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Studies on potential use of tin(IV) porphyrin in a role of proteins' label

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ABSTRACT

We present an electrochemical and optical characterization of 5,10,15,20-tetraphenylporphyrin tin(IV) dichloride (Sn–tpp) in terms of its potential use as a hybrid proteins' label. Our research comprised Sn–tpp and Sn–tpp in the presence of model proteins selected as to mimic a receptor or surface blocking agents: bovine serum albumin, ovalbumin, and immunoglobulin G. In the course of the study, we determined optimal conditions for analysis by means of differential pulse voltammetry, ultraviolet–visible spectrophotometry, and spectrofluorimetry. In electrochemical detection, the influence of the working electrode, solvent, and supporting electrolyte was examined. Displacements of the received signals along the potential axis (a shift of the potential) and changes in signal intensities due to the addition of proteins were observed and analyzed. Simultaneously, the suitability of Sn–tpp as a label in optical detection mode was assessed by using spectroscopic techniques. The obtained results prove Sn–tpp to be applicable in dual and triple detection systems. Such an approach will improve the reliability of the analysis and, at the same time, will allow for widening the range of the linear response with some overlapping ranges of concentrations.

Unique features of porphyrins and metalloporphyrins make them applicable in a variety of analytical methods. As complexing agents, porphyrins allow for determination of cations based on absorption and emission spectra [1–3]. Due to selective complexation of anions, they also find application in high-performance liquid chromatography [4,5] and potentiometry [6–8]. For instance, the system in the focus of our research, 5,10,15,20-tetraphenylporphyrin tin(IV) dichloride (Sn–tpp),[1] has been applied as an ionophore selective for salicylate anion [9].

Porphyrins themselves can be determined using their spectrometric, luminescent, and voltammetric characteristics. There is also the possibility of using metalloporphyrins containing cations of radioactive isotopes, leading to their determination through the radioactivity measurements, which have been used mainly in immunoassays and immunosensors [10]. Another advantage of porphyrins is related to high stability of the ring system [11] and their susceptibility to modifications, which enables easily giving them various desirable properties by changing metal cations or introducing functional groups to rings. Moreover, some of them exhibit interesting catalytic properties [12], which also give additional possibilities for their determination.

Analyses conducted for clinical, environmental, and biochemical purposes often require determination of a wide variety of biological compounds. However, a signal sufficient for direct detection of an analyte is rarely achieved in reactions occurring between biospecies. To this end, an analysis using biomolecules' labeling is needed. Despite the fact that bioparticles' labeling is time-consuming and requires elaborated methods of coupling, it ensures sensitive determination of the analyte by asserting low detection limits. Labels find particular application in affinity biosensors, where they are chiefly used when conjugated with specific antibodies or aptamers in a sandwich assay system.

Depending on detection techniques, a variety of labeling methods may be employed, leading to different ways of signal generation. For optical determination, the labeling agents of choice are nanoparticles [13–16], quantum dots [17,18], and fluorescent dyes [19,20]. When electrochemical detection is applied, one turns to redox-active compounds such as ferrocene [21–23], methylene blue [24–26], ferricyanides [27–29], and various ruthenium complexes [30,31]. In radioimmunoassays, radioactive isotopes are used [32]. Finally, indirect detection is possible with the use of enzymes [33–35].

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[1] *Abbreviations used:* Sn–tpp, 5,10,15,20-tetraphenylporphyrin tin(IV) dichloride; UV–Vis, ultraviolet–visible; Mn–tpp, 5,10,15,20-tetraphenylporphyrin manganese(III) chloride; tpp, tetraphenylporphyrin; PhCN, benzonitrile; DMSO, dimethyl sulfoxide; (TBA)I, tetrabutylammonium salt of iodide; (TBA)ClO_4, tetrabutylammonium salt of perchlorate; (TBA)tetraphenylborate, tetrabutylammonium salt of tetraphenylborate; (TOA)Br, tetraoctylammonium salt of bromide; BSA, bovine serum albumin; OVA, ovalbumin (chicken egg albumin); IgG, immunoglobulin G; EDC, 1-ethyl-3-(3-dimethylpropyl)carbodiimide hydrochloride; DPV, differential pulse voltammetry; THF, tetrahydrofuran.

