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Antimicrobial activity of chemically modified dextran derivatives

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ABSTRACT

Cationic amphiphilic dextran derivatives with a long alkyl group attached to the reductive end of the polysaccharide chain and quaternary ammonium groups attached as pendent groups to the main dextran backbone were synthesized and tested for their antimicrobial properties against several bacteria and fungi strains. Dependence of antimicrobial activity on both polymer chemical composition (dextran molar mass, length of end alkyl group and chemical structure of ammonium groups) and type of microbes was highlighted by disc-diffusion method (diameter of inhibition zone) and broth microdilution method (minimum inhibitory concentrations). Polymers had antimicrobial activity for all strains studied, except for *Pseudomonas aeruginosa* ATCC 27853. The best activity against *Staphylococcus aureus* (Minimun Inhibitory Concentration 60 μg/mL) was provided by polymers obtained from dextran with lower molecular mass (M_n = 4500), $C_{12}H_{25}$ or $C_{18}H_{37}$ end groups, and *N*,*N*-dimethyl-*N*-benzylammonium pendent groups.

1. Introduction

Infections caused by bacteria are an increasing threat to human safety, causing a large number of deaths every year (Gabriel, Som, Madkour, Eren, & Tew, 2007). The complex epidemiological situation is worsened by the bacterial resistance developed against traditional antibiotics and the lack of new and more active antibiotics (Davies & Davies, 2010; Wright, 2012). Therefore, it is essential to continuously develop antimicrobial agents with novel modes of action to face the evolving resistance. Antimicrobial polymers are a class of new antimicrobial agents with a rapid expansion in the last decade (Kenawy, Worley, & Broughton, 2007; Munoz-Bonilla & Fernandez-García, 2012; Siedenbiedel & Tiller, 2012; Sobczak, Debek, Oledzka, & Kozłowski, 2013; Timofeeva & Kleshcheva, 2011). In comparison with conventional agents of low molecular weight, polymeric antimicrobials have advantages such as longer-term activity, non-volatilization, inability to permeate the skin, longer circulatory time and reduced residual toxicity to the environment (Wang et al., 2015; Waschinski et al., 2008). Several classes of materials such as biocidal polymers, biocide-releasing polymers and antibiotic-conjugated polymers were developed (Siedenbiedel & Tiller, 2012; Strassburg et al., 2015). Amphiphilic cationic polymers are macromolecular counterparts to quaternary ammonium compounds, a well known class of broad-spectrum antibacterial agents, which are constantly applied as disinfectants in medical, industrial, or household areas. Antimicrobial activity of both types of cationic derivatives is based on a similar mechanism: the positively charged molecules adsorb on negatively charged microbial cell surface, diffuse through the cell wall and interact with the cytoplasmic membrane, leading to an irreversible damage of the cell membrane integrity and eventually to the cell death. This action mechanism is considered less prone to acquired resistance (Jennings, Minbiole, & Wuest, 2015; Munoz-Bonilla & Fernandez-García, 2012).

Chemical composition and relative position of cationic and hydrophobic groups have a decisive influence on cationic amphiphilic polymers antimicrobial activity. Polymers with both groups located on the same polymer side groups (pendent type) or with cationic and hydrophobic groups located on different side chains (random copolymers) (Oda, Kanaoka, Sato, Aoshima, & Kuroda, 2011) were designed and tested for their antimicrobial activity. Some recent studies have shown that antimicrobial activity of a polymer can be tuned and enhanced by separation of a quaternary ammonium group and a hydrophobic group (a long alkyl chain) by placing them at the opposite ends of a polymer chain (poly(methyloxazoline)) (Krumm et al., 2014; Waschinski et al., 2008). This chemical structure and the length of the end alkyl groups determine the degree of cell wall penetration by

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the polymer. The results provide interesting opportunities for the development of new biologically active polymers. Recently, we designed and prepared new cationic amphiphilic polymers which combine the pendent type structure with that placing the hydrophobic group at the end of the polymer chain (Nichifor, Mocanu, & Stanciu, 2014). The new polymers are based on a biocompatible and biodegradable polysaccharide, dextran, which can be selectively modified at its reductive chain end and at its numerous OH groups. These selective chemical modifications provided dextran derivatives carrying a hydrophobic alkyl chain at the reducing end and quaternary ammonium groups with moderate amphiphilicity attached as pendent groups to dextran backbone. The polymers form aggregates in aqueous solutions, and their self-assembling ability depends on the alkyl chain length and cationic group amphiphilicity (Nichifor et al., 2014). In the present work, we tested the activity of some of these new polymers as antimicrobial agents, using several bacterial and yeast strains. The influence of polymer chemical composition (dextran molar mass, end alkyl chain length, pendant quaternary ammonium group structure) on antimicrobial activity was followed.

