialidase-expressing bacteria living in the same niche (Shakhnovich et al., 2002), or, as hypothesized by Sohanpal et al. (2004, 2007), by host sialidases that are activated in the course of inflammation. The latter process is part of the normal recycling of sialic acid and there has been a recent suggestion that the host cells might use free sialic acid to help them cope with oxidative stress (Iijima et al., 2007).

Regardless of the mode of release of free monomeric sialic acid, bacteria must then capture this resource using specific transporters (Fig. 1). The one exception to this is *Neisseria gonorrhoeae*, which appears to have obviated the need for uptake by secreting the enzymes that sialylate its LPS and by using the activated form of sialic acid, CMP-Neu5Ac, from the host as a substrate (see next section). Our understanding of sialic acid transport in bacteria has until recently been quite poor and, at the molecular level, limited to the characterization of the NanT sialic acid transporter from *E. coli* K-12 (Vimr et al., 2004). This is a classical secondary transporter of the major facilitator superfamily (MFS) and is essential for Neu5Ac uptake by *E. coli* (Fig. 1) (Vimr et al., 2004). The route of sialic acid uptake in *H. influenzae* and *P. multocida* has recently been established; it is dependent on a tripartite ATP-independent periplasmic (TRAP) transporter, SiaPQM (Steenbergen et al., 2005; Allen et al., 2005; Severi et al., 2005). This family of transporters are unusual as they are secondary transporters like NanT, but utilize an extracytoplasmic solute receptor (ESR) protein in the transport mechanism (Fig. 1). The ESR component of the *H. influenzae* sialic acid transporter, SiaP, binds sialic acid with high affinity and specificity (Severi et al., 2005), and the structure of this protein revealed a fold not dissimilar to that seen in other ESR proteins from ATP-binding cassette (ABC) transporters (Muller et al., 2006). These data suggest that SiaPQM functions as a high-affinity transporter in vivo, which would be consistent with the scavenging requirements of *H. influenzae*. The sialic acid transporter from *Haemophilus ducreyi* (SatABCD) has also been characterized; interestingly, it is not a TRAP transporter but rather is an ABC transporter and hence presumably of high affinity (Fig. 1) (Post et al., 2005). Therefore it appears that bacteria have evolved multiple routes to capture sialic acid from the environment, supporting the importance of this process in vivo. This is certainly true for organisms such as *H. influenzae* and *H. ducreyi*, which are unable to synthesize sialic acid, and rely on their respective transporters for the provision of sialic acid to the LPS sialylation pathways (Severi et al., 2005; Allen et al., 2005; Post et al., 2005). As we explain below, LPS sialylation is an important virulence factor for some pathogens, which again stresses the importance of sialic acid uptake in the lifestyle of these bacteria.

There has also been a recent characterization of the Neu5Ac-inducible porin NanC (YjhA) from *E. coli* K-12, which is essential for growth on Neu5Ac when both the general OmpC and OmpF porins are not expressed (Fig. 1) (Condemine et al., 2005). However, the occurrence of this porin is not widespread and the role of Neu5Ac-specific transport across the outer membrane of bacterial pathogens has not been established.

In addition to ‘preparing the supply line’ for making sialic acid-modified cell surfaces, some bacteria, such as *H. influenzae* and *E. coli*, can also utilize the transported sialic acid as a carbon and nitrogen source (Vimr et al., 2004; Severi et al., 2005). The pathway by which this occurs has been well characterized and uses the N-acetylmuraminate aldolase NanA that cleaves Neu5Ac to ManNAc and pyruvate (Fig. 1). ManNAc is then ultimately converted to fructose 6-phosphate and ammonia, via the action of the NanK, NanE, NagB and NagA proteins, where it can enter central metabolism (Fig. 1) (Vimr et al., 2004). How these bacteria maintain the balance between the catabolic and anabolic pathways is not clearly defined, nor is it clear how sialic acid catabolism contributes to virulence. There is evidence that in *H. influenzae* sialic acid catabolism competes with the LPS sialylation pathway for sialic acid that has been transported into the cell (Fig. 1), as demonstrated by the LPS hyper-sialylation phenotype and increased fitness in an animal model of a nanA mutant (Vimr et al., 2000). In *E. coli* K1 catabolism has the potential to compete with the polysialic acid (PSA) synthesis pathway (Fig. 1); however, whereas the *neu* genes are expressed constitutively the *nan* genes are induced by Neu5Ac (Vimr et al., 2004), and the hypothesis of tight coupling of Neu5Ac synthesis and activation by NeuB/NeuA might explain the lack of a futile cycle of Neu5Ac synthesis and degradation, and implies that the catabolic operon is expressed only when exogenous sialic acid is made available (Vimr et al., 2004). Interestingly Chang et al. (2004) have shown that the *nanAT* genes are required for *E. coli* colonization of the mouse colon, where sialic acid-rich mucin appears to be an important nutrient source in vivo. However, studies on the contribution of sialic acid catabolism to virulence using animal models must be interpreted with caution as the distribution and nature of sialic acid may be significantly different in the human host, which affects any possible extrapolation.

