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Microbiology

Amino Acid-Based Biodegradable Polycations with Antimicrobial Activity

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(Presented by Academy Member Tengiz Zaalishvili)

ABSTRACT. The bactericidal activity of arginine-based polycations – polyesterurethane (PEUR- EG_2-Arg-EG_4) and polyesterureas (PEU - 1-Arg-2 and 1-Arg-3) toward the bacteria: *Bacillus subtilis, Staphilococcus aureus, Mycobacterium album, Pseudomonas fluorescens, Esherichia coli* was studied in *in vitro* experiments. Biodegradable polycations – polyesterurethane (PEUR - EG_2-Arg-EG_4) and polyesterureas (PEU - 1-Arg-2 and 1-Arg-3) were synthesized on the base of bis-nucleophilic monomers - bis-(~-amino acid)~|μ-alkylene diesters and bis-electrophilic monomers: activated bis-carbonates and activated diol-bis-carbonates by the method of low-temperature polycondensation in solution. The key monomers for the synthesis of arginine-containing polymers -bis-(~-amino acid)~|μ-alkylene diesters contain two, easily hydrolizable ester bonds and are stable in the form of salts of di-p-toluene sulfonic acid. The arginine-based polymers were studied according to the standard methods. Their yield was satisfactory, and reduced viscosity varied in the range of 0.1-0.2dl/g (M_w~ 8-10KDa). The bactericidal activity of polycations was studied using the Photocolorimetric method. It was estimated that the studied polycations reveal bactericidal activity which depends on their chemical structure. Correlation between hydrophobicity level of polycations and their bactericidal activity was established. The bactericidal activity of polycations was exposed mainly in logarithmic phase of bacterial development.

New arginine-containing polymers reveal significantly lower cytotoxicity towards the eucariotic cells than the polycations widely spread in genetic ingineering. The arginine-containing biodegradable polycations with hydrophobic fragments are able to form micro- and nanoparticles, which makes them very attractive as bactericidal preparations of a new generation and physiologicaly active containers for purposeful drug delivery systems. *© 2017 Bull. Georg. Natl. Acad. Sci.*

Key words: polycations, antimicrobial activity, arginine, biodegradable polymers

Recently the antimicrobial substances are of great interest both from scientific and practical points of view. Demand for antimicrobial compounds is very high in different fields, like medicine, food industry; special textiles, water cleaning, etc. [1]. Three main groups of organic cations were distinguished as the antibacterial compounds: bactericidal peptides of natural origin and their analogues, low molecular non-

peptide substances, and bactericidal polymers. Bactericidal peptides are the unique group of molecules with wide spectrum of antimicrobial activity and represent the significant constituent of the innate immune system [2]. Synthesized substances with high antimicrobial properties were obtained according to the properties of natural antimicrobial peptides (AMP) [3, 4]. As the synthesis of AMP is expensive and very laborious, moreover, peptides are easily degraded by enzymes and become inactive, the idea of their commercialization appear to be unsuccessful. Well-known antibiotics belong to the low molecular bactericides. Derivatives of guanidine make a large group of low molecular bactericides, e.g. chlorhexidine [5]. Big library of guanidine derivatives was created and their antimicrobial testing was carried out [6]. Toxicity and short-term effect are the negative properties of low molecular antibacterial agents.

