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Microcantilevers Modified by Specific Peptide for Selective Detection of Trimethylamine

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Abstract

We report a biosensor based on a microcantilever that is modified by a specific peptide for highly selective detection of trimethylamine (TMA). The assay is based on binding-induced bending of the peptide functionalized microcantilevers. The sensor is selectively responsive to TMA. The amplitude of microcantilever bending at equilibrium is a function of the concentration of TMA with a dynamic range from 8 ppm to 800 ppm. The detection limit is approximately 8 ppm. There is a good intra-sensor and an acceptable inter-sensor reproducibility as evidenced by the standard deviation of 5% and 15%, respectively.

1. Introduction

TMA is a gas at room temperature with an irritating, fishy, and ammonia-like odor. It is listed as a substance under restriction control according to the regulations on the air pollution control act.^{1,2} In the field of quality control in food industry, the amount of volatile basic substances such as TMA is a useful indicator of spoilage in fresh and lightly preserved seafood; it increases during the breakdown of seafood, such as fish and shrimp.^{3,4,5} In the field of medical diagnosis, an increase in the concentration of TMA in the breath of patients can be used as a sign of virerinic disease.⁶ Therefore, detection of TMA is of interests in environmental protection, food industry, and medical diagnosis.

Although TMA is a gas at room temperature, TMA in rotten seafood are dissolved in the sample lysates. Therefore, detection of TMA in water, other than in air, was used as a standard method for measuring the level of TMA.^{7,8} Traditionally, TMA is determined by gas chromatography (GC),⁹ high performance liquid chromatography (HPLC),¹⁰ ion mobility spectrometry (IMS),¹¹ and mass spectrometry (MS).¹² However, these methods require instrumentations that are emphatically non-portable, quite expensive, and very complex, which is not well suited to the field analysis of TMA samples.

Micro-electro-mechanical systems offer unique opportunities in the design of cost-effective analytical methods. One example is microcantilevers (MCLs), which could be made extremely sensitive to chemical and physical changes.^{13,14,15} MCLs can be mass produced

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through a typical lithography process, and can be readily integrated into a MEMS device. To date, extremely sensitive chemical vapor sensors based on MCLs have been demonstrated using selective coatings on cantilevers.^{16,17} Requirements such as sensitivity, small size, low cost, and simultaneous detection of multiple species make the MCL sensor approach very attractive.

The unique characteristic of MCLs is their ability to deflect due to molecular adsorption- or binding-induced change in surface tension, which produces the upward or downward bending of the MCLs. By recording the deflection magnitude of the MCLs, the concentration of target biological or chemical species can be measured. This characteristic of MCLs qualifies them for detecting species both in air and solution. From molecular point of view, the binding or adsorption result in electrostatic repulsion¹⁸ or attraction¹⁹, steric effects²⁰, conformation change of proteins²¹, and intermolecular interactions²² between molecules on the cantilever surfaces that alter the surface stresses on the cantilever. This is achieved by confining the adsorption to one side of the MCL.

Recently, Yang et al reported a microcantilever-based sensor for detection of TMA²³. This microcantilever is modified by a layer of carboxylic acid group and the sensitivity is as low as 10 ppm, which is appropriate for detection of TMA in early stage of food spoilage. However, the selectivity of this sensor is low because a variety of chemicals can interact with carboxylic acid. In contrast to the low selectivity of small molecule-based chemical sensors, biosensors based on specific proteins or peptides are generally more selective to specific analytes. One such molecular recognition molecule for TMA is a peptide with the following sequence: Cys-Pro-Ser-Ala-Asn-Asn-Ser-Thr-Val-Lys-Glu-Gly-Cys-Gly. This peptide is a fragment of a natural olfactory receptor protein which can selectively bind a TMA molecule.²⁴ In this work, we demonstrate that a MCL modified by a layer of this peptide can be used for the detection TMA with high sensitivity and selectivity.

2. Experimental

2.1. Materials

In our experiments, we used commercially available silicon MCLs (μ Mash, CSC12, San Jose, CA). The dimensions of the rectangular silicon MCLs were 90 μ m in length, 35 μ m in width, and 1.0 μ m in thickness. One side of these cantilevers was covered with a thin film of chromium (20 nm) and followed by a 20 nm layer of gold, both deposited by e-beam evaporation. Since gold film does not stick well to SiO₂, the thin chromium layer was used to improve adhesion. On the uncoated side of the commercial microcantilever was silicon with a 12–19-Å-thick naturally grown SiO₂ layer, which is called “native oxide”. The shape of the MCLs used in this work is shown in Figure 1.