**Deploying sialic acid onto the battlefield – synthesis of sialic acid-containing cell-surface features**

Once obtained (by either de novo biosynthesis or acquisition from the host), sialic acid can be incorporated into cell-surface macromolecules that modulate the pathogen’s interaction with the host (see next section).
The obligatory first step in this process is the conversion of sialic acid into the activated form CMP-Neu5Ac (catalysed by CMP-sialic acid synthetases), which is then added to appropriate acceptors by linkage-specific sialyltransferases (Fig. 1). As mentioned above, N. gonorrhoeae relies instead on an outer-membrane-associated sialyltransferase to scavenge CMP-Neu5Ac directly from the secretions of the host (Fig. 1) (Shell et al., 2002).

A significant part of our knowledge about the incorporation of sialic acid into bacterial macromolecules has come from the analyses of the processes for synthesis of the PSA capsules of E. coli and N. meningitidis. In E. coli, NeuA activates Neu5Ac prior to its incorporation into the K1 and K92 capsules while the N. meningitidis orthologue carries out the analogous function relating to both capsule and LPS synthesis (Fig. 1). In E. coli, NeuS functions as the main polysialyltransferase adding Neu5Ac to oligosialic acid receptors to form the PSA capsule, which is then exported through the Kps system (Vimr et al., 2004). Amongst the other sialyltransferases, the Lic3A-homologue CpsK that adds a terminal O-acetylation (Claus et al., 2004; Steenbergen et al., 2006; Deszo et al., 2005), an emerging and exciting new feature of sialic acid metabolism also seen in unencapsulated organisms (see below). In E. coli the O-acetyltransferases NeuO and NeuD modify PSA and monomeric Neu5Ac, respectively, the latter of which can be deacetylated by NeuA acting as a bifunctional enzyme (Fig. 1) (Steenbergen et al., 2006; Deszo et al., 2005; Bergfeld et al., 2007). These new discoveries highlight the huge potential diversity of capsule structures that may be presented on the cell surface by varying the patterns of acetylated sialic acid (King et al., 2007). The only Gram-positive bacterium reported to produce a sialic acid-containing capsule is S. agalactiae, which possesses a sialyltransferase (CpsK) that adds a terminal O-acetylation of the PSA capsule to galactose within the capsule’s oligosaccharide repeat (Chaffin et al., 2005). Again, recent work has shown that this Neu5Ac residue can be modified by O-acetylation (Lewis et al., 2006).

Sialylation of the LPS is also catalysed by linkage-specific sialyltransferases. Both N. meningitidis and N. gonorrhoeae sialylate their LPS through the outer-membrane zn-2,3 sialyltransferase Lst (Fig. 1), although the enzyme is expressed at a higher level in the gonococcus (Packiam et al., 2006). LPS sialylation is also a feature of several pathogenic members of the Pasteurellaceae, including H. ducreyi, H. influenzae, Haemophilus somnus and P. multocida. H. influenzae has the greatest number of characterized LPS sialyltransferases, with up to four being present in any given strain (Fox et al., 2006). The main sialyltransferase is Lic3A (Fig. 1), which adds zn-2,3-Neu5Ac (Hood et al., 2001), and has been shown to be absolutely required for bacterial survival in the middle ear in an animal model of otitis media (Bouchet et al., 2003). Amongst the other sialyltransferases, the Lic3A-homologue Lic3B is a bifunctional enzyme that can add mono- or disialic acid to the LPS acceptor (Fig. 1) (Fox et al., 2006). C. jejuni also possesses mono- or bifunctional LPS sialyltransferases transferring either zn-2,3-Neu5Ac or disialic acid (Gilbert et al., 2000). It is of note that the terminal sialic acid residue in the disialylated LPS can also be modified by an O-acetyltransferase (Fig. 1) (Houliston et al., 2006). Sialic acid, also in its O-acetylated form, can be included as part of the oligosaccharides that are repeated to form the O-antigen of LPS in E. coli (Ali et al., 2006), although the enzymes required for this process are not well studied.

While sialylated LPS and PSA capsules have obvious benefits to bacteria in particular host compartments, for example in evading the immune response as we describe below, their presence may not always be desirable when the organism is within other compartments either inside or outside the host. Some pathogens, such as Neisseria spp., H. influenzae and C. jejuni, regulate expression of cell-surface features by stochastic genetic mechanisms relying on rapid and reversible on to off switching of the expression of key biosynthetic genes, the so-called ‘phase variation’ (van der Woude & Baumler, 2004). Phase variation also modulates O-acetylation of the PSA capsule of E. coli K1 (Deszo et al., 2005; Bergfeld et al., 2007). Such variable expression makes it likely that any population of bacteria will be expressing alternative glycoforms and that any one expression pattern might confer a fitness advantage to the relevant bacteria under any given conditions.

