PHYSICAL CHEMISTRY 2016

13th International Conference on
Fundamental and Applied Aspects of
Physical Chemistry

Proceedings
Volume II

B E L G R A D E
September 26-30, 2016

ISBN 978-86-82475-33-0
Title: Physical Chemistry 2016 (Proceedings)
Editors: Željko Čupić and Slobodan Anić
Published by: Society of Physical Chemists of Serbia, Studentski trg 12-16, 11158, Belgrade, Serbia.
Publisher: Society of Physical Chemists of Serbia
For Publisher: Slobodan Anić, President of Society of Physical Chemists of Serbia
Printed by: "Jovan", Printing and Publishing Company; 200 Copies
Number of pages: 3+434; Format B5; printing finished in September 2016

Text and Layout: "Jovan"

200 - *Copy printing*

A NEW COLORED SUBSTRATE FOR SCREENING OFBETA-GLUCANASES-DEGRADING MICROORGANISMS

D. M. Jakovljević[1], A. N. Đurić[2], B. D. Kekez[2], G. Đ. Gojgić-Cvijović[1], V. P. Beškoski[1,2] and M. M. Vrvić[1,2]

[1]*ICh TM – Department of Chemistry, University of Belgrade, Njegoševa 12, P.O. Box 473, 11000 Belgrade, Serbia (djakovlj@chem.bg.ac.rs)*
[2]*Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, P.O. Box 51, 11158 Belgrade, Serbia*

ABSTRACT

Novel chromogenic macromolecular substrate was synthesized by covalently coupling of branched β-glucan isolated fromthe cell-wall of baker's yeast *S. cerevisiae*with anthraquinone reactive dye Remazol Brilliant Blue R (RBBR).

New material could potentially have many applications, i.e. for evaluation of endo- and exo-1,3/1,6 glucanases or for screening of β-glucan-degrading microorganisms.

INTRODUCTION

One of the most studied β-glucan from the microbial origin is the β-glucan isolated from the cell wall of yeast *Saccharomyces cerevisiae*. The basic structural characteristic of this polysaccharide is the main linear backbone consisting of (1,3)-linked β-glucopyranose units. Some of these residuses are substituted through O-6 position with single β-glucopyranosyl units, as shown in Figure 1. [1,2]. It is known that glucan from *Saccharomyces cerevisiae* show varied biological activity: immunomodulatory properties,antioxidant effects and protective effect of the aging of cells [3]. Its non-digestibility and, in some degree, fermentation by intestinal microbial flora affects to be considered as a prebiotic [4].

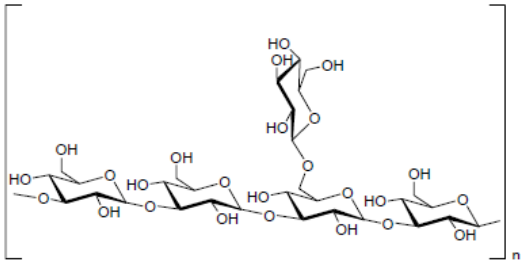

Figure 1. Structure ofβ-glucan

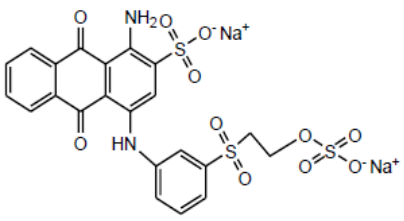

Figure 2. Remazol Brilliant Blue R

Reactive dye Remazol Brilliant Blue R (Fig. 2) belongs to the class of anthraquinone dyes that have wide commercial application [5]. Ethylsulfonyl group of RBBR in alkaline conditions forms vinyl sulfonyl group which undergo addition with carbon nucleophiles of polysaccharide.

The aim of this work was to synthesize and *characterize* a new polymeric material obtained bycovalently coupling of branched β-glucan isolated from the cell wall of baker's yeast *S. cerevisiae* and reactive dye Remazol Brilliant Blue R. This material can be used as potential colored macromolecular substrate for specific assays for selective determination of endo- and exo-1,3/1,6-β-glucanases or for screening of β-glucanases-forming microorganisms.

EXPERIMENTAL

β-glucan used in this work was isolated from the cell-wall of active dry baker's yeast (*Saccharomyces cerevisiae*) commercial product made by Fermentation Industry "Alltech-Fermin", Senta, Serbia and purified by repetitive alkaline/acid treatment [1,2]. Other reagents and solvents were purchased from commercial sources and used as supplied. β-D-glucan was stained with RBBR following the procedure of Khalikova nad Usanov with some modifications [6]. The RBBR dyeaqueous solution (0.2 g/6.0 mL) was added dropwise to a vigorously mixed suspension of β-glucan in water (0.5 g/ 40 mL) at 60°C (magnetic stirrer). After 30 min of stirring, 5.5 g of NaCl was added, and mixing was continued for next hour. The mixture was then alkalized with NaOH (0.5 g/10 mL of water) and the reaction mixture was heated to 80°C. Anhydrous Na_2CO_3 (0.5 g) was added, and extensive stirring was continued for 2 h at 80°C. After cooling, the stained product was centrifuged (3000 rpm, 10 min) and washed with a 96% ethanol. The colored substrate was resuspended in distilled water, dialyzed and lyophilized. Resulting product was insoluble in distilled water, but was soluble in DMSO after autoclaving.Characterization of samples was performed by FT-IR spectra in ATR mode on Thermo Nicolet 6700 FT-IR Spectrophotometer (500-4000 cm^{-1}). UV-VIS spectra were recorded on Shimadzu UV-1280 Spectrophotometer.Elemental analyses were performed on automated analyzer Vario EL III CHNS/O from Elementar Co.

