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Controllable synthesis and antimicrobial activities of acrylate polymers containing quaternary ammonium salts

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ABSTRACT

A series of acrylate polymers containing quaternary ammonium salts (PDMAEMA-BC) having tunable molecular weights were synthesized via atom transfer radical polymerization (ATRP), and the effect of the degree of polymerization (DP) on the antimicrobial activity against bacteria (*Escherichia coli*, *Staphylococcus albus*), pathogenic fungi (*Candida albicans*) and phytopathogenic fungi (*Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *cubense* race 4) was systematically assessed. The antimicrobial properties against *E. coli*, *S. albus* and *C. albicans* were characterized using the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) or minimum bactericidal concentration (MBC) values, whereas the antimicrobial activities against *R. solani* and Foc4 were evaluated using the effective concentration (EC_{50} and EC_{90}), MIC and MFC values. The results indicated that the PDMAEMA-BC homopolymers showed better antimicrobial activities than the corresponding monomer, i.e., the acrylate quaternary ammonium salt monomer (DMAEMA-BC), and the optimal antimicrobial activities were obtained for moderate PDMAEMA-BC chain lengths. These results help to understand the antimicrobial mechanism of polymeric quaternary ammonium salts and highlight their potential application as fungicidal agents for controlling both human and plant diseases.

1. Introduction

When various crops are planted in the field, they are confronted with serious infection by phytopathogenic fungi. *Rhizoctonia solani* form sclerotia when the external conditions are not suitable for their development. In addition, *Fusarium oxysporum* can survive for up to 30 years in the absence of bananas, and non-host weed species that are infected by the pathogen act as inoculum reservoirs [1]. The fungi are usually killed by spraying high levels of low molecular weight antimicrobial agents because traditional antimicrobial agents are easily washed away and thus need to be repeatedly sprayed. Furthermore, low molecular weight antimicrobial agents are toxic to the environment and have poor chemical stability [2]. To properly solve this problem, antimicrobial agents are designed based on polymers that contain antimicrobial functional groups. Their advantages, including better efficacy, low toxicity, no volatility, high chemical stability and prolonged lifetimes, have attracted a great deal of attention [2–4].

The mode of action of low molecular weight cationic biocides has been summarized as follows: (i) adsorption onto the bacterial cell surface; (ii) diffusion through the cell wall; (iii) binding to the cytoplasmic membrane; (iv) disruption of the cytoplasmic membrane; (v) release of cytoplasmic constituents, such as K^+ ions, DNA, and RNA; and (vi) death of the cell [5]. According to our previous studies, the bactericidal mechanism is very complicated and involves several targets in fungal cells, including the disruption of cellular structures, such as the cell wall and plasma membrane; the induction of lipid peroxidation; mitochondrial dysfunction and interference with genomic DNA [6].

Cationic antimicrobials are well-known in the development of self-sterilizing surfaces and are used in many applications, such as hospital surfaces, surgical equipment, protective hospital clothes, medical implants, wound dressings, food packaging materials, and everyday consumer products [7]. Among these cationic antimicrobials, quaternary ammonium compounds are probably the most explored and widely deployed [8–10]. The antimicrobial activity of polymers containing quaternary ammonium salts was associated with complex factors, such as molecular weight, the types of counter anions, charge density, alkyl chain length and steric hindrance, the hydrophilic–hydrophobic balance, and the type of bacteria species [2,11]. The molecular weight

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plays an important role in determining the antimicrobial properties. Homopolymers of quaternary ammonium salts exhibited far better antimicrobial activities than the corresponding monomers [12,13]. Additionally, Ikeda and his co-workers investigated the antimicrobial activity of polymethacrylate containing pendant biguanide units and found that polymer samples with low and high molecular weights exhibited lower bactericidal activity against *S. aureus* than those in the intermediate range [14]. Chen and co-workers determined that the antimicrobial properties of quaternary ammonium functionalized poly (propyleneimine) dendrimers have a parabolic dependence on molecular weight [3]. Huang and co-workers prepared polypropylene (PP) coated with a non-leachable biocide by chemically attaching poly (quaternary ammonium) (PQA) to the surface of PP and found that polymers with relatively high molecular weight (M_n > 10 kDa) showed almost 100% killing efficiency (against *E. coli*), while shorter PQA chains (M_n = 1.5 kDa) demonstrated less activity with the same grafting density [15].

