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Labile lead phytochelatin complex could enhance transport of lead ions across biological membrane

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ABSTRACT

In connection with studies of transport of hazardous metals across model phospholipid membranes (PLMs), behavior of complexes of lead with phytochelatin PC₂ has been examined. Using differential potentiometric stripping analysis (PSA, Chronopotentiometry) with different values of constant negative current, the release of free lead ions confirmed their labile behavior. Similar conclusion resulted from voltammetry on silica gel modified carbon paste electrode. Diffusion controlled transport across a PLMs containing ionophore calcimycin (A23187) revealed 26 times more lead ions transported in case of Pb-phytochelatin complexes than in case of free lead ions. For labile behavior, complex with two Pb atoms at phytochelatin molecule is responsible.

1. Introduction

Lead is a toxic metal occurring naturally in relatively small extent, but due to huge men activities, it represents one of main contaminants of the environment, mainly of soil, of groundwater, and of surface waters. Pb²⁺ is the most common and reactive form of lead, forming mononuclear and polynuclear oxides and hydroxides. Entering of lead into plants, as the beginning of food chain, poses danger of toxicity for animal and human population [1].

Each plant cell and some of its sub-organelles are surrounded by phospholipid (PL) membranes (PLMs), composed mainly from different fatty acids, glycerol, and ethanolamine, choline, inositol, cholesterol and of some other basic molecules. Such PL membranes are created of a thin layer of amphiphatic molecules of PLs. They are organized spontaneously so that their hydrophobic tails are separated from surrounding polar liquids by hydrophilic heads [2].

Cholesterol represents other substantial and important part of plasma membrane and in low concentrations of the membranes of intracellular organelles [3]. This biomolecule does not form bilayer by itself, however, it is nonhomogeneously distributed in PL membranes [4]. Cholesterol positively effects on stability of biomembranes [2]. Moreover, various polysaccharides and partly or fully built-in peptides are incorporated into these relatively flat membranes.

Only very small and simple molecules (e.g., oxygen, water) can be transported spontaneously across such very thin membranes. Transport of most of other molecules and species must be realized via various ion

channels, which are formed by pore-forming membrane proteins. They are responsible for establishing of membrane potentials and similar electrical signals, which enable the flow of species across membranes and regulate cell or compartment volumes. In some cases, it is necessary to supply some energy (e.g., in the form of adenosine triphosphate (ATP)) required for transport. Via such ion channels, most of ions are moved, e.g., biologically important Na⁺, K⁺, Ca²⁺ cations, transport mechanisms and composition of which have been elucidated in detail, and on the other hand, toxic Cd²⁺, Pb²⁺ cations, transports of which have been studied, nevertheless, remained unelucidated up to now [5–9].

As it has been confirmed, transport process of various ions can be affected by presence of complexes of low molecular weight organic acids (e.g., oxalic, citric, malic) [10–13] or phytochelatin [14].

Plants have developed variety of mechanisms of defense against toxic influence of heavy metals - adsorption at cell walls, production of low molecular weight organic acids (e.g., oxalic, citric), and of sulfur containing ligands, especially phytochelatin [15–18]. Phytochelatin – polypeptides of structure [(γ-Glu-Cys)_n-Gly] (where “γ-Glu” means γ-glutamic acid, “Cys” means cysteine, and “Gly” means glycine) - PC_n - were studied mainly with the influence of cadmium ions on different types of plants. Although lead ions are in the list of activators of phytochelatin synthesis, only few cases of Pb phytochelatin occurrence were described lately [1,19,20].

Properties of various types of phytochelatin (PC₂, PC₃, PC₄) [21] and metallothioneins [22] have been investigated in last decades

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intensively using different methods, e.g., NMR, UV–vis spectroscopy [23], electrospray ionization tandem mass spectrometry (ESI MS-MS) [21]. Different electrochemical methods have been relatively frequently used for these purposes, often in combination with other analytical techniques [24–31]. First of all, Voltammetry and potentiometric stripping analysis (PSA, chronopotentiometry) can yield us very valuable data in this research too [24,25,27].

To elucidate transport processes across biological membranes, synthetically prepared phospholipid membranes have been utilized, e.g., in the form of black membranes, vesicles, or supported membranes with incorporated ion channels [32,33] and characterized by electrochemical impedance spectroscopy (EIS) [5,27,28,34].

In previous studies of cadmium ion transport across model PL membrane containing ionophore A23187 (calcimycin) [5,14], we found enhanced transport of cadmium ions when a labile complex Cd-malic acid was present. Such influence of labile complexes in diffusion-controlled transport was studied also with diffusion gradient technique (DGT) and documented with higher levels of cadmium in concerned plants [35].

Pistia stratiotes is one of plants where intense synthesis of phytochelatin was described [36]. Moreover, in some countries, this plant is used as a component of animal feed. In this connection, lead levels in rats were analyzed and its high levels were found in all samples from the experimental group, which feed contained *pistia* [37]. Therefore, the behavior of lead phytochelatin complexes and transport of lead across PLMs is next question to study. The ionophore calcimycin is effective also for transport of lead ions [32] and voltammetric study with the application of multivariate curve resolution suggested formation of two Pb-phytochelatin complexes, reduced at hanging mercury drop electrode (HMDE). The peak potential difference of these two complexes higher than 200 mV [38] witnesses their different stabilities.

