MOLECULAR CHARACTERISATION AND DIAGNOSIS

Detection and characterisation of the genes encoding glyoxalase I and II from *Neisseria meningitidis*

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Glyoxalase enzymes I and II are involved in a detoxification process consisting of conversion of reactive dicarbonyl compounds (e.g., methylglyoxal) to less reactive hydroxy acids. The structural gene for meningococcal glyoxalase I (*gloA*) was identified by screening an expression library with a rabbit antiserum. The meningococcal *gloA* gene consisted of 138 deduced amino acids, with a calculated mol. wt of 15.7 kDa. The DNA and deduced protein sequence of *gloA* was compared to known sequences of glyoxalase I enzymes and showed high homology with *gloA* of several eukaryotic and prokaryotic species. Insertion of a *gloA*-containing plasmid in *Escherichia coli* increased the host organism's tolerance to methylglyoxal from <2 mM to >4 mM, thus demonstrating its functional identity. A databank search also revealed the presence of a putative *gloB* gene, encoding glyoxalase II (GlxII), in the recently released genomic sequences of *Neisseria meningitidis* and *N. gonorrhoeae*.

Introduction

Glyoxalase I (GlxI; *S*-D-lactoylglutathione methylglyoxal lysase) and glyoxalase II (GlxII; *S*-2-hydroxyacylglutathione hydrolase) are the enzyme components of the glyoxalase system. GlxI catalyses the formation of *S*-D-lactoylglutathione from hemithioacetal, formed non-enzymically or enzymically from methylglyoxal (2-oxo-aldehydes) and reduced glutathione, as shown in Fig. 1 [1]. GlxII catalyses the hydrolysis of *S*-D-lactoylglutathione to D-lactic acid and regenerates reduced glutathione as shown in Fig. 1.

The physiological role of the glyoxalase system is still not completely defined, but it is evident that it represents a detoxification mechanism that inactivates the electrophilic 2-oxo-aldehydes. Many compounds, both aliphatic and aromatic, containing the di-carbonyl function have been tested and found to serve as substrates for the enzyme. The primary physiological

substrate of the glyoxalase system is methylglyoxal (MG). The latter is formed non-enzymically from dihydroxyacetone phosphate and glyceraldehyde-3-phosphate [2]

GlxI has been found in a wide variety of species, including mammals, prokaryotes and plants. The molecular mass of the enzyme from the different sources varies between 20 and 48 kDa. The enzyme derived from yeasts and bacteria appears to be monomeric, whereas the mammalian enzyme is a dimer with two identical subunits. The active site of GlxI contains a glutathione recognition site, general base catalyst and a proximate zinc ion, Zn²⁺ [3]. GlxII has also been found in most tissues of mammals, as well as in bacteria and plant species. To date, neither of these two enzymes has been reported in meningococci. This report describes the detection and characterisation of GlxI and a putative GlxII of *Neisseria meningitidis* following the screening of a genomic expression library.

Materials and methods

The methods for bacterial growth, genetic library screening, DNA sequencing and analysis were as described by Kizil *et al.* [4].

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Fig. 1. Detoxification of methylglyoxal.

Assay for glyoxalase I activity

A stock solution of 1 M methylglyoxal (MG; Sigma) was prepared in Luria Bertani Broth (LBB; Oxoid) and filtered through a 0.45- μm filter. A series of 180- μl volumes of LBB, containing increasing concentrations of (0–16 mM) of methylglyoxal, was put in rows of a 96-well microtitration plate. A 20- μl inoculum of each overnight culture (10^8 organism/ml) was added to each of these and incubated at 37°C (stationary). These *Escherichia coli* were transformants containing pBlue-script with or without meningococcal *gloA*. At different time intervals, 20- μl samples were taken in duplicate from each well, diluted (10^1 – 10^4) in phosphated-buffered saline (PBS) and 20- μl volumes of each dilution were seeded on to blood agar. The plates were incubated overnight, then the numbers of cfu/ml were counted and the $\log_{10}$ cfu of the average of the duplicates was documented. Where a clear reduction in cfu counts was detected, the test was repeated to obtain six cfu counts and their p value was calculated by a Sign test, as described previously [5].

Results and discussion

In a recent investigation, a meningococcal expression library was screened in this laboratory with rabbit polyclonal antibodies raised against meningococcal proteins [4]. Several clones of recombinant DNA were identified and rescued, *in vivo*, into pBlue-script plasmids and fully sequenced. One of these meningococcal DNA fragments (clone C1) contained an open reading frame (ORF) that showed a high degree of DNA and deduced amino-acid homology with the *gloA* gene of several bacterial and eukaryotic species [4]. This ORF (named meningococcal *gloA*) consisted of

138 deduced amino acids with an estimated mol. wt of 15.7 kDa (acc. no. Y14298). The deduced protein sequence showed homology with GlxI of *Salmonella typhimurium* (67%) [6], *Lycopersicon esculentum* (52%) [7], *Pseudomonas putida* (50%) [8] and *Homo sapiens* (47%) [9]. Fig. 2 shows the protein sequence alignment of the GlxI of these organisms, which confirms the presence of several regions of sequence identity among them. These regions were analysed by a Basic Local Alignment Search Tool (BLAST) search of the NCBI non-redundant database, with the default parameters [10].

