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SYNTHESIS AND ANTIFUNGAL ACTIVITY OF PULLULAN-AMPHOTERICIN B CONJUGATE

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Abstract

Polyene antibiotics are antifungal compounds produced by *Streptomyces* strains. In order to decrease toxicity and improve water solubility various pharmaceutical formulations have been prepared. The aim of this work was to prepare conjugate of polysaccharide pullulan and polyene antibiotic amphotericin B. Increasing reactivity of carbohydrate polymer was achieved by introducing of aldehydo groups into polymer chain. Resulting polyaldehydo glucan was coupled with amino groups of amphotericin B to give Schiff base structures. Obtained conjugate is soluble in water and shows significant activity against *Candida albicans*.

Introduction

Polyene antibiotics belong to the class of macrocyclic lactone group of antibiotics. Beside macrocyclic ring, they have series of conjugated double bonds in their molecule, which is essential for their activity.

Although these molecules are very efficient fungicides, their use is limited due to numerous unwanted effects, mostly nephrotoxicity [1]. Toxicity is a consequence of weak water solubility on physiological pH value and affinity toward cholesterol. The main strategies for overcoming these deficiencies are physical and chemical modifications during production of appropriate antibiotic formulations. One of the successful chemical modifications is coupling reaction between antibiotic and polysaccharide molecules, which results in greater water solubility. That makes apsorption in gastrointestinal tract better while antimicrobial activity remains similar and toxicity is reduced.

Introduction of aldehydo functions into polysaccharide molecule is possible to obtain by periodate oxidation [2]. That enables coupling reaction with compounds which have available amino functions, like polyene antibiotics. In this paper is described such modification of pullulan, a fungal exopolysaccharide produced by *Aureobasidium pullulans* species. It is a linear molecule which consists of α-(1→6)-linked maltotriosyl units. It is biodegradable, nontoxic and readily soluble in water [3,4].

Results and Discussion

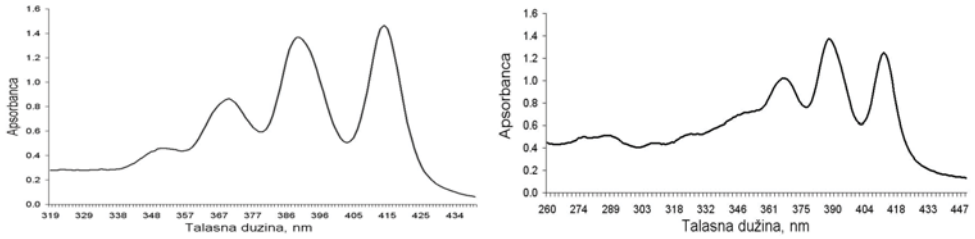
Periodate oxidation of pullulan. Oxidation of pullulan to polyaldehyde derivative was done with oxidizing agent (sodium periodate) in aqueous solution. Excess periodate and iodate ions were removed from reaction mixture by dialysis and after

that, oxidized glucan was liophilized. Aldehydo groups in polymer were determined by titration with standardized iodine solution [5]. Oxidation was done at room temperature (20 °C). In these conditions, samples of 30% oxidized pullulan were obtained.

Synthesis of pullulan-amphotericin B conjugates. Coupling reaction between oxidized pullulan and amphotericin B was done in borate buffer (pH 11) in the dark, with continual stirring, at 40 °C, during 48 h period.

UV and IR spectroscopy of conjugates. Coupling reaction was monitored by UV and IR spectroscopy, by comparing spectra of pure polysaccharide and conjugate. UV-VIS spectra was obtained on GBC Cintra 40 spectrophotometer. FT-IR spectra was obtained on Nicolet 6700 FT-IR apparatus in ATR technique or in KBr.

