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Iron-regulated membrane proteins and bacterial virulence

E. GRIFFITHS
National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, England

Abstract. The amount of iron that might be readily available to bacteria in body fluids is extremely small. This iron restricted environment induces phenotypic changes in the metabolism and in the composition of the membrane proteins of bacteria growing *in vivo.* These changes are now providing a fresh insight into the capabilities of bacteria to multiply in host tissues and are suggesting new possibilities for targetting therapeutic and prophylactic measures.

Keywords. Bacterial virulence; iron-regulated membrane proteins.

Introduction

The ability of an invading pathogen to multiply successfully under the conditions found in the host is an essential factor in any infection. Here bacteria must produce the full complement of virulence determinants required for pathogenicity. It is, of course, well known that bacteria can alter their metabolism rapidly in response to environmental changes, and they are capable of existing in a variety of physiological states which can be quite different from one another. However, in the past the effect of the host environment on pathogenic bacteria has generally been ignored. We thus find that most investigations into bacterial virulence have been carried out with organisms grown in *vitro* under conditions that do not necessarily reflect microbial behaviour *in vivo;* this is likely to give at best only a partial picture of bacterial characteristics associated with virulence and with immune responses important for protection. The situation is now changing rapidly and we are becoming more and more aware that specialized determinants are induced only when a pathogen encounters its host.

Host factors that might be expected to influence bacterial characteristics and their ability to multiply *in vivo* include temperature, pH, osmotic pressure, oxygen tension and the availability of essential nutrients. Perhaps the best-understood property of the environment encountered by pathogens in host tissues, and its effects on bacterial properties and growth, is the availability of iron. Our understanding of the way the host normally restricts the availability of this metal and the effect of its restriction on bacterial metabolism and multiplication has increased enormously in recent years and there is now a considerable literature on the relationship between iron and pathogenicity (Bullen and Griffith, 1987).

Availability of iron *in vivo*

Although there is a considerable amount of iron present in the body fluids of humans and animals, it is known that the amount of free iron which might be readily available to bacteria, is normally extremely small (Bullen and Griffiths, 1987). Most is found intracellularly, in ferritin, haemosiderin or haem, and extra-

cellular iron in body fluids is attached to high affinity iron binding glycoproteins, transferrin in serum and lymph, and lactoferrin in external secretions and milk. A related protein called ovotransferrin is found in avian egg white. These proteins bind iron extremely tightly and ensure that virtually no free iron is available to invading bacteria.

Nevertheless, pathogenic bacteria can multiply successfully under these conditions to establish extracellular infections. They must therefore be able to adapt to this iron-restricted environment and produce mechanisms for assimilating protein-bound iron or for acquiring it from liberated haem. So far little is known about the availability of iron inside cells, although it seems in some cells at least, iron is freely available (Lawlor *et al*., 1987).

Siderophore-mediated iron uptake systems

The best-understood systems whereby bacterial pathogens assimilate iron from host iron-binding proteins are those which depend on the production of soluble, low-molecular mass, high, affinity iron-chelating compounds known as siderophores (Bullen and Griffiths, 1987; Crosa, 1989). Some siderophores are able to remove iron from iron-binding proteins and the best understood of these are those used by enteric organisms. For example *Escherichia coli, Salmonella typhimurium* and *Klebsiella pneumoniae* produce a phenolate chelator called enterobactin (enterochelin) under conditions of iron restriction. Some strains produce, in addition, a hydroxamate chelator called aerobactin. In particular many strains of *E. coli* and *K. pneumoniae* that cause septicaemias produce aerobactin, the genes for which can be located in the chromosome or on a plasmid (Crosa, 1989). It is not entirely clear why acquiring the ability to make this second siderophore confers a selective advantage on bacteria that can already make enterobactin although there have been a number of hypotheses (Bullen and Griffiths, 1987; Crosa, 1989). Mutants unable to produce relevant siderophores are unable to multiply in the presence of an iron-binding protein.

An integral part of the high-affinity iron-uptake systems based on chelators is the production of outer membrane proteins which act as receptors for ferric siderophores, as well as mechanisms for the release of chelator-bound iron (Bullen and Griffiths, 1987; Crosa, 1989; Ecker *et al*., 1986; Neilands, 1982). The strict requirement for outer membrane receptor proteins in siderophore-mediated iron uptake is shown by the fact that mutants lacking such proteins are completely devoid of transport activity (Crosa, 1989; Neilands, 1982, Grewel *et al*., 1982). Although the outer membrane receptors are siderophore specific, inner membrane components, also necessary for siderophore-mediated iron transport are less specific.

