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Design in biotechnology: can living organisms in bioassays be replaced on enzymes?

Key words: bioassays, luciferase, bioluminescence, enzymatic biosensors.

Historically, the application of bioluminescence in toxicology began with the usage of luminous bacteria and they are still widely used. As opposed to other test objects such as paramecia, algae, and so on, the luminous bacteria assay is faster (< 30 min). However, as with other organisms, luminous bacteria is petulant. The failure to maintain the stable state of bacterial culture during measurements and storage results in low accuracy of measurement, a clear disadvantage of this method caused by the "petulance". The bacteria react to the toxic substances either by decreasing or by increasing the luminous intensity, often leading to ambiguous interpretation of results. Because of these shortcomings the luminous bacteria assay didn't show reliable results. The new approach to develop the bioluminescent enzymatic biosensors, toxicity bioassays and reagents has been described. To solve the problem of how to detect, identify, and measure the contents of the numerous chemical compounds in environmental monitoring, food product monitoring, and medical diagnostics, the bioluminescent enzymatic toxicity assays were proposed, wherein the NAD(P)H:FMN-oxidoreductase +luciferase substitutes for living organisms. The immobilized reagent Enzymolum was introduced to facilitate and accelerate the development of cost-competitive enzymatic systems for use in biosensors. Prototype biosensors offer cost advantages, versatility, high sensitivity, rapid response, extended shelf and flexible storage conditions. Due to the coupling with luciferase, it is possible to design new enzymatic bioassays in toxicology and combine them into a set. The set includes key enzymes of metabolic processes such as LDH, trypsin and others. The set of bioluminescent enzymatic toxicity assays was used for monitoring natural and laboratory aquatic ecosystems, soil contamination, as well as for toxicity analysis of pesticides and sanitary assessment of nanomaterials. The new possibilities of enzymatic bioassays are discussed.

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