Chemicals used in these experiments, including TMA, histamine (HI), hypoxanthine (HX), acetone, ethanol, ethyl acetate, DMF, methanol, toluene, hexane, and chloroform were used as received from Alfa-Aesar Inc. (Ward Hill, MA) The peptide used in this biosensor were commercially synthesized from SBS Genetech Co. Ltd (Beijing, China) and supplied as HPLC-purified preparation.

2.2. Deflection Measurement

The deflection experiments were performed in a flow-through glass cell using a four-quadrant atomic force microscopy (AFM) with integrated laser and position sensitive detector (Digital Instruments, Santa Barbara, CA). For continuous flow-through experiments, initially, the buffer solution was circulated through the cell using a syringe pump.

A schematic diagram of the apparatus used in this study was previously reported.²⁵ A constant flow rate was maintained during each experiment. The flow rate was controlled using a syringe pump (KD Scientific, Holliston, MA) equipped with a low-pressure liquid chromatography injector valve and injection loop (Upchurch Scientific, Oak Harbor, WA). Before each measurement, a MCL was equilibrated in 0.01 M phosphate buffer until a stable baseline was achieved (all at 25°C; 5 mL/h flow rate for flow experiments). Experimental solutions containing different concentrations of TMA were injected directly into the flowing fluid stream via a low-pressure injection port sample loop arrangement with a loop volume of 0.5 ml. This arrangement allows for continuous exposure of the cantilever to the desired solution without disturbing the flow cell or changing the flow rate. Since the volume of the glass cell, including the tubing, was only 0.3 ml, a relatively fast replacement of the liquid in contact with the cantilever was achieved. MCL deflection measurements were determined using the optical beam deflection method. The bending of the cantilever was measured by monitoring the position of a laser beam reflected from the gold-coated side of the cantilever onto a four-quadrant AFM photodiode. We define bending toward the gold side as ‘bending up’ and towards silicon side as “bending down. In case adsorption occurs on the gold surface, in general, the downward bending is caused by repulsion or expansion of molecules on the gold surface, which is so called compressive stress; vice versa, the upward bending is caused by attraction or contraction of molecules on the gold surface, which is called tensile surface stress. The cantilever was immersed in the buffer solution until a baseline was obtained and the voltage of the position-sensitive detector was set as background corresponding to 0 nm.

MCL based detection systems are prone to drift problems. We observed similar drifting problems in these experiments. The drifting problem, however, was alleviated after equilibrating the prepared cantilevers in the buffer solutions for over 1 hour. Typically, a good baseline (< 0.001 N/s surface stress noise) was obtained in one to two hours after equilibrating the cantilevers in the buffer solutions. In these experiments, we injected the sample after a good baseline was obtained. For a given cantilever the experiments were repeated at least three times.

The surface stress change in the figures were calculated according to Eq. (1).²⁶

$$\Delta Z = \left(\frac{3(1-\nu)L^2}{ET^2} \right) \delta s, \quad (1)$$

where Δz is the observed deflection at the end of the MCLs, ν and E are Poisson's ratio (0.2152) and Young's modulus (156 GPa for silicon) for the substrate, respectively. t and l are the thickness and length of the MCLs, respectively, and δs is the differential surface stress on the MCLs.

2.3. Modification of MCLs by SH-containing peptide

In general, molecular recognition molecules can be self-assembled or covalently linked to one side of the microcantilevers²⁷. It is well known that sulfhydryl-containing long chain molecules, including DNA and proteins, can form a monolayer on a gold surface through the formation of Au-S bonds.²⁸ In this work, a cysteine on the N-terminal of the peptide provides a natural sulfhydryl group to bring the peptide on the gold side of MCLs. The formation of a peptide monolayer on MCLs was conducted by immersing gold-coated silicon MCLs in a 0.01M peptide solution in phosphate buffer (PB, pH 7.0) for 24h. All gold-coated MCLs were firstly treated with piranha (3 parts H₂O₂/7 parts H₂SO₄) prior to peptide immobilization.

