Application of Synthetic Peptides with Molecular Imprinting for Improving Odorant Detection

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Abstract

The olfactory receptor can recognize and discriminate large number of different odorant molecules. Mimicking the binding domain on olfactory receptor, a novel method for odorants detection, is developed by integrating the functional synthetic peptides from combinatorial chemistry and molecular imprinting technology. The molecular imprinting technology is applied to enhance the sensitivity and specificity of binding ability of functional peptide with the target odorant. We have previously reported that the potential amino acid candidates corresponded to six positions of hexapeptide are identified using the positional scanning synthetic peptide combinatorial libraries (PS-SPCL) and molecular imprinting approach. In the present studies, 32 individual defined hexapeptides representing the hexameric combinations of the most active amino acid residues were synthesized and tested. These peptides were mixed with the target odorant to prepare the molecularly imprinted peptides, then were coated on a piezoelectric quartz crystal. Four imprinted defined peptides BA-10, BA-18, BA-24 and BA-26 exhibiting the highest binding affinity to odorant butyric acid were selected. The sensitivity of the butyric acid with the four potent imprinted peptides and non-imprinted peptides were examined. The selectivity of different functional group odorants with the four butyric acid imprinted peptides were also studied. The sensitivity and selectivity between the imprinted peptides, BA-10, BA-18, BA-24 and BA-26 and the odorant butyric acid were significantly enhanced by the effect of the molecular imprinting. The combination of peptide library and molecular imprinting technology enhance the sensitivity and specificity of binding ability for odorant detection and can be used for development of odorant sensing tools.

Keywords: synthetic peptide, molecular imprinting, odorant

1. Introduction

The mammalian olfactory system can recognize and discriminate large number of different odorant molecules.[1] The odor discrimination occurs during the association of odorous ligands with specific receptors on olfactory sensory neurons. The receptor protein plays an important role in odorant recognition and cell signaling. The literature reports suggest that the extracellular loop and transmembrane domain might be the major part of the odorant-binding domain in olfactory receptors.[2, 3] The design of functional polymers that can selectively recognize molecules has become an active area of research in recent years.[4, 5]

The recognition and subsequent complementary binding between a substrate and an odorant molecule is the key step in the odor discrimination process. To mimic the olfactory sensing system, utilizing synthetic peptides as sensing materials capable of binding to specific odorant is of significant interest to study. The peptide library that is composed of thousands or millions of peptide sequences offers a high throughput for target drug or compound screening.[6, 7] However, a major limitation of the peptide library screening approach is low selectivity and sensitivity to the target compound. Molecular imprinting is a methodology for the creation of selective recognition sites in molecules.[8, 9] The recognition sites constitute an "induced molecular memory" capable of selectively recognizing the imprint species. Since the conformations of peptides are considered as one of the key factors for absorption effect between peptides and odorant molecules, integrating the molecular imprinting technology shall create the selective recognition sites for the template molecule.[10, 11] These recognition sites have a complementary shape and size with the template compound which can enhance the sensitivity and specificity of binding ability of functional peptide with the target odorant. An imprinted functional peptide can form a specific configuration with a complementary target odorant and increase the binding ability with the target odorant. Based on these rational concept, our strategic approach is to use molecular imprinting process preparing synthetic peptide receptor which has the specific recognition for the target odorant.

The objective of our research is to develop a novel method for odorants detection by integrating the functional synthetic peptides from combinatorial chemistry and molecular imprinting technology. The butyric acid is selected as a target odorant to investigate the sensitivity and specificity effect of imprinted functional peptide versus non-imprinted peptide on their sensing ability for odorant detection. The imprinted peptides with high binding ability of butyric acid are selected by the positional scanning synthetic peptide combinatorial libraries (PS-SPCL) methodology.[12, 13] The potential amino acid candidates corresponded to each

amino acid position of polypeptide can be identified. To define the optimal binding sequences of peptides, all possible combination of potential amino acid candidates of peptides were reconstructed and tested.

In our previous study result, the top two amino acid candidates corresponded to six positions of hexapeptide C X_1 X_2 X_3 X_4 X_5 X_6 have identified with the positional screening synthetic peptide combinatorial libraries of the 114 imprinted hexapeptide libraries.[14]

In the present work, 32 individual defined hexapeptides representing the hexameric combinations of the most active amino acid residues were synthesized and their binding affinity with odorant butyric acid were evaluated.

2. Method

This study integrates the odorant detection and molecular imprinting technologies to prepare synthetic peptide receptor. The hexapeptides were synthesized by