2. Experimental part

2.1. Materials

2.1.1. Chemicals

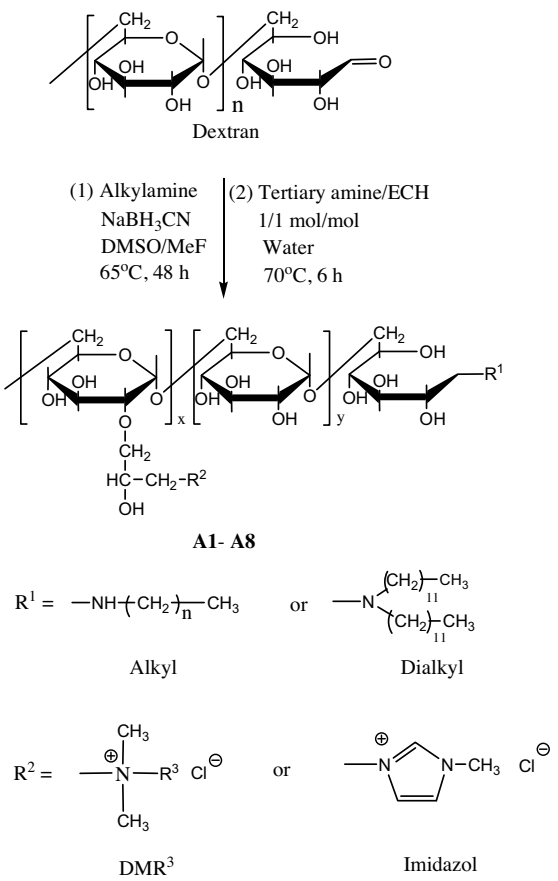
Dextran samples from *Leuconostoc mesenteroides* with molecular weights M_r (as indicated by the supplier) 6000 (D6) and 9000–11000 (D10) were purchased from Sigma. The number-average molar masses (M_n) determined by size exclusion chromatography were 4500 (D6) and 8000 (D10). All the other reagents were from Aldrich and used as received. DMSO and *N*-methylformamide (MeF) were dried on molecular sieves.

2.1.2. Microorganisms

Gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Sarcina lutea ATCC* 9341), Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and pathogenic yeasts (*Candida albicans* ATCC 90028, *Candida glabrata* ATCC MYA 2950, *Candida parapsilosis* ATCC 22019) used as reference strains were obtained from the Culture Collection of the Department of Microbiology, Faculty of Pharmacy, "Gr. T. Popa" University of Medicine and Pharmacy, Iasi, Romania. *Staphylococcus aureus* species of clinical provenience were isolated from surgical wounds (*S. aureus* 65, *S. aureus* 68, *S. aureus* 100) and blood culture (*S. aureus* 4828). *S. aureus* 68 and *S. aureus* 100 are methicillin resistant (MRSA), the other two species are methicillin sensitive (MSSA).

2.2. Polymer synthesis

Polymer synthesis was realized by a procedure described in detail elsewhere (Nichifor et al., 2014). In a first step, dextran (D6 or D10) with an alkyl end group was obtained by reductive amination of dextran reductive end with a large excess of dodecyl, octadecyl or di(dodecyl)amine, using a mixture DMSO-MeF as a solvent and $NaCNBH_3$ as a reducing agent. The product was purified by repeated precipitation from DMSO in methanol. The integrals of the specific peaks found in ^{1}H NMR spectrum (DMSO-d_6, Bruker Avance DRX 400 spectrometer) were used to calculate the modification degree with formula: $100(A_{CH3}/3)/(A_{dex}/DP_{dex})$, where A_{CH3} and A_{dex} are the integrals of the peaks assigned to the methyl protons of the alkyl chain (0.85 ppm,) and anomeric protons of dextran (4.7 ppm), respectively, and DP_{dex} is the dextran degree of polymerization. The obtained values were in the range 92–98%. The complete reduction of intermediate unstable Schiff base to amine group was proved by UV analysis (325–330 nm) performed on 0.1 wt% aqueous solutions. In the second step, the end modified polymers were reacted with an equimolar mixture of a tertiary amine (*N*,*N*-dimethyl-*N*-octylamine, *N*,*N*-dimethyl-*N*-benzylamine or 1-methylimidazol) and epichlorohydrin, in order to attach quaternary ammonium groups to the dextran main chain. The final polymers **A1–A8** were obtained by precipitation from water-methanol mixture 1/1 v/v in acetone. The general chemical structure depicted in Scheme 1 was confirmed by the presence of several peaks (^{1}H NMR, D_2O) assigned to pendent groups (-C<u>H</u>-OH at 4.2 ppm and -N-<u>CH</u>$_3$ at 3.1 ppm) besides the peaks characteristics to dextran and end group. The amino group content (expressed as mol%) was determined by elemental analysis (content in chloride ions measured by potentiometric titration with $AgNO_3$), using formula $DS = x = 100(162 \cdot Cl)/(3550 - Cl \cdot Mp)$ where *Cl* and *M*p are cloride content, in wt%, and molecular weight of attached pendent group, respectively.



Scheme 1. Synthesis pathways and general chemical structure of cationic amphiphilic dextran samples **A1–A8**. (1) Reductive amination of dextran end aldehyde group; (2) Chemical modification of dextran OH groups with formation of pendant quaternary ammonium groups. Detailed chemical composition of each sample is given in Table 1.

2.3. Methods

2.3.1. Polymer characterization

The onset of polymer aggregation, critical aggregation concentration (*CAC*), was determined by fluorescence measurements in the presence of pyrene as a fluorescent probe (Nichifor et al., 2014). Steady-state fluorescence emission spectra were obtained with a LS 55 Perkin Elmer fluorescence spectrometer, using an excitation wavelength of 337 nm. Zeta potential and size of aggregates formed in 1 wt% aqueous solutions of cationic polymers was measured with a Zetasizer Nano-ZS, ZEN-3500 model (Malvern Instruments) with