The difference in cell structure between fungi and bacteria leads to differences in the antimicrobial activities of quaternary ammonium salts against bacteria and fungi. Therefore, it is very essential to conduct research on the relationship between the antifungal activity and molecular weight of poly quaternary ammonium salts. To this end, in this paper, we synthesized a series of acrylate polymers containing quaternary ammonium salts (PDMAEMA-BC) with tunable molecular weights via ATRP and systematically assessed the antimicrobial activities against two typical phytopathogenic fungi (*Rhizoctonia solani* (*R. solani*), whose main morphology is a mycelium and *Fusarium oxysporum* f. sp. *cubense* race 4 (Foc4), whose main morphology is a spore), Gram-negative bacteria (*Escherichia coli* (*E. coli*)), Gram-positive bacteria (*Staphylococcus albus* (*S. albus*)) and pathogenic fungi (*Candida albicans* (*C. albicans*)) using DMAEMA-BC and PDMAEMA-BC with various molecular weights.

2. Experimental

2.1. Materials

2-(dimethylamino)ethyl methacrylate (DMAEMA, 99%), ethyl 2-bromoisobutyrate (EtBriB, 98%), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA, 98%) and copper(I) bromide (CuBr, 99%) were supplied by Shanghai Macklin Biochemical Technology Co. Ltd. (Shanghai, China). Benzyl chloride (BC, 99%) was supplied by Aladdin Reagent Co. Ltd. (Shanghai, China). Beef extract was supplied by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Peptone was supplied by Guangdong Ring Kay Microbial Technology Co., Ltd. (Guangzhou, China). Agar was supplied by MYM Biological Technology Company (Shanghai, China). RPMI-1640 liquid medium was supplied by HyClone Company of America (Utah, USA). All microorganisms were kindly supplied by the Fungus Laboratory, Department of Plant Pathology, South China Agricultural University.

2.2. Synthesis of poly(2-(dimethylamino)ethyl methacrylate) containing quaternary ammonium salts (PDMAEMA-BC)

Poly(2-(dimethylamino)ethyl methacrylate) containing quaternary ammonium salts (PDMAEMA-BC) was prepared in a two-step process, i.e., first, the controlled synthesis of PDMAEMA, followed by quaternization of PDMAEMA to afford PDMAEMA-BC, as shown in Scheme 1.

PDMAEMA was polymerized via ATRP technology, and a brief description of the polymerization procedure is as follows: 10.0 g (0.06 mol) of DMAEMA and pre-determined amounts of EtBriB and PMDETA were dissolved in 50 mL of isopropanol in a 200 mL Schlenk flask. The reaction system was subjected to three freeze-pump-thaw cycles. Next, a calculated amount of CuBr was quickly added into the flask under a nitrogen atmosphere. During ATRP polymerization, certain polymer molecular weights were targeted by selecting appropriate initial monomer and EtBriB concentrations. The ratio of $[DMAEMA]_0/[EtBriB]_0$ (mol/mol) were set at 4/1, 8/1, 15/1, 30/1, 60/1, 70/1 and 80/1 to yield a theoretical degree of polymerization (DP) of 4, 8, 15, 30, 60, 70 and 80, respectively. The mixture was stirred at 60 °C for 8 h under a nitrogen atmosphere. The reaction was stopped by exposing it to air and dried under vacuum at 60 °C (approximately 70% yield). The catalyst was removed by neutral alumina column chromatography with acetone as the eluent. For convenience, the products were named PDMAEMA-n, and n represents the DP of PDMAEMA.

