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Title: Measurement of acetylcholinesterase activity by electrochemical analysis method utilizing organocatalytic reactions

Background and Purpose: In recent years, electrochemical sensing methods for enzyme activity measurement have been investigated. However, many of these methods rely on sensors that detect hydrogen peroxide (H_2O_2) generated as a product of enzymatic reactions. As a result, they are not suitable for reaction systems that do not produce H_2O_2 and often require multiple reaction steps to generate H_2O_2, which presents a significant limitation. In contrast, electrochemical approaches using nortropine-*N*-oxyl (NNO) allow the direct oxidation of substrates or products of enzymatic reactions to produce a current signal. This strategy effectively overcomes the limitations of conventional methods and provides a more direct and versatile solution for enzyme activity measurement.

Research Outline: It was confirmed that NNO promotes the electrochemical oxidation of choline chloride, and based on this property, it was demonstrated that the hydrolysis of acetylcholine by AChE could be monitored in real time. Measurements performed at varying AChE concentrations showed that the current value increased in proportion to enzyme activity, yielding a good linear relationship. These results suggest that this method is suitable for quantifying a broad range of AChE activities. While traditional methods for measuring AChE activity mainly rely on colorimetric or fluorometric techniques, the present method offers the advantages of rapid and simple evaluation through electrochemical measurement.

Future Prospects: This approach is applicable to a variety of enzymatic reactions involving the formation or loss of hydroxy and amino groups, and is expected to be developed in the future.

Reference:

Measurement of acetylcholinesterase activity by electrochemical analysis method utilizing organocatalytic reactions. *RSC Adv.* 2025, 15, 32464-32469. doi: 10.1039/d5ra04585a.