FEMS Microbiology Letters 250 (2005) 195–200

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

Peptidoglycan precursor pools associated with MraY and FtsW deficiencies or antibiotic treatments

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Received 13 January 2005; received in revised form 9 June 2005; accepted 6 July 2005

First published online 15 August 2005

Edited by R.A. Bonomo

Abstract

Blocking peptidoglycan synthesis in *Escherichia coli* with moenomycin or vancomycin led to the accumulation of UDP-MurNAc-pentapeptide and of its immediate upstream precursors, whereas with cephaloridine or penicillin G the pool of UDP-MurNAc-pentapeptide decreased. With MraY and FtsW deficiencies the decrease of UDP-MurNAc-pentapeptide was accompanied by an increase of the upstream nucleotide precursors and the appearance of UDP-MurNAc-tetrapeptide.

Keywords: Peptidoglycan precursors; FtsW; Moenomycin; Vancomycin; β-lactams

1. Introduction

The assembly of the disaccharide-peptide monomer unit of bacterial peptidoglycan proceeds by a well defined linear sequence of cytoplasmic and membrane reactions involving nucleotide precursors and lipid intermediates [1]. After translocation to the outer side of the cytoplasmic membrane its polymerization involves transglycosylation and transpeptidation reactions [2,3]. The nucleotide precursors have been shown to accumulate by interference with a downstream step mainly by an antibiotic or a conditional lethal mutation [ref. in [4–6]]. In particular, the blocking of a membrane or polymerization step will lead to accumulation of the last cytoplasmic precursor, UDP-MurNAc-pentapeptide, as initially established with penicillin-treated *Staphylococcus aureus* [7].

In *Escherichia coli* the accumulation of precursors upstream of the pentapeptide nucleotide has been observed with conditional lysis mutants [8]. Surprisingly, no mutants had been found with an increased UDP-MurNAc-pentapeptide pool under the non-permissive conditions, although the large collection considered most certainly contained mutants in the membrane or polymerization steps. Moreover, penicillin G, an inhibitor of the transpeptidation reactions [3], has not been shown to lead to increases of the UDP-MurNAc-pentapeptide pool [8,9]. On the other hand, UDP-MurNAc-pentapeptide accumulation has been observed when the two immediate downstream transferases MraY and MurG catalysing the formation of lipids I and II are blocked. This was accomplished by protein E of ΦX174 for the first [10] and by use of a conditional mutant for the second [11]. Furthermore, the inhibition of

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0378-1097/$22.00
doi:10.1016/j.femsle.2005.07.005

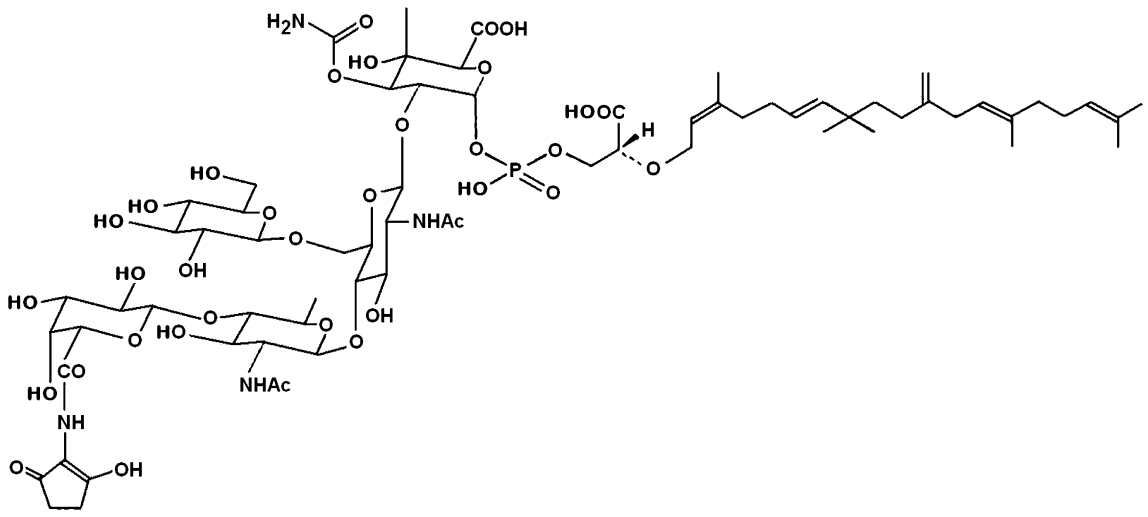

Fig. 1. Moenomycin A.

transglycosylation by moenomycin [2] (Fig. 1) promotes an increase of both the nucleotide precursor and lipid intermediate pools [9]. Blocking any of the three steps before transpeptidation seems to lead to an accumulation.

The translocation of lipid II through the membrane takes place between the MurG step and transglycosylation by a yet unknown mechanism. Recently, a number of reasons [[12–15] and ref. therein] have led to the suggestion that transmembrane protein FtsW [14–16] located in the septum of growing bacteria [17] might be involved in this process. In the present work, the precursor pool levels of conditional mutants of MraY and FtsW were compared after induction of the deficiencies. This led to complex responses differing from the previous observed variations of the UDP-MurNAc-pentapeptide pool and prompted us to study more thoroughly the effects of antibiotics inhibiting the polymerization steps.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The three *E. coli* K-12 derivatives used were grown at 37 °C under strong aeration and cells were harvested at 4 °C. MraY deficient strain DBYC2 [18] and FtsW deficient strain DBWC2 [19] kindly provided by W. D. Donachie and K. Begg, respectively, were grown in 2YT medium [20] supplemented with the appropriate antibiotics: for the *mraY* mutant ampicillin at 50 μg ml^{-1} and chloramphenicol at 25 μg ml^{-1}; for the *ftsW* mutant the same plus kanamycin at 25 μg ml^{-1}. The MraY and FtsW deficiencies were induced by transfer at an appropriate dilution of exponential phase cells growing in an arabinose-containing medium to a prewarmed glucose-containing medium devoid of antibiotics. Strain HfrH was used as wild type reference [21].

2.2. Chemicals and analytic procedures

UDP-MurNAc-L-Ala-D-Glu (UDP-MurNAc-dipeptide), UDP-MurNAc-L-Ala-γ-D-Glu-*meso*-A_2pm (UDP-MurNAc-tripeptide) and UDP-MurNAc-L-Ala-γ-D-Glu-*meso*-A_2pm-D-Ala-D-Ala (UDP-MurNAc-pentapeptide) were obtained as previously described [21]. UDP-MurNAc-L-Ala-γ-D-Glu-*meso*-A_2pm-D-Ala (UDP-MurNAc-tetrapeptide) was obtained from UDP-MurNAc-pentapeptide by action of the DD-carboxypeptidase from *Actinomura sp. strain R39* as described for the lysine-containing UDP-MurNAc-tetrapeptide [22]. Moenomycin and vancomycin were provided by Aventis and Ely Lilly, respectively. Protein contents were determined according to Bradford with bovine serum albumin as a standard [23].

2.3. Extraction and quantification of the UDP-MurNAc-peptide precursors

Extraction of the UDP-MurNAc-peptide precursors and their quantification were carried out as previously described [21].

3. Results and discussion

3.1. Growth of the MraY and FtsW deficient strains

To establish the essential nature of MraY and FtsW, Boyle et al. [18,19] constructed strains DBYC2 and DBWC2 by insertional inactivation of the chromosomal