The enteric bacteria produce several new outer membrane proteins under iron-restricted conditions but so far only some have been identified as ferric siderophore receptors. For example, an 81 kDa protein in *E. coli* functions as the receptor for ferric enterobactin (Neilands, 1982; Hollifield and Neilands, 1978), and a colicin V plasmid-encoded 74 kDa protein functions as the receptor for aerobactin (Grewel *et al*., 1982; Bindereif *et al*., 1982). Most of the work on the iron-regulated outer membrane proteins of *E. coli* has been carried out with laboratory strains such as *E. coli* K12. However, it is now known that pathogenic strains produce similar new

proteins when grown *in vitro* in the presence of iron-binding proteins, and *in vivo* during infection (Griffiths *et al.,* 1983; Chart and Griffiths, 1985; Chart *et al.,* 1988). Results also show considerable qualitative and quantitative variation in the expression of iron-regulated membrane proteins by different strains of pathogenic *E. coli*; some strains produce larger amounts of these proteins than *E. coli* K12, and some produce iron-regulated proteins not seen in laboratory strains (Griffiths *et al.,* 1983; Chart *et al.,* 1988).

Although much of our understanding of iron-sequestering systems based on siderophores and outer membrane protein receptors, and of their role in bacterial virulence, has come from studies on enteric bacteria, there is increasing evidence that similar systems play an equally important role in the pathogenicity of other bacteria (Bullen and Griffiths, 1987; Crosa, 1989). Iron-regulated siderophores, membrane proteins and other iron-regulated properties have been found in many pathogens and the list continues to grow (Poole and Braun, 1988; Williams *et al.,* 1988; Carniel *et al.,* 1989; Domingue *et al.,* 1989; Fernandez-Beros *et al.,* 1989; Paul *et al.,* 1989). In many cases the function of the iron-regulated membrane proteins is unknown, although some are known to be ferric siderophore receptors. The synthesis of several factors, like bacterial toxins, is also regulated by iron (Bullen and Griffiths, 1987; Crosa, 1989).

Siderophore-independent receptor mediated iron-uptake systems

The best-understood systems used by pathogenic bacteria to assimilate iron from host iron-binding proteins are those which depend on the production of siderophores and outer membrane ferric-siderophore receptors. However, the fact that such systems are the best-understood at present does not mean that they are the most common, nor indeed the most efficient systems for sequestering iron *in vivo.* Some pathogens, like the *Neisseria* and *Haemophilus* species, use a mechanism which depends on the direct interaction between the bacterial cell surface and the iron-binding protein in a manner analogous to the reaction occurring between transferrin and the mammalian cell (Crichton and Charloteaux-Wauters, 1987; Huebers and Finch, 1987); no siderophore is involved. Perhaps the most significant feature of these systems, which distinguishes them from the known siderophore-mediated mechanisms, is the highly specific nature of the process (Mickelsen and Sparling, 1981; Mickelsen *et al.,* 1982; Simonson *et al.,* 1982; Schryvers and Morris, 1988; Morton and Williams, 1989; Schryvers and Lee, 1989). Thus the iron uptake system of *N. meningitidis* is highly specific for human transferrin and human lactoferrin and discriminates against lactoferrin and transferrin from other species. This has obvious implications for explanations of host specificity of *N. meningitidis* and for the development of an animal model (Schryvers and Gonzalez, 1989).

Although molecular mechanisms involved in iron-uptake by these organisms are not understood, it is thought that membrane receptors specific for transferrin or lactoferrin are involved. Recently, iron-regulated lactoferrin- and transferrin-binding proteins have been identified in the membrane of *Neisseria* and *Haemophilus* species (Schryvers and Morris, 1988; Schryvers, 1989; Schryvers and Lee, 1989). Of interest is the considerable molecular and antigenic heterogeneity of the meningococcal transferrin-binding protein in the many different clinical isolates of *N. meningitidis* (Schryvers and Lee, 1989; Griffiths *et al.,* 1990).

Molecular mechanisms regulating iron-controlled functions

In *E. coli* the expression of chromosomal and plasmid encoded systems controlled by iron, including some toxins, are negatively regulated *via* a global repressor protein called Fur, which uses ferrous iron as a co-repressor (Bagg and Neilands, 1987); Fur is the product of a regulatory gene *fur.* A *fur*-like system operates in several pathogens, including *Corynebacterium diphtheriae,* where it controls the synthesis of diphtheria toxin as well as other iron responsive determinants (Tai and Holmes, 1988). Binding sites for the Fur repressor protein, called the "iron-box", have been identified in several iron-regulated promoters (Bagg and Neilands, 1987; Poole and Braun, 1988; Tai and Holmes, 1988). The consensus sequence contains a highly AT-rich palindrome and deletions that disrupt the palindromic structure make the promoter unresponsive to regulation by iron (Calderwood and Mekalanos, 1988). An additional global regulatory system, based on the undermodification of several transfer RNAs, also appears to operate in *E. coli* and *S. typhimurium* (Bullen and Griffiths, 1987). Much less is known about the molecular mechanisms of iron regulation of virulence determinants in other organisms.

Conclusion

Without doubt, current developments are rapidly increasing our understanding of the critical role of iron, or the lack of it, in infection, and are producing a fresh insight into the capacity of bacteria to multiply *in vivo* and to cause disease. This information has important practical implications for vaccine production and for the development of new therapeutic measures for treating bacterial infections. For example, the identification and characterization of determinants expressed only in certain niches in the host, such as those produced under iron restricted conditions, offer the possibility of producing novel or improved vaccines containing important host-induced antigens. These may be much more relevant to host protection than antigens found on organisms grown in the usual laboratory media.

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