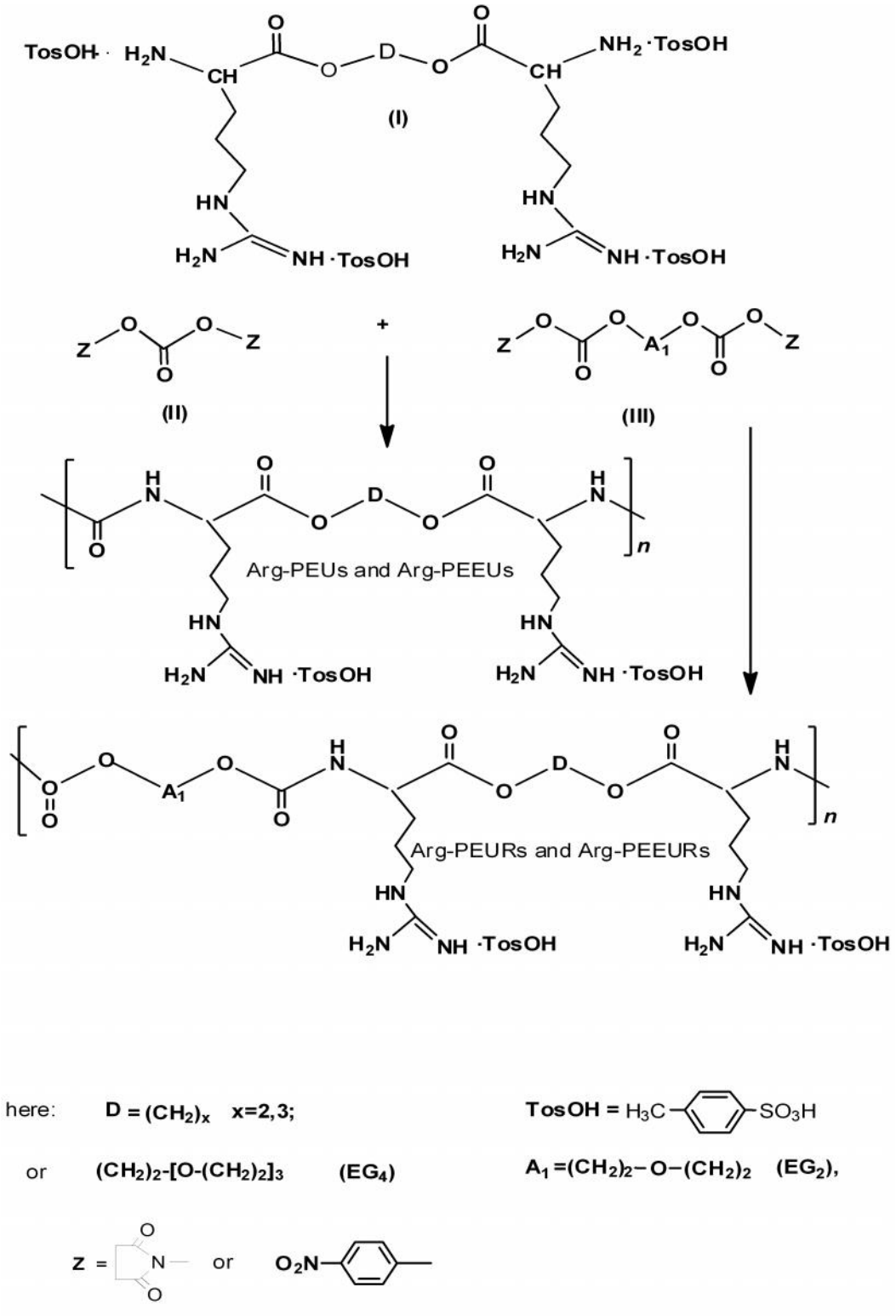
Ability of film- and products forming, and prolongation of bactericidic activity is characteristic of polymeric bactericides. They are used in coating of medical materials and device surfaces, to prevent formation of a biofilm [7]. Polymeric biocides are toxic towards the human cells [8]. They are non-biodegradable and hard to eliminate from the body. Development of a new, biodegradable bactericidal polymers of polycationic nature, with wide spectrum of properties and tolerant to eucariotic cell, is very important today. Among recently obtained biodegradable polymers on the base of natural amino acids AA-BB type ones are very popular and their synthesis and investigation represents the pioneering work of Georgian scientists [9-11]. It was supposed that arginine-based biodegradable polymers (ABP) would possess bactericidal properties, and this supposition was proved in our previous experiments [12]. Scientists of Cornell University have demonstrated that new, arginine-containing polymers reveal significantly lower cytotoxicity towards the eucariotic cells than the polycations widely spread in genetic ingineering [13]. Similar result was obtained by us while studying the cytotoxicity of ABP towards 4T1 Cells [14]. Accordingly biomedical application of ABP is prospective.

The key monomers for the synthesis of arginine-containing polymers are bis-(a-amino acid)α,ω-alkylenediesters(I) (scheme 1), which contain two, easily hydrolizable ester bonds. Monomers (I) are stable in the form of salts of di-p-toluene sulfonic acid. Activated bis-carbonates (II) and diol-bis-carbonates (III) were applied as bis-electrophilic monomers for the synthesis of desirable ether-ester polycations (polyester ureas and polyether-ester urethanes respectively, Fig. 1). Polymers were studied according to the standard methods. Their yield was satisfactory, and the reduced viscosity varied in the range of 0.1-0.2dl/g. This presumably corresponds to the molecular weight of about 8-10KDa.

Arginine-based polymers -PEUs: 1-Arg-2 and 1-Arg-3, as well as PEEUR - EG_2-Arg-EG_4 were taken for investigation of bactericidal properties. Influence of the selected polymers was studied both on gram-positive and gram-negative bacteria: *Bacillus subtilis* (strain *C*-226), *Staphilococcus aureus* (strain K-422), *Mycobacterium album (*strain *L*-166); *Esherichia coli* (strain Sh-44). *Pseudomonas fluorescens* (strain MI-72).

