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Manganese porphyrins — Studies on their potential use for protein labeling

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ABSTRACT

The presented work aims at characterization of electrochemical and optical behavior of 5,10,15,20-Tetraphenyl-21H, 23H-porphyrin manganese(III) chloride (Mn-tpp) in terms of its potential usage as a hybrid label for particles of biological origin. The results demonstrate the possibility of Mn-tpp application in triple detection system, as it was proved to be successfully determined in presence of proteins by means of differential pulse voltammetry, UV–Vis spectrophotometry and spectrofluorimetry.

In case of electrochemical detection, a variety of parameters that may affect the nature of the generated signal was optimized. The selection of supporting electrolyte was also taken into consideration with particular attention, employing tetrabutylammonium salts with different counterions (borate, bromide, iodide and perchlorate).

Subsequently, the efforts were undertaken to examine the protein's (mimicking the receptor or surface blocking agent) impact on the derived signals. In the role of model bioparticles bovine serum albumin (BSA), chicken egg albumin (CEA) and immunoglobulin G (IgG) were employed. After addition of protein, displacement of received signals along the potential axis (a shift of the potential) and changes in signals intensities were observed.

Simultaneously, research on Mn-tpp was carried out using spectroscopic techniques in order to assess its suitability for use as a label also in optical detection mode.

1. Introduction

Protein detection plays currently a crucial role in a number of clinical and biochemical analyses. The elaboration of methods enabling determination of proteins is also relevant in numerous applications in analytical chemistry [1].

The direct monitoring of the reactions occurring between the two bioparticles seems to lead to the most comfortable and undemanding detection format as it involves no molecule modification. However, it is in fact difficult to fulfill because of the limited sensitivity of the analytical methods that could ensure the direct determination of proteins after their binding. Therefore, methods of bioparticle labeling enabling the generation of sufficiently large change of the analytical signal are being sought, and thereby allow not only the detection of associated proteins, but also the quantification on the basis of changes in the label concentration. The considerable diversity of labels used, demonstrates how great is the need to develop a sufficiently sensitive and stable tracer for molecules of biological origin.

Labels find particular application in affinity biosensors, where they are chiefly used when conjugated with specific antibodies or aptamers in sandwich assay system. A wide variety of labels is being used, depending on the detection mode. When electrochemical detection is applied, ferrocene [2–6], methylene blue [7–11], ferricyanides [12–17] and ruthenium complexes [18–21] are most commonly used as labeling agents. In case of optical determination, nanoparticles [22–26], quantum dots [27–29] and fluorescent dyes [30,31] are employed. Radioactive isotopes are also widely utilized, mainly in radioimmunoassays [32,33]. Enzymes constitute a widely used group of compounds, which catalytic activity allows for indirect determination of analyte through determination of respective products of enzymatic reaction [34–38]. Thus, particular label is predominatingly dedicated to the appropriate detection technique.

Porphyrins and metalloporphyrins represent a class of compounds, which appears to be a very promising labeling tool, since its presence may be monitored using triple detection system, thereby improving the reliability of the analysis. By dint of their unique properties, porphyrins are applicable in a wide variety of analytical methods. The absorption and emission spectra are their most useful property, as they allow for spectroscopic determination of various compounds, especially metal ions e.g. Cd(II) [39], Cu(II) [40,41] or Pb(II) [42]. Metalloporphyrins find also application in potentiometry, playing a role of selective electroactive reagents for anions [43,44] and in HPLC as complexing agents for the determination of transition metals and as stationary phases in immobilized metal ion chromatography [45,46]. There is also a possibility to use metalloporphyrins containing cations of radioactive isotopes in their structure, leading to its determination through the radioactivity measurements [47].

The unique spectroscopic and luminescent properties of porphyrins and metalloporphyrins, as well as the capacity to undergo redox

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reactions were utilized in presented studies, as they allow porphyrins to be characterized based on both the optical detection mode (fluorescence and absorption properties), and also determined using electrochemical techniques. These features make them excellent candidates for the role of hybrid proteins' labels. Commonly used quantum dots, which also have similar properties, so can be determined using spectroscopy and electrochemistry, exhibit however certain limitations. A potential advantage of porphyrins over quantum dots is that they need no disintegration step and therefore may be used repeatedly, as electrochemical measurements by their reversibility allow for porphyrin's multiple usage.

The assumption of this study was to investigate the properties of 5,10,15,20-Tetraphenyl-21H, 23H-porphyrin manganese(III) chloride (Mn-tpp) in terms of their potential usage as proteins' tracer. Mn-tpp is one of the selected metalloporphyrins predestined to become proteins' label applicable in triple detection system. To this end, the electrochemical studies were carried out in order to both precisely characterize redox properties of the examined compound and to determine the optimal conditions for conducted analysis. A variety of parameters that may affect the nature of the generated signals was optimized. Simultaneously, by means of spectrophotometry (UV–Vis) and spectrofluorimetry, spectroscopic characterization of Mn-tpp was carried out. Subsequently, using the same techniques the protein influence on mentioned properties, taking into account type and range of changes in absorption and fluorescence spectra and the effect on porphyrin redox properties, was analyzed.

