Journal of Medicinal Chemistry and Drug Discovery

Available online at http://www.jmcdd.com
October - November, 2013, Vol. 1, No.1, pp 65-75

Research Article

MUR-A: A CRITICAL TARGET BEHIND NEW ANTIBACTERIAL DRUG DISCOVERY

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(**Received**: October 26, 2013; **Accepted**: November 23, 2013)

Abstract

The bacterial cell wall represents an attractive target site for antibiotic research as it is a fundamental structure for bacterial survival. The enzyme MurA (UDP-N-acetylglucosamine enolpyruvyl transferase, accomplishes an initial step in the cytoplasmic biosynthesis of peptidoglycan precursor molecules. It catalyzes the transfer reaction of phosphoenolypyruvate (PEP) to the 3'hydroxyl' group of UDP-N-acetyl glucosamine (UNAG) generating enolpyruvyl-UDPN-acetyl glucosamine (EP-UNAG) and inorganic phosphate. The broad-spectrum antibiotic fosfomycin – to date still the only known MurA inhibitor with clinical relevance - acts as an analogue of the substrate PEP by irreversible alkylation of the Cys115 thiol group .The MurA-dependent metabolites are of vital importance for bacteria, and the enzyme is therefore in the focus of anti bacterial drug discovery.

Introduction

An antibacterial is a compound or substance that kills or slows down the growth of bacteria. We usually associate the beginning of the modern antibacterial era with the names of Paul Ehrlich and Alexander Fleming. Infectious diseases are the leading causes of human morbidity and mortality for most of human existence.[1] Antibacterials are probably one of the most successful forms of chemotherapy in the history of medicine. They save countless lives and make enormous contribution to the control of infectious diseases since the beginning of antibacterial era. Perhaps most of us born since the Second World War don't know how much enthusiasm, dedication, and hardship have been put in antibacterial drug discovery, and take the success of antibacterial agents too much for granted. Therefore, let's first look back what the human did to combat the infections before antibacterial era and how the out- standing scientists discovered so many efficient antibacterial agents used clinically today and led us enter the antibacterial era.[2,3]As serious infectious diseases and multidrug resistance are emerging

repeatedly, new anti-biotics are needed badly to combat these bacterial pathogens, but the progress of discovery seems relatively slow. Most chemical scaffolds of antibiotics used now were just introduced between the mid-1930s and the early 1960s. There are many reasons for this. The first is scientific. We have discovered the easy-to-find antibiotics. Now we have to work harder and think more cleverly to find new drugs. Another reason is commercial. Antibiotics are used much less than other drugs and the new antibiotic are just used to treat serious bacterial infections at most of the time. So antibiotics have a poor return on investment. In 2008 only five major pharmaceutical companies still kept their Enthusiasm in antibacterial discovery. It is most important to delink research and development costs from drug pricing and the return from investment on antibacterial discovery. If the government could establish some subsidies and financial assistance schemes to compensate the cost, more drug companies will be attracted to this area.[4, 5]

Role of Mur enzymes in peptidoglycan biosynthesis

The biosynthetic pathway of peptidoglycan is a complex two-stage process. The first stage, which occurs in the cytoplasm, is the formation of the monomeric building block N-acetylglucosamine–N-acetylmuramyl pentapeptide. The first committed step in the pathway is the transfer of an enolpyruvate residue from phosphoenolpyruvate (PEP) to position 3 of UDP-N-acetylglucosamine. This reaction is catalysed by MurA. This is followed by a MurB-catalysed reduction of the enolpyruvate moiety to D-lactate, yielding UDP-N-acetylmuramate. A series of ATP-dependent amino acid ligases (MurC, MurD, MurE and MurF) catalyse the stepwise addition of the pentapeptide side-chain on the newly reduced D-lactyl group, resulting in the formation of UDP-N-acetylmuramyl pentapeptide.[6-9]

