Microbial Pathogenesis 99 (2016) 253–263

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Host environmental signals and effects on biofilm formation

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ARTICLE INFO

Article history:
Received 23 March 2016
Accepted 18 August 2016
Available online 21 August 2016

Keywords:
Biofilm
Insulin
Catecholamine
Vitamin K
Dehydroepiandosterone (DHEA)
Progesterone
Estrogen
Escherichia coli
Candida albicans
Staphylococcus aureus
Catheter-associated infections
Quorum compounds

ABSTRACT

Biofilm matrix formation is a phenotype linked to the ability to survive a hostile host environment that includes the presence of antimicrobial peptides and serum factors. Multiple hormones and other host derived factors have been shown to function as exogenous quorum signaling compound homologs that inform microbes of their *in situ* presence, thus triggering a shift from a planktonic to the sessile biofilm phenotype. The focus of this review is to describe the impact various host-derived factors have on the initial steps required for biofilm formation, i.e., adherence to host surfaces and multiplication in the host.

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1. Introduction

Whether members of the host microbiome or pathogen, microbes evolved with their respective hosts. With few exceptions, the ability to form biofilms is a basic requisite skill these organisms, whether bacteria or fungi, require to colonize and survive the host environment. This process of biofilm formation and colonization encompasses the processes of adherence, formation of an extracellular matrix and eventual departure. A signal is required for organisms to alter their phenotypic behavior from that of a sessile

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http://dx.doi.org/10.1016/j.micpath.2016.08.015
0882-4010/

population to that of a colonizing biofilm. To date most of the focus in biofilm studies has been on the signaling compounds produced by the organisms themselves as the population size reaches a sustainable level, i.e., quorum signals. However, biofilm phenotype development, maturation rate and extent are dependent on a multitude of environmental signals. These signals include either chemical and physical indicators such as available nutrients, pH, and temperature, in addition to quorum compounds or their mimics. Since microbes are constantly processing multiple signals in their decision making process, from an evolutionary perspective, recognition of endogenous quorum signaling compounds is but a piece of the biofilm puzzle. Various studies have shown that a broad spectrum of host factors affect prokaryote and eukaryote biofilm formation and subsequent colonization. These factors include peptide hormones, e.g. insulin, steroid hormones including progesterone, estrogen, dehydroepiandrosterone (DHEA), monoamines, e.g. catecholamine and essential vitamins, including vitamin K. Recent evidence with insulin, which has been shown to be both an endogenous quorum signaling compound and an exogenous signal, indicates that microbes utilize the chemical signals produced by, or available in, the host. These chemical signals act as a microbial navigation, or GPS system, that is a signpost to the microbe indicating their location, in addition to acting as a warning to the population of the need for phenotypic change if they are to optimize their survival in a hostile host environment. An essential behavior for these pathogens and members of the host microbiome is the ability to colonize the host which requires the ability to attach to host surfaces and multiply on host tissue. A universal effect these host factors have is the acceleration, or inhibition, of this sessile growth. The focus of this chapter is to examine the role various host-connected chemical signals play in modulating microbial phenotype expression, specifically that of biofilm formation and its related steps.

2. Insulin

Insulin is a polypeptide hormone that is secreted by the beta cells associated with the pancreatic Islets of Langerhans [3]. Insulin is encoded by a single gene, but undergoes post-translational cleavage to form two polypeptide chains, the A-chain and the B-chain (Fig. 1). In humans, insulin secretion is most potently stimulated by elevations in blood glucose; a secondary secretagogue is an elevation in blood amino acids. Insulin binds to receptors on most, if not all, cell types. Insulin binding to its specific cell surface receptor triggers a cascade of events, ultimately regulating many biochemical pathways, as well as regulating transport of glucose and amino acids in some tissues. In general insulin stimulates anabolic pathways and inhibits catabolic pathways.

