PRION PROTEIN ELECTROCHEMICAL STUDY AND ITS INTERACTION WITH QUANTUM DOTS

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INTRODUCTION

Infectious prion called PrP^{Sc} is a modified form of the naturally occurring prion (PrPC), which is present in mammalian cells. Infectivity of the protein is determined by the conformational change in the structure of the α-helix of the PrPC to conformation of β-sheet PrP^{Sc}. Prion diseases are usually diagnosed clinically and subsequently confirmed by histopathological examination of brain tissue post - mortem. The only reliable molecular marker for prion diseases is the presence of abnormal forms of PrP^{Sc}, pathologically conformationally altered, which are accumulated in the central nervous system and to a lesser extent in lymphoreticular tissues. Currently, there is no diagnostic test for the rapid and reliable detection of prion diseases in animals or humans from body fluids. New diagnostic techniques aimed at increasing the sensitivity and specificity of PrPSc detection and identification of novel surrogate markers are now being intensively developed [8, 12]. In terms of the concentration of modified prion proteins in body fluids is very low, it is necessary to look for ways to increase the sensitivity of the assay. In recent years, it shows that different methods of labeling biomolecules using fluorescence or other marks, it is very advantageous for the detection of biomolecules with reducing the detection limit by several orders of magnitude. Quantum dots, semiconductor materials, the size of units to tens of nanometers, are one group of these brands. Excellent optical properties of QDs predestinate them in usage for imaging and as optical probes for detection of peptides, proteins, nucleic acids and other biomolecules [1, 6, 7, 10, 13].. The interaction between β-sheet breaker prion protein and CdTe quantum dots (QDs) was studied by differential pulse voltammetry connected with adsorptive transfer stripping technique.

Fig. 1: Quantum dots

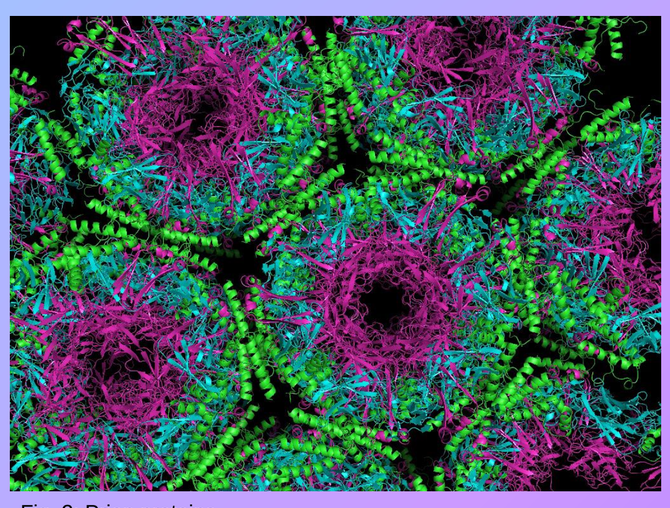

Fig. 2: Prion proteins

MATERIAL & METHODS

QD were prepared according to Duan et al. [5]. In a typical synthesis, 4 ml of cadmium chloride solution (CdCl2, 0.04 mol/l) was diluted to 42 ml with ultrapure water, and then trisodium citrate dihydrate (100 mg), Na2TeO3 (0.01 mol/l, 4 mL), MPA (119 mg), and NaBH4 (50 mg) were added successively under magnetic stirring. The molar ratio of Cd2+/MPA/Te was 1:7:0.25. 10 ml of the resulting CdTe precursor was put into a Teflon vessel. A CdTe QDs were prepared at 95°C and times 30 min. under microwave irradiation (400 W). After microwave irradiation, the mixture was allowed to cool to lower than 50 °C and the CdTe QDs sample was removed. Working standard solutions were prepared daily by diluting the stock solutions. Electrochemical measurements were performed using AUTOLAB Analyser (EcoChemie, Netherlands) connected to VA-Stand 663 (Metrohm, Switzerland), with a standard cell with three electrodes. The working electrode was a hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2. The reference electrode was an Ag/AgCl/3M KCl electrode and the platinum electrode was the auxiliary one. The supporting electrolyte was acetate buffer (0.2 M sodium acetate trihydrate, ajust to pH = 5 using acetic acid).. Differential pulse voltammetric measurements were carried out under the following parameters: deoxygenating with argon 60 s; start potential -1.5 V; end potential 0 V; a modulation time 0.057 s, a time interval 0.2 s, a step potential of 1.05 mV/s, a modulation amplitude of 250 mV, E_{ads} = 0 V. All experiments were carried out at room temperature (22–24 °C). The DPV samples analysed were deoxygenated prior to measurements by purging with argon (99.999%) saturated with water for 120 s.

RESULTS & DISCUSION

We mixed the prion proteins in the ratio 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9 and 1:10 and left them to interact for 24 hours in the dark at 35 °C. After the interaction, this mixture was analyzed by adsorptive transfer technique (adts) in conjunction with differential pulse voltammetry. With A constant concentration of prion protein and increasing concentrations of quantum dots, the voltammograms provide three peaks (fig. 3a). With increasing concentrations of quantum dots there was an increase of the peaks signals and the fourth peak begins to create. The results show that the peaks 1, 3, 4 are directly proportional to the rising concentration, which is an important characteristic for the assessment of the concentration of prion proteins (fig. 3b). Consider the complex quantum dots – prion in the way of constant concentration of quantum dots and increasing concentrations of the prion protein, is evident that only three peaks (peak 1, 2 , 3) at all concentration range are noticeable. Peak 4 is no longer shown (fig. 3b). Electrochemical signals of peak 1 and 3 increase linearly with the increasing concentrations of the prion, which shows the dependence on the concentration of prion protein (fig. 3d). Electrochemical signal of peak 2 decreases with increasing concentration of prions. From the peak heights is clear that peak 1 is the most sensitive one for evaluation the concentration of the complex quantum dots - prion. The calibration curve was measured in the concentration range from 1.10^{-5} to 4 μg/ml (75 fg/5 ml to 20 ng/ml). In whole concentration range, the resulting dependence has A logarithmic trend. Strictly linear part of the calibration curve was found in the concentration range of 0.05 ng/ml to 4 ng/ml. The limit of detection (3S/N) was determined by 1 fg in 5 ul.

