

# Review of Tissue Enzyme Sensors

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**Abstract** – A wide variety of enzyme sensors have been developed over the past decade. This paper is a review of tissue enzyme biosensors that have been fabricated and tested. Three different transduction techniques will be discussed. The application and importance of plant and animal tissue in biosensing will also be covered. The three sensors in this review include a chemiluminescent sensor that uses porcine kidney tissue to determine if lactic acid is present in a sample, a fiber optic sensor using corn tissue to determine levels of pyruvate in a sample, and a potentiometric sensor that uses yellow squash tissue to catalyze the amino acid L-Glutamate.

**Index Terms** –Biosensor, Enzyme, Animal Tissue, Plant Tissue

## INTRODUCTION

Enzymes are the most commonly used biological components [1]. They are the catalysts of biochemical reactions and are responsible for bringing about almost all of the chemical reactions in living organisms. Chemical reactions would occur too slowly for the pace of metabolism if enzymes were not present. All known enzymes are proteins made up of chains of amino acids linked together by peptide bonds as shown in Figure 1 [2].

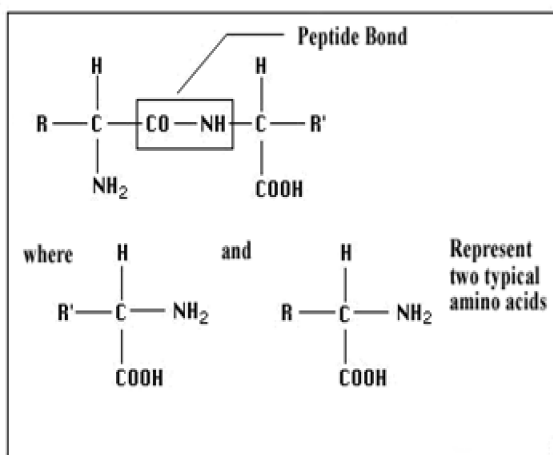
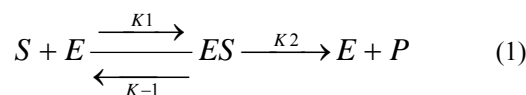


Figure 1: Typical protein structure [2].

Enzymes are an important diagnostic and research tool due to their selectivity relative to the reactions they catalyze. The four types of specificity associated with enzyme reactions are explained below.

- Absolute: an enzyme will catalyze only one specific reaction.
- Group: only molecules that have specific functional groups, such as amino, phosphate, and methyl groups, will be affected by the enzyme.
- Linkage: a particular type of chemical bond, regardless of the rest of its molecular structure, will be acted upon by the enzyme.
- Stereochemical: an enzyme will target a particular steric or optical isomer [2].

The basic enzyme reaction is shown in equation (1) [1].



Where S is the substrate concentration, E is the enzyme concentration catalyzing the reaction, ES is the complex concentration formed from the enzyme and substrate, P is the product concentration, and  $k_1$ ,  $k_{-1}$  and  $k_2$  are rates of reaction [1]. The rate of enzyme-catalyzed reactions is affected by temperature and pH level. Like most chemical reactions, raising the temperature will increase the activity of the reaction. An enzyme has an optimal temperature at which it will catalyze quickest. An enzyme also has an optimum pH value where it is most active. Extremely high or low pH levels generally result in complete loss of activity for most enzymes [2].

## BASIS FOR TISSUE ENZYME BIOSENSOR

A biosensor is comprised of a sensing element and a transducer. It converts the biological response into a measurable signal [1]. The sensing element is the biological material, referred to as the substrate, which the enzyme will act upon. The reaction between the sample and the enzyme is measured by the transducer.

Most of the tissue-based biosensors have coupled tissues either to amperometric electrodes or

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potentiometric transducers [3]. Potentiometry measures a cell's electric potential at zero current. In amperometry, current is measured as a potential increases. The current increases when the electric potential reaches the reduction potential of oxygen. The peak current is directly proportional to the concentration of oxygen [1].

Another transduction method incorporates optical sensors to measure and analyze samples. One type of photometric behavior used in enzyme biosensors is chemiluminescence (CL). CL measures optical emissions from energized molecules to determine analyte concentration. Since it relies on the detection of electromagnetic radiation produced by a reaction, there is no need for an external light source [8]. The chemiluminescence emission intensity is proportional to the concentration of the element being tested [3].

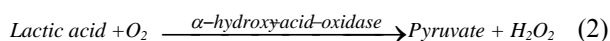
The usage of natural plant tissue and animal tissue based biosensors has had a major impact on biosensor development [3]. Tissue enzymes require less preparation than pure enzymes and exist in their natural environment thus eliminating the need to be extracted and purified. Also, the lifetime of a tissue enzyme is much longer than a pure enzyme making them more cost effective [1]. Examples of tissue material and uses is shown in Table 1.

Table 1: Biosensors based on enzyme tissue use and related materials [1].

