Biosensors

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Turning Tryptophanase into Odor-Generating Biosensors**

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Abstract: *An odor-based sensor system that exploits the metabolic enzyme tryptophanase (TPase) as the key component is reported. This enzyme is able to convert an odorless substrate like S-methyl-L-cysteine or L-tryptophan into the odorous products methyl mercaptan or indole. To make a biosensor, TPase was biotinylated so that it could be coupled with a molecular recognition element, such as an antibody, to develop an ELISA-like assay. This method was used for the detection of an antibody present in nM concentrations by the human nose. TPase can also be combined with the enzyme pyridoxal kinase (PKase) for use in a coupled assay to detect adenosine 5′-triphosphate (ATP). When ATP is present in the low μM concentration range, the coupled enzymatic system generates an odor that is easily detectable by the human nose. Biotinylated TPase can be combined with various biotin-labeled molecular recognition elements, thereby enabling a broad range of applications for this odor-based reporting system.*

We use our sense of smell every day to detect threats such as fire or spoiled food. The doping of natural gas with mercaptans for leak detection is an elegant example of the engineering of safety systems that exploit our sense of smell. Surprisingly, there were no reports of taking advantage of our olfactory sense for the design of a biosensor until Mohapatra and Phillips recently reported an odor-based biosensor that uses two small molecules and glucose oxidase to detect enzymes as analytes.[1] The first small molecule is a glucose derivative specifically designed to be a substrate for the enzyme to be detected. The presence of this enzyme leads to the generation of glucose; glucose then reacts with glucose oxidase to produce hydrogen peroxide, which in turn reacts with the second small molecule to generate volatile ethanethiol. This approach is particularly useful for detecting enzymes through the use of specialized substrates. However, it is not clear how it could be adapted to achieve the detection of other types of analytes, which is the focus of the current study.

We have been interested in exploring tryptophanase as the key component in the construction of odor-based biosensors (Figure 1a).[2] Tryptophanase, denoted TPase in this report, is a metabolic enzyme that is capable of converting L-tryptophan and *S*-methyl-L-cysteine into indole and methyl mercaptan, respectively (Figure 1b).[3] Indole and methyl mercaptan can be easily detected by the human nose, with threshold concentrations of 0.02 ppb (1.0×10^{-12} M) and 0.2 ppb (1.0×10^{-11} M), respectively.[4] Therefore, the combination of TPase/L-tryptophan or TPase/*S*-methyl-L-cysteine offers an attractive strategy for producing odors to which the human nose is extremely sensitive. When linked to a molecular recognition element (such as an antibody or aptamer), TPase should allow for the odor-based detection of a wide variety of molecular targets. To demonstrate such an application, we herein describe the coupling of an antibody to TPase and the use of the resulting biosensor to detect a protein target.

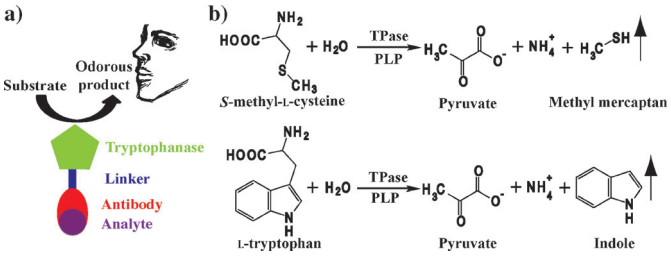

Figure 1. Use of tryptophanase (TPase) to construct an odor-based sensor. a) Concept. Linking TPase to a molecular recognition component allows the reporting of a target through odor generation. b) Odor-generating reactions catalyzed by TPase.

TPase is also an enzyme that requires pyridoxal phosphate (PLP) as a coenzyme; therefore, an analyte that can be linked to the production of PLP would also be compatible with a TPase-derived odor-based detection system. To this end, we also developed an odor-based biosensor for adenosine triphosphates (ATP), an important biological cofactor.

We first investigated the biotinylation of TPase in order to establish a simple and general method for coupling a chosen antibody to TPase. The biotinilyated TPase can be linked to a biotinylated antibody through avidin, a tetrameric biotin-binding protein. The biotinylation of TPase was accomplished

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by using *N*-hydroxysulfosuccinimide biotin as a biotinylated reagent.