Insulin is a highly conserved phylogenetically ancient protein recognized and produced by organisms from all six taxonomic kingdoms. *Burkholdia* sp. along with *E. coli* have receptors for mammalian insulin. In addition, *E. coli* insulin bioactivity is blocked by both rabbit anti-guinea pig insulin and anti-insulin receptor antibodies (rabbit anti-guinea pig insulin receptor) to a degree comparable to that measured for certain vertebrates (chickens) [4–9]. The highly conserved nature of this protein in evolution, and its constitutive production and excretion throughout *E. coil*'s growth cycle argues that its presence has a fundamental function. This argument is further supported by the presence of an apparent regulatory mechanism, i.e., pitrylysin (insulin-degrading enzyme) production [10,11]. In addition to production of microbial insulin, *E. coli* responds to human recombinant insulin (Humulin®). Both mammalian and *E. coli* insulin induce phenotypic changes analogous to that reported for autoinducing quorum signaling compounds, e.g. homoserine lactones (AI-1), autoinducer-2 (AI-2: furanosyl-borate diester), autoinducer-3 (AI-3; peptide ~127 amino acid catecholamine mimic) and indole [12–14]. Thus, human insulin functions as an interkingdom quorum signaling compound inducing phenotypic behavioral changes. However, unlike the classical quorum autoinducers described by Bassler and others, it appears that insulin changes the amplitude of the glucose response enabling multiple dynamic states and stochastic switching between alternative dynamic states and phenotypes [15–19]. Mammalian insulin, an *E. coli* insulin mimic, regulates growth kinetics, sessile (biofilm formation) and planktonic (motility and chemotaxis) behavior dependent on the presence or absence of glucose [2,20–22]. How insulin regulates behavior is still largely unknown. The effects of insulin and glucose on phenotypic changes are concentration specific, as was previously measured for growth kinetics [21].

The most fundamental phenotypic response to recombinant human insulin (Humulin®) by Gram-positive and Gram-negative bacteria occurs in the form of altered generation time (increased or decreased) dependent on insulin concentration and whether glucose is present and at what concentration [21]. Growth of *E. coli* is inhibited at high insulin and glucose levels (1.0% or 5.0% glucose with 400 μU/ml or 200 μU/ml), but growth is enhanced at low insulin and glucose levels (200 μU/ml insulin with 0.1% or 0.5% glucose and 0.5% glucose with 20 μU/ml insulin). In addition, insulin significantly increases the lag growth phase of bacterial cultures [21]. Beyond effects on replication, recombinant human insulin alone promotes planktonic population phenotypes and functions as a chemorepellent. However, when combined with glucose, insulin enhances biofilm formation *via* an alteration in phenotype associated with the initial step in biofilm formation, attachment to surfaces [23–30].

Mammalian insulin with glucose affects the association of *E. coli* with glass (silica) an electronegative surface, as well as adherence to buccal epithelial and uroepithelial cells (Table 1; Fig. 2). Microscopically, mid-logarithmic *E. coli* cells grown in insulin and glucose

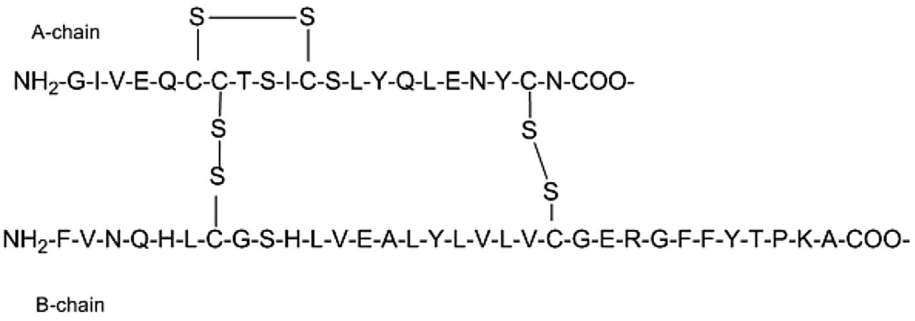

Fig. 1. Human insulin. The sequence is represented using the single letter amino acid abbreviations.