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Short communication

SHORT COMMUNICATION

Molecular weight dependent antistaphylococcal activities of oligomers/polymers synthesized from 3-aminopyridine

CAHIT AKGUL* and MEHMET YILDIRIM

Department of Chemistry, Faculty of Sciences and Arts, Canakkale Onsekiz Mart University, Terzioglu Campus, Canakkale, Turkey

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Abstract: The main aim of this study was to investigate the relationship between molecular weight and the antistaphylococcal activity of oligomers/polymers synthesized from 3-aminopyridine. Different oligomers/polymers were synthesized from 3-aminopyridine by changing the oxidative polycondensation reaction conditions. They were characterized by size exclusion chromatography and their antibacterial activities were compared by employing standardized susceptibility assays. The obtained experimental results demonstrated that 3-aminopyridine had no antistaphylococcal activity. However, as a result of polymerization, strong antistaphylococcal activity was obtained. Oligomers/polymers synthesized from 3-aminopyridine had varying degrees of antistaphylococcal activity and the maximum activity was obtained from relatively very short oligomers. It was therefore concluded that polymerization of 3-aminopyridine is required for antistaphylococcal activity and strength of this activity depends on the molecular weights of the synthesized molecules.

Keywords: antibacterial; 3-aminopyridine; oligomer; *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus is one of the bacterial strains that can cause serious infections. Whilst several antibiotics, including penicillin, methicillin and vancomycin, have been successfully employed to eradicate *S. aureus* infections, bacterial resistance against common antibiotics has increased dramatically over the past few decades.[1] Thus, the need for the discovery and development of novel antibacterial agents is of paramount importance. The basic mechanisms of antibacterial action include inhibition of cell wall synthesis, inhibition of protein synthesis, alteration of the cell membrane, inhibition of nucleic acid synthesis and antimetabolite activity.[2] Bacterial cell wall and membrane structures are the

* Corresponding author. E-mail: cahitakgul@yahoo.com
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main targets of several commercial antibiotics.[3] However, intracellular bacterial components also carry the potential to be promising targets for antimicrobial compounds.[4] The search for novel antibacterial agents and new targets for antimicrobial agents will undoubtedly continue to be among the priorities of researchers in the future.

Synthetic polymeric materials have been widely utilized for multiple purposes, including the exploitation of their *in vivo* and *in vitro* antimicrobial activities.[5,6] Since many properties of polymers are dependent on their molecular weight, it is reasonable to think that their antibacterial activities can also be molecular weight dependent.[7] In a previous study, it was shown that oligo-3-aminopyridine has strong antibacterial activity against gram positive bacterial species.[8] This observation led to the notion that the bioactivity might be molecular weight dependent. Thus, in this study, novel oligomers/polymers were prepared from 3--aminopyridine by changing oxidative polycondensation reaction conditions and their molecular weights determined using chromatographic techniques. Then, the antibacterial activities of these monomers/oligomers/polymers were comparatively analyzed to determine whether the bioactivity was molecular weight dependent.

EXPERIMENTAL

Materials

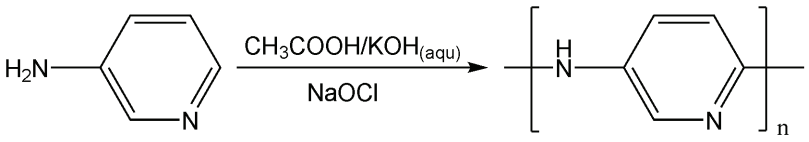
Blank, gentamicin and vancomycin discs were obtained from Oxoid Ltd. (Basingstoke, UK) Mueller–Hinton broth, Mueller–Hinton agar (MHA), 3-aminopyridine, KOH and CH_3COOH were supplied by Merck KGaA (Darmstadt, Germany) and were used as received. The dimethylformamide (DMF–HPLC grade) used for the size exclusion chromatography was from Merck. A 30 % aqueous solution of sodium hypochlorite (NaOCl) was supplied by Paksoy Chemical Co. (Turkey).

Bacterial strains

Gram-positive *S. aureus* (ATCC 25923) from the Refik Saydam National Public Health Agency, Ankara, Turkey, was used as a representative strain.