To demonstrate protein detection through the use of an antibody, we selected anti-rabbit IgG (ARIgG) as the antibody, which specifically recognizes rabbit IgG (RIgG) as the target. To achieve target detection, we devised a detection procedure that we termed TPase-linked immunosorbent assay (TLISA; Figure 2a). Briefly, RIgG was allowed to

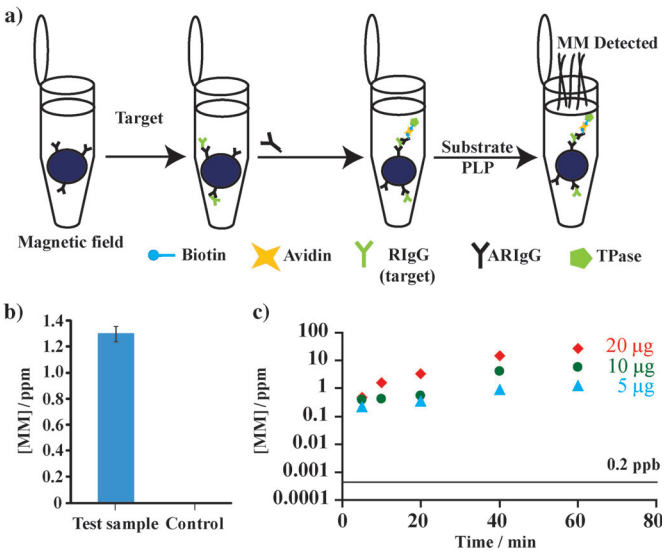

Figure 2. TPase-linked immunosorbent assay (TLISA) for protein detection using an antibody. a) Schematic of TLISA. The target is RIgG and the select selected antibody is ARIgG. The biosensor was assembled by combining biotinylated TPase, avidin, and biotinylated ARIgG. The addition of *S*-methyl-L-cysteine (or L-tryptophan) and PLP leads to the formation of methyl mercaptan (or indole). b) Methyl mercaptan (MM) concentration in the headspace generated for a test sample (containing 440 µg RIgG) and a control (no RIgG) after one hour. Each experiment was done in triplicate. c) Concentration of methyl mercaptan (MM) as a function of time for samples containing different amounts of TPase, 15 mM *S*-methyl-L-cysteine and 0.1 mM PLP in KDP buffer. Each data point is the average of three measurements. The straight line represents the detection threshold for the human nose (0.2 ppb).

bind to magnetic beads (MB) containing ARIgG. After washing and blocking with super block T20 buffer, the MB were incubated with pre-assembled TPase–Avidin–ARIgG reporter and then washed. This was followed by the addition of *S*-methyl-L-cysteine (or L-tryptophan) and PLP, which led to the generation of methyl mercaptan (or indole) that can be detected by a human nose. For quantification purposes, a photoionization detector (ToxiRAE Plus PID) was also used to analyze methyl mercaptan in the headspace. It is noteworthy that the photoionization detector has a limit of detection of 0.1 ppm, which means that the human nose is about 500 times more sensitive than this instrument.

The concentration of methyl mercaptan reached 1.3 ppm (Figure 2b) in the test sample containing RIgG. This concentration is 6500 times higher than the threshold level for the human nose. By contrast, no methyl mercaptan was detected when RIgG was absent (Control in Figure 2b). We also tested L-tryptophan as the substrate (to give indole as the product), and comparable results were obtained (see the Supporting Information). These findings show that TLISA can report the presence of a target analyte of interest with high sensitivity.

The ideal biosensor should have a fast response time. To determine how long it would take to generate a measurable signal, we examined the effect of different amounts of TPase on the production of methyl mercaptan from *S*-methyl-L-cysteine (Figure 2c). As expected, increasing the amount of TPase led to faster generation of methyl mercaptan. Even 5 µg TPase can generate 100 ppb methyl mercaptan (500 × the threshold level for the human nose) in less than 5 min (Figure 2c), thus indicating that our system has a quick response time. A similar test was also done with L-tryptophan (see the Supporting Information).

Next, we performed odor-based experiments to determine the minimum amount of TPase that can actually be detected by the human nose as a way to measure the sensory threshold of the TPase-based sensing system. Following established protocols,[5] we prepared a pre-reaction mixture that contained *S*-methyl-L-cysteine (1 mM) and PLP (0.1 mM). This mixture was then used to set up a series of testing sample sets, each of which consisted two identical blanks (i.e. without TPase) and a positive sample with varying amounts of TPase (note: having two blanks served to decrease the probability of identifying the positive sample by guessing). Each panelist was asked to evaluate the entire collection of sample sets in order of sequentially increasing concentrations of TPase and identify the positive samples.