Substrate	Biocatalytic Material: Enzyme Tissue	Sensing Element
Glutamine	Porcine kidney cells	NH <sub>3</sub>
Adenosine	Mouse small-intestine mucosal cells	NH <sub>3</sub>
AMP	Rabbit muscle	NH <sub>3</sub>
Guanine	Rabbit liver	NH <sub>3</sub>
Hydrogen Peroxide	Bovine liver	O <sub>2</sub>
Glutamate	Yellow squash	CO <sub>2</sub>
Pyruvate	Corn kernel	CO <sub>2</sub>
Urea	Jack bean meal	NH <sub>3</sub>
Phosphate/fluoride	Potato tuber/GOD	O <sub>2</sub>
Dopamine	Banana pulp	O <sub>2</sub>
Tyrosine	Sugar beet	O <sub>2</sub>
Cysteine	Cucumber leaf	NH <sub>3</sub>
Glutamine	Porcine kidney mitochondria	NH <sub>3</sub>

### a) Animal Tissue

A sensor to detect lactic acid using porcine kidney tissue as the recognition element has been developed by Fangqiong Wu, Yuming Huang, and Chengzhi Huang at Southwest Normal University, Beibei, Chongqing, PR China. The porcine kidney tissue oxidizes the lactic acid under the catalysis of  $\alpha$ -hydroxy acid oxidase present in the tissue. This produces hydrogen peroxide which can react with luminol in the presence of potassium ferricyanide to generate a chemiluminescent signal [3]. The reaction is shown in equation (2).



A flow sensing system, as shown in Figure 2, was used to detect the lactic acid. Two peristaltic pumps were used to deliver the sample solution as well as a doubly deionized water solution mixed with the chemiluminescence reaction reagents. Polytetrafluoroethylene (PTFE) tubing was used to connect all components in the flow system. The advantages of PTFE tubing are its low coefficient of friction, high chemical resistance, and high and low temperature resistance [4]. The H<sub>2</sub>O<sub>2</sub> produced by the enzyme-catalyzed oxidation from the porcine kidney tissue was injected into the water carrier stream then mixed with the luminescent reagents. A photomultiplier tube (operated at -600V) of the Type IFFL Flow-Injection Chemiluminescence Analyzer was used to detect the chemiluminescent signal produced in the flow cell. The signal was then recorded by a computer to be further analyzed [3].

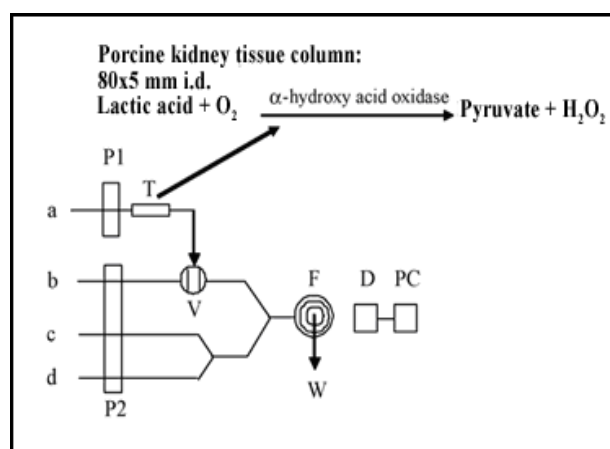


Figure 2: Schematic diagram of lactic acid flow system used: (a) sample; (b) H<sub>2</sub>O; (c) 80  $\mu$ mol/L luminol (pH 10); (d) 50  $\mu$ mol/L potassium ferricyanide; (P1) pump 1; (P2) pump 2; (T) tissue column; (V) valve; (W) waste; (D) detector; (PC) personal computer; (F) flow cell [3].

This method was successfully used to analyze lactic acid in plasma and milk samples. The results showed there was a linear relationship between the chemiluminescent intensity and a lactic acid concentration in the range of 1-1000  $\mu\text{mol/L}$  [3]. It is shown in Figure 3 that passing of lactic acid solution into the porcine kidney tissue column could produce  $\text{H}_2\text{O}_2$  which elevated the radiation from oxidation of luminol by potassium ferricyanide [3].

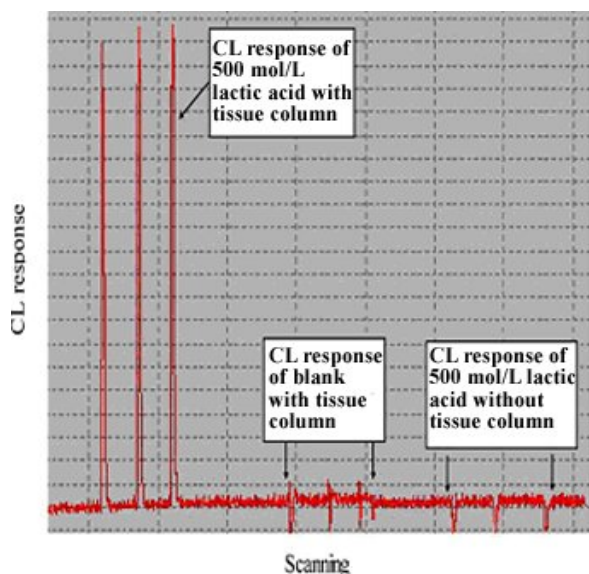
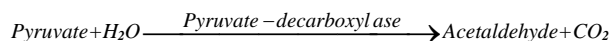


Figure 3: CL response of 500  $\mu\text{mol/L}$  lactic acid with and without tissue column, and blank CL response with tissue column.