Synthesis of oligomers/polymers from 3-aminopyridine

3-Aminopyridine (AP) was converted into its oligomer/polymer derivatives (OAP) through oxidative polycondensation (OP) reactions in an aqueous alkaline or acid medium using NaOCl (30 %) as the oxidant, as described in the literature (Scheme 1).[9] The polymerizations were performed in 250 mL three-necked round-bottom flasks which were fitted with a condenser, thermometer and magnetic stirrer. Alkaline and acid media were realized using aqueous solutions of KOH and CH_3COOH, respectively. The polymerizations performed in acid medium were terminated *via* neutralization by the addition of 1 M aqueous KOH solution and the oligomers/polymers were precipitated. In addition, OAP-1, which was synthesized in alkaline medium, was precipitated in the reaction medium without neutralization. The oligomer/polymer syntheses were performed under different reaction conditions to obtain compounds of various molecular weights (Table I).



Scheme 1. Oxidative polycondensation reaction of 3-aminopyridine.

TABLE I. Experimental conditions of the oxidative polymerization reactions and the results of the size exclusion chromatography (SEC) of the oligomers and polymers synthesized from 3-aminopyridine (monomer and oxidant concentrations used in the experiments: $[AP]_0$ = = 0.266 mol L^{-1} and $[NaOCl]_0$ = 0.120 mol L^{-1})

Compound	*t* / °C	Time, h	Media	M_n	M_w	*PDI*	Yield, %
OAP-1	25	1	alkaline[a]	700	1950	2.79	69
OAP-2	25	1	acid[b]	1450	2500	1.72	65
OAP-3	90	1	acid	37000	59350	1.60	54
OAP-4	25	5	acid	16400	18500	1.13	59

[a]($[KOH]_0$ = 0.0625 mol L^{-1}; [b]$[CH_3COOH]_0$ = 1.131 mol L^{-1}

Molecular weight determination of the oligomers/polymers

The number average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity index (*PDI*) were determined by the size exclusion chromatography (SEC) technique (Shimadzu) at 25 °C. For the SEC investigations, an SGX (100 Å and 7 nm diameter loading material) 3.3 mm i.d.×300 mm column was used; eluent: DMF (0.4 mL min^{-1}), polystyrene standards were used for calibration. A refractive index detector (RID) was used to analyze the products.

Antibacterial susceptibility testing using disc diffusion assay

The oligomers/polymers synthesized from 3-aminopyridine were dissolved in DMSO to obtain a concentration of 100 μg per 20 μL and 100 μg per disc were used in the disc diffusion assay.[10] Briefly, the microorganisms were grown on MHA plates and then 3 mL of MHB was inoculated with 3 well separated colonies for each strain. After incubation at 37 °C, the concentrations of suspensions were adjusted to 10^8 cells mL^{-1}. Each bacterial suspension was spread over the surface of the MHA using sterile cotton swabs. 100 μg per 20 μL of the chemicals were pipetted onto filter paper disks (6 mm in diameter). The discs were then placed onto the inoculated agar surface. After keeping at room temperature for ≈30 min, the plates were incubated at 37 °C for 20 h. Discs containing gentamicin (10 μg) and vancomycin (30 μg) were included as the positive controls and DMSO-only (20 μL) the negative control. The results are expressed as the diameter of inhibition zones and the presented values are the average of three separate experiments.

*Determination of the minimum inhibitory concentration (*MIC*)*

The broth macrodilution assay was performed, as recommended by NCCLS, to determine the minimum inhibitory concentration (*MIC*).[11] Bacterial strains were cultured overnight at 37 °C and suspended in MHB to obtain a final inoculum density of 10^6 cells mL^{-1} and 0.5 mL of this suspension was added to 0.5 mL of susceptibility test broth containing serial 2-fold dilutions of the chemicals. Two tubes were also included to check the sterility of the media. All the tubes were incubated at 37 °C for 20 h. The *MIC* was considered as the lowest concentration at which the chemical prevented visible bacterial growth.

RESULTS AND DISCUSSION

It was demonstrated in this study that the average molecular weights of the synthesized oligomers depended on the employed reaction conditions. These observations were similar to those of previous studies reporting that many factors, such as temperature, reaction time, initial concentration of monomer, acidic or basic medium and the oxidant type, can affect the yields of oxidative polymerization reactions.[12] The polymerization conditions and the size exclusion chromatography (SEC) results calculated from the chromatograms are given in Table I, from which it can be seen that oligomers with different molecular weights were obtained in the four different sets of employed reaction conditions. These values clearly show that when the alkaline medium was used, the average molecular weight of the OAP was relatively low (see Table I, OAP-1). However, when the acid medium was used under the same conditions, such as monomer and oxidant concentrations, temperature, and the reaction time, the average molecular weight was clearly higher. When the temperature was increased from 25 to 90 °C, with the other conditions being constant, the number average molecular weight (M_n) and weight average molecular weight (M_w) values increased from 1450 and 2500 to 37000 and 59350 g mol^{-1}, respectively (see Table I, OAP-2 and OAP-3). However, when the temperature and other conditions were constant but the reaction time was increased from one to five hours, the M_n and M_w values increased from 1450 and 2500 to 16400 and 18500 g mol^{-1}, respectively (see Table I, OAP-2 and OAP-4). All other reaction conditions are summarized in Table I.