The best estimate threshold (BET) for each panelist was calculated as the geometric mean of the highest concentration that was not recognized and the next concentration identified correctly. The BET for the entire panel (five people in our case) was determined as the geometric mean of individual BETs. The BET for TPase assay was found to be 3.6 nM (Table S3 in the Supporting Information). We applied the same method, but with varying amounts of RIgG, for determining the BET for the TLISA assay, which was found to be 85 nM (Table S4).

Coupled enzymatic assays are widely used in bioanalysis and this strategy is particularly useful for analyzing enzyme-catalyzed reactions for which the substrate and product are difficult or impossible to measure.[6] The idea is to use a secondary enzyme to transform the product of the first reaction into a product that can be readily measured. Motivated by the wide use of coupled enzymatic assays and the general importance of ATP in the context of biological systems, we decided to develop a coupled enzymatic assay that uses odor as a readout for the presence of ATP. ATP is the universal energy source in living organisms. It disappears in less than two hours after cell death, and the amount of ATP per cell is generally constant.[7] ATP detection is often used as a general assay for monitoring drinking-water cleanliness[8] and detecting food spoilage,[9] as well as for hygiene monitoring in hospitals.[10]

TPase is a tetrameric enzyme, the activity of which activity is dependent on PLP.[11] Moreover, we found that the activity of TPase can be modulated by the concentration of PLP (Figure S3 in the Supporting Information). Because PLP can

be synthesized from pyridoxal by pyridoxal kinase (PKase) using ATP as a cofactor, an odor-based sensor for ATP can be conveniently constructed by coupling PKase and TPase and their respective substrates pyridoxal and *S*-methyl-L-cysteine (Figure 3a).

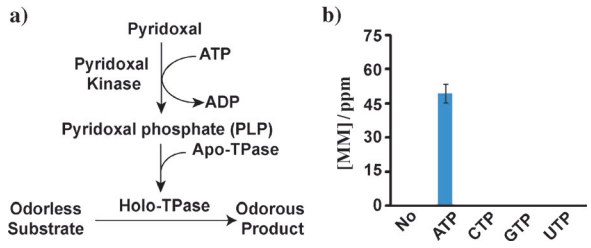

Figure 3. ATP detection by using a coupled enzymatic odor-based sensor. a) Coupled enzyme reactions. Pyridoxal kinase converts pyridoxal into PLP, which serves as a cofactor to activate TPase to produce methyl mercaptan (MM). b) The specificity of the ATP sensor. Samples (700 μL) contained 0.2 mM of the respective nucleoside triphosphate, 40 μg TPase, 50 μg PKase, 0.1 mg mL^{-1} pyridoxal, and 15 mM *S*-methyl-L-cysteine (or L-tryptophan). Each data point is the average of three measurements.

To validate the coupled assay and to demonstrate its specificity towards ATP, we performed the following experiment. First we prepared parallel samples containing PKase, TPase, pyridoxal, and *S*-methyl-L-cysteine. Each solution was then mixed with ATP, guanosine triphosphate (GTP), cytidine triphosphate (CTP), or uridine triphosphate (UTP). For the control sample, no nucleoside triphosphate was added. Finally, the methyl mercaptan concentration in the headspace was measured with the ToxiRAE Plus PID and the results are shown in Figure 3b. As expected, only ATP was able to induce the production of methyl mercaptan. The concentration of methyl mercaptan in the headspace was 49.1 ppm, a value much larger than the 0.2 ppb detection limit for the human nose. A similar experiment was performed with L-tryptohpan as the substrate (see the Supporting Information).

We also determined the BET for ATP with a panel of 5 people and with *S*-methyl-L-cysteine as the substrate. The BET was found to be 0.32 μM (Table S5).

In summary, we have developed an odor-based sensor system that exploits TPase as the key component. This enzyme is able to convert the substrates *S*-methyl-L-cysteine and L-tryptophan into methyl mercaptan and indole, respectively, both of which can be easily detected by the human nose. The catalytic power of the enzyme ensures rapid signal generation. In addition, the use of biotinylated TPase allows easy coupling of TPase to a molecular recognition element, such as an antibody, for the development of an ELISA-like assay. Moreover, we have shown that TPase can be coupled to PKase for the creation of a system that is capable of detecting ATP, which may find use in monitoring water and food quality. The system presented herein is modular in nature and the ability to combine the biotinylated TPase reporter with virtually any molecular recognition element should allow for a broad range of applications for this odor-based reporting system.

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