In our early experiments screening and testing of the polycations(ABP) by disco-diffusive method was performed. Degree of biocidity of (ABP) was determined and experimental biopolymers and their minimal concentrations were selected [12]. In the presented work we used the Photocolorimetric method to study the bactericidal activity of arginine-based polycations. The minimal concentration (MIC) of the preparation with antibacterial activity was determined following the density of the biomass accumulated during the whole growth period of the culture (optical density was measured at 500nm wave length on the Photoelectrocolorimeter (PhEC-2-UkhL-4.2.Ru). Beef-extract broth (from Sigma-Aldrich Chemie GmbH) was used for cultivation.

Table 1 demonstrates the influence of different



Scheme 1. Synthesis of arginine-containing ether-ester polycations

Table 1. Effect of 1-Arg-2 and 1-Arg-3 on the density of microorganisms' biomass (PhEC, after 24h development)

N	Test-culture	Substance						Test-culture on beef-extract broth
		1-Arg-2			1-Arg-3			
		Concentration, mg/ml						
		0. 10	0.01	0.001	0.1	0.01	0.001	
1.	*Bacillus subtilis*	0.005	0.007	0.27	0.08	0.20	0.24	0.27
2.	*Mycobacterium album*	0.60	0.62	0.68	0.59	0.60	0.62	0.80
3.	*Escherichia coli*	0.707	0.80	1.05	0.62	0.64	0.68	1.30
4.	*Pseudomonas fluorescens*	0.31	0.37	0.46	0.35	0.37	0.38	0.52
5.	*Staphilococcus aureus*	0.58	0.59	0.59	0.508	0.508	0.52	0.60

concentrations of polycations - 1-Arg-3 and 1-Arg-2 on microorganisms' growth and development. They inhibited growth of the following microorganisms: *Bacillus subtilis, Staphylococcus aureus, Mycobacterium album, Pseudomonas fluorescens, Escherichia coli*, but at different levels. The maximal inhibitory effect was observed at 10^{-1}mg/ml concentration of the substances. Both 1-Arg-3 and 1-Arg-2 had the strongest effect on *Escherichia coli*, and the less influence was observed on *Staphylococcus aureus*. The decreasing of the toxic substance concentration (10^{-2}, 10^{-3}mg/ml) caused weakening of the inhibitory effect and at the concentration of 10^{-3}mg/ml the biomass density was almost equal to the initial meaning. The increas of hydrophobicity caused some changes of the inhibitory ability of PEUs and the inhibitory effect of 1-Arg-3 was stronger towards *Escherichia coli, Staphylococcus aureus,* then of1-Arg-2, presumably because of higher hydrophobicity of 1-Arg-3. The inhibitory effect of polyether-ester urethane EG_2–Arg-EG_4 (10^{-1}mg/ml) on microorganisms' growth was higher compared to the above mentioned toxicants, presumably caused by inclusion of ethylene glycol chains in polyether-ester urethane structure; but in contrast to PEUs, the biocidity of the latter was not selective (Fig. 1-5). The character of the growth of test-microorganisms during 24 hours, without polycation and with it was studied to determine the developmental phase of the microorganism at which the inhibitory effect of EG_2–Arg-EG_4 was evident (Fig. 1-5, curves 1 and Fig. 1-5, curves 2 respectively). It was established that the characters of microorganisms' development in time were different. The logarithmic phase of *Escherichia coli* and *Mycobacterium album* prolonged for 18-19 hours, while for *Bacillus subtilis* it made for more than 22 hours, in case of *Pseudomonas fluorescens* the logarithmic phase prolonged for 16-18 hours, and for *Staphylococcus aureus* 18 hours. The duration of other phases of microorganisms' growth (steady state and dying phase) was individual for a particular microorganism. Curves 2 in Fig. 1-5 demonstrate the dynamics of EG_2-Arg-EG_4 influence during the microorganisms' growth and development. Concentration of the added EG_2-Arg-EG_4 was 0.1mg/ml. From the obtained results it is clear that the inhibitory effect of polycation EG_2-Arg-EG_4 on *Escherichiacoli* growth was revealed 10 hours later (Fig. 1, curve 2). In case of *Pseudomonas fluorescens* its effect began 4 hours later, i.e. at the beginning of the logarithmic phase and prolonged during the whole phase (more than 16 hours) (Fig. 2, curve 2). Inhibition of the growth of *Bacillus subtilis* was not evident during the first 10 hours, but growth process was significantly weak during further 8 hours of microorganism cultivation (Fig.3, curve 2). The biocidic effect of EG_2-Arg-EG_4 on growth of *Mycobacterium album* was stronger compared to experimental test-culture (Fig.4, curve 2). Under the influence of polycation growth of microorganism culture was absent during first 6 hours. Later the growth process was very slow. Inhibition of the growth of *Staphyococcus aureus* began 6 hours later and prolonged till the end of cultivation (Fig. 5, curve 2). Analysis of the obtained results