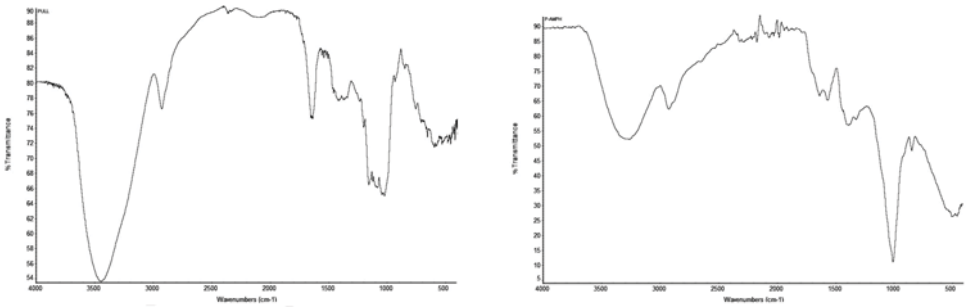
UV spectra of pullulan and oxidized pullulan do not have absorption maxima because polysaccharides do not have any chromophore groups in their molecule. After coupling reaction, spectrum of product occurs to be significantly different (Figure 1b). By comparing spectrum of synthesized conjugate with spectrum of pure amphotericin B (Figure 1a), it can be seen that characteristic absorption peaks (on 365 nm, 387 nm and 412 nm) overlaps. Based on this, it can be concluded that aldehydo groups that are introduced in polysaccharide molecule are successfully coupled with antibiotic.



a) *b)*

Fig.1: UV spectra of amphotericin B (a) and pullulan-amphotericin B conjugates (b)

FT-IR spectroscopic data suggest on chemical bonding between oxidized pullulan and amphotericin B, too. FT-IR spectrum of native pullulan (Figure 2a) shows wide absorption bands typical for polysaccharides. FT-IR spectrum of pure amphotericin B has bands on 2850 cm^{-1} and 2915 cm^{-1}, which correspond to symmetrical and asymmetrical vibrations of C-H bonds from CH_2 groups, band on 1695 cm^{-1} which is from ester C=O function and absorption band on 1012 cm^{-1} from polyene series [6]. FT-IR spectrum of conjugate (Figure 2b) shows combined absorption bands typical for both pullulan and antibiotic.



a) *b)*

Fig.2: FT-IR spectra of pullulan (a) and pullulan-amphotericin B conjugates (b)

Antifungal activity of conjugates. Antifungal activity was determined by spot-test on *Candida albicans* strain ATCC 24433. Minimal inhibitory concentration (MIC) represents the smallest concentration of solution which leads to growth inhibition after 24 h incubation at 28 ºC. For 30% oxidized pullulan-amphotericin B conjugate, calculated MIC was 1.56, and for pure antibiotic in this test 0.78 μg/ml.

Conclusion

The synthesis of pullulan-amphotericin B conjugate was performed by reaction coupling between oxidized polysaccharide and antibiotic. On the basis of its spectral characteristics it was concluded that obtained conjugate have combined spectral properties of both - polysaccharides and polyene antibiotics. Conjugate is soluble in water, which is indication for its potential use in making different nontoxic formulations. It possesses significant antifungal activity against *C. albicans* strain.

Acknowledgement.

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***UV and IR spectroscopy of conjugates*. Coupling reaction was monitored by UV and FT-IR spectroscopy.**
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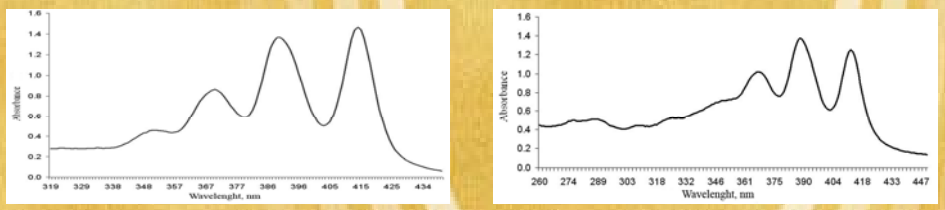

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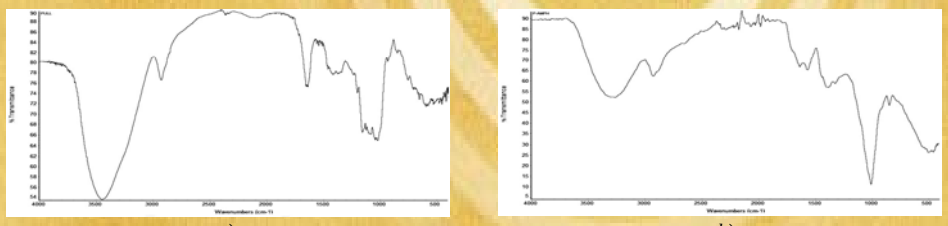

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