Microbial Pathogenesis 52 (2012) 85–91

Contents lists available at SciVerse ScienceDirect

Microbial Pathogenesis

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

Sugar inhibits the production of the toxins that trigger clostridial gas gangrene

M.B. Méndez [1], A. Goñi [1], W. Ramirez [1], R.R. Grau*

Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Hospital Provincial del Centenario, Universidad Nacional de Rosario, CONICET, Rosario, Argentina

ARTICLE INFO

Article history:
Received 26 March 2011
Received in revised form
24 October 2011
Accepted 27 October 2011
Available online 4 November 2011

Keywords:
Gas gangrene regulation
Clostridium perfringens
Toxin production
Carbon signalling
Wound healing
Sugar
Carbon catabolite repression

ABSTRACT

Histotoxic strains of *Clostridium perfringens* cause human gas gangrene, a devastating infection during which potent tissue-degrading toxins are produced and secreted. Although this pathogen only grows in anaerobic-nutrient-rich habitats such as deep wounds, very little is known regarding how nutritional signals influence gas gangrene-related toxin production. We hypothesize that sugars, which have been used throughout history to prevent wound infection, may represent a nutritional signal against gas gangrene development. Here we demonstrate, for the first time, that sugars (sucrose, glucose) inhibited the production of the main protein toxins, PLC (alpha-toxin) and PFO (theta-toxin), responsible for the onset and progression of gas gangrene. Transcription analysis experiments using *plc-gusA* and *pfoA-gusA* reporter fusions as well as RT-PCR analysis of mRNA transcripts confirmed that sugar represses *plc* and *pfoA* expression. In contrast an isogenic *C. perfringens* strain that is defective in CcpA, the master transcription factor involved in carbon catabolite response, was completely resistant to the sugar-mediated inhibition of PLC and PFO toxin production. Furthermore, the production of PLC and PFO toxins in the *ccpA* mutant strain was several-fold higher than the toxin production found in the wild type strain. Therefore, CcpA is the primary or unique regulatory protein responsible for the carbon catabolite (sugar) repression of toxin production of this pathogen. The present results are analyzed in the context of the role of CcpA for the development and aggressiveness of clostridial gas gangrene and the well-known, although poorly understood, anti-infective and wound healing effects of sugars and related substances.

1. Introduction

Clostridium perfringens is considered to be the most ubiquitous and widely distributed pathogen in nature [1,2]. Two key features that allow for its wide distribution and pathogenicity are its ability to produce numerous virulence-related factors and its ability to form highly resistant dormant spores [1,3,4]. Virulent *C. perfringens* isolates produce as many as thirteen different protein toxins [1–3] and a similar number of other virulence-related proteins (i.e., extracellular matrix binding proteins) [1,5] that are important for the development of different diseases (food poisoning, antibiotic-associated diarrhea and fatal gas gangrene) that are produced by this pathogen [2,4,6].

Clostridium gas gangrene (clostridial myonecrosis) is an acute and devastating infection that develops after the entrance of vegetative *C. perfringens* cells or dormant spores into the body through an injury or predisposing illness (i.e., diabetes, colon cancer) [4,7–9]. Once they are established at the site of infection, *C. perfringens* vegetative cells or their germinated spores grow quickly, resulting in a remarkable invasion and destruction of healthy living tissue due to the action of potent extra-cellular protein toxins that cause the necrosis of the host tissue [1,6,7,10–12].

Two toxins are essentials for the onset and progression of clostridial gas gangrene: alpha-toxin, also known as PLC, which is an exo-enzyme with phospholipase C and sphingomyelinase activities [10,11] and theta-toxin or perfringolysin O (PFO), a thiol-activated cytolysin [6,8]. Both toxins are thought to act synergistically to create an anoxic environment and provide essential nutrients inside the host's infected tissues as gas gangrene disease progresses (several inches per hour), despite appropriate antibiotic therapy [6–11].

Even though the clinical importance and sanitary relevance of human gas gangrene infection, our knowledge of the signals affecting toxin production in the gas gangrene process remains sparse. Many bacteria regulate virulence gene expression in response to cell population density, a phenomenon known as quorum sensing

* Corresponding author. Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, CONICET, Suipacha 531, Subsuelo Sala 9, 2000 Rosario, Argentina. Tel.: +54 341 4353377.
E-mail addresses: robertograu@fulbrightmail.org, robertograu.conicet@gmail.com (R.R. Grau).
[1] These authors contributed equally to this work.

0882-4010/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micpath.2011.10.008