3. Results and discussions

3.1. Effect of Monolayer Formation Time on the Bending Amplitude

Our previous work showed that the formation time of the monolayer affects the bending amplitude of MCLs, and subsequently the sensitivity of the MCL sensors.²⁹ It is expected that it would require a longer time for the formation of a compact layer of the peptide on the gold surface due to repulsion of charged groups in the peptide relative to other long chain neutral compounds. To optimize the sensitivity toward TMA, the deflection of MCLs due to TMA adsorption were measured as a function of the SAM formation time, and the results are shown in Figure 2. The results indicate that the bending response of MCLs is insignificant when the MCLs are treated for less than 4h in the peptide solution. The deflection magnitude of the MCLs increases with a longer monolayer formation time and it reaches a plateau when the MCL has been treated for more than 24h. Based on this observation, all the cantilevers were prepared by an immersion into the peptide solution for 24 hours for the measurement of TMA. The results suggest that the larger bending amplitude corresponding to longer monolayer formation time is due to the formation of a more compact monolayer of the peptide.

3.2. Deflection profiles and detection limit of the MCLs to TMA

We paid attention to the concentration between 8 ppm to 800 ppm because this arrange is what of interests to the food industry for seafood spoilage.^{30,31} When solutions that contain various concentrations of TMA were injected into the fluid cell, the MCLs bent up toward the Au side with different amplitudes as shown in Figure 3. The MCL bending reached equilibrium within 500s. For each measurement, a 0.5 mL aliquot of TMA solution in 0.01 M of phosphate buffer was switched into the fluid cell, where the MCL was held. It took 300–500 s for the injected TMA solution to flow through the fluid cell, considering the retention time due to the dead volume. After that, the original phosphate buffer solution was circulated back into the fluid cell. It was observed that the MCL deflection gradually returned to its original position as the solution composition returned to the original phosphate buffer solution. It was also observed that at different concentrations, the cantilever bent at different amplitudes, and the bending of the cantilever at different TMA concentrations. The MCL deflection was fully reversible.

Repeated exposure of the same 800 ppm solution of TMA to the same peptide-modified MCL caused similar deflection amplitudes and bending rates, as shown in Figure 4. The standard deviation is within 5%, indicating good measurement-to-measurement reproducibility. Exposure of an 800 ppm solution of TMA to five different MCLs prepared under the same conditions also caused similar deflection amplitudes and the standard deviation was within 15% indicating an acceptable MCL-to-MCL reproducibility (Figure S1 in the supporting information). Control experiments were performed by exposing an unmodified MCL to an 800 ppm solution of TMA. No deflection of the MCLs was observed (also shown in Figure 4).

3.3. Sensitivity, Selectivity, and Stability of the Peptide-Modified MCL

Figure 5 shows the bending response of peptide modified MCLs to various concentrations of TMA from 8 ppm to 800 ppm. The larger MCL deflection suggests larger surface stress change on the MCL surface at higher TMA concentrations. The detection limit was approximately 8 ppm (0.0048 N/m surface stress as compared to the noise level of 0.001 N/m), which is approximately the same as that of the carboxylic acid-modified MCL sensor.²¹ However, the selectivity of this sensor is dramatically improved as shown in Figure 6. The MCLs have no or only slight responses to histamine (HI), hypoxanthine (HX), acetone, ethanol, ethyl acetate, DMF, methanol, toluene, hexane, and chloroform. The potential

interference of contaminating chemicals on the peptide modified MCL was evaluated in this study. Besides TMA, HI^{32,33} and HX^{34,35} are the main chemical spoilage indicators, commonly used for fish quality assessment. As shown in Figure 6, the surface stress changes on the MCL surface are 0.035 ± 0.005 N/m and 0.040 ± 0.006 N/m upon exposure to 800 ppm TMA and 800 ppm TMA + 800 ppm HI + 800 ppm HX, respectively. It showed that the HI and HX do not have a significant interference on the detection of TMA by using the peptide modified MCL. The high selectivity of this sensor to TMA is due to the high specificity of this peptide to TMA than to any other solvents.²⁴

The peptide modified MCLs demonstrated excellent stability for more than 1 months when stored in the buffer solution at 4 °C or stored in a dry state at room temperature as evidenced by the similar profiles and bending amplitude in Figure 3.