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A step toward more efficient detection of proteins is offered by a combination of several techniques encompassing a different range of the linear response of the analyte. The resulting dual or triple detection systems lead to improved reliability of the analysis and broadened range of concentrations in which the compound can be determined. Porphyrins, with their capacity to undergo redox reactions and their unique optical response properties, are promising candidates for hybrid proteins' labels in multiple detection systems.

The aim of this work was to present an investigation of Sn–tpp in terms of its potential use as proteins' label. In the course of preliminary studies, approximately 30 different porphyrin types were screened as candidates. Sn–tpp was selected as one of the most promising compounds because it enables the generation of three independent analytical signals.

In the first stage of the research, we carried out electrochemical studies to precisely characterize redox properties of the examined compound and to determine optimal conditions for the conducted analysis. Simultaneously, by means of ultraviolet–visible (UV–Vis) spectrophotometry and spectrofluorimetry, spectroscopic characterization of Sn–tpp was performed. In the second stage, the same techniques were applied to establish the influence of proteins on electrochemical and optical response. It is shown that we can control the proteins' impact on Sn–tpp signals, which was a necessary step before we proceeded to the conjugation with proteins. The obtained results were compared with a similar study conducted for 5,10,15,20-tetraphenylporphyrin manganese(III) chloride (Mn–tpp), which is also a compound predestinated for the role of hybrid biomolecules' label [36].

Materials and methods

Reagents

To obtain Sn–tpp, metalation of tetraphenylporphyrin (tpp) was performed in accordance with the following procedure. Tpp (0.1 g) in the acidic form and 5-fold molar excess of $SnCl_2$ (0.2 g) were heated in 10 ml of benzonitrile (PhCN) to a temperature of 160 °C for 20 h. The reaction progress was monitored by UV–Vis spectrophotometry and thin layer chromatography. After the reaction, 30 ml of hexane was added and the reaction flask was left for 12 h in a freezer. The Sn–tpp was purified using column chromatography on alumina with dichloromethane as eluent.

All measurements, both electrochemical and spectroscopic, were performed in dimethyl sulfoxide (DMSO) obtained from Sigma. The supporting electrolytes were tetrabutylammonium salts of iodide [(TBA)I], perchlorate [(TBA)ClO_4], and tetraphenylborate [(TBA)tetraphenylborate] and tetraoctylammonium salt of bromide [(TOA)Br], all of which were received from Sigma. The commercially available proteins bovine serum albumin (BSA), ovalbumin (OVA, chicken egg albumin), and immunoglobulin G (IgG) were obtained from Sigma.

Conjugation procedure

The conjugation procedure was carried out using carboxylated derivative of tetraphenylporphyrin tin(IV) dichloride through its binding with amino groups of proteins' side chains. To this end, the activation of porphyrin's carboxyl groups was first carried out using 2 mM 1-ethyl-3-(3-dimethylpropyl)carbodiimide hydrochloride (EDC) in DMSO for 2 h. Subsequently, the protein (BSA, OVA, or IgG) prepared in carbonate buffer was added to solution of porphyrin and was left for overnight reaction. For the reaction, 10-fold molar excess of porphyrin relative to number of amino side groups present in protein chains was employed. After the conjugation reaction, the solution was subjected to lyophilization and the lyophilisate was dissolved in water and centrifuged to separate undissolved particles containing unreacted porphyrin. The obtained compounds were characterized by means of electrochemical and optical techniques to confirm the possibility of using Sn–tpp in the role of hybrid proteins' label.

Measurements

The electrochemical experiments were carried out using a potentiostat (CHI660A, CH Instruments, 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 oxidation potential of Fe^{2+}/Fe^{3+} (in the form of potassium hexacyano complex) in DMSO solution containing 10% water with respect to water solution was shifted by 154 mV toward negative potentials, and the value of the reference electrode's potential was constant during performed measurements. The measurements were carried out using differential pulse voltammetry (DPV) maintaining the following parameters: 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 and a PerkinElmer Lambda 25 spectrophotometer. Spectrofluorimetric spectra were obtained on a Varian–Cary Eclipse spectrofluorimeter. All samples were adjusted to blank of pure DMSO.

