Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.yu • www.shd.org.yu/JSCS

J. Serb. Chem. Soc. 72 (12) 1271–1274 (2007) UDC **Gerardia savaglia*:639.61/.62:547.567:547.96
JSCS–3659 *Short communication*

SHORT COMMUNICATION

Chemical modification of the lectin of the marine coral *Gerardia savaglia* by marine quinone avarone

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(Received 3 August 2007)

Abstract: The quinone avarone, isolated from the marine sponge *Dysidea avara*, possesses the ability to chemically modify proteins. In this work, modification of lectin isolated from the coral *Gerardia savaglia* by avarone was examined. The techniques used for studying the modification were: SDS PAGE, isoelectric focusing and hemagglutination testing. The results of the SDS PAGE indicate dimerization of the protein. A shift of the pI toward lower value occurs upon modification. The change of the hemagglutination activity of the protein confirms that chemical modification of *G. savaglia* lectin by avarone changes its ability to interact with the membrane of erythrocytes.

Keywords: avarone, quinone, *Gerardia savaglia* lectin, covalent modification.

INTRODUCTION

Lectins are (glyco)proteins of plant or animal origin possessing an ability to specifically bind carbohydrate moieties. Such properties make them useful tools for the isolation and characterization of polysaccharides and glucoconjugates,[1,2] as well as in biomedical research.[3–5] Marine organisms are rich sources of lectins.[6,7] One of them, *Gerardia savaglia* lectin (GSL), was isolated from Adriatic sea coral and partially characterized.[8] This mannose specific lectin showed hemagglutinating activity toward human and some animal red blood cells, moderate mitogenic activity and a feature to bind glycoproteins present in the nuclei from CV-1 monkey kidney cells. A sesquiterpenoid hydroquinone avarol was isolated from the Mediterranean sponge *Dysidea avara* and oxidized to quinone avarone.[9,10]

In this work, the ability of avarone to chemically modify proteins, previously examined in case of some enzymes,[11,12] was tested. The significance of this study lies especially in the fact that modification of a marine lectin by a compound

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doi: 10.2298/JSC0712271P

from the marine environment has been investigated, so that the results might give insight into marine ecological relations.

The chemical modification of GSL by avarone was studied by hemagglutination testing, SDS PAGE and isoelectric focusing.

EXPERIMENTAL

Avarol was isolated from the extract of the sponge *Dysidea avara*, collected in the Bay of Kotor (Montenegro) and oxidized to avarone with silver oxide.[10]

Gerardia savaglia lectin was isolated from crude coral extract in a pure form by affinity and gel chromatography.[8] The activity of the isolated lectin was examined by hemagglutination testing.[13]

Modification of *Gerardia savaglia* lectin was performed in 20 % EtOH. The final volume was 3.75 ml and the final concentrations of the lectin and avarone were 1 mg/ml and 0.5 mg/ml, respectively. The pH value of the mixture was adjusted to 8.5 with $NaHCO_3$. The reaction was performed at r.t. for 48 h with stirring. Subsequently, the mixture was centrifuged at 14000 rpm and the pellet was washed three times with ethanol. The pellet was then resuspended in 50 mM acetate buffer, pH 5.5 and prepared for hemagglutination testing, SDS PAGE and isoelectrofocusing.

Hemagglutination testing was performed on sheep erythrocytes.[13]

SDS PAGE was performed under reducing conditions. The molecular masses of the denatured protein samples were determined on 10 % polyacrylamide gel using molecular markers of 14.4 kD, 18 kD, 29 kD, 45 kD and 66 kD.[14]

Isoelectrofocusing was performed on 7.5 % polyacrylamide gel under non-reducing conditions with an ampholite range from 3−10 and a calibration pI kit.[15]

RESULTS AND DISCUSSION

After mixing avarone and the lectin solution, a change of color of the reaction mixture from light yellow to violet and pellet formation were visible after approximately 24 h.

The protein modification was examined by SDS PAGE. Reaction of avarone and *Gerardia savaglia* lectin gave a visible band at 30 kD (Fig. 1), which suggests dimerization of the protein (molecular mass of the monomer is 14.8 kD). Isolelectricfocusing showed four bands at pH values 8.7, 8.5, 8.3 and 7.3, indicating a shift of the pI value of the protein to lower pI values upon modification (Fig. 2). These results suggest that the side chains of basic amino acids, such as lysine amino groups, are involved in the reaction of the protein and the quinone.

