Spectroscopic Approaches for Understanding Graphene Family Material interactions with Enzymes

Bashiru Ibrahim[1], Divya Singh[1], Swaroop Chakraborty[1], Andrea Rossi[2], Francesco Pellegrino[2], Damjana Drobne[3], Eugenia Valsami-Jones[1]

1. Introduction

Graphene family materials (GFMs) are structural or chemical variants of graphene, a two-dimensional allotrope of carbon, that has attracted significant attention in recent years due to its remarkable properties and applications in various fields including biomedicine. Such applications include cellular imaging, drug delivery, biosensing and tissue engineering [1]. Despite the potential of graphene and GFMs in biomedicine, knowledge of their interaction with enzymes remains poorly understood. This interaction can occur through a range of mechanisms such as physical absorption, hydrogen bonding and π-π stacking interactions [2]. Such interactions can change the structural conformation of enzymes, impacting their stability, activity, specificity, and overall biological function [3]. Therefore, it is essential to investigate the interaction of GFMs with enzymes [4]. In the current study, circular dichroism (CD) spectroscopy was used to study effects of GFMs interactions with cholinergic enzyme-acetylcholinesterase (AChE). The technique provides an insight into the conformational structure of the enzyme and can elucidate the structural alterations induced by GFMs. The enzyme is a serine hydrolase that plays a critical role in degrading acetylcholine (ACh) and terminating neurotransmission. It is involved in cellular growth, apoptosis, drug resistance, response to signals and inflammation [5]. Understanding how GFMs change the structural composition and function of AChE can offer valuable insights into potential applications of GFMs in the treatment of neurological disorders.

2. Methodology

The studied GFMs were provided by the University of Torino, where they were synthesised using the Hummers modified method, which involves stirring high quality graphite in a mixture of concentrated sulphuric acid and phosphoric acid together with a strong oxidizing agent (potassium permanganate). The materials were characterised by dynamic light scattering (DLS), Fourier-transform infrared spectroscopy (FTIR) and UV-visible spectroscopy. The interaction of GFMs with AChE was studied by circular

[1] School of Geography, Earth and Environmental Sciences, College of Life and Environmental Sciences, University of Birmingham, Birmingham B15 2TT United Kingdom
*b.ibrahim@bham.ac.uk
[2] Department of Chemistry, University of Torino, Via Pietro Giuria, 7, 10125 Torino, Italy.
[3] Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia

dichroism (CD). The CD spectra were recorded in a 1 mm path length quartz cell for the far UV (190 - 300 nm). The measurements were performed with step size of 1.0 nm, a spot width of 1.5 nm and an average time of 2s. The CD spectra of GFMs in 100 mM phosphate buffer (pH 8.0) were recorded and subtracted from the enzyme spectra. The enzyme was dispersed with GFMs (0 – 200 µg/ml) directly into the cuvette, and the spectra were corrected for dilution. The mean ellipticity was calculated using Dicroweb software [6].

3. Results and Discussion

The results revealed that the materials have hydrodynamic diameters ranging from 430-4,370 nm when dispersed in ultrapure water. Zeta potential measurements of the synthesised materials indicated negatively charged surfaces (-21-38 mV), suggesting good stability and dispersion. The absorption spectra (260-270 nm) confirmed the formation of GFMs using UV-vis. FTIR showed the formation of the following broad bands: 3212 cm^{-1} matching the O-H stretching vibrations that are typical of hydroxyl and carboxyl functional groups; 1719 cm^{-1} corresponding to C=O stretching vibrations implying the presence of carbonyl and carboxyl groups; 1622 cm^{-1} showing the contribution of cyclic aromatic groups; 1050 cm^{-1} matching the C-O stretching vibrations, which are the typical absorption bands of ethers.