Results and discussion

The preliminary studies were designed to select those metalloporphyrins that are able to guarantee three independent analytical signals and, thus, can be used as hybrid proteins' labels. To this end, we analyzed porphyrin complexes containing cations of various metals, such as Co, Cr, Fe, In, Mn, Ni, Sn, and Zn, to choose those exhibiting interesting redox, fluorescent, and UV–Vis properties at the same time. Only some of them fulfill this requirement. For instance, iron porphyrin showed usable electrochemical signals, with few intense peaks (one of which is situated near 0 V potential), but due to paramagnetic properties it exhibits no fluorescence. On the other hand, zinc porphyrin, despite appropriate optical response, is less electrochemically usable due to low negative potential values.

On the basis of obtained outcomes, Sn–tpp was selected among others as one of the most promising porphyrins to play the role of hybrid label. In its case, the analysis involving all three detection techniques allowed obtaining well-defined and stable signals of high intensities.

Electrochemical characterization

Choosing the best possible conditions for electrochemical measurements is crucial because even slight changes in signal intensities may hinder quantitative determination of the analyte or the corresponding label in the case of indirect detection.

To choose the best possible conditions for Sn–tpp measurements, we first examined the influence of the working electrode and the applied solvent. In Figs. 1 and 2, we present voltammograms obtained for oxidation of Sn–tpp on two types of electrodes and in various solvents, respectively.

Of the tested electrodes, the glassy carbon electrode provided well-defined signals with high intensities. In comparison, much

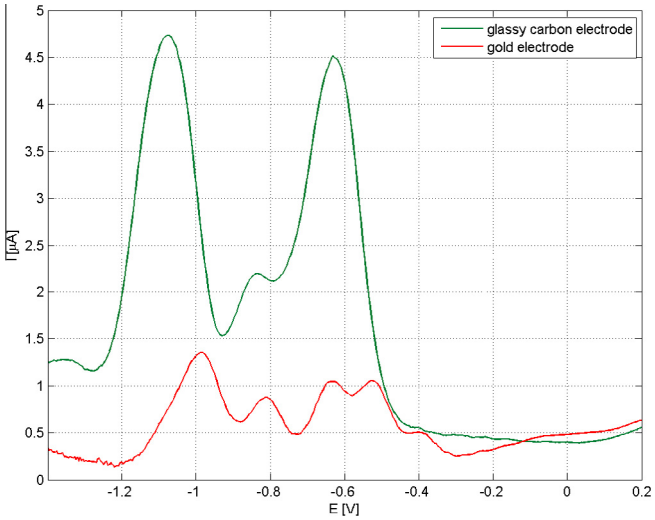

Fig.1. Differential pulse voltammograms of 1 mM Sn–tpp obtained on glassy carbon electrode and gold electrode in DMSO containing 0.01 M (TOA)Br.

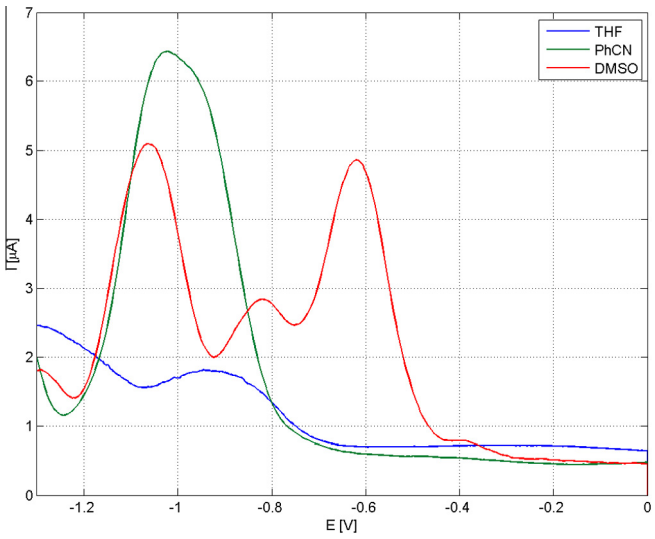

Fig.2. Differential pulse voltammograms of 1 mM Sn–tpp obtained on glassy carbon electrode in different solvents containing 0.01 M (TOA)Br.

poorer performance was found for the gold electrode of a comparable working area.

The influence of different solvents on tin–porphyrin complexes has been reported only for tin(II) complexes [37]. In this study, three different solvents were verified with respect to their effect on the quality of the obtained signal: PhCN, DMSO, and tetrahydrofuran (THF). We proved DMSO to provide the most intense current signals in the widest potential range without the introduction of background interference. Furthermore, DMSO retains proteins' activity, allowing for its subsequent applications; therefore, it is employed for analyses involving proteins, especially immunoassays [38,39]. Following the above findings, we chose DMSO for a preparation of all samples.