The antibacterial activities of the synthesized oligomers/polymers were assessed using the standard disc diffusion assay and broth macrodilution assay. The results of these assays are summarized in Table II. Neither 3-aminopyridine (AP) monomer itself nor its relatively longer oligomers or polymers showed any antibacterial activity against *S. aureus*. In a previous study, a smaller oligomer of 3--aminopyridine (OAP) was synthesized, characterized and the M_n, M_w and polydispersity index (*PDI*) values were found to be 250 and 800 g mol^{-1} and 3.20, respectively.[9] When 50 μg of this OAP was used, the inhibition zone was 17 mm in the disc diffusion assay and the *MIC* was found to be 25 μg mL^{-1} in broth macrodilution assay.[8] These values for this oligomer were included in Table II just for comparison. No inhibition of bacterial growth was observed in cultures containing 3-AP, OAP-2, OAP-3, OAP-4 at concentrations of up to 1 mg mL^{-1} (Table II). When all these molecular weight values of the previous and the current study and the corresponding antibacterial activities of the oligomers/polymers are considered, it can easily be seen that the maximum antibacterial activity was obtained with relatively very short oligomers synthesized from 3-aminopyridine. It can imply that short oligomers of 3-aminopyridine can easily diffuse into bacterial cells and show their antibacterial activities inside the cell. On the contrary, since relatively longer oligomers and polymers of 3-aminopyridine

cannot cross the cell wall and/or cell membrane structures of bacterial cells, they cannot inhibit bacterial growth under similar growth conditions. The requirement of polymerization of 3-aminopyridine for this antistaphylococcal activity could entail that its oligomers can mimic some intracellular components. However, the exact inhibitory mechanism remains to be elucidated and requires much more detailed investigations.

TABLE II. Antibacterial activities of oligomers/polymers synthesized from 3-aminopyridine

Compound	Diameter of inhibition zone[a], mm	*MIC* / μg mL^{-1}
3-AP	NI	–[b]
OAP	17	25
OAP-1	13	100
OAP-2	NI	–
OAP-3	NI	–
OAP-4	NI	–
Gentamicin (10 μg)	19	
Vancomycin (30 μg)	17	
DMSO (20 μL)	NI	

[a]Includes the diameter of the disc (6 mm) and 100 μg disc was used for each compound. Values are average of three separate experiments. NI: No zone of inhibition was observed; [b]no inhibition of bacterial growth with concentrations up to 1 mg mL^{-1}

CONCLUSIONS

From all these experimental results, it can be concluded that polymerization is required for 3-aminopyridine to possess antistaphylococcal activity. The antibacterial activity of the oligomers/polymers synthesized from 3-aminopyridine are molecular weight dependent and the maximum antibacterial activity was supplied by relatively, very short oligomers. In addition, the molecular structures and properties of short oligomers can be thought essential for antibacterial activity to be exhibited, since 3-aminopyridine monomer itself has no inhibitory effect on bacterial growth.

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И З В О Д

ЗАВИСНОСТ АНТИСТАФИЛОКОКНЕ АКТИВНОСТИ ОЛИГОМЕРА И ПОЛИМЕРА СИНТЕТИСАНИХ ОД 3-АМИНОПИРИДИНА ОД ЊИХОВЕ МОЛЕКУЛСКЕ ТЕЖИНЕ

CAHIT AKGUL и MEHMET YILDIRIM

Department of Chemistry, Faculty of Sciences and Arts, Canakkale Onsekiz Mart University, Terzioglu Campus, Canakkale, Turkey

Циљ ове студије је био да испита везу између молекулске тежине и антистафилококне активности олигомера и полимера синтетисаних од 3-аминопиридина. Различити олигомери и полимери су синтетисани од 3-аминопиридина променом услова реакције оксидативне поликондензације. Окарактерисани су гел хроматографијом, а антибактеријске активности су

упоређиване стандардним тестовима. Експериментални резултати су показали да 3-аминопиридин нема антистафилококну активност. Са друге стране, полимеризацијом се стиче јака активност. Олигомери и полимери су имали различиту активност и најјачу су испољили релативно кратки олигомери. Закључено је да је полимеризација 3-аминопиридина неопходна за постизање антистафилококне активности и да та активност зависи од молекулске тежине синтетисаних молекула.

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