3.4. Kinetic of the Microcantilever Bending

The binding site of the peptide for TMA has not been discussed in the reported literature.²² We propose that the recognition of TMA came from the interaction of TMA with $-\text{COO}^-$ group of glutamic acid (Glu) in the peptide, and the sequence other amino acids provide a specific secondary structure for selectively binding of TMA molecule. When TMA is adsorbed on the peptide surface, the cantilever bent upward due to the release of repulsion between COO^- groups in the peptide molecules, as shown in Figure 7.

It was anticipated that the Langmuir adsorption model could be used to describe the absorption of TMA on the peptide covered surface. The rate of formation of a fraction of a monolayer, θ , is proportional to the concentration of the reacting species in solution and to the fraction of the surface remaining free of sorbant, $1-\theta$. Thus, the MCL bending vs the time follows the relationship¹⁷

$$\delta \propto 1 - \exp(-kt) \quad (1)$$

Where δ is the surface stress, k is the reaction rate, and t is the time.

The k was calculated to be 0.23 s^{-1} using a non-linear curve-fitting method to fit the observed experimental data.

Langmuir model is the simplest, yet the most popular model for adsorption of a single layer of analytes on a surface. Since a surface stress is generated from the adsorption, the binding molecules on the surface should not be independent because of the interactions between adjacent binding molecules, thus the Langmuir model may not be appropriate for the binding study. However, our experimental results showed that the adsorption fit the Langmuir model very well. We hypothesize that this is due to the small surface stress change based on adsorption of a layer of TMA on the surface, i.e. weak interactions between adjacent binding molecules. As a result, the effect of adjacent binding molecules can be negligible, and the binding molecules on the surface can be considered as independent, which is the same as those occur on surfaces of other substrates where the Langmuir model has been used.

As a matter of fact, there is no difference between the surface of the microcantilevers and the surface of other substrates regarding the binding study, and thus the Langmuir model can be applied for the microcantilever surface if it can be applied to the surface of other substrates. In other word, the surface stress observed on the microcantilever surface should also occur on a surface of any other substrates, which has long been neglected because few devices can be used to detect this surface stress change due to the adsorption. From the best

of our knowledge, it has not been studied under what surface-stress-change the Langmuir model can or cannot be used. A systematic study in this concept will result in new knowledge on binding-induced surface stress changes, which may help improve the sensitivity of microcantilever sensors.

4. Conclusions

In summary, a special peptide was immobilized on a MCL surface for detection of TMA in the liquid phase at room temperature. The detection limit for TMA is approximately 8 ppm. The MCLs showed high selectivity to TMA.

It is well known that the mammalian olfactory system can recognize and discriminate a large number of different odorant molecules. The detection of chemically distinct odorants presumably results from the association of olfactory receptor proteins.³⁶ Our work showed that the special peptide modified MCLs may provide opportunities for selective identification of specific chemicals. This approach has the advantage that it might be applicable to a large number of proteins or a peptide of olfactory receptor proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
SEM image of MCLs (μ Mash, CSC12). The middle cantilever is used in all the experiments.

Figure 2.

The maximum surface stress change of peptide-coated MCLs when exposed to 800 ppm TMA in 0.01M phosphate buffer. The MCLs were prepared by immersing the MCLs into a 0.01 M solution of the peptide in 0.01M phosphate (pH 7.0) buffer for different periods of time.

Figure 3. Dynamics of bending amplitude for a peptide modified MCL as a function of TMA concentration in 0.01 M phosphate buffer (the injection point is indicated with arrows).

Figure 4.

Three replicates of bending responses as a function of time for a peptide modified MCL following injection of an 800 ppm of TMA in 0.01 M phosphate buffer (the injection point is indicated with arrows) at 5 mL/h flow rate. Control experiments were performed by exposing an unmodified MCL to an 800 ppm solution of TMA.

Figure 5. Correlation between maximum bending amplitudes of a peptide modified MCL and TMA concentration in a 0.01 M phosphate buffer.

Figure 6. Comparison of bending responses for a peptide modified MCL upon exposure to 800 ppm TMA and a variety of other chemicals at the same concentration at 5 ml/hr flow rate.

Figure 7.
Schematic representation of cantilever upward-bending due to the release of repulsion between COO^- in the peptide molecules.