Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2020, **30**, 211–213

Mendeleev Communications

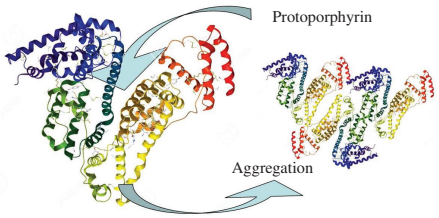
Albumin aggregation promoted by protoporphyrin *in vitro*

Natalya Sh. Lebedeva, Elena S. Yurina, Yury A. Gubarev,* Aleksey N. Kiselev and Sergey A. Syrbu

G. A. Krestov Institute of Solution Chemistry, Russian Academy of Sciences, 153045 Ivanovo, Russian Federation. E-mail: yury.gu@gmail.com

DOI: 10.1016/j.mencom.2020.03.027

Protoporphyrin upon its binding with serum albumin changes its secondary structure due to the conversion of part of α helices into β-folding. This process results in the association of albumin globules *in vitro*.

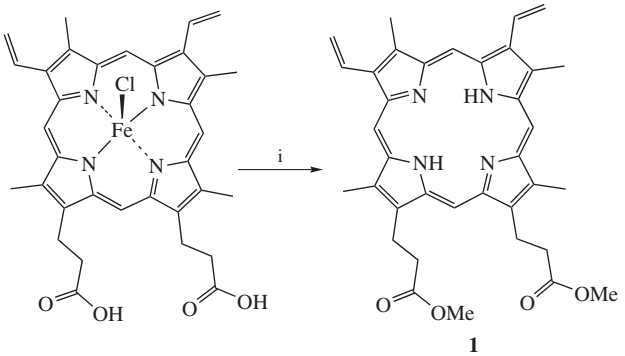


Keywords: porphyrin, albumin, aggregation, UV-VIS spectroscopy, IR spectroscopy, fluorescence.

Protoporphyrin belongs to the blood group porphyrins and its complex with Fe^{2+} called hemin represents a prosthetic group for a number of proteins including those involved in transport and storage of oxygen (*e.g.* hemoglobin, myoglobin), electron transfer, drug and steroid metabolism (*e.g.* cytochromes) as well as signal transduction (*e.g.* nitric oxide synthase, guanylate cyclase). The content of protein-free hemin in a body is controlled by hemoxygenase.[1] Lack of heme or problems in the synthesis of protoporphyrin result in severe diseases such as anemia and porphyria.[2] For their treatment, drugs containing synthetic hemin[3,4] are typically used. On the other hand, an excess of protein-free porphyrin also leads to various pathologies, including temporary thrombosis, liver failure and hemorrhagic diathesis.[5,6] Moreover, an excessive amount of hemin may cause lysis of erythrocyte membranes especially in sickle-shape cell anemia,[7] oxidation of low-density lipoproteins[8] and formation of fatty acid hydroperoxides. These processes lead to renal failure associated with intravascular hemolysis, hemorrhagic damage of the central nervous system and atherogenesis.[8] Localization as well as concentration of protoporphyrin and heme should be strictly regulated in an organism, since any deviation from their optimal amount causes a pathological condition. In turn, the porphyrin content is influenced by the effectiveness of enzymes catabolizing heme as well as by the efficiency of transport systems that ensure the circulation of hydrophobic porphyrin in cells, intercellular fluid and bloodstream. Some systems for the heme and protoporphyrin transportation in mammals have been discovered and extensively explored.

The data on the transport proteins causing the passage of tetrapyrrole macroheterocyclic compounds through the cell membrane, such as HCP1 proteins, FLVCR, Abcg2 and Abcb6, as well as of extracellular heme-binding proteins, such as hemopexin, haptoglobin and serum albumin, is summarized in the review.[9] According to the results of our works[10–12] on the interaction of exogenous synthetic macroheterocyclic compounds with serum albumin, it was hypothesized that the interaction of endogenous porphyrins with albumin can lead to the protein aggregation. This work was aimed at the experimental verification of the hypothesis about the protein aggregation mechanism. The acquired results can afford a detection of the molecular-level reason for β-folding of globular proteins, which is an 'identity card' for diseases like amyloidosis and hypoalbuminemia.[13]

The investigation of the interaction of hydrophobic protoporphyrin **1** with BSA† was carried out in a medium containing 0.5 M NaCl with DMF added to the concentration not exceeding 0.19 M. The synthesis of protoporphyrin **1** is shown in Scheme 1. The chosen concentration of NaCl eliminated the effect of polyelectrolyte protein swelling, while DMF provided the solubility



1

Scheme 1 *Reagents and conditions*: i, MeOH, pyridine, CH_2Cl_2, Mohr's salt, AcCl.

† Bovine serum albumin, fraction V (BSA) was purchased from Acros Organics (USA).

Protoporphyrin IX dimethyl ester **1**. Hemin (5.0 g, 7.6 mmol) and pyridine (5.0 ml) were placed in a three-necked flask, then MeOH (300 ml), CH_2Cl_2 (300 ml) and Mohr's salt (20.0 g, 51 mmol) were added. Acetyl chloride (150 ml, 2.1 mol) was gradually added under stirring and cooling, while the temperature was kept below 35 °C. The mixture was stirred for 1 h and then diluted with H_2O (500 ml). The bottom organic layer was separated, washed with aqueous ammonia (25%, 50 ml), then with H_2O (200 ml) and dried over anhydrous Na_2SO_4. The product was purified by chromatography on silica gel 60 mesh using CH_2Cl_2 as the eluent. Yield 4.48 g (99%).

UV-VIS and fluorescence spectra were recorded using an AvaSpec-2048 spectrophotometer (Avantes, Netherlands) at 25°C. A monochromatic LED UVTOP-295 (Sensor Electronic Technology, USA) was employed as an excitation light source in the fluorescence experiments. IR spectra were recorded on an Avatar 360 IR-Fourier spectrometer (Thermo Nicolet, USA) in KBr pellets.

Italian Oral Surgery, 30 (2020) 211-213. doi:10.1016/j.mencom.2020.03.027

Italian Oral Surgery, 30 (2020) 211-213. doi:10.1016/j.mencom.2020.03.027