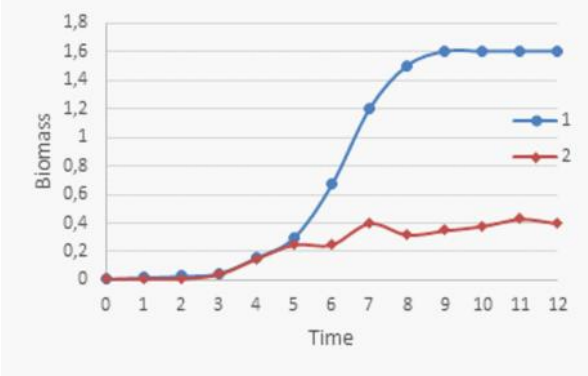


Fig. 1. The inhibition of *Esherichia coli* growth by PEEUR EG_2-Arg-EG_4.

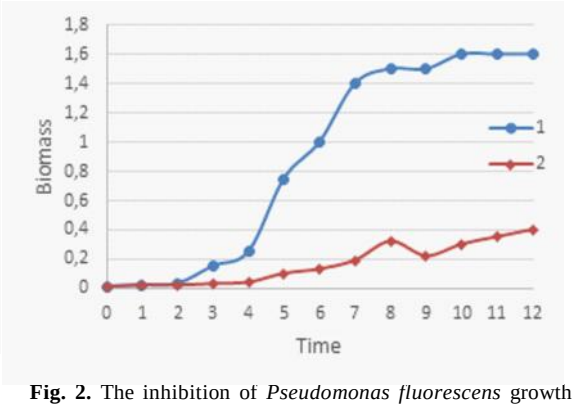


Fig. 2. The inhibition of *Pseudomonas fluorescens* growth by PEEUR EG_2-Arg-EG_4.

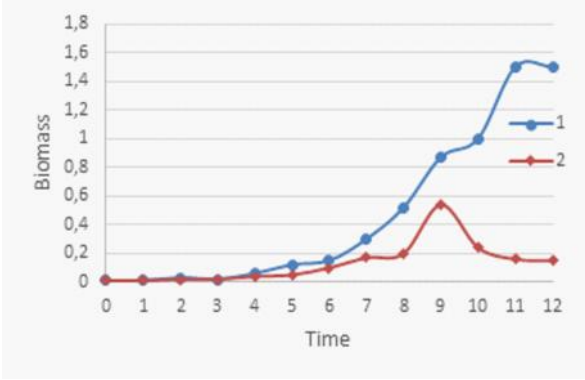


Fig. 3. The inhibition of *Bacillus subtilis* growth by PEEUR EG_2-Arg-EG_4.

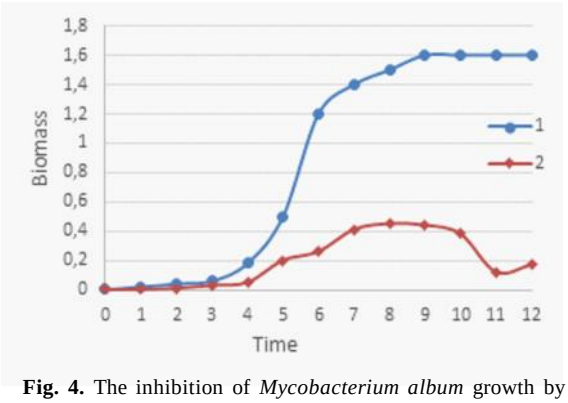
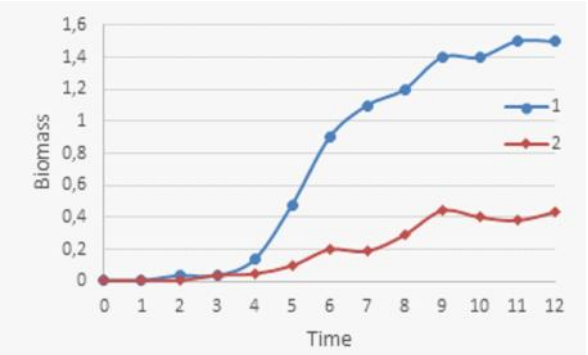


Fig. 4. The inhibition of *Mycobacterium album* growth by PEEUR EG_2-Arg-EG_4.



