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"***

Foreword

Dear Colleagues

Welcome to the 8th Conference of the Serbian Biochemical Society, entitled "*Coordination in Biochemistry and Life*".

The title of this year's Conference refers to an important place of coordination chemistry in biochemistry and biomedicine, but also to a need to coordinate the efforts towards new knowledge with fellow scientists from other fields in order to reach more. The collaboration within FEBS3+ (Croatia, Hungary, Slovenia, and Serbia) Meeting Programme continues with the invited lecture of our dear colleague Tantos Ágnes from Research Center for Natural Sciences, Budapest, Hungary. For the first time we have 'Diaspora Lecture' that will be delivered by Miloš Filipović, a top 'product' of Serbian biochemistry who is now affiliated at the Université de Bordeaux. We have more than forty PhD students from Serbia, Hungary, and Belarus with poster presentations, and for the first time the Conference is held outside the capital. It believe that we are getting better each year, and that we are prepared for future challenges.

I would like to express my gratitude to the members of the Scientific Board who suggested lecturers, to all respected colleagues who accepted the invitation, and to our dear hosts from the University of Novi Sad.

Editor of the Proceedings
Ivan Spasojević

Biliverdin-copper complex at the physiological pH

Milena Dimitrijević[1*], Jelena Bogdanović Pristov[1], Milan Žižić[1], Dalibor Stanković[2,3], Danica Bajuk-Bogdanović[4], Marina Stanić[1], Snežana Spasić[5], Wilfred Hagen[6], Ivan Spasojević[1]

[1]Department of Life Sciences, Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia
[2]The Vinča Institute of Nuclear Sciences, University of Belgrade
[3]Innovation Center of the Faculty of Chemistry, University of Belgrade
[4]Faculty of Physical Chemistry, University of Belgrade
[5]Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade
[6]Department of Biotechnology, Delft University of Technology, Delft, The Netherlands

**e-mail: milena.dimitrijevic@imsi.bg.ac.rs*

Biliverdin (BV) is a degradation product of heme catabolism, which is rapidly converted to bilirubin (BR) by BV reductase [1]. Biliverdin and unconjugated BR, commonly named bile pigments, have important function in biochemical processes. The presence of copper and other biological and toxic transitional metals at significant concentrations in bile implies the possibility that metal complexes with bile pigments can be formed [2]. Consequently, our interest was to study the complex of BV with copper in physiological conditions – phosphate buffer with pH 7.4.

UV-Vis spectrophotometry was applied to investigate formation/degradation of complex of BV with copper ions and to check stoichiometry by titration, showing that BV interacted with Cu^{2+} in 1:1 stoichiometry. Mass spectroscopy analysis confirmed this – ion at *m/z* 643.36 was detected. The results of Raman spectroscopy of BV were in good agreement with previous reports [3]. Comparing spectra of BV and BV-Cu complex, the following differences were observed: a new band at low wave number is emerged for the complex may be attributed to Cu-N bond vibration; the band which was shifted to lower energies implicates increased stability of BV in the complex; intensity changes imply a more planar structure of BV in the complex, while stronger bands in complex imply higher delocalization of π-electrons and consequently a higher stability of the BV structure. Pertinent to this, it has been proposed that complexes of BV model compounds with Cu^{2+} may show unusual electronic structures that exhibit a significant ligand radical character. ^{1}H NMR spectrum of BV in phosphate buffer had a poor resolution of signals, which may originate from aggregation, but this was of little relevance here, since the addition of copper ions led to a very strong effect – the complete loss of almost all lines. The loss of signals represents the result of strong paramagnetic effects that may come from an unpaired e^- that is delocalized in pπ orbitals of the ring/ligand influencing all protons in the

complex. The EPR spectrum of Cu^{2+} ($S = 1/2$; $I = 3/2$) in phosphate buffer shows that Cu^{2+} is weakly coordinated in an axial symmetry with one $g_{\perp}$ line and four lines coming from hyperfine coupling along $g_{\parallel}$. The addition of BV in equimolar concentration led to the loss of Cu^{2+} signal. The remaining signal in the $[BV]/[Cu^{2+}] = 1$ system was broad, and did not show hyperfine structure. The *g*-value of the isotropic signal of BV-Cu complex was significantly lower than the average *g*-value of Cu^{2+} in the phosphate buffer indicating delocalization of the spin away from the metal nucleus. Similar EPR signals have been reported previously [4]. Parallel-mode EPR showed no signal. Furthermore, the spectra were run over a wide field range and no half field lines were observed, either in parallel or in perpendicular mode. These results are consistent with $S = 0$ for the copper center. Further, redox properties of the complex were examined. BV showed a well-defined anodic peak. The $[BV]/[Cu^{2+}] = 2$ system showed two additional oxidation peaks at much lower potentials than BV. The former potential corresponds to the oxidation of Cu^{1+}, as we have shown previously [5]. There was a slight consumption of O_2 in $[BV]/[Cu^{2+}] = 1$ system, which may be explained by traces of 'free' copper. However, in the presence of an excess of copper ($[BV]/[Cu^{2+}] = 0.5$), the consumption of O_2 was significant. This implies that 'free' Cu^{2+} reacts with the complex and 'shuttles' an e^- to O_2. The complex was susceptible to oxidizing agents but not to reducing agents.

Considering the results obtained we conclude that, at physiological pH, BV builds a complex with copper ions in 1:1 stoichiometry. The formation of complex involves the rearrangement of electronic structure which provides increased energetic stability and strong paramagnetic effects. We believe that a complex with a highly delocalized unpaired e^- and the formal $BV^{\bullet+}$-Cu^{1+} character best suites the outlined properties, but other structures of the complex cannot be completely ruled out. The presented results may shed new light on long-standing issues of BV chemistry and catalysis in biological systems.

Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (III43010).

References

1. Zhang L. Heme Biology: The Secret Life of Heme in Regulating Diverse Biological Processes. World Scientific Publishing Company, Singapore, 2011.
2. Suzuki N. On bilirubin-metal complex compounds in relation to black pigments of gallstones. Tohoku J Exp Med 1966;90:195-05.
3. Celis F, Campos-Vallette M, Gómez-Jeria J, Clavijo R, Jara G, Garrido C. Surface-enhanced Raman scattering and theoretical study of the bilichromes biliverdin and bilirubin. Spectrosc Lett 2016:49:336-42.
4. Peeks M, et al. Electronic delocalization in the radical cations of porphyrin oligomer molecular wires. J Am Chem Soc 2017;139:10461-71.
5. Božić B, et al. Mechanisms of redox interactions of bilirubin with copper and the effects of penicillamine. Chem Biol Interact 2017; 25:129-34.