Next, different supporting electrolytes were examined. We selected tetrabutylammonium and tetraoctylammonium salts containing different counterions: borate, bromide, iodide, and perchlorate. Various ions were taken into consideration because they may interact differently with porphyrin rings or metal cation contained in their structure. Voltammograms for oxidation of Sn–tpp using several types of electrolytes are depicted in Fig. 3. Two well-defined peaks could be distinguished, and both observed processes were fully reversible [40]. Potential values obtained in the presence of bromide salt amount to −1.04 V (peak corresponding to anion radical formation at porphyrin π-ring system) and −0.63 V (formation of dianion).

We found different anions in the supporting electrolyte to cause shifts and changes in signal intensities reflecting varying strengths of the porphyrin–ion complexation. The dianion formation peak is shifted toward more negative potentials in the following order: bromide > iodide > perchlorate > borate (borate corresponds to the lowest potential value). This reflects increasing strength of complexation; the higher the porphyrin–anion stability constant, the more negative potential recorded. The total shift between the two extreme peaks, bromide and borate, was 14.7 mV. For the anion radical peak, the order of salt anions remains unchanged, with a similar maximum difference of peak positions (10.4 mV). The signal intensities for both dianion and anion radical peaks decrease in the following order: perchlorate > iodide > bromide > borate. The total height difference between perchlorate and borate amounts to 0.547 μA for the dianion peak and 0.307 μA for the anion radical peak. Finally, all of the applied electrolytes ensure clearly defined signals of sufficient intensities; therefore, their use is valid from the perspective of analytical purposes. Similar shifts of potentials were observed for Mn–tpp, and the strength of manganese cation complexation set the anions in the following order: borate > bromide > iodide > perchlorate.

In the next step, the proteins' impact on the derived signals was evaluated because their presence may lead to apparent changes in the nature of signals derived from the label. To asses this effect, porphyrin–protein incubation was conducted prior to the measurement, leading to noncovalent conjugation. Because some literature reports concerning the impact of tin porphyrins on various proteins were documented [41–44], in the framework of this study we also took efforts to verify proteins' influence on Sn–tpp for this particular application and to enable quantitative estimation of occurring interactions on the analytical signals. We selected two types of model proteins of known size and amino acid sequences—BSA and OVA—and IgG. With the help of the DPV technique, we also appraised the minimal concentration of the porphyrin that can be used to achieve signals of sufficient intensity in the Sn–tpp/protein system.

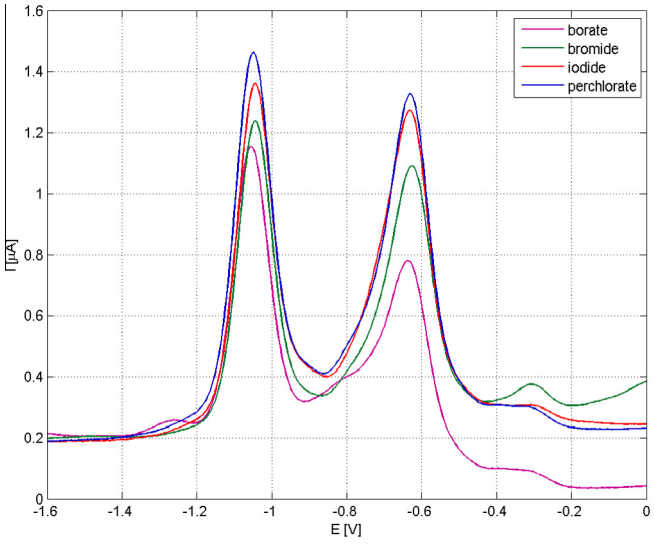

Fig.3. Differential pulse voltammograms representing oxidation of 0.25 mM Sn–tpp obtained on glassy carbon electrode in DMSO containing electrolytes with different anions, all at a concentration of 0.01 M.

We demonstrate a versatile impact of the examined proteins on electrochemical behavior of Sn–tpp. Regardless of the type of the added biomolecules, no significant change was observed for the peak at −0.63 V (anion radical formation peak). In the case of the signal at −1.05 V, the interactions between proteins and the porphyrin π-ring system promote a decrease in its intensity. At its expense, additional redox reactions appear. This behavior is strongly affected by the supporting electrolyte applied (as presented in Fig. 4).