2. Experimental

2.1. Reagents and materials

The 0.1 M KCl supporting electrolyte solutions were prepared from KCl Suprapur, purchased from Merck, Czech Republic. The p.a. solvents were obtained from Ing. Petr Švec - PENTA, Czech Republic. All other chemicals used were of analytical purity grade. Borate buffer pH 8.2 was prepared from Suprapur sodium tetraborate acidified by HNO₃, suprapur (Merck, Czech Republic). The AAS standard solution of Pb²⁺ (1000 mg L⁻¹ in 2% HNO₃) was purchased from Analytica, Czech Republic. The solution pH was adjusted to desired value using NaOH, suprapur (Merck, Czech Republic). For the preparation of supported PLMs (s-PLMs) 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (Avanti Polar Lipids, USA) was used.

Phytochelatin PC₂ [(γ-Glu-Cys)₂-Gly] was obtained from Genosphere Biotechnologies, France.

The used ionophore A23187 (Calcimycin, Calcium Ionophore, Antibiotic A23187, Calcium Ionophore A23187) was purchased from Sigma-Aldrich, Czech Republic.

For all measurements, deionized water from Milli-Q-Gradient, Millipore, Czech Republic (conductivity < 0.05 μS cm⁻¹) was used.

2.2. Instrumentation and procedures

2.2.1. Voltammetry a potentiometric stripping analysis (chronopotentiometry)

Voltammetric and differential PSA measurements were carried out by the PC-controlled voltammetric analyzer *ECO-TRIBO polarograph* (Polaro-Sensors, Czech Republic), equipped with *POLAR.PRO* software v. 5.1 (Polaro-Sensors, Czech Republic) and with *MultiElchem* v. 3.1 software (J. Heyrovský Institute of Physical Chemistry of CAS, v.v.i., Czech Republic). *Pen-type hanging mercury drop electrode* (Polaro-Sensors, Czech Republic) and SiO₂-modified carbon paste electrode

were used as the working electrodes. Ag/AgCl/KCl_{sat} electrode (Elektrochemické Detektory, Czech Republic) was a reference electrode and the platinum wire (diameter 1 mm) wire served as a counter electrode. The measurements were performed at laboratory temperature (25 ± 2 °C).

The values of pH were measured using pH-meter Jenway 3505 (Bibby Scientific Ltd., UK).

Silica gel-modified carbon paste electrode, which offers possibility to accumulate electroactive analytes [39] was prepared by hand mixing of graphite powder with paraffin oil and 10% w.w. of silica gel.

The paste was packed into a Teflon electrode body with a stainless steel piston, serving for renewing of the paste surface as well as for an electric contact [40,41]. The area of this used electrode amounted to 7.1 mm². The new surface was smoothed on a card paper.

2.2.2. Electrochemical impedance spectroscopy

The EIS measurements were realized using potentiostat PGSTAT302N + FRA2 module, equipped with software Nova 1.11 (both Metrohm, Czech Republic). The electrochemical impedances were determined using Ag/AgCl electrodes (silver wire, diameter 1 mm, electroplated by silver chloride), which have been used as a working and a reference electrodes. Platinum wire, diameter 1 mm, served as the auxiliary electrode. The Nyquist plots (the dependence of the imaginary part (Z'') on the real part (Z') of impedance) recorded in 0.1 M KCl with applied amplitude 0.005 V provided satisfactory results in the frequency range of 0.1–1000 Hz.

2.3. Transport of lead ions across model membrane

Because ionophore calcimycin acts as a divalent cation ionophore, it has been used for simulation of real ion channels and model transporter of dications. This ionophore was described as effective transporter of cadmium ions across PLMs as well as of lead ions [32,33,42,43]. Therefore, the same electrochemical cell developed earlier for experiments with transport of cadmium ions across the PLMs [44] was used. Such cell consists from two glass columns, separated by two Teflon parts with holes (0.07 cm²), between which the polycarbonate membrane is inserted. Two separated compartments represent then extracellular (*Electrolyte 1*) and intracellular (*Electrolyte 2*) milieu, each of them containing 3 mL of 0.1 M KCl solution. The electrodes were placed into the holes in the top of glass compartments (one silver wire coated with silver chloride as working electrode was immersed into the *Electrolyte 1*, the other silver wire coated with silver chloride as reference electrode and platinum wire as auxiliary electrode were immersed into the *Electrolyte 2*) [44].

2.4. Model supported phospholipid membrane

The s-PLMs were formed by self-assembling of PLs in the holes of the IsoporeS Membrane Filters (Millipore, USA) polycarbonate, hydrophilic 8.0 μm, and the supporting membrane thickness amounted to 7–22 μm. For PLM preparation, 2 mg of DPPC, 20 μL of 1 · 10⁻⁶ M calcimycin solution in ethanol (p.a. ethanol 99.88%) and 100 μL of n-heptane were dissolved at a hot water bath. Then a volume of 15 μL of such prepared DPPC solution was applied to one side of the polycarbonate support, the solvent was let to evaporate, and then another volume of 15 μL was applied to the other side of the support. After 30 min., both sides were simultaneously exposed to aqueous electrolyte (0.1 M KCl).

Formation of PLM was monitored by EIS measurement. After steady state has been reached (approximately 1 h), changes of *Electrolyte 1* were realized (addition of Pb ions or the exchange of 2 mL of KCl solution for that containing Pb-phytochelatin complexes) and transport across PLM, monitored by EIS measurement, was allowed for exactly 1 hour. Subsequently, transported Pb ions were determined in *Electrolyte 2* (after its acidification with HNO₃) using anodic stripping voltammetry on HMDE.

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