A large number of antibiotics in clinical use, mostly B-lactams and glycopeptides, act by inhibiting the later steps in peptidoglycan biosynthesis. However, the earlier steps of the biosynthesis of cytoplasmic peptidoglycan precursor are poorly exploited as antibacterial targets: none of the enzymes involved in these steps is inhibited by known antibiotics or synthetic chemicals of therapeutic usefulness, except for MurA, which is inhibited by fosfomycin. The murA to murF genes are all essential in bacteria. In addition, Mur proteins are highly conserved among various bacterial species, and common structural motifs can be identified. For this reason, a potential Mur inhibitor would be expected to be bactericidal and to have a wide spectrum, which validates the choice of these important bacterial enzymes as targets for the development of new inhibitors.[10]

Structure, catalysis and inhibitors of MurA

Structure and catalytic mechanism The crystal structures of Escherichia coli MurA complexed with UDP-N-acetylglucosamine and fosfomycin and of unliganded Enterobacter cloacae MurA are both known. MurA has two globular domains connected by a double-stranded linker According to E. coli MurA numbering, the first domain containing the catalytic site Cys-115 comprises residues 22–229, and the second domain comprises residues 1–21 and 230–419. The main-chain fold of each domain is very similar, with three parallel internal helices surrounded by three helices and three four-stranded b-sheets. The overall protein fold is very similar to that of the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, the only other known enolpyruvyl transferase. The catalytic site of MurA is situated in a deep cavity between the two globular domains. The uridinyl ring of UDP-N-acetylglucosamine is sandwiched between two hydrophobic surfaces created by Arg-120 and Pro-121 on one face and by Leu-124 on the other. These interactions, combined with the hydrogen bonds involving uridinyl base atoms N1 and O1, provide good binding specificity for this base. The kinetic mechanism of MurA transferase has been shown to involve the generation of a non-covalently bound tetrahedral phospholactoyl-UDP-N-acetylglucosamine ntermediate. The structure and chirality of this adduct were determined from the structure of the complex between the fluoromethyl tetrahedral ketal analogue and the C115A mutant of MurA.

This addition–elimination mechanism for MurA Kinetic data from another study suggested a mechanism involving the formation of a phospholactoyl–enzyme tetrahedral intermediate preceding the formation of the phospholactoyl–substrate intermediate. In this covalent enzyme intermediate, Cys-115 is attached to the C-2 of PEP to form an O-phosphothioketal intermediate. However, it was proved that the covalent intermediate is dispensable for catalysis and appears to be off the main catalytic pathway. The formation of the covalent O-phosphothioketal enzyme intermediate from MurA of E. cloacae was confirmed by solution-state and timeresolved solid-state nuclear magnetic resonance (NMR) spectroscopy. Small-angle X-ray scattering (SAXS) and fluorescence spectroscopy were used to study conformational changes in MurA upon binding to its substrates. Binding of the sugar nucleotide to the free enzyme results in substantial conformational changes, which can be interpreted as the transition from an open to a closed form. PEP also appears to induce a structural change upon its addition to the free enzyme, but this change was less pronounced than that observed upon binding with the sugar nucleotide substrate. Another site-directed mutagenesis study performed on E. cloacae MurA demonstrated that Lys-22 is crucial for enzymatic activity and for the formation of the phospholactoyl–enzyme tetrahedral intermediate. Recent phylogenetic analysis revealed two distinct classes of MurA transferases. The first class of transferases occurs throughout all bacteria except Gram-

positive Mycoplasma spp. The second type exists as a duplicate gene copy only in the low-G+C Gram-positive bacteria. Structurally and functionally, both types are highly similar, and one enzyme can substitute for the other.[11]