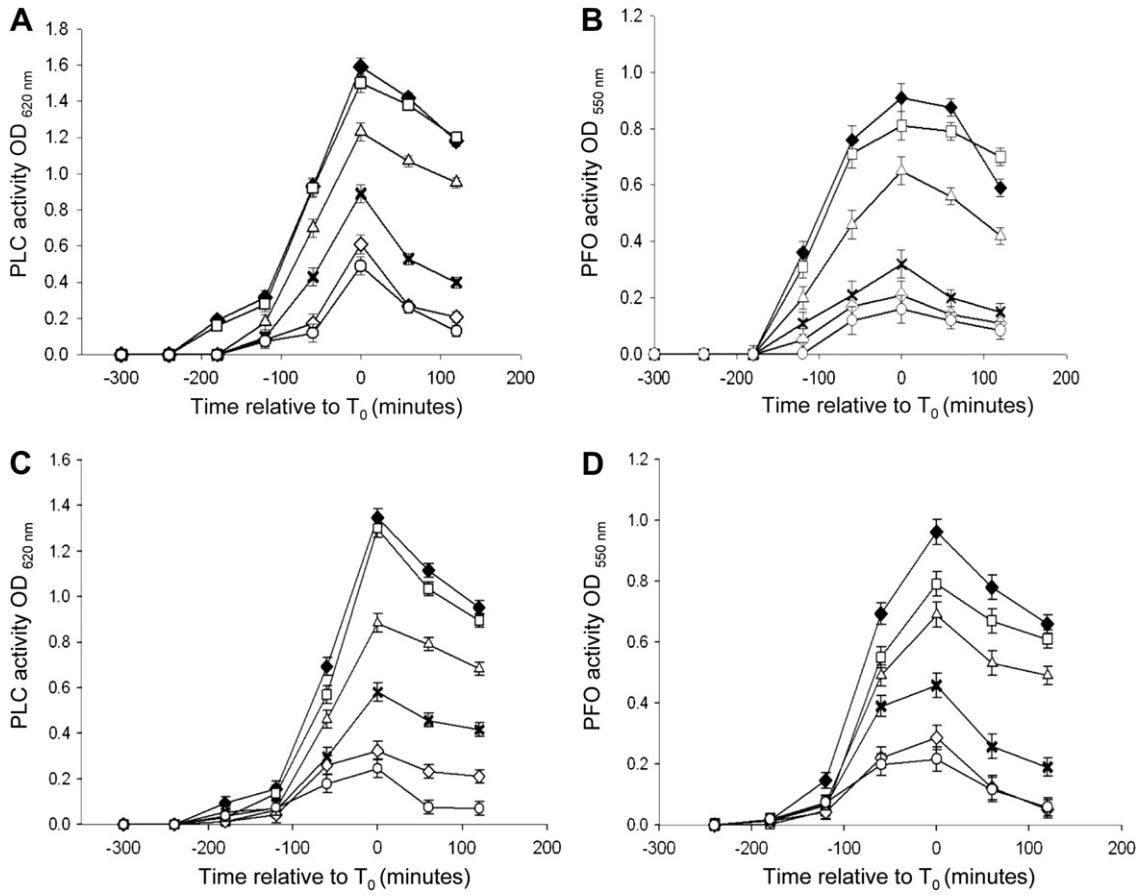

Fig. 1. Sugars regulate PLC and PFO production in *C. perfringens*. Dose-dependent responses of PLC (alpha-toxin) phospholipase activity (A, C) and PFO (theta-toxin) hemolytic activity (B, D) of cell-free supernatants of *C. perfringens* strain 13 grown in TY broth with (white symbols) or without (black symbol) glucose (A–B) or sucrose (C–D) supplementation. The assayed sugar concentrations were as follow: 0.25% (-□-), 0.5% (-△-), 1.0% (-×-), 2.0% (-◇-), and 3% (-○-). Samples were taken at the indicated times and enzymatic reactions were developed as indicated in the Methods section. The addition of glucose or sucrose, at the different concentrations, did not affect the vegetative growth while the final cellular yield of *C. perfringens* strain 13 cultures grown in the presence of sugars was slightly higher (data not shown). T_0 represents the end of the exponential phase of growth. A representative set of results obtained from three independent experiments is shown.

(QS), and environmental signals [13–15]. Recent studies have demonstrated the key role of different QS mechanisms on the toxin production of the human gas gangrene-producer *C. perfringens* strain 13 [5,16–19]. Intriguingly, these QS regulatory systems act positively on *C. perfringens* toxin production while the nature of putative signals acting negatively on toxin production remains completely unknown.

An unexplored global regulatory network that could regulate the homeostasis of gas gangrene-related toxin production is carbon availability [20]. Effectively, sugars and related substances (i.e. honey and molasses) have been used since millenary times to promote wound healing and prevent wound infection [21–26]. The explanation of the role of sugars in the treatment of wounds to prevent infection and accelerate healing is complex and perhaps impossible to reduce to a single mechanism. In a recent study, we demonstrated that sugars (glucose, sucrose and others carbon catabolites) mediated the carbon catabolite repression (CCR) of gliding motility in *C. perfringens*, a social behavior that would have a significant role during gangrene dispersion throughout tissues [4]. CCR is a widespread phenomenon in bacteria where the expression of a number of genes is regulated by the presence of a preferred carbon source such as sucrose or glucose [20]. Because toxin production is crucial for the development of *C. perfringens* gas gangrene [6–12], we explored the possibility that sugars (CCR) might constitute an important nutritional signal that regulates gas gangrene-related toxin production in *C. perfringens*.

Here, we report the effects of CCR, as mediated by sucrose and glucose, on the expression and activity of the major gas gangrene-associated toxins, PLC (alpha-toxin) and PFO (theta-toxin). We also demonstrate that the carbon catabolite control protein CcpA constitutes the unique and primary transcription factor responsible for the inhibition of toxin production in the presence of sugars. These results shed new light to the beneficial effects of sugars and related substances to prevent wound infection and accelerate its healing.

2. Results and discussion

2.1. *C. perfringens* alpha- and theta-toxin production is inhibited in the presence of sugars

To determine the effect of the presence of sugars on PLC and PFO toxin production, we first examined the effect of different concentrations (ranging from 0.25% to 3.0%) of sucrose and glucose, sugars (carbon catabolites) that are rapidly metabolized by *C. perfringens* [1,4]. For the experiments described in this work, we used the *C. perfringens* strain 13 because it is a natural isolate able to cause experimental gas gangrene, it can be easily genetically manipulated, the sequence of its genome is completed and published and is widely used as a reference strain of *C. prefringens* for research studies around the world [1,4,5,16]. Sugar (sucrose or glucose) was added at the beginning of each experience to TY