Our results further revealed a distinct increase in the absorption spectra of AChE enzyme upon interaction with GFMs, suggesting the formation of enzyme-GFMs complexes. Interestingly, the concentration of GFMs (ranging from 0 to 200 µg/ml) did not significantly change the conformational structure of the enzyme (Figure 1). However, analysis using Dicroweb software revealed slight changes in the α-helix content of the enzyme, and β-sheet indicating subtle alterations in their secondary structure elements. Furthermore, the addition of GFMs led to significant peaks shift in the original spectra of AChE, which could be related to changes in the conformational structure of the enzyme.

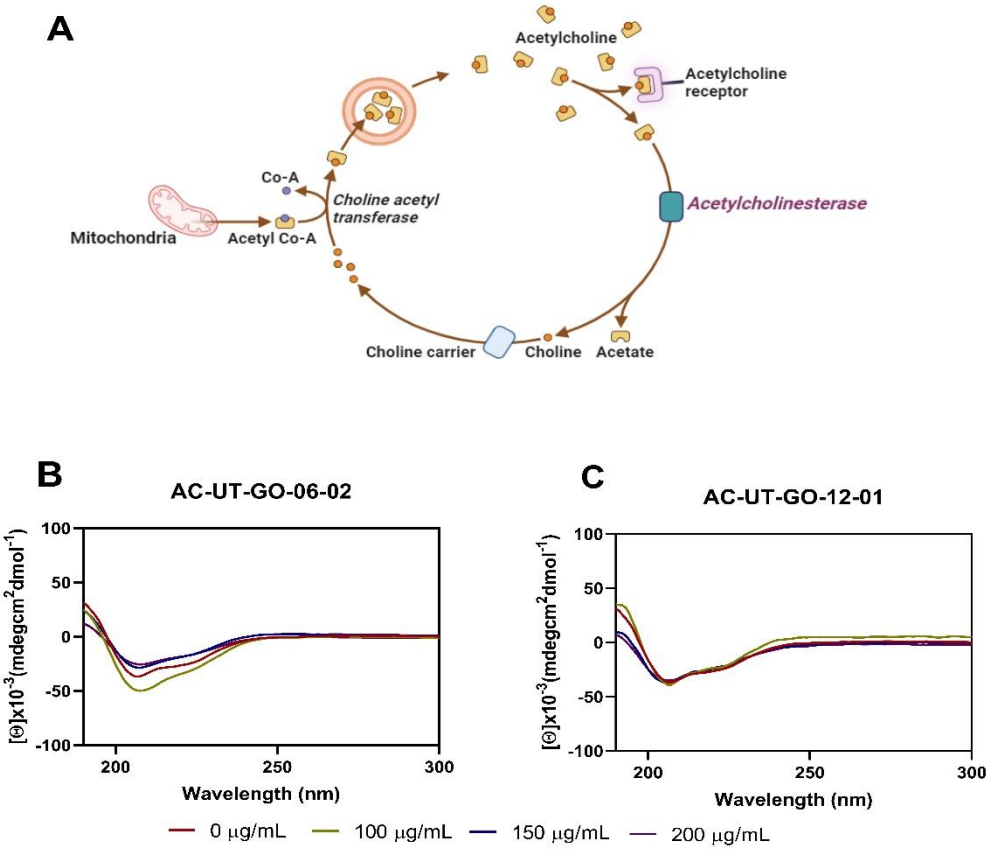

Figure 1: GFMs-AChE interaction (A) Schematic figure showing intermediates related to the biosynthesis of Acetylcholine and (B-E) mean residue ellipticity of AChE (0.5 mg/ml) titrated with increasing concentrations of GFMs (0-200 µg/ml) at room temperature.

4. Conclusions

In conclusion, our study highlights the ability of GFMs to interact with AChE and induce subtle structural changes. Through spectroscopic analysis, we elucidated the dynamics of enzyme-GFMs interactions and their effects on AChE structure. Further investigation into the underlying mechanisms of these interactions could pave the way for the development of novel biomedical applications harnessing the unique properties of GFMs.

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6. References

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