# Serbian Biochemical Society

**President:** Marija Gavrović-Jankulović
**Vice-president:** Suzana Jovanović-Šanta
**General Secretary:** Milan Nikolić
**Treasurer:** Milica Popović

## Organization Committee

Suzana Jovanović-Šanta
Milan Nikolić
Ivana Beara
Nataša Simin
Željko Popović
Jovana Šućur
Milica Popović

## Scientific Board

Mihajlo B. Spasić
Marija Gavrović-Jankulović
Ivan Spasojević
Neda Mimica-Dukić
Aleksandra Stefanović
Marko Živanović
Polina Blagojević

## Proceedings

**Editor:** Ivan Spasojević
**Technical secretary:** Jelena Nestorov
**Cover design:** Zoran Beloševac
**Publisher:** Faculty of Chemistry, Serbian Biochemical Society
**Printed by:** Colorgrafx, Belgrade

# Serbian Biochemical Society
# Eighth Conference

with international participation

University of Novi Sad – Rectorate Hall
16.11.2018. Novi Sad, Serbia

"***Coordination in Biochemistry and Life"***

# Isolation, characterization and biological activity of R-phycoerythrin from red macroalgae *Porphyra* spp.

**Ana Simović*, Milan Nikolić, Simeon Minić**

*Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Belgrade, Serbia*

**e-mail: simovic.ana@gmail.com*

Red algae *Porphyra* spp. are traditionally used in cuisine and medicine of Eastern Asia countries. Porphyra algae, popularly known as "nori", are rich in proteins, dietary fiber, pigments, inorganic elements, vitamins, polyunsaturated fatty acids and mycosporine-like amino acids. In addition to exceptional nutritional value, a number of studies have shown beneficial physiological effects of these compounds, such as immunomodulating, anticancer, antihyperlipidemic and antioxidative activities, which is why nori has gained recognition as a superfood [1].

R-phycoerythrin (R-PE) is the most abundant pigment in *Porphyra* spp. It is a water-soluble, intensely pink to red colored phycobiliprotein with yellow fluorescence. It's composed of apoprotein portion and covalently bound open-chain tetrapyrrole chromophores, red phycoerythrobilins and yellow-orange phycourobilins. Commercially, R-PE is mostly used as a fluorescent probe, with emerging application as an industrial dye. This protein gets increased recognition as a nutraceutical with pronounced antioxidative, anticancer, immunomodulatory and anti-aging potential [2].

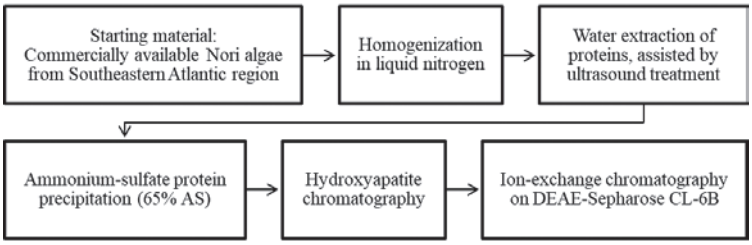


**Figure 1.** Schematic diagram of the isolation and purification protocol for R-PE from *Porphyra* spp.

In this study, we firstly isolated and purified R-PE from commercially available nori algae dried flakes, by the procedure optimized in our laboratory (Figure 1). By the same protocol, we also isolated Porphyra's less abundant purple phycobiliprotein R-phycocyanin (R-PC), which was used for comparison purposes. Identities of isolated proteins were confirmed by SDS-PAGE (14% gel; not shown) and by standard spectroscopic methods (Figure 2), based on positions of the peaks in the UV-visible absorption and fluorescence

emission spectra. Obtained purity index was 3.8 for R-PC ($A_{620}/A_{280}$) and 5.7 for R-PE ($A_{565}/A_{280}$), suggesting analytical/standard purity for both proteins.