#### b) Plant Tissue

A pyruvate selective sensor constructed with a slice of corn tissue and an optical based  $\text{CO}_2$  sensor has been developed by Xiaoying He and Garry A. Rechnitz at the University of Hawaii. The reaction that takes place is shown in equation (3).



Catalyzation of pyruvate occurs by a reaction with pyruvate decarboxylase which causes the release of  $\text{CO}_2$  that is measured by introducing a pH sensitive fluorescent dye. The corn is taken, when frozen, and sliced into 0.2 to 0.3 mm strips which are then fixed to the end of a modified pipette as shown in Figure 4 [5]. The sensor tip is then put into an activating buffer solution for two hours to increase sensitivity with the target molecule.

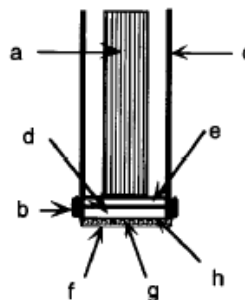


Figure 4: Schematic Diagram of the corn tissue based fiber optic sensor. (a) common end of the bifurcated fiber; (b) plastic ring; (c) pipette tip; (d) indicator solution; (e) glass slide; (f) dialysis membrane; (g) slice of corn kernel tissue; (h) gas permeable membrane [6].

The optical components used consist of a light source, a sensing unit, and a photodetection system [6]. Incident light is shone through the setup into the fiber optic tip and is photomultiplied to achieve amplification. The biosensor, while stable because of the plant tissue, shows marked decrease in performance after seven days.

Another plant enzyme based biosensor involves using the tissue of a yellow squash to catalyze the amino acid L-glutamate in a reaction that produces  $\text{CO}_2$  gas. Figure 5 shows the L-glutamate structure as well as the 4-aminobutyrate structure and  $\text{CO}_2$  produced after the reaction.

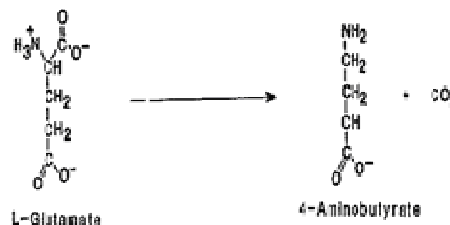


Figure 5: Catalyzation of L-glutamate by yellow squash tissue [7].

The  $\text{CO}_2$  is measured using potentiometric methods. Figure 6 shows the potentiometric rate processes and the various factors that influence them such as:

1. Diffusion across the membrane
2. The reaction rates of the substrate and enzyme
3. Diffusion in the biocatalytic layer

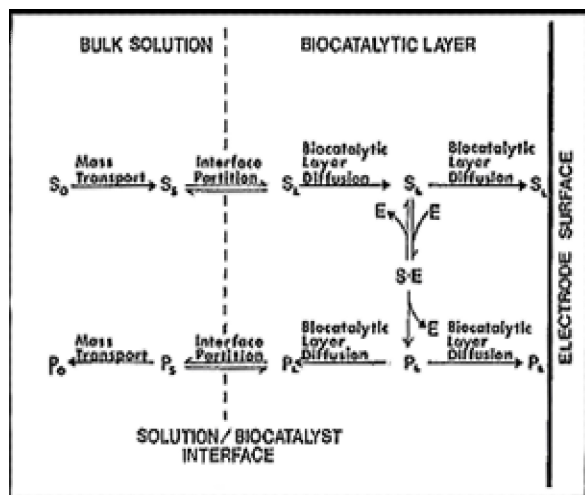


Figure 6: Potentiometric Biosensor Rate processes [6].

This biosensor uses the immobilized enzymes on a substrate of bovine serum albumin (BSA)-glutaraldehyde. The sensor requires a phosphate buffer, a 40% glycerol solution to stabilize the enzyme and a pyridoxal-5'phosphate (PLP) to activate the enzyme. The biological material in this sensor can be stable for as much as one month at 4° C or as little as seven days at room temperature.

## CONCLUSION

The wide variety of plant and animal tissue enzymes play an important role in biosensing. An enzyme's ability to increase the rate of reaction of a specific material allows for faster transduction times and greater specificity. The advantages of tissue enzymes is that they require less preparation than pure enzymes, do not need to be extracted and purified, and have a longer lifespan than pure enzymes. Common transduction techniques for a tissue enzyme reaction include chemiluminescence, optics, and potentiometrics. It has been shown that lactic acid can be detected with the use of porcine kidney tissue, pyruvate can be detected with the use of corn, and L-glutamate can be detected with the use of yellow squash tissue.

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