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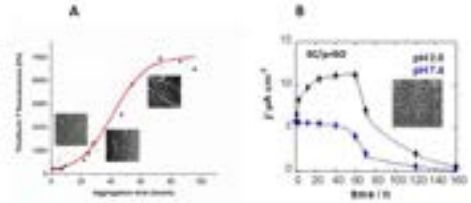
Electrochemical sensors coated with prGO coated enable to capture different aggregation behaviors of proteins and peptides

Alina Vasilescu
International Centre of Biodynamics, Romania

Statement of the Problem: Protein instability due to misfolding and aggregation is of big concern for protein-based therapeutics because it impacts the bioavailability and immunogenicity of such drugs. Simple and cost-effective analytical methods, indicating the presence or absence of protein aggregates, are consequently of high importance.

Methodology & Theoretical Orientation: Porous reduced graphene oxide (prGO) coated electrodes have been investigated for the early and sensitive identification of protein aggregation. The detection principle lies in following the change in the oxidative current of the proteins. The sensor architectures studied included glassy carbon electrodes with drop cast prGO and disposable, screen printed electrodes modified with prGO using the layer-by-layer deposition technique. The studies focused on the protein lysozyme and the pharmaceutical polypeptide calcitonin having the ability to form aggregates in different conditions of pH and temperature. Parallel experiments were performed by fluorescence with thioflavin T, size exclusion chromatography and Atomic Force Microscopy Imaging.



Findings: Comparing the oxidation peak of lysozyme by differential pulse voltammetry for different electrode architectures allowed validating the higher sensitivity of the prGO-coated interfaces versus bare ones. Moreover, the modified electrodes allowed detecting in a fast and reliable manner the changes in the protein structure occurring at pH 2 and pH 7.4, as per processes leading to the formation of amyloid and amorphous aggregates, respectively (Fig.1). Screen printed electrodes modified with prGO enabled to differentiate between the amyloid-type aggregation of calcitonin (2 mg mL-1) in citrate buffer and no amyloid formation in acetate buffer. These electrodes were also applied to the analysis of a pharmaceutical drug product of low potency, Miacalcic (8.3 µg mL-1 calcitonin), where no aggregation was observed.

Conclusion & Significance: Electrochemical sensors coated with prGO coated enable to capture different aggregation behaviors of proteins and peptides and represent a complementary tool for biopharmaceutical analysis.

Biography

Alina Vasilescu is an analytical chemist with expertise in the development and validation of novel analytical methods. Her experience encompasses both academic research and analytical research in the pharmaceutical industry. She currently works as a Senior Researcher at the International Centre of Biodynamics in Bucharest, where she coordinates several research projects, focussing on the development of (bio)sensors for practical applications. Study of protein aggregation is a primary research area and she collaborates with other groups for including nanomaterials in the development of novel sensors.

avasilescu@biodyn.ro

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