1. In pure growth medium;
2. In growth medium containing PEEUR (0.1mg/mL)

Fig. 5. The inhibition of *Staphylococcus aureus* growth by PEEUR EG_2-Arg-EG_4.

makes clear that the biocidic effect of the experimental polycations towards the tested microorganisms was revealed mainly in different periods of the logarithmic phase and did not step over the steady state.The experimental polycations revealed biocidic effect on both gramm-positive and gramm-negative microorganisms. Among them 1-Arg-2 and 1-Arg-3 revealed selective inhibitory effect towards some microorganisms.

It must be mentioned that arginine-containing biodegradable polycations with hydrophobic fragments are able to form micro- and nanoparticles, which makes them very attractive as bactericidal preparations of new generation and physiologically active containers for purposeful drug delivery systems.

მიკრობიოლოგია

ბიოდეგრადირებადი პოლიკატიონები ამინომჟავების საფუძველზე ანტიმიკრობული აქტიურობით

დ. ხარაძე*, თ. მემანიშვილი*, ქ. მამულაშვილი, თ. ომიაძე*, ლ. ყირმელაშვილი*, ზ. ლომთათიძე****

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(წარმოდგენილია აკადემიის წევრის თ. ზაალიშვილის მიერ)

შესწავლილია არგინინის საფუძველზე მიღებული პოლიკატიონების: პოლიეთერესტერურეთანის -PEUR (EG_2-Arg-EG_4) და პოლი(ესტერშარდოვანების) –PEU (1-Arg-2 და 1-Arg-3) ბაქტერიციდული აქტიურობა *in vitro* ექსპერიმენტებში ბაქტერიების: Bacillus subtilis, Staphilococcus aureus, Mycobacterium album, Pseudomonas fluorescens, Esherichia coli მიმართ. ბიოდეგრადირებადი პოლიკატიონები პოლიეთერესტერურეთანი -PEUR (EG_2-Arg-EG_4) და პოლი(ესტერშარდოვანები) –PEU (1-Arg-2 და 1-Arg-3) მივიღეთ ბის-ნუკლეოფილური მონომერების - ბის(~-ამინომჟავა)~,μ-ალკილენ დიესტერებისა და ბის-ელექტროფილური მონომერების: გააქტიურებული ბის-კარბონატების და გააქტიურებული დიოლ-ბის-კარბონატების საფუძველზე დაბალტემპერატურული პოლიკონდენსაციის მეთოდით ხსნარში. არგინინშემცველი პოლიმერების სინთეზის საკვანძო მონომერები ბის(~-ამინომჟავა)~,μ-ალკილენ დიესტერები შეიცავენ ორ ადვილად ჰიდროლიზებად ბმას და მდგრადებია დი-p-ტოლუოლსულფომჟავა მარილების სახით. პოლიმერები შევისწავლეთ არგინინის საფუძველზე სტანდარტული მეთოდების გამოყენებით. მათი გამოსავლიანობა იყო დამაკმაყოფილებელი, დაყვანილი სიბლანტე ვარირებდა 0,1-0,2 დლ/გ (Mw ¼ 8-10KDa) ფარგლებში. პოლიკატიონების ბაქტერიციდული აქტივობა შევისწავლეთ ფოტოკოლორიმეტრული მეთოდით. დადგენილია, რომ შესწავლილ პოლიკატიონებს ახასიათებთ ბაქტერიციდული აქტიურობა და იგი დამოკიდებულია მათ ქიმიურ სტრუქტურაზე. გამოვლენილია კორელაცია პოლიკატიონების ჰიდროფობურობის ზრდასა და ბაქტერიციდულ აქტიურობას შორის, აგრეთვე ისიც, რომ პოლიკატიონების ბაქტერიციდული აქტიურობა ძირითადად ვლინდება ბაქტერიების განვითარების ლოგარითმული ფაზის პერიოდში. ახალი არგინინშემცველი პოლიკატიონები გაცილებით ნაკლებ ციტოტოქსიკურია ეუკარიოტული უჯრედების მიმართ გენურ ინჟინერიაში ფართოდ გამოყენებულ პოლიკატიონებთან შედარებით. არგინინშემცველ პოლიკატიონებს ჰიდროფობური ფრაგმენტებით მიკრო- და ნანონაწილაკების წარმოქმნის უნარი აქვთ, რაც მათ მიმზიდველს ხდის ახალი თაობის ბაქტერიციდული ნაერთებისა და ფიზიოლოგიურად აქტიური კონტეინერების დასამზადებლად სამკურნალო პრეპარატების მიზანმიმართული მიწოდების სისტემებისათვის.

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