Furthermore, results of secondary structures analysis (from far-UV CD spectra data) suggest a high content of α-helices in R-PE (72%) and R-PC (66%), in accordance to literature data [3]. Thermal stability monitoring (by CD spectroscopy) and melting point ($T_m$) determination results indicate that R-PE ($T_m$ ~76,0°C) is notably more stable than R-PC (Tm ~55°C), which makes it a good candidate for application in food industry.

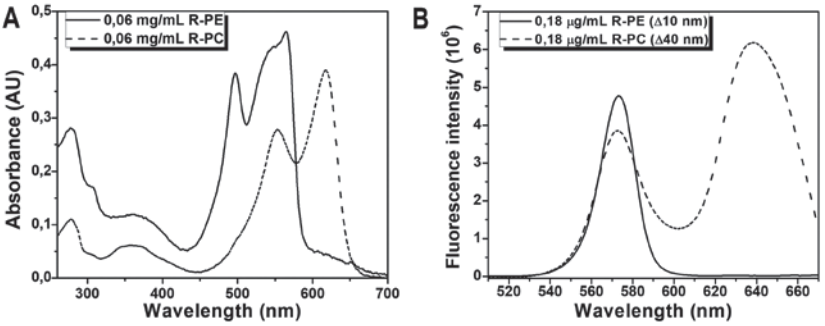


**Figure 2.** (A) UV-VIS absorption spectra of purified R-PE (with characteristic maxima at 498 and 565 nm) and R-PC (553 and 618 nm); (B) synchronous fluorescence spectra of R-PE (Δλ 10 nm; maximum at 573 nm) and R-PC (Δλ 40 nm; maxima at 573 and 638 nm).

Finally, we evaluated R-PE bioactivity in terms of its ability to bind physiologically important, redox active $Cu^{2+}$ and $Zn^{2+}$ ions, and protein antioxidant and free radicals scavenging activities. UV-VIS and CD spectroscopy data revealed binding of metal ions to R-PE, without significant impact on protein secondary structure. Binding constants determined by fluorescence quenching method were: 7.4 x $10^5$ $M^{-1}$ ($Cu^{2+}$) and 1.2 x $10^3$ $M^{-1}$ ($Zn^{2+}$). Results from *in vitro* assay systems [DPPH–, ABTS–, hydroxyl radical–, and superoxide anion radical–scavenging activity, ferric ion reducing ability of plasma (FRAP) assay, ferrous ion-chelating activity (FICA), and reducing power (RP) assay] showed that R-PE exhibit concentration-dependent antioxidant potential similar to, if not better than that found in R-PC. Our results support observed health-related benefits and importance of further research on this phycobiliprotein.

## References


1. Fleurence J, Levine I (eds) Seaweed in health and disease prevention. 1st ed. Academic Press, Cambidge, 2016.
2. Sonani RR, Rastogi RP, Patel R, Madamwar D. Recent advances in production, purification and applications of phycobiliproteins. World J Biol Chem 2016;7:100-9.
3. D'Agnolo E, Rizzo R, Paoletti S, Murano R. R-phycoerythrin from the red alga Gracilaria longa. Phytochemistry 1994;35:693-6.

CIP- Каталогизација у публикацији
Народна библиотека Србије

577.1(082)

SRPSKO biohemijsko društvo. Konferencija sa međunarodnim učešćem (8 ; 2018 ; Novi Sad) Coordination in biochemistry and life : [proceedings] / Serbian Biochemical Society, Eighth conference with international participation, 16.11.2018., Novi Sad, Serbia ; [editor Ivan Spasojević]. - Belgrade : Faculty of Chemistry : Serbian Biochemical Society, 2017 (Belgrade : Colorgrafx). - 198 str. : ilustr. ; 25 cm Tiraž 200. - Str. 11: Foreword / Ivan Spasojević. - Bibliografija uz većinu radova.

ISBN 978-86-7220-096-6 (FOC)

a) Биохемија - Зборници

COBISS.SR-ID 269798412