Contact with the enemy – alteration of host responses by sialic acid-coated bacteria

As sialoglycoconjugates can be predominant components of mammalian cell surfaces, the incorporation of sialic acid into bacterial cell-surface features (be those sialic acid-containing capsules or sialylated LPS) is hypothesized to allow bacterial pathogens to disguise themselves as host cells and thus circumvent and/or counteract the host’s immune responses through a strategy of ‘molecular mimicry’ (Harvey et al., 2001).

The PSA capsule of N. meningitidis serogroup B and E. coli K1 is poorly immunogenic and this is thought to be a consequence of its being structurally identical to the PSA chains of the mammalian neuronal cell adhesion molecule, NCAM (Vimr & Lichtensteiger, 2002). The exact mechanisms by which the PSA capsule helps evade the host immune response are not clear, but in meningococci this capsule is required for resistance against the killing effect of human serum, possibly by hindering the insertion of the complement membrane-attack complex (MAC) in the bacterial membrane (Vimr & Lichtensteiger, 2002). The PSA capsule of E. coli K1 does not confer serum resistance,
but it inhibits opsonization and phagocytosis in vivo (Fig. 1) (Vimr & Lichtensteiger, 2002). The sialylated capsule of S. agalactiae has similar inhibitory effects on phagocytosis and acts by impairing C3 deposition on the cell surface, thus preventing activation of the complement alternative pathway (Marques et al., 1992).

LPS sialylation also inhibits the complement alternative pathway in both N. gonorrhoeae and in non-typable H. influenzae (NTHi), although these two pathogens use different mechanisms to this effect (Figueira et al., 2007; Ram et al., 1998). Gonococcal sialylated LPS increases the binding to the bacterial cell surface of factor H (fH), an anti-activator of the complement alternative pathway (Fig. 1) (Ram et al., 1998). This strategy mimics the effect that sialylation has on some eukaryotic cell membranes, which self-protect from C3 attack by recruiting fH, and results in higher serum resistance (Ram et al., 1998). To date it is not known whether the sialylated LPS is an actual binding site for fH (Fig. 1) (Madico et al., 2007). Interestingly, the positive effect of LPS sialylation on fH binding requires the presence of the gonococcal porin PorB (Fig. 1), suggesting that sialylated LPS and PorB might constitute a composite epitope, or that sialylation might cause a conformational change in the LPS that unmasks novel sites in PorB (Madico et al., 2007). In NTHi, LPS sialylation inhibits deposition of C3 without entailing fH binding (Fig. 1) (Figueira et al., 2007). Restoration of virulence to an avirulent, sialic acid-free mutant of NTHi inoculated into complement-depleted chinchillas demonstrated a role for the complement in eliminating unsialylated H. influenzae in vivo (Figueira et al., 2007). Circumstantial evidence suggests that one target of C3 on unsialylated cell surfaces might be LPS, raising the possibility that sialylation acts by masking those sites (Figueira et al., 2007).

There is evidence that the sialylated bacterial surfaces of C. jejuni, N. meningitidis and S. agalactiae (LPS for the former two species and capsule for the latter) can interact with sialic acid-specific lectins of the Siglec family, which are normally expressed on the surface of cell types of the immune system (Avril et al., 2006; Carlin et al., 2007; Jones et al., 2003). This has led to the hypothesis of direct cellular interactions between these cell types and the bacterial cells, which may represent yet another way by which bacterial cell-surface sialylation might modulate the host’s immune response (Avril et al., 2006; Carlin et al., 2007; Jones et al., 2003).

Concluding remarks

We have attempted in this short review to introduce some of the interesting new research that is elucidating how bacteria build sialic acid-containing structures and the roles of these in pathogenesis. In an interesting variation on this theme, the group of Ian Blomfield (Sohanpal et al., 2007, 2004) has demonstrated a different way by which sialic acid may modify bacterial cell surfaces. In uropathogenic strains of E. coli, free Neu5Ac inhibits the phase-variable expression of type 1 fimbriae, which are known virulence factors mediating adherence to and invasion into epithelial cells (Sohanpal et al., 2004). This is achieved by antagonizing the activator effect of key N-acetylsugar-responsive transcription factors (NanR and NagC) on the fimB gene, which is in turn a regulator of the expression of the fim operon (Sohanpal et al., 2004). As sialic acid is produced by the host in the course of inflammation, it is possible that a sialidase-negative bacterium such as E. coli recognizes free sialic acid as an indicator of inflammation, and downregulates expression of adhesins in response (Sohanpal et al., 2004, 2007). These recent discoveries along with new work on sialic acid transport, sialic acid acetylation and the mechanisms of serum resistance continue to further our understanding of sialic acid in biology and specifically of how it is exploited by pathogenic bacteria for a variety of different purposes in vivo.

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