Bioorganic Chemistry 80 (2018) 639–648

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

Development of dilipid polymyxins: Investigation on the effect of hydrophobicity through its fatty acyl component

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ARTICLE INFO

Keywords:
Polymyxin
Lipopeptides
Amphiphiles
Antibiotic
Adjuvant
Efflux
Antimicrobial resistance

ABSTRACT

Continuous development of new antibacterial agents is necessary to counter the problem of antimicrobial resistance. Polymyxins are considered as drugs of last resort to combat multidrug-resistant Gram-negative pathogens. Structural optimization of polymyxins requires an in-depth understanding of its structure and how it relates to its antibacterial activity. Herein, the effect of hydrophobicity was explored by adding a secondary fatty acyl component of varying length onto the polymyxin structure at the amine side-chain of L-diaminobutyric acid at position 1, resulting to the development of dilipid polymyxins. The incorporation of an additional lipid was found to confer polymyxin activity against Gram-positive bacteria, to which polymyxins are inherently inactive against. The dilipid polymyxins showed selective antibacterial activity against *Pseudomonas aeruginosa*. Moreover, dilipid polymyxin **1** that consists of four carbon-long aliphatic lipids displayed the ability to enhance the antibacterial potency of other antibiotics in combination against *P. aeruginosa*, resembling the adjuvant activity of the well-known outer membrane permeabilizer polymyxin B nonapeptide (PMBN). Interestingly, our data revealed that dilipid polymyxin **1** and PMBN are substrates for the MexAB-OprM efflux system, and therefore are affected by efflux. In contrast, dilipid polymyxin analogs that consist of longer lipids and colistin were not affected by efflux, suggesting that the lipid component of polymyxin plays an important role in resisting active efflux. Our work described herein provides an understanding to the polymyxin structure that may be used to usher the development of enhanced polymyxin analogs.

1. Introduction

Polymyxins are a class of antibacterials used as drugs of last resort to treat multidrug-resistant (MDR) Gram-negative bacterial infections that are non-responsive to conventional antibiotic treatments [1]. Concerns with polymyxin's nephrotoxicity and neurotoxicity as well as the availability of less toxic alternatives hampered their widespread clinical usage in the past. However, a rejuvenated interest in polymyxins have been observed recently due to the alarming increase of MDR pathogens that are impervious to most antibiotics, but also due to improved understanding of polymyxin's pharmacokinetic/pharmacodynamic properties and how they relate to alleviating toxicity [1,2]. Polymyxin B and E, also known as colistin (Fig. 1A) are currently used in the clinic as monotherapy or as part of combination therapy with other antibiotics when standard treatment options fail [3].

The general structure of polymyxins consist of a cyclic heptapeptide core attached to a linear tripeptide with an acylated N-terminus to a fatty acid (Fig. 1A). A segregated hydrophobic and hydrophilic domains bestow polymyxins an amphiphilic nature. Polymyxin's hydrophilicity is due to the polar amine side-chains of L-2,4,-diaminobutyric acid (Dab) and hydroxyl side-chains of L-threonine (Thr). These polar side-chains, but also the peptide/amide backbone, are responsible for polymyxins' lipopolysaccharide (LPS) binding that consequently result to displacement of divalent cation bridges and outer membrane destabilization [4]. The majority of polymyxins' hydrophobic character is from the fatty acid acylated to its N-terminus. This lipid component is believed to be crucial in polymyxins' insertion into the destabilized outer membrane and its transit to periplasmic space [5], but also is responsible for disrupting the inner membrane stability [6,7]. Hydrophobicity is also imparted by L-leucine (Leu) and either D-phenylalanine (phe) in polymyxin B or D-leucine (leu) in colistin. These hydrophobic amino acids are believed to play a crucial role in LPS binding [4]. Overall, the amphiphilic nature of polymyxins results to membrane disruption that leads to intracellular component leakage and bacterial cell death. Notably, polymyxins exhibit poor activity against Gram-positive bacteria as they do not bind favorably to lipoteichoic acid

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https://doi.org/10.1016/j.bioorg.2018.07.018
Received 17 May 2018; Received in revised form 12 July 2018; Accepted 15 July 2018
Available online 19 July 2018