The specific hemagglutinating activity of the resuspended pellet towards sheep erythrocytes was about 10-fold lower (160 hemagglutination units (HU)/mg protein) than the activity of the unmodified lectin (1706 HU/mg), which confirms the involvement of the same amino acid residues in the protein polymerization and interaction with the membrane of the erythrocytes. The identity of the modified residues remains to be determined. At present, there is no conclusive evidence whether the modification occurs in the binding site or in other regions resulting in a conformational change and concomitant decrease in activity.

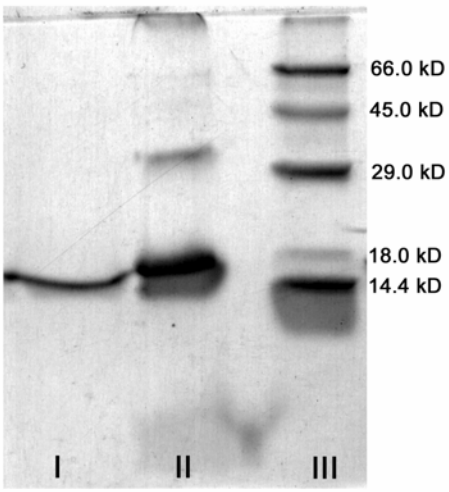

Fig. 1. SDS PAGE electrophoregram. I: Unmodified *G. savaglia* lectin. II: Modified *G. savaglia* lectin. III: Molecular weight markers.

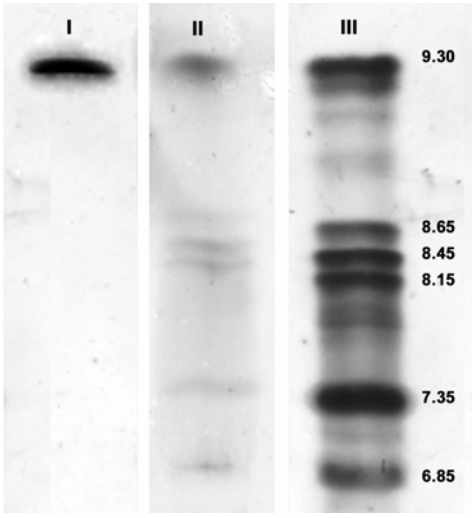

Fig. 2. Isoelectrofocusing gel. I: Unmodified *G. savaglia* lectin. II: Modified *G. savaglia* lectin. III: pI calibration kit markers. For clarity, only the region in which modified bands occur is shown.

CONCLUSIONS

The results presented in this work lead to the conclusion that avarone could chemically modify *Gerardia savaglia* lectin and that this modification altered its characteristics and biological activity.

The biological functions of lectins, especially those isolated from marine organisms, are still not completely understood. Marine lectins are involved in cell recognition and aggregation and in the process of the elimination of foreign organisms.[16,17] Although additional evidence is necessary, the obtained results could indicate covalent modification of the lectins as a possible manner of action in these processes, essential for the survival of these sedentary organisms. On the other hand, since compounds similar to avarone are present in algae,[18,19] ingestion of these algae could impair the interactions in which lectins are involved in the coral, so that a toxic effect to the animal could occur.

Acknowledgement. This work was partially supported by the Ministry of Science of the Republic of Serbia (Project No. 142026).

И З В О Д

ХЕМИЈСКЕ МОДИФИКАЦИЈЕ ЛЕКТИНА МОРСКОГ КОРАЛА *Gerardia savaglia* МОРСКИМ ХИНОНОМ АВАРОНОМ

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Аварон, хинон изолован из морског сунђера *Dysidea avara*, поседује способност да хемијски модификује протеине. У овом раду испитивана је модификација лектина изолованог из корала *Gerardia savaglia* авароном. Технике за праћење хемијске модификације биле су: SDS PAGE, изоелектрично фокусирање и хемаглутинациони тест. Резултати SDS PAGE упућују на димеризацију протеина. Долази до померања pI вредности протеина. Промена хемаглутинационе активности *G. savaglia* лектина авароном утицала је на његову способност интеракције са мембраном еритроцита.

(Примљено 3. августа 2007)

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