However, the outcomes presented in this study refer to the application of Mn-tpp as a simplified system that will require subsequent modification in order to conjugate a protein. After the functionalization of Mn-tpp with amino or carboxyl groups, the creation of covalent bound with protein's chains will become possible.

2. Experimental

2.1. Reagents

All measurements, both electrochemical and spectroscopic, were performed in dimethyl sulfoxide (DMSO) obtained from Sigma. 5,10,15,20-Tetraphenyl-21H, 23H-porphyrin manganese(III) chloride (Mn-tpp) was used as received from Mid-Century Chemicals. The supporting electrolytes were tetrabutylammonium salts of iodide ((TBA)I), perchlorate ((TBA)ClO_4), tetraphenylborate ((TBA)tetraphenylborate) and tetraoctylammonium salt of bromide ((TOA)Br), all of which received from Sigma. Commercially available antibody — immunoglobulin G (IgG) from rabbit serum and proteins: bovine serum albumin (BSA) and chicken egg albumin (CEA) were obtained from Sigma.

2.2. Measurements

The electrochemical experiments were carried out using potentiostat CHI660A (CH Instruments Co., USA) in a conventional three-electrode cell consisting of glassy carbon working electrode, Ag/AgCl reference electrode and gold-wire counter electrode. The 1 M KCl solution was applied as an internal electrolyte of the reference electrode. The measurements were performed using differential pulse voltammetry (DPV) maintaining the same parameters of: potential increment = 0.004 V, amplitude = 0.05 V, pulse width = 0.1 s and pulse period = 0.2 s for all analyzed samples. Deaeration of all solutions was accomplished by passing a stream of nitrogen through the solution for 5 min before each measurement.

UV–Vis absorption spectra were obtained using classical 1 cm quartz cells by means of Perkin Elmer — Lambda 25 spectrophotometer. Spectrofluorimetric spectra were obtained on VARIAN — Cary Eclipse spectrofluorimeter. All samples were adjusted to blank of pure dimethyl sulfoxide.

3. Results and discussion

3.1. Electrochemical characterization

In the first stage of research electrochemical properties of Mn-tpp were examined. Preliminary studies designed to predict the best possible parameters that may affect the nature of the generated signal including material of working electrode and applied solvent were performed (data not shown). On the basis of these initial outcomes glassy carbon working electrode was chosen as guaranteeing the best performance. As for the solution composition, on one hand the solvent, which could provide the most intense current signals, in the widest potentials range, was sought. On the other, solvent in which the signal is derived solely from the porphyrin, and therefore devoid of background interference was chosen. The preliminary measurements were therefore carried out with the use of benzonitrile (PhCN), dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF). The solvent, which proved to be appropriately adjusted to ensure the optimal analysis conditions, was DMSO and so all samples were prepared in it. Dimethyl sulfoxide is furthermore the solvent, which is most suitable in the view of the subsequent application of protein to remain its activity and is applied for analyses involving particles of biological origin, especially in immunoassays [48,49].

The essential part of electrochemical research relates to the examination of influence of different supporting electrolytes. Compounds, which were intended for this purpose, were tetrabutylammonium or tetraoctylammonium salts containing different counterions: borate, bromide, iodide and perchlorate, which were taken into consideration, as various ions may interact differently with porphyrin rings or metal cation contained in their structure. Voltammograms obtained for respectively oxidation and reduction of Mn-tpp are depicted in Fig. 1. In both cases, three well-defined peaks can be distinguished, and all three processes are fully reversible. Considering oxidation, when ranging from the less negative potentials, first peak at the potential of −0.32 V corresponds to the reversible oxidation of Mn(II) to Mn(III); second at −1.35 V can be attributed to the formation of anion radical; the third oxidation peak at −1.87 V accounts for the formation of dianion (potential values obtained in the presence of perchlorate salt). The observed characteristics and processes assigned to it are in accordance with the results presented in [50].

Furthermore, it should be highlighted that dependently on the anion applied in the supporting electrolyte, shifts in potential and differs in signal intensities have occurred. For peak corresponding to manganese ion oxidation (at −0.32 V), examined ions are set in an order borate > bromide > iodide > perchlorate with a decrease in the potential, which reflects in the increase of interaction between salt's anion and porphyrin complexing center. The shifts are therefore the result of manganese ion (present in the metalloporphyrin center) complexation by different anions forming parts of supporting electrolytes. Furthermore, this feature of metalloporphyrin has been used for construction of anion-selective sensors based on Mn-tpp [51–53] (and other metalloporphyrins). From the analytical point of view, all applied electrolytes excluding borate are appropriate, as they ensure clearly defined analytical peaks and sufficient intensities of signals.

Subsequently, the bioparticles' impact on the derived signals was evaluated on the basis of displacement of received signals along the potential axis (a shift of the potential). Also the changes in the intensity, so in the height of registered peaks, were taken into account. To this end, two types of model proteins of known size and amino acid sequences and immunoglobulin G were employed.

The influence of the bioparticles was examined due to the interactions, which may occur between protein and metalloporphyrin, such as: complexation (coordination bond), electrostatic interactions, hydrogen bonds, van der Waals interactions or π-stacking interactions. The assumption of presented study was to use solutions