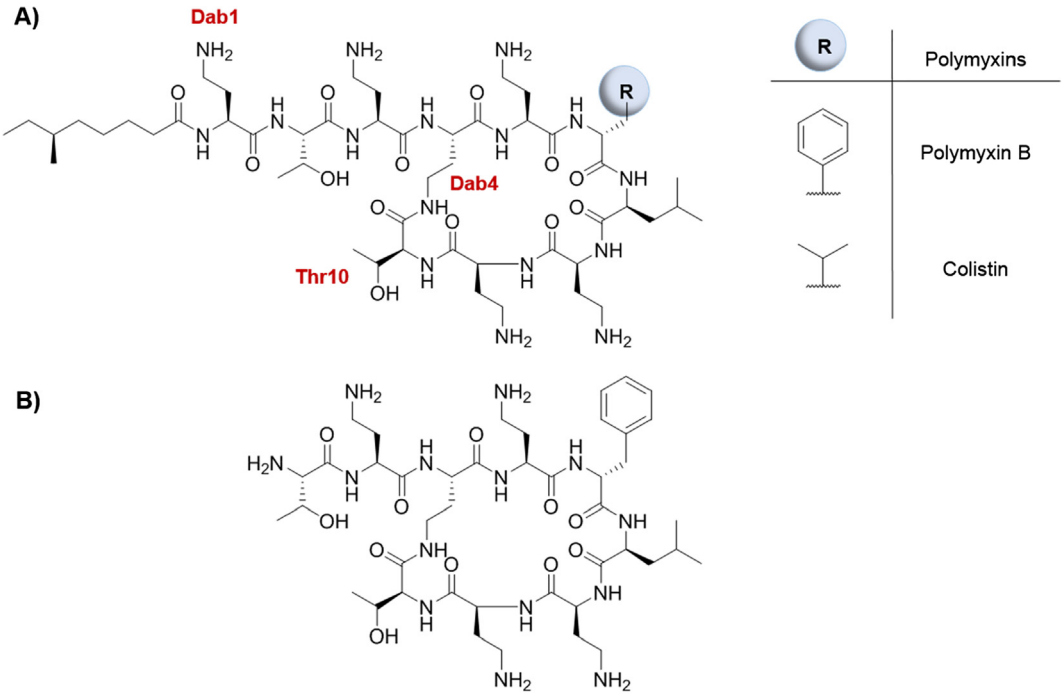

Fig. 1. Polymyxin structures: (A) polymyxin B & colistin; (B) polymyxin B nonapeptide (PMBN).

studded in the cytoplasmic membrane [8].

Structure-activity relationship (SAR) studies have generated a wealth of knowledge on the parameters which are critical/non-critical for polymyxins' antibacterial activity. For instance, alanine scanning of polymyxin B revealed several amino acid side-chains that are not crucial for activity [9]. It also has been elucidated that aliphatic hydrocarbon lipids of seven to nine carbons-long are optimal for antibacterial activity [4]. However, several aliphatic hydrocarbon non-classical isosteres such as adamantyl and aromatic functional groups may yield derivatives with similar antibacterial activity to polymyxins but with less nephrotoxicity [4,10]. Removal of the lipid and Dab at position 1 (Dab1) yields polymyxin B nonapeptide (PMBN) (Fig. 1B), which is known to permeabilize the outer membrane yet lacks the ability to kill bacteria [11]. In fact, PMBN is known to enhance the cellular entry and therefore antibacterial activity of antibiotics that suffer limited outer membrane penetration [12]. The absence of antibacterial activity in PMBN is due to the lack of lipid component crucial for interaction of polymyxins with lipid bilayers of the outer- and inner membranes of Gram-negative bacteria. Several SAR studies confirmed that hydrophobicity at certain structural points of PMBN is crucial for its outer membrane sensitization and LPS binding properties [13,14]. A derivative of PMBN called SPR741 is currently being evaluated in clinical studies as an adjuvant to enhance the efficacy of antibiotics in combination against Gram-negative pathogens [15,16]. For instance, SPR741 potentiated an extensive panel of antibiotics against *Enterobacteriaceae* and *Acinetobacter baumannii*, but not against *Pseudomonas aeruginosa* [17]. Two recently completed phase-1 clinical studies (https://clinicaltrials.gov/ct2/show/NCT03022175; https://clinicaltrials.gov/ct2/show/NCT03376529) showed SPR741 to be well-tolerated in healthy volunteers up to a single dose of 800 mg or multiple dose up to 600 mg every 8 h for 14 consecutive days.

It is evident that hydrophobicity plays a critical role in the antibacterial activity of polymyxins [4]. A recent study revealed that remodelling of outer membrane through *pagL*-induced lipid A deacylation resulted to decreased membrane interaction and penetration of polymyxins [18]. The removal of an acyl group from the typically hexa-

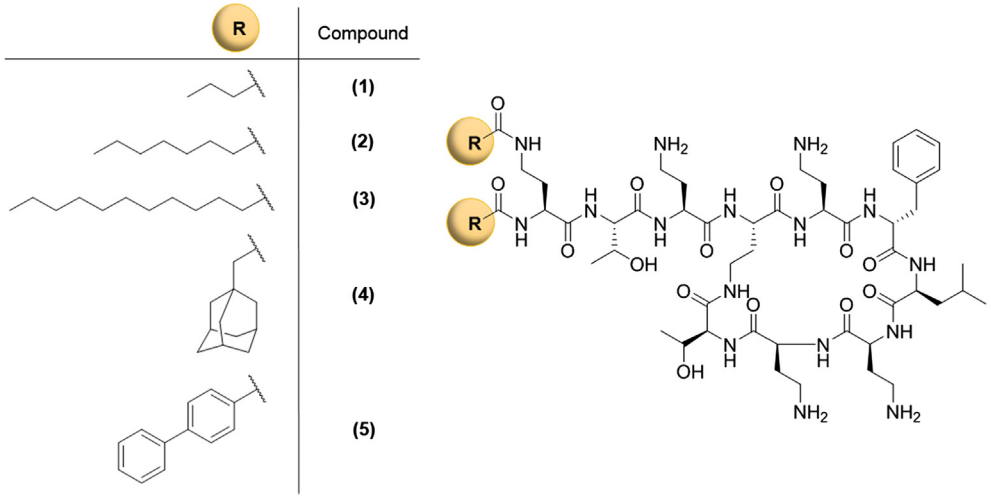

Fig. 2. Synthesized dilipid polymyxins.