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Further insights into the effect of pH on the fluorescence and structure of green fluorescent protein (GFP)

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The Enhanced Green Fluorescent Protein (EGFP) has intense and natural fluorescence, and is biocompatible with a diversity of biological systems, which makes it promising for use in the development of biosensors. However, this commercial application is limited, mainly due to the high cost and lack of knowledge about EGFP stability under stress conditions. Although studies have been done into EGFP stability at different pH, they mostly only show the presence or lack of fluorescence, with no in-depth structural evaluations or analysis of the reversibility of the process. Bridging this knowledge gap can allow the development of novel biocompatible pH-biosensors for medical use, which can help in monitoring different diseases that are known for altering the pH of the affected areas, such as certain tumors and synovial diseases. Hence, the objective of this work was to evaluate the effect of pH on the fluorescence activity and structure of EGFP to assist in the development of biosensors.

In this study, EGFP was exposed to different pH for 30 min and evaluated by circular dichroism, fluorescence spectroscopy (2D and 3D), intrinsic fluorescence, small-angle X-ray scattering (SAXS) in well-plates, and with size-exclusion chromatography (SEC-SAXS). Then, the pH of each sample was adjusted until the solution reached neutrality (pH 7.4), and after 60 min, EGFP was again evaluated by the same techniques. It was determined that EGFP is highly stable at neutral-alkaline pH (7.4 to 13.0), has a small fluorescence quenching at slightly acidic pH (6.0 and 5.0) and total quenching at pH ≤ 4.0. At pH 6.0, the fluorescence was almost completely recovered with the return of the pH to neutral, however, from pH values of 5.0 to 2.0, the fluorescence was only partially recovered. In addition, at pH 6.0 there was no change in the secondary and tertiary structure of EGFP (as observed by CD, SAXS, and SEC-SAXS) because the fluorescence quenching was only the result of reversible changes caused by protonation, considering the isoelectric point of the protein is 6.2. Between pH 5.0 to 2.0, the results indicate that there were structural changes at tertiary and secondary levels, hence EGFP recovery was only partial. Therefore, it is possible to conclude EGFP fluorescence is highly dependent on pH, exhibiting reversible changes in conformation between pH 6.0 and 7.0, and irreversible structural changes at pH ≤ 5.0. These properties make EGFP a very promising biomolecule for the development of novel acidic-to-basic pH-biosensors.

Keywords: Green Fluorescent Protein, pH Stability, Biosensors, Circular Dichroism, SAXS.

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