Available online at www.sciencedirect.com
SCIENCE DIRECT
Microbes and Infection 8 (2006) 1569–1578
Microbes and Infection
www.elsevier.com/locate/micinf

Original article

Differences in gene expression levels and in enzymatic qualities account for the uneven contribution of superoxide dismutases SodCI and SodCII to pathogenicity in *Salmonella enterica*

Nara Figueroa-Bossi, Serena Ammendola [1], Lionello Bossi*

Centre de Génétique Moléculaire, CNRS, 91198 Gif-sur-Yvette, France

Received 4 July 2005; accepted 17 January 2006
Available online 18 April 2006

Abstract

Most *Salmonella enterica* serovars produce two periplasmic [Cu,Zn] superoxide dismutases, SodCI, which is prophage encoded, and SodCII, encoded by a conserved chromosomal gene. Both enzymes were proposed to enhance *Salmonella* virulence by protecting bacteria against products of macrophage oxidative burst. However, we previously found SodCI, but not SodCII, to play a role during mouse infection by *S. enterica* serovar Typhimurium. Here we have extended these findings to another serovar of epidemiological relevance: sv Enteritidis. In both serovars, the dominant role of SodCI in virulence correlates with its higher levels in bacteria proliferating in mouse tissues, relative to SodCII. To analyze the basis of these differences, the coding sequences of *sodCI* and *sodCII* genes were exchanged with the reciprocal 5′-regions (in serovar Typhimurium). The accumulation patterns of the two proteins in vivo were reversed as a result, indicating that the regulatory determinants lie entirely within the regions upstream from the initiation codon. In the construct with the *sodCI* gene fused to the *sodCII* 5′-region, SodCI contribution to virulence was reduced but remained significant. Thus, both, high-level expression and some unidentified qualities of the enzyme participate in the phenotypic dominance of SodCI over SodCII in *Salmonella* pathogenicity.

Keywords: SODC; GIFSY-2; Prophage; Salmonella; Typhimurium; Enteritidis

1. Introduction

Superoxide anion is a reduced form of molecular oxygen generated by a variety of biological processes associated with aerobic metabolism [1]. Superoxide is toxic to cells, as it rapidly inactivates iron–sulfur proteins and is the precursor of highly reactive agents such as hydroxyl radical and peroxynitrite that can damage cell membranes, proteins and DNA [1]. In all aerobic organisms, from bacteria to humans, accumulation of these reactive species is thwarted by the activity of superoxide dismutases (SOD), a large family of metal-cofactored enzymes which scavenge superoxide radicals [2]. Most bacteria produce multiple forms of SOD, which differ in the nature of the metal used as cofactor and in their cellular localization. Typically, SODs that carry manganese or iron as cofactor are localized in the cytoplasm [3,4], whereas forms cofactored by copper and zinc are synthesized with a signal sequence and exported to the periplasm or to the outer membrane [5,8–13]. The extracytosolic location of [Cu,Zn] SODs suggests that these enzymes play a role in the protection against superoxide produced outside the bacterium. This being a situation encountered by bacterial pathogens exposed to the oxidative burst of professional phagocytes, considerable attention has been given to the potential role of [Cu,Zn] SODs in virulence. In most organisms analyzed, these studies have shown that inactivation of the [Cu,Zn] SOD gene, *sodC*, results in increased susceptibility to the oxidative burst in vitro and attenuation of virulence in animal models [6–9]. However, exceptions to this pattern have been reported, causing

* Corresponding author. Tel.: +33 1 69 82 3811; fax: +33 1 69 82 3877.
E-mail address: bossi@cgm.cnrs-gif.fr (L. Bossi).
[1] Present address: Dipartimento di Biologia, Università di Roma Tor Vergata, 00133 Rome, Italy.

1286-4579/$ - see front matter © 2006 Elsevier SAS. All rights reserved.
doi:10.1016/j.micinf.2006.01.015

the involvement of [Cu,Zn] SOD (SodC) in pathogenesis to remain equivocal [10–12].