It was important to confirm the functional attributes of the identified meningococcal gene. Therefore, the effect of the meningococcal *gloA* gene on the sensitivity of an *E. coli* strain (XL1-Blue) to methylglyoxal (MG) was tested. As shown in Fig. 3a, the growth rate of *E. coli* containing pBlue-script plasmid (no meningococcal DNA insert, negative control) was inhibited in the presence of MG in the medium. MG at 4 mM and 16 mM concentrations completely inhibited the growth of the cells after 2 and 1 h, respectively, with no subsequent recovery. In the presence of 2 mM MG, $\log_{10} > 4$ growth inhibition was observed in the first 3 h, which was sustained over 24 h.

The *E. coli* strain transformed with pSKC1 (containing meningococcal *gloA*), showed greater resistance to MG (Fig. 3b). In 2 mM MG, there was very little, if any, reduction in growth rate. At a concentration of 4 mM MG, there was an early reduction of $\log_{10} > 3$ in growth rate ($p < 0.01$), which recovered during the subsequent 22 h, reaching levels comparable to the control ($\log_{10} < 1$, below control). At 16 mM MG, growth reached complete inhibition very rapidly (within 2 h) ($p < 0.001$). However, growth was observed to

193

recover transiently during the next hour, but this recovery was not sustained (Fig. 3b).

Based on sequence comparison, GlxI enzyme appeared to contain a flexible peptide loop near the active site that is structurally (and functionally) analogous to the catalytic loop of chicken muscle triosephosphate isomerase (TIM) [11]. This region includes residues 100–111 in man, which correspond to residues 56–65 in meningococci. Furthermore, Glu-100 of human GlxI (corresponding to Glu-56 in meningococci) might be the active site base involved in the enediol-proton-transfer mechanism proposed for GlxI enzyme.

GlxI is dependent on bivalent metal ions for catalytic activity. The essential metal of the native enzyme has

Fig. 3. Growth curve of *E. coli* strain XLI-Blue containing (a) pBluescript (negative control) or (b) pSKC1 in the presence of increasing concentrations of methylglyoxal; △, no MG (negative control); ▲, 2mM; ●, 4mM; □, 16mM.

been identified as zinc [12]. Previous studies also suggest that Zn^{2+} appears to bind three-to-five (often four) co-ordination ligands, and the types of ligands consist of histidine, cysteine, glutamate, aspartate or water [13]. Clugston *et al.* [6] also identified possible zinc-binding residues, based on the available GlxI sequences and their alignments. In *S. typhimurium* Zn^{2+} ligands were identified as His-74, Cys-86, Asp-115, Asp-117 and Glu-122 [6], which appear to be conserved in meningococci (Fig. 2).

The amino-acid sequence of the meningococcal GlxI was also used to search the genomic sequence of *N.*

gonorrhoeae (University of Oklahoma, Ok, USA) and a putative GlxI with 97% (395 of 406) identity to meningococcal GlxI, was identified.

It was of interest to see whether *N. meningitidis* possesses *gloB*, the gene encoding GlxII. The first *gloB* gene was isolated from human liver and had a mol. wt of 28 kDa [14]. Recently, a *gloB* gene was also identified and characterised in the yeast *Saccharomyces cerevisiae*, with a mol. wt of 31 kDa and 59% identity at protein level to its human counterpart [15]. Here, the human GlxII amino-acid sequence was obtained and used to search the genomic sequence data released for *N. meningitidis* (Sanger, Wellcome). A putative *gloB* gene was identified with 250 deduced amino acids and an estimated mol. wt of 27.8 kDa. Again, a high level of conservation was observed and the deduced protein showed 35% identity with human GlxII. The functional attributes of this gene can be determined following cloning and expression of biologically active forms of the encoding protein and tested in an isolated system, such as *E. coli*. However, ideally, the function of GlxI and GlxII would be better characterised by testing the ability of isogenic *gloA* and *gloB* deletion mutants of *N. meningitidis* to survive increasing concentrations of methylglyoxal, compared to the wild-type strain. These experiments were beyond the objectives of the present study. In conclusion, this is the first identification of the presence of the glyoxalase system in *N. meningitidis* and *N. gonorrhoeae*.

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