^{1}H NMR (400 MHz, D_2O, δ, ppm): 0.97 (s, C−CH_3), 1.98 (s, C−CH_2−C), 2.37 (s, N(CH_3)$_2$), 2.77 (t, N−CH_2−CH_2), 4.20 (t, O−CH_2−CH_2). FT-IR (KBr, cm^{-1}): 2863–2947 (ν_{CH}), 1732 ($\nu_{C=O}$), 1020,1047 (ν_{C-N}).

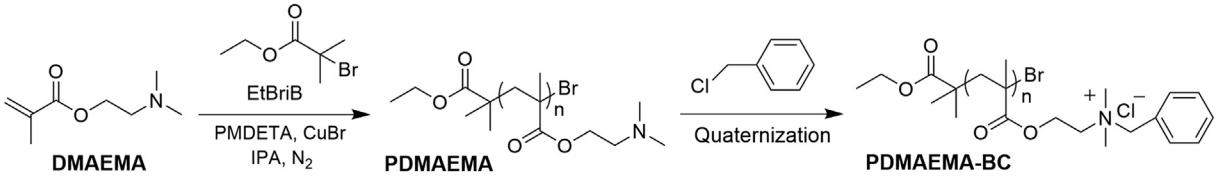
PDMAEMA-BC was synthesized in a quaternization reaction between PDMAEMA and benzyl chloride (BC), as briefly stated in the following: 6.0 g of PDMAEMA and 4.8 g of BC were dissolved in 20 g of an ethyl alcohol/methylbenzene (1/1, m/m) mixture in a 100 mL Schlenk flask. The mixture was subjected to three freeze-pump-thaw cycles and then stirred at 70 °C under a nitrogen atmosphere for 24 h; subsequently, the mixture was precipitated with anhydrous diethyl ether, and the precipitate was washed with anhydrous diethyl ether three times and dried under vacuum at 40 °C overnight (approximately 90% yield). For convenience, the products were named PDMAEMA-BC-n, and n represents the DP of PDMAEMA-BC.

^{1}H NMR (400 MHz, D_2O, δ, ppm): 1.06 (s, C−CH_3), 3.14 (s, N^+(CH_3)$_2$), 3.87(t, CH_2−CH_2−N^+), 4.35–4.76 (m, N^+−CH_2−Φ, O−CH_2−CH_2), 7.59 (s, Φ−H). FT-IR (KBr, cm^{-1}): 2870–2979 (ν_{CH}), 1726 ($\nu_{C=O}$), 1041 (ν_{C-N}), 768 ($\gamma_{Φ-H}$).

2.3. Synthesis of an acrylate quaternary ammonium salt monomer (DMAEMA-BC)

An acrylate quaternary ammonium salt monomer (DMAEMA-BC) was synthesized via a quaternization reaction between DMAEMA and BC. 6.0 g of DMAEMA and 4.8 g of BC were dissolved in 20 g of an ethyl alcohol/methylbenzene (1/1, m/m) mixture in a 100 mL Schlenk flask. The mixture was subjected to three freeze-pump-thaw cycles and then stirred at 40 °C under a nitrogen atmosphere for 24 h; subsequently, the mixture was precipitated with anhydrous diethyl ether, and the precipitate was washed with anhydrous diethyl ether three times and dried under vacuum at 40 °C overnight (approximately 90% yield).

^{1}H NMR (400 MHz, D_2O, δ, ppm): 1.98 (s, C−CH_3), 3.18 (s, N^+(CH_3)$_2$), 3.83–3.86 (t, CH_2−CH_2−N^+), 4.65(s, N^+−CH_2−Φ), 4.73–4.75 (t, O−CH_2−CH_2), 5.82, 6.21 (d, CH_2=C(CH_3)), 7.62 (s, Φ−H). FT-IR (KBr, ν, cm^{-1}): 2853–2970 (ν_{CH}), 1722 ($\nu_{C=O}$),1635 ($\nu_{C=C}$), 1041 (ν_{C-N}), 768 ($\gamma_{Φ-H}$).



Scheme 1. Synthetic route to PDMAEMA and PDMAEMA-BC.