Salmonellae are enteric bacteria that infect a wide range of animals from reptiles to mammals. Most of the isolates from warm-blooded animals are grouped into a single species, *Salmonella enterica*, and classified according to their antigenic formula into serovars. *Salmonella* serovars differ greatly in host range and in the type of disease they cause. Some can infect hosts as distant as birds and humans; others show a variable degree of adaptation to specific hosts [13]. Broad-host range serovars Typhimurium and Enteritidis cause gastroenteritis in most of their hosts and a systemic disease resembling typhoid fever in rodents [13]. The pathogenicity of *Salmonella* is tightly linked to its ability to survive and replicate inside host macrophages. Bacteria have evolved a variety of strategies for eluding or countering the oxidative burst of macrophages. One of these strategies relies upon the detoxifying activity of periplasmic [Cu,Zn] SODs. Most *S. enterica* serovars harbor two (occasionally three) genes for such enzymes [14,15]. The *sodCI* gene is carried by a prophage and presumably horizontally acquired [15,16]. The *sodCII* gene lies within a conserved portion of the genome and is the orthologue of the sole *sodC* gene of *Escherichia coli* K12. Mature SodCII protein shares 85% identity with *E. coli* SodC but only 62% identity with SodCI. The two *Salmonella* enzymes also differ in their quaternary structure; SodCI is a homodimer, whereas SodCII, by analogy to its *E. coli* orthologue, is most likely a monomeric protein [17]. A number of independent studies have substantiated the contribution of SodCI enzyme to *Salmonella* pathogenicity. Mutants of this protein in serovars Typhimurium, Dublin and Choleraesuis were attenuated in mice and more susceptible to killing by activated macrophages [6,7,16]. Similar analyses with *sodCII* mutants have yielded conflicting results. Some authors have found *sodCII* mutants less virulent than the respective parental strains [14,18,19]; others have provided evidence for the lack of such effects [20,21]. These discrepancies remain unexplained, although they appear to correlate with the type of allele used. The evidence linking SodCII to pathogenicity largely rests on the attenuated phenotype of a single allele, which results from plasmid DNA insertion in the distal portion of the gene [14,18,19]. In contrast, the analysis of *sodCII* deletion mutants provided no evidence for the involvement of the protein in *Salmonella* virulence [20,21]. It was suggested recently that the truncated form of the SodCII protein resulting from DNA insertion might produce a dominant toxic phenotype [20] (see Section 4). Results presented below further strengthen the notion that SodCI, but not SodCII, contributes to virulence in *S. enterica*. These findings raise the question of the basis for the differential roles of the two enzymes in mouse infection. The analysis of bacteria growing inside host cells showed SodCI levels to be much higher than SodCII levels, suggesting that differential accumulation of the two proteins in vivo accounts for their uneven contribution to virulence [21]. On the other hand, in a more recent study, SodCI retained its predominant role even when expressed from the *sodCII* promoter, suggesting that unique properties of the enzyme are responsible for the phenotypic dominance [20]. This study did not assess the effect of the promoter exchange on in vivo expression levels and thus could not determine whether SodCI specific properties related to enzyme function or protein stability in vivo. To address this point, in the present work, the structural portions and regions preceding the initiation codon of *sodCI* and *sodCII* genes were exchanged in strains in which these genes are fused to epitope tags, and the levels of both proteins measured in vitro and in vivo. In parallel, similar constructs made without tags were used to assess the effects of the exchange on the contribution of either enzyme to virulence. This work showed that the determinants of SodCI and SodCII differential accumulation in vitro and in vivo lie entirely in the region upstream from the coding sequence. In agreement with the previous report [20], SodCI still contributed to virulence in the hybrid configuration in spite of being poorly expressed in vivo. However, this contribution was noticeably lower than that in the native context suggesting the need of high-level expression for the protein to optimally fulfill its task during infection. This conclusion was further supported by the detection of a small, but significant participation of SodCII in virulence in the strain carrying the *sodCII* gene fused to *sodCI* 5′-region.

2. Materials and methods

2.1. Strains and growth conditions

S. enterica strains used in this work (Table 1) were isogenic derivatives of serovar Enteritidis strain LK5 or serovar Typhimurium strain ATCC14028. Strains were constructed by transduction using phage P22 HT 105/1 *int-201* as described [21]. Bacteria were cultured in LB broth (1% bacto tryptone (w/v), 0.5% Difco yeast extract (w/v), 0.5% NaCl (w/v)) solidified by the addition of 1.5% (w/v) Difco agar when needed. Antibiotics were used at the following concentrations: spectinomycin dihydrochloride, 80 mg/l; chloramphenicol, 10 mg/1; kanamycin monosulphate, 50 mg/l; sodium ampicillin, 50 mg/l. Liquid cultures were grown in New Brunswick gyratory shakers, and growth was monitored by measuring the optical density at 600 nm with a Milton–Roy Spectronic 301 spectrophotometer.

2.2. Plasmids

Plasmids pKD3, pKD4 and pKD13 [22] pSUB11 [23], pSUB13 and pBT22-spec were used as DNA templates for the polymerase chain reaction (PCR). Plasmid pSUB13 is a derivative of plasmid pKD13 carrying the FLAG epitope sequence adjacent to the *flp*-deletable cassette. Plasmid pBT22-spec is a ColE1 plasmid containing the spectinomycin-resistance *aadA* gene. Plasmid pkD46 [22] was the source of λ Red recombinase.

2.3. Use of the λ Red recombination method: general aspects and nomenclature

Most of the constructs used in this work were made by the λ Red recombination protocol [22,24] as implemented by Ho