TOWARD ENZYME-BASED COLORIMETRIC BIOSENSORS BASED ON AN INEXPENSIVE ENZYME, PAPAIN

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Abstract:
Traditional chromatographic methods, capillary electrophoresis, mass spectrometry, and immunoassay are effective for various small molecule determinations but have certain limitations such as complexity, time-consuming sample preparation, and the requirement of expensive instruments. In recent years, the enzyme-based biosensor has emerged as the alternative method with simplicity, high sensitivity, and multi-residue detection. However, it still employed the expensive enzyme including acetylcholinesterase. This research proposes an overview of recent advances in the development of biosensors exploiting the inhibition of papain by various small molecules in food and environmental samples including insecticides, secondary fertilizers, and dietary supplements. Papain, an inexpensive and common enzyme found mainly in papaya resin, belongs to a proteolytic enzyme group capable of digesting large-sized proteins into smaller molecules. The activity of papain was easily monitored by the hydrolysis of *Nα*-benzoyl-arginine-*p*-nitroanilide **(**BAPNA**).** The presence of yellow color produced by a product, *p-nitroaniline,* can be measured at 430 nm**.** The concentration of papain-inhibited molecules is linearly related to the degree of inhibition. The papain biosensors exhibited detection limits and linearity at ppm level. These biosensors were stable for a period of 2 to 120 days. The progress in the development of a papain-based biosensor enriched the possibilities for an on-site colorimetric protocol in the determination of inhibiting small molecules.

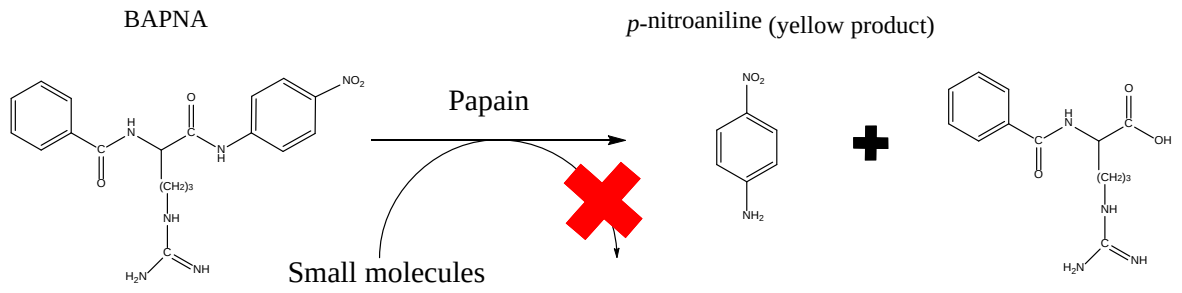

Figure 1. Principle of small molecule determination based on papain inhibition system