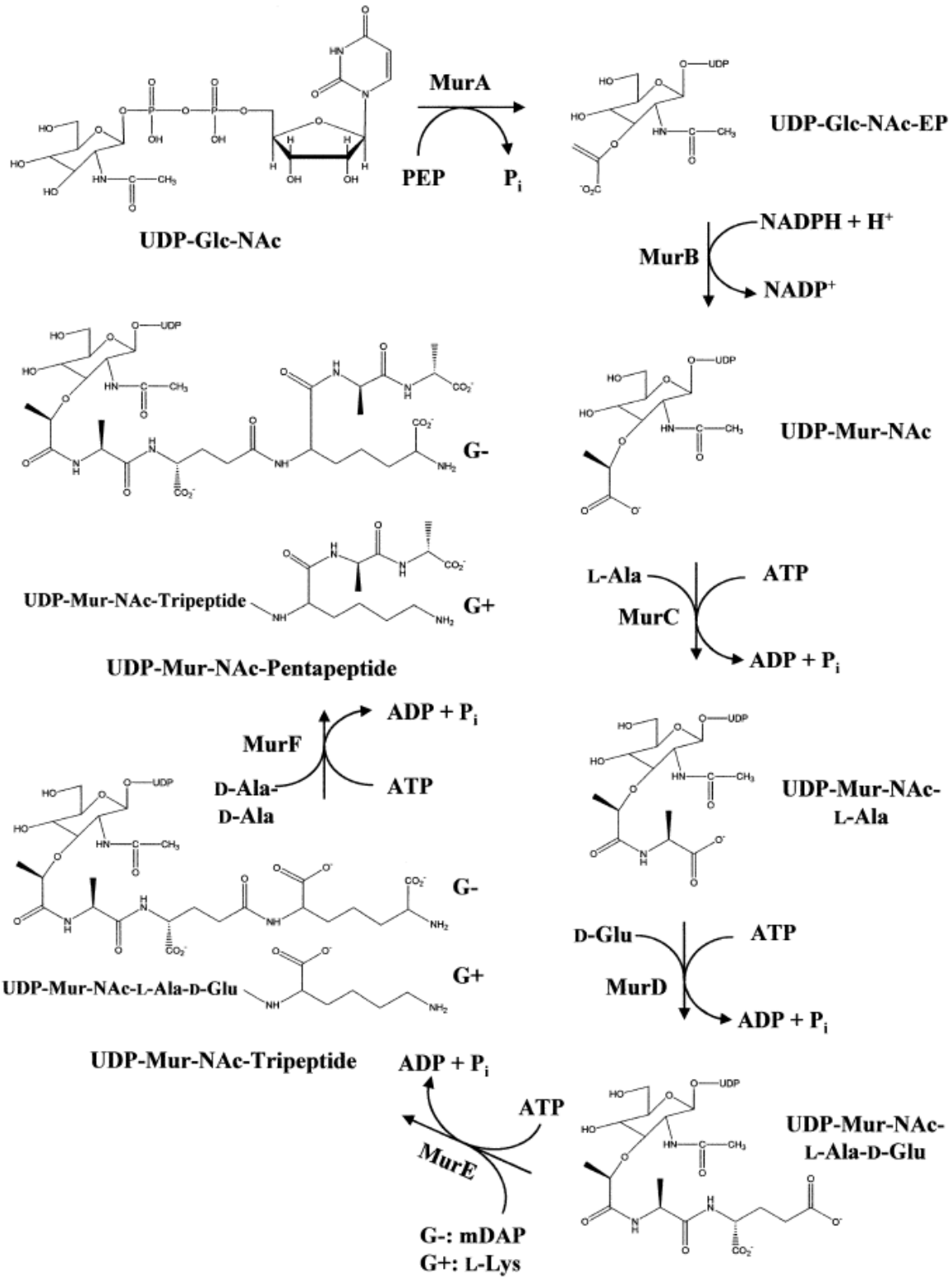
UDP-Glc-NAc
MurA
PEP
Pi
UDP-Glc-NAc-EP
MurB
NADPH + H+
NADP+
UDP-Mur-NAc
L-Ala
ATP
MurC
ADP + Pi
UDP-Mur-NAc-Tripeptide
G+
G-
UDP-Mur-NAc-Pentapeptide
MurF
D-Ala-
D-Ala
ATP
ADP + Pi
UDP-Mur-NAc-L-Ala
D-Glu
ATP
MurD
ADP + Pi
UDP-Mur-NAc-L-Ala-D-Glu
UDP-Mur-NAc-Tripeptide
MurE
ATP
ADP + Pi
UDP-Mur-NAc-L-Ala-D-Glu
G-: mDAP
G+: L-Lys

Fig. 1. Conversion of UDP-N-glucosamine to UDP-N-acetylmuramyl pentapeptide by the sequential action of MurA to MurF enzymes. UDP-GlcNAc, UDP-N-acetylglucosamine; UDP-Glc-NAc-EP, UDP-N-acetylglucosamine enolpyruvate; UDP-Mur-NAc, UDP-N-acetylmuramic acid; mDAP, meso-diaminopimelic acid; G+, Gram-positive bacteria; G–, Gram-negative bacteria.