For iodide, bromide, and borate salts, we observed different response depending on the type of protein (Fig. 4A). IgG led to a decrease of nearly 43% in the intensity of the −1.05-V peak. An additional peak at more negative potentials (−1.23 V) also occurred, which indicates additional redox processes. Analogous behavior was observed for OVA. In contrast, the addition of BSA in the presence of iodide anion caused a 70% decrease of the −1.05-V signal as well as formulation of other signals of low intensities and no analytical utility.

When perchlorate salts were applied as supporting electrolytes, we observed the same effect for all types of proteins, that is, a significant diminution of signal at −1.05 V accompanied by the occurrence of extra signals of low intensities (Fig. 4B).

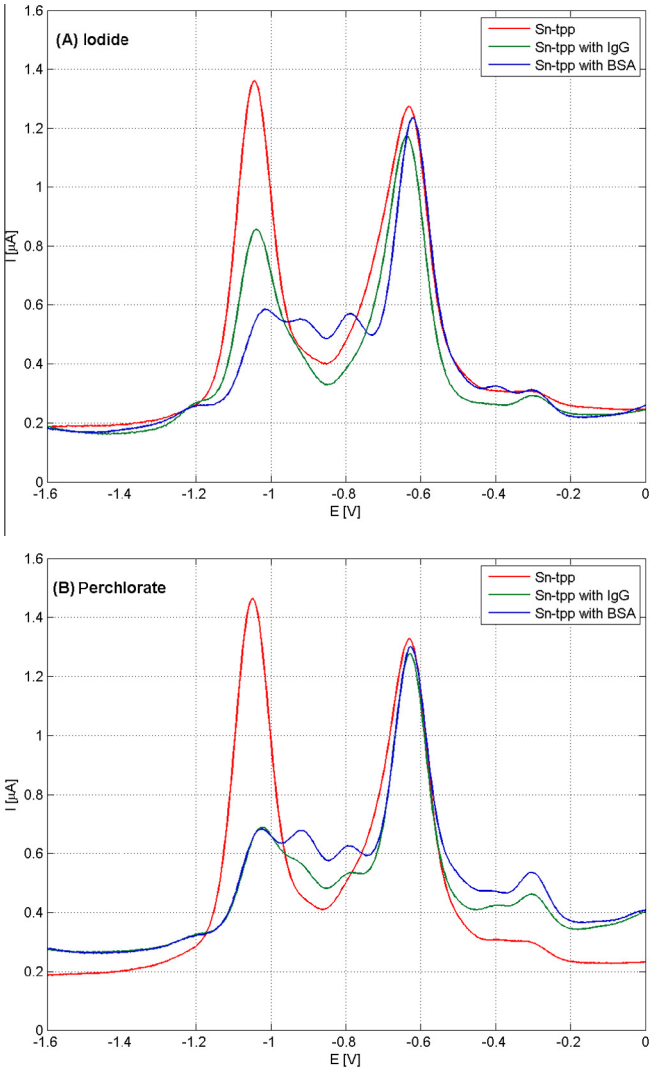

Fig.4. Differential pulse voltammograms representing oxidation of 1 mM Sn–tpp in the presence of IgG or BSA (molar ratio porphyrin/BSA = 10:1) obtained on glassy carbon electrode in DMSO solution containing 0.01 M tetrabutylammonium iodide (A) and 0.01 M tetrabutylammonium perchlorate (B).

The effects caused by the presence of proteins included (i) the decrease of signal intensities and (ii) the formation of additional signals that can be attributed to the creation of Sn–tpp complexes with proteins and anions coming from the supporting electrolyte. The observed patterns of electrochemical response point toward the ability of Sn–tpp to simultaneously engage in complexes with both proteins and anions (the coordination number of Sn(IV) usually equals six [45]). It should be stressed that the presence of these effects does not hinder the possibility of using Sn–tpp as a label. As we have shown, the peak at −0.63 V remains almost unchanged, causing Sn–tpp to retain its analytical utility.

For the stable signal at −0.63 V, we used DPV to establish the detection limit and linear response range of Sn–tpp, obtaining values of 0.75 μM and 1 to 500 μM, respectively. As a comparison, in the case of Mn–tpp, the detection limit amounted to 40 μM, but the range of linear response was slightly wider at 50 to 700 μM. Finally, we confirmed the immutability of these parameters in the presence of proteins at the assumed amount of protein equivalent to the amount of porphyrin in its detection limit (1:1 molar ratio).

