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Spectrophotometric Detection of Histidine Using Enzyme Reaction and Examination of Reaction Conditions

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Abstract : The measurement of amino acid content is reported to be useful for the diagnosis of several types of diseases, including lung cancer, gastric cancer, colorectal cancer, breast cancer, prostate cancer, and diabetes. The conventional detection methods for amino acid are high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS), but they have several drawbacks as the equipment is cumbersome and the techniques are costly in terms of time and costs. In contrast, biosensors and biosensing methods provide more rapid and facile detection strategies that use simple equipment. The authors have reported a novel approach for the detection of each amino acid that involved the use of aminoacyl-tRNA synthetase (aaRS) as a molecular recognition element because aaRS is expected to a selective binding ability for corresponding amino acid. The consecutive enzymatic reactions used in this study are as follows: aaRS binds to its cognate amino acid and releases inorganic pyrophosphate. Hydrogen peroxide (H_2O_2) was produced by the enzyme reactions of inorganic pyrophosphatase and pyruvate oxidase. The Trinder's reagent was added into the reaction mixture, and the absorbance change at 556 nm was measured using a microplate reader. In this study, an amino acid-sensing method using histidyl-tRNA synthetase (HisRS; histidine-specific aaRS) as molecular recognition element in combination with the Trinder's reagent spectrophotometric method was developed. The quantitative performance and selectivity of the method were evaluated, and the optimal enzyme reaction and detection conditions were determined. The authors developed a simple and rapid method for detecting histidine with a combination of enzymatic reaction and spectrophotometric detection. In this study, HisRS was used to detect histidine, and the reaction and detection conditions were optimized for quantitation of these amino acids in the ranges of 1–100 µM histidine. The detection limits are sufficient to analyze these amino acids in biological fluids.
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