Inhibitors

MurA is the only cytoplasmic step inhibited by a clinically used antibacterial agent. Fosfomycin a naturally occurring broadspectrum antibiotic, is the best known inhibitor of MurA. [12] Fosfomycin has been the drug of choice for the treatment of paediatric gastrointestinal infections resulting from Shiga-like toxin-producing Escherichia coli (STEC) in Japan, andthe early administration of this antibiotic is critical for the effective treatment of STEC infections. Fosfomycin is also among the first-line agents for the treatment of bacterial infections of the urinary tract, which is a common health problem, particularly in women. Inhibition of MurA enzyme by fosfomycin is competitive, the antibiotic acting as an analogue of PEP and forming a covalent bond with the active cysteine residue of the enzyme. Fosfomycin is tightly packed between the enzyme and UDP-N-acetylglucosamine, making hydrogen bonds with several different segments of the polypeptide chain. There are also hydrogen bonds between the fosfomycin hydroxyl group and the C-3 hydroxyl of the UDP-N-acetylglucosamine sugar ring and between one of its phosphonate oxygen atoms and the amide nitrogen of UDP-N-acetylglucosamine. Time-dependent inactivation of MurA by fosfomycin was found to be greatly accelerated by the presence of UDP-N-acetylglucosamine, which suggests that active site conformational changes induced by UDP-N-acetylglucosamine are essential for inactivation. AXS and fluorescence spectroscopy showed that fosfomycin did not affect the structure of the free enzyme or the sugar nucleotide-bound enzyme. However, more recent fluorescence spectroscopy studies conducted by the same researchers showed evidence of fosfomycininduced structural changes in the case of the UDPN-acetylglucosamine-liganded enzyme with a time dependence similar to that observed for the inactivation process There is a high frequency of development of fosfomycin resistance. Fosfomycin enters bacterial cells by active transport through the L-a-glycerophosphate (a-GP) uptake system and the glucose-6-phosphate (G6P) uptake system. Chromosomally encoded fosfomycin-resistant strains have an impairment in fosfomycin uptake a low-affinity transferase enzyme or overproduction of the enzyme Fosfomycin resistance encoded by plasmids results from enzymatic modification of the antibiotic in some clinical isolates of Serratia marcescens, Klebsiella pneumoniae, E. cloacae and Staphylococcus epidermidis Owing to the importance of fosfomycin in the treatment of STEC infections, the emergence of fosfomycin-resistant STEC isolates has become a significant problem in antibiotic therapy for these

infections. A recent report suggests that the fosfomycin resistance in these STEC isolates results from concurrent effects of alteration of the a-GP uptake system and/or the G6P uptake system and of the enhanced transcription of the murA gene. [13,14] It has been suggested that the resistance of Mycobacterium tuberculosis to fosfomycin results from the presence of an aspartate residue instead of the cysteine residue at the active site of the enzyme. Replacement of this aspartate residue by a cysteine reversed the resistance of the enzyme to inhibition by fosfomycin . In addition, replacement of the active Cys 115 in the E. coli enzyme counterpart by aspartate and, to a much lesser degree, by glutamate maintained enzymatic activity but conferred complete resistance to the time-dependent inactivation. Three novel inhibitors of the E. coli MurA enzyme, a cyclic disulphide, a purine analogue and a pyrazolopyrimidine, were identified recently. When preincubated with MurA, these compounds showed IC50s lower than that of fosfomycin. [15] The presence of UDP-N-acetylglucosamine during preincubation lowered the IC50 at least fivefold, suggesting that, like fosfomycin, the three compounds may interact with the enzyme in a specific fashion that is enhanced by UDP-N-acetylglucosamine. [16] The three compounds have no apparent structural similarity to fosfomycin. The three compounds exhibited antibacterial activity, but not caused specifically by MurA inhibition, as inhibition of DNA, RNA and protein synthesis was observed in addition to the inhibition of peptidoglycan biosynthesis. The minimum inhibitory concentrations (MICs) of the compounds against test strains were of the same order as fosfomycin. The compounds are proposed to be tightly, but not covalently, associated with MurA. They seem to bind to the MurA enzyme at or near the site at which fosfomycin binds.[17]