RESULTS AND DISCUSSION

The biopolymeric colored substrate was synthesized by coupling reaction of β-glucan isolated from the cell-wall of baker's yeast *S. cerevisiae* with reactive dye RBBR. FT-IR spectrum of pure β-glucan contained a strong broad absorption at 3000–3500 cm^{-1} corresponding to ν (OH), the peaks at 2950-2850 cm^{-1} attributable to (νCH/CH_2), two partially overlapped absorptions at 1024 and 1048 cm^{-1} arising to ring and (C–OH) side group stretching, and the peak at 890 cm^{-1} assigned to the β-glycosidic (C1–H) deformation mode.However, in the FT-IR spectrum of β-glucan-dye derivative (Fig. 3.) typical absorptions at 670-870 cm^{-1}(γ C-H), 1650-1450 cm^{-1} (ν C=C of aromatic), 1530 cm^{-1} and 1380 cm^{-1} (ν C-N), 1037 cm^{-1} and 1120 cm^{-1} (ν S=O)indicate the presence of anthraquinone ring, C-N and S=O vibrations, as a result of the modification of β-glucan with RBBR.

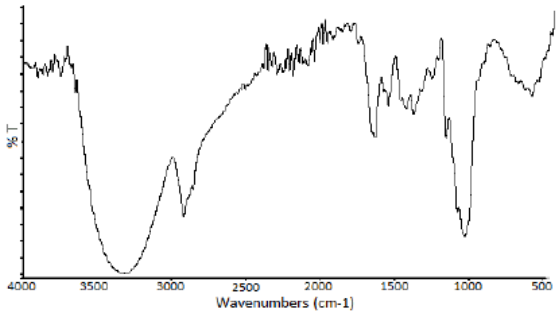

Figure 3. FT-IR spectrum of covalently coupled β-glucan-RBBR

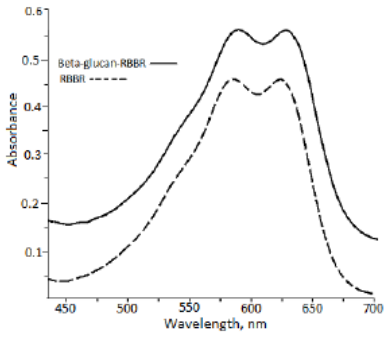

Figure 4. UV-VIS spectra of RBBR and β-glucan-RBBR derivative

The elemental analysis of β-glucan yields: C, 44.4; H, 6.2. The obtained values C and H for glucan are in accordance with the values of neutral glycans. In coupled product the content of RBBR is reflected by the increase of nitrogen content after reaction of β-glucan with dye. The elemental analysis of β-glucan-RBBR yields: C, 46.3; H, 6.7; N, 0.6. UV-VIS spectra (region 450-700 nm) of RBBR and synthesized substrate were shown in Fig.4. The free RBBR ($1.6x10^{-4}$mM/mL) in DMSO showed two absorption maxima at 585 nm and 625 nm. The spectrum

of β-glucan coupled with RBBR showed a sameshape of the spectrum, with maxima slightly blue-shifted (up to 5 nm) compared to the RBBR, which indicates that contribution to the UV-VIS spectrum of synthesized stained biopolymer comes from the anthraquinone segments. This is in accordance with previously published data [6].

CONCLUSION

The synthesis of new colored polymeric substrate was performed by covalent coupling of the branched β-glucan isolated from the cell-wall of *S. cerevisiae* with reactive anthraquinone dye Remazol Brilliant Blue R. The coupled derivative was characterized by elemental analysis, FT-IR and UV-VIS data. Synthesized material can be used as potential colored macromolecular substrate for screening of β-glucanases-degrading microorganisms.

Acknowledgement

This work was supported by the Ministry of Education and Science of the Republic of Serbiathrough Project III 43004.

REFERENCES

[1] B.A. Stone, A.E. Clarke, Chemistry and Biology of (1,3)-β-Glucans, La Trobe University Press, Australia,1992, pp. 47–49.
[2] D. Zlatković, D. Jakovljević, Dj. Zeković, M.M.Vrvić, J. Serb. Chem. Soc. 2003, **68**, 805-809.
[3] Đ. Zeković, S. Kwiatkowski, M.M. Vrvić, D. Jakovljević, C.A. Moran, Crit Rev. Biotechnol. 2005, **25**, 205-230.
[4] O.B. Laugier, S.D. Spasić, V. Mandić D. Jakovljević, M.M. Vrvić, Int. J. Food Sci. Technol. 2012, **47**, 369-375.
[5] C. Fleischmann, M. Lievenbrück, H. Ritter, Polymers, 2015,7, 717–746.
[6] E.F. Khalikova, N.G. Usanov,Appl.Biochem. Microb. 2002, **38**, 89–93.