Optical characterization

To assess the effect of proteins on optical properties of Sn–tpp, we recorded absorption and emission spectra for different concentrations of the applied biomolecules. Shifts of the absorption and fluorescence maxima or changes in the signal intensities were observed.

Fig. 5 presents the UV–Vis spectra of Sn–tpp at different concentrations of OVA (the amount of 1 mg corresponds to the molar ratio porphyrin/OVA = 1:1). The absorbance maximum at 425 nm corresponding to the Soret band becomes slightly shifted toward lower frequencies with the increase of biomolecules' concentration, with the total shift amounting to 2 nm. This is a result of interaction between the protein (e.g., phenylalanine rings) and the π-ring system of porphyrin or metal cation complexed in its coordination center. The increase of amount of the added protein is followed by the decrease in absorbance intensity (amounting to 0.14 absorbance unit, which is 15.6% of the initial signal, in the analyzed range of biomolecules' concentration). This reflects the differences in molar absorption coefficients resulting from complexation of protein (ligand-complexing tin may be displaced by protein). With the increase of proteins' amount, the signal baseline is increasing, which is a result of incomplete dissolution of

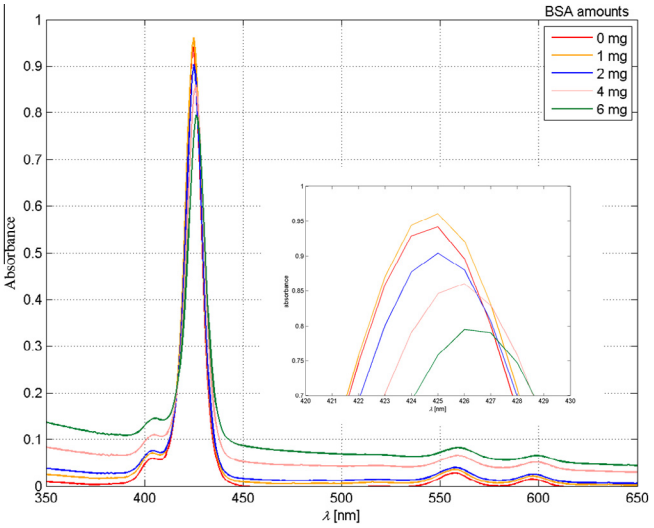

Fig.5. UV–Vis spectra of 20 μM Sn–tpp with BSA of different concentrations obtained in DMSO solution containing 0.01 M tetrabutylammonium iodide.

protein at its high concentration. The same results were obtained for BSA and IgG. Comparing porphyrins with tin and manganese cations in the coordination center, we observed the same correlation in the Soret band intensities; however, shifts toward lower frequencies were more apparent in the case of Sn–tpp [36]. The detection limit for Sn–tpp obtained by UV–Vis spectrophotometry was 2.5 nM with a linear range of response up to 5 μM (in the case of Mn–tpp: 0.5 nM with a linear range of response up to 20 μM). We also confirmed that the addition of an equivalent amount of protein or antibody did not alter the limit of detection, as was the case for electrochemical detection.

The impact of electrochemical supporting electrolytes on absorption spectra was also examined. This study was conducted to verify whether one single sample of protein labeled with Sn–tpp could be applied in systems with different detection modes. We found that electrolytes do not affect the characteristic of the spectrum. Therefore, the same sample may be analyzed in both electrochemical and optical detection modes without a modification of its composition, thereby minimizing its consumption. The only necessary step is a dilution of the sample before spectrometric measurements in order to adjust to linear response range.

The fluorescence may also provide an excellent basis for the detection and determination of porphyrins with appropriate sensitivity. For this reason, we conducted the spectrofluorimetric analysis of Sn–tpp. The effect of proteins and salts on the spectra was evaluated. First, unlike the case of Mn–tpp, we observed a significant impact of the salts on the Sn–tpp fluorescence spectra. For each of the four examined electrolytes, a decrease of the emission intensity was found, with the largest decline in the case of bromide. Second, we found that the addition of proteins caused only slight negligible changes of signal intensities, as was also reported for manganese porphyrin [36]. The fluorescence spectra for Sn–tpp at different concentrations of OVA are presented in Fig. 6.

Lastly, we verified the simultaneous influence of different anions of supporting electrolyte and the presence of proteins. The addition of iodide and perchlorate did not cause any effect on the spectra. Intensities of analytical signals at 604 and 654 nm remained the same as for porphyrin with the mentioned ions only. In contrast, the combination of proteins with bromide ion led to an increase of the signals with respect to the porphyrin in bromide solution only. In this case, the anion is probably displaced from the complex with porphyrin and substituted with the protein ligand. The increase of the proteins' amount resulted in a further increase of emission intensity, as depicted in Fig. 7. These conclusions apply to both examined types of albumins and to IgG. The detection limit for this technique is 0.025 μM, with linearity of the response up to 10 μM, regardless of the proteins' presence.

The obtained results revealed that Sn–tpp emission is susceptible to the influence of anions whose presence cannot be avoided in real samples. This excludes the possibility of using fluorescence in competitive assays, where the sample matrix is present throughout the analysis. Moreover, the composition of the matrix is usually difficult to predict. Therefore, in the case of competitive assays, UV–Vis spectroscopy and voltammetry should be applied. We emphasize that the range of linear responses provided by these two techniques completely covers the range that is guaranteed by fluorescence. In sandwich assays, there are no limits to the application of spectrofluorimetry for Sn–tpp detection. This is due to the fact that after binding the analyte sensor's surface is rinsed in order to wash unbound components of the matrix.

Conjugation with proteins

We conjugated Sn–tpp with model proteins to confirm whether it can be exploited for proteins' labeling and determined using several detection techniques. The carboxylated derivative of tin porphyrin was employed, which enabled the creation of covalent bond with amino groups of proteins' amino acids.

The conjugation reaction was carried out in DMSO using EDC as activating agent. After the reaction, the solution was subjected to lyophilization and the lyophilisate was dissolved in water because the porphyrin becomes water soluble when bound to protein. Such a procedure allowed for separation of water-insoluble unbound porphyrin. In contrast, the application of water-soluble porphyrins has more restrictions in terms of selection of a separation method because all of the reagents and products are soluble in the same medium. Therefore, this approach provides suitable conditions for porphyrin analysis and facilitates conjugates' separation.

However, the application of DMSO does not preclude the determination of proteins, which may become denatured in organic solvents. The procedure that we propose requires the application of DMSO only during the conjugation reaction when mixed solvent of water/DMSO (1:1, v/v) is employed to enable dissolution of porphyrin. As we have noted, the obtained conjugates are water

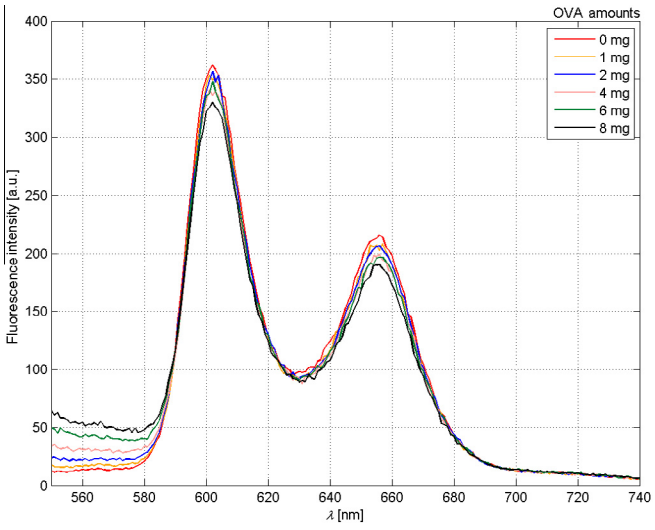

Fig.6. Emission spectra of 10 μM Sn–tpp with OVA of different concentrations obtained in DMSO solution. λ_{ex} = 390 nm.

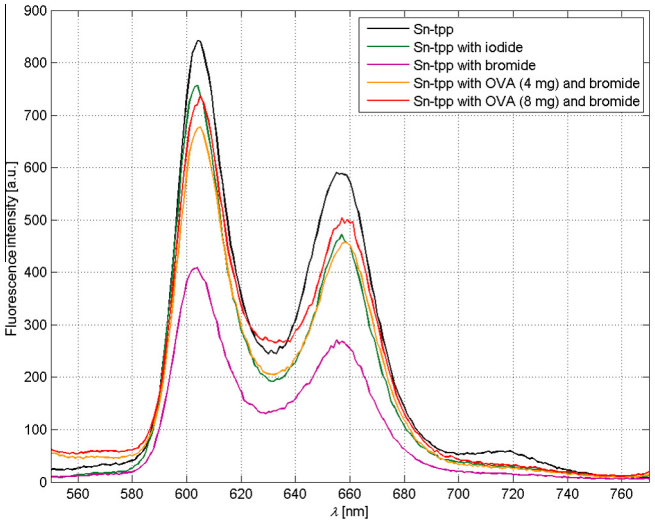

Fig.7. Emission spectra of 10 μM Sn–tpp in the presence of supporting electrolytes and different amounts of OVA (in the case of bromide) obtained in DMSO solution. λ_{ex} = 390 nm.

soluble, so both the reaction between detected receptors in assay (e.g., antigens, antibodies) and the detection of bound labels may be performed in water solutions.

The conjugates were characterized using UV–Vis spectrophotometry, and the efficiencies of the conjugation reaction calculated for the Soret band amounted to 76.5, 11.4, and 56.8% for BSA, OVA, and IgG, respectively. These values were confirmed by spectrofluorimetry with corresponding values of: 73.2, 10.6, and 52.3%.

The electrochemical measurements revealed some changes in the nature of Sn–tpp response after the conjugation. The analytically usable peak at −0.63 V corresponding to dianion formation is shifted to a more negative potential of −0.86 V, and this effect was not observed in the case of the aforementioned noncovalent conjugation. The determination of porphyrin label using DPV also confirmed the obtained reactions' efficiencies. The characterization of obtained conjugates demonstrates that Sn–tpp can be successfully applied as proteins' label detectable with three detection techniques.

It is also worth mentioning that the stoichiometry of conjugation reaction may vary; depending on the type and structure of protein, the amount of porphyrin varies. This results in various detection limits; for example, if 1 protein molecule binds 10 molecules of porphyrin instead of 1, the detection limit will be one order of magnitude lower. For this reason, the calibration in necessary for each system independently and no general conclusion can be drawn.

The porphyrin label may also be applied in different assay formats and modes. In the case of optical techniques, the surface on which receptor layers will be immobilized only needs to be permeable to radiation (e.g., microwell plate, optical fiber). For electrochemical determination, the destruction of a sandwich assay by changing conditions—ionic strength, pH, or the addition of surfactant before label determination—may be necessary.

Conclusion

In this work, we verified the possibility of application of tin(IV) porphyrin as hybrid proteins' labels. To this end, a characterization of Sn–tpp in terms of its electrochemical and optical response was conducted.

First, we determined optimal conditions for electrochemical analysis of Sn–tpp. DMSO and glassy carbon were selected as the best solvent and material of the working electrode, respectively. We paid particular attention to the influence of the supporting electrolyte. Bromide, borate, iodide, and perchlorate salts were examined. We proved that the supporting electrolyte affects both positions and intensities of the obtained signals and, therefore, that its impact needs to be taken into consideration.

Second, we verified the effect of proteins on the electrochemical signals obtained from the porphyrin. Two types of albumins—BSA and OVA—and IgG were compared. We demonstrated that the addition of proteins does not impede the determination of porphyrin because one of the peaks remains analytically usable. Examined porphyrin was characterized by a detection limit of 0.75 μM, which did not change after the addition of protein.

In the third part of our study, we examined the possibility of Sn–tpp determination in the optical detection mode. We proved the efficacy of the UV–Vis analysis. The only effect exerted by the proteins on absorption spectra was a slight decrease in the absorbance intensity with an increase of proteins' concentration. In the case of spectrofluorimetry, a strong effect of anions was observed, which limits the application of this technique only to sandwich assays. The detection limits for Sn–tpp were 2.5 nM for UV–Vis spectrophotometry and 0.025 μM for spectrofluorimetry.

Sn–tpp was found to meet all of the criteria to be used as a label for proteins in dual and triple detection systems. These results provide a point of reference that is essential for subsequent characterization of the obtained conjugates. By the use of functionalized Sn–tpp for creation of covalently bound protein–porphyrin conjugates, we proved that the porphyrin label can be successfully determined using three detection techniques.

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