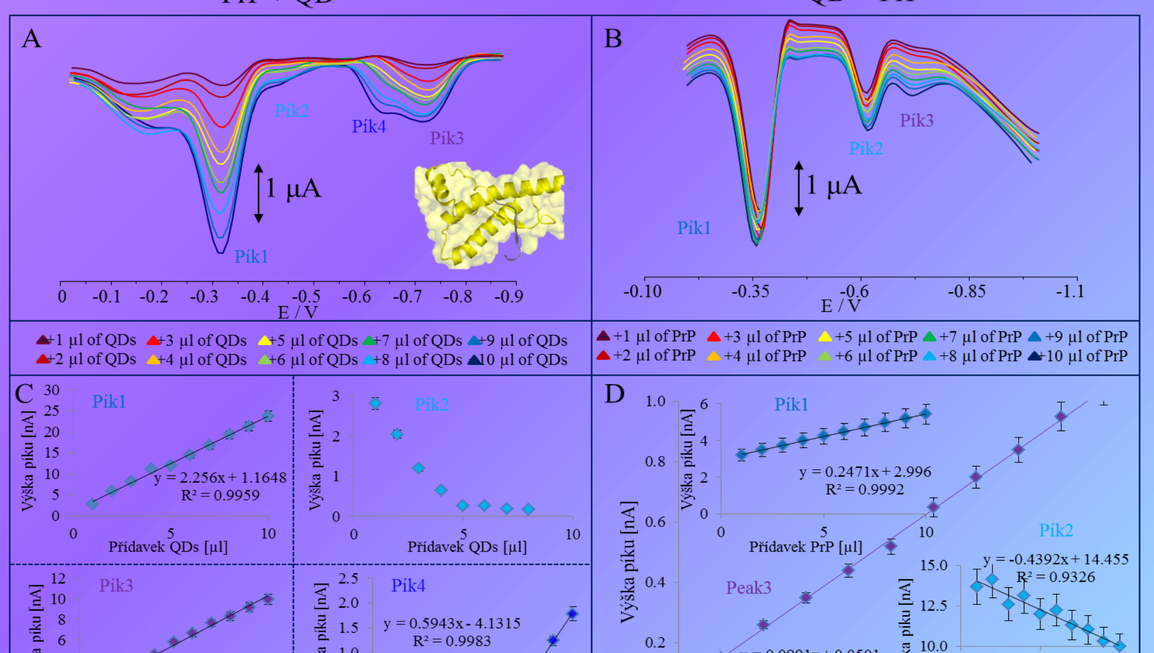

Fig. 3: Electrochemical signal dependence on the concentration of quantum dots–prion complex. (A) At constant concentration of prion and increasing concentration of QDs. (B) At constant concentration of QDs and increasing concentration of prion protein. (C, D) The individual peaks dependences on the electrochemical signal intensity.

Fig. 4: Electrochemical analyzer with 3-electrode system

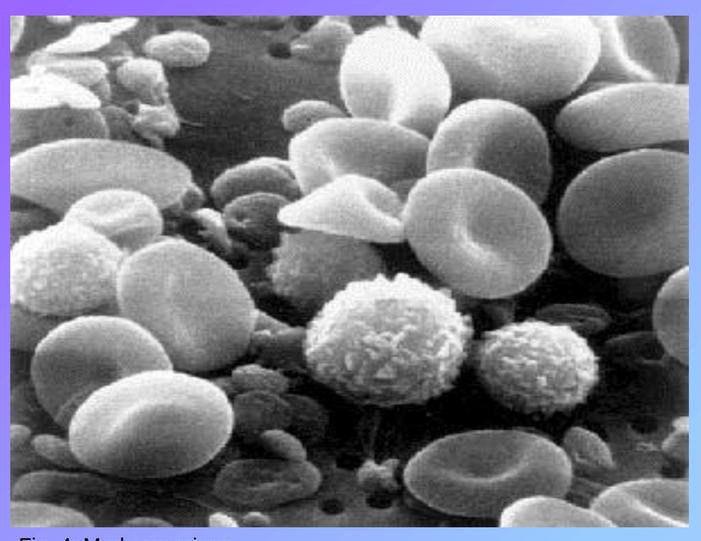

Fig. 4: Mad cow prions

CONCLUSION

Ouantum dots, the miniature, light-emitting crystals are auspicious fluorescent markers for cellular and biomolecular labelling. In comparison with organic dyes and fluorescent proteins, the quantum dots have unique optical and electronic properties. In this study was found out that the quantum dots also have electrochemical properties, due to this fact they can be used as an excellent electroactive marker for prion proteins determination. Complex QD-protein is very stable and it can be quantitated in very small volumes. This discovery may open new possibilities for determination of these proteins not only in animal tissues but also residues on surgical instruments and other types of potentially contaminated materials.

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