Conclusion

The extensive use of antibiotics in hospitals and community since their introduction into medical practice has created major evolutionary pressures in bacteria to develop various resistance mechanisms. This phenomenon has led to increased morbidity, mortality and health care costs. The search for new antibacterial agents directed towards novel targets became highly imperative. The biosynthetic pathway of cytoplasmic peptidoglycan precursor is currently gaining much interest as a target site for antibacterial

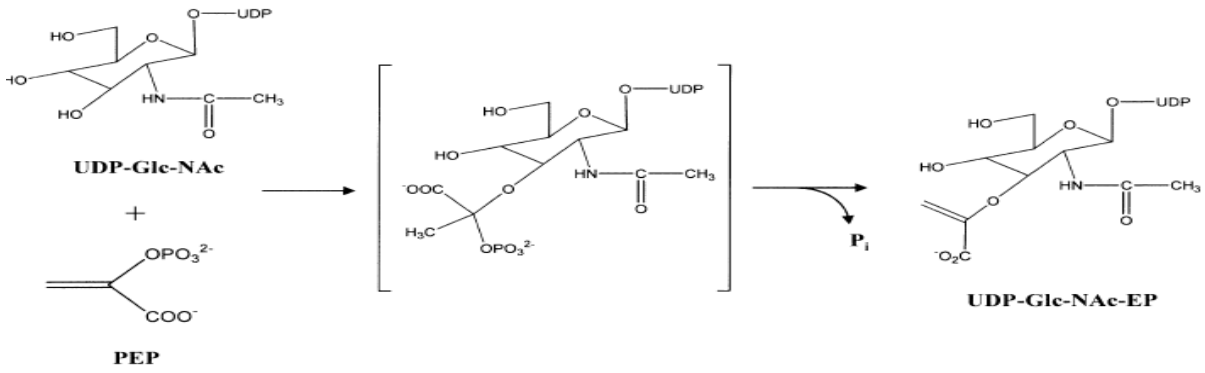

Fig. 2. Addition–elimination mechanism for MurA involving the formation of a tetrahedral phospholactoyl-UDP-N-acetylglucosamine adduct .

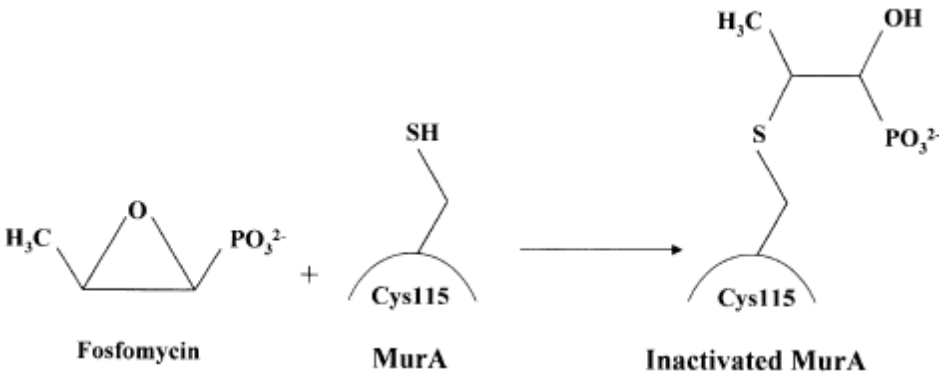

Fig. 3. Inactivation of MurA by fosfomycin as a result of the covalent linkage between Cys-115 of MurA and fosfomycin.

Many inhibitors of the Mur cytoplasmic enzymes have been reported, but none has yet led to the development of a clinically utilized therapeutic agent. This review offers an overview of the structural features and the catalytic functions of MurA essential bacterial enzymes, as well as the progress made to date in the conception of Mur inhibitors. A better understanding of the structural characteristics and the elucidation of the mechanisms of Mur enzymes would provide valuable insight into the search and rational design of a new generation of specific bacterial cell wall inhibitors.

Acknowledgement:

Author wish to express his sincere thanks to Mr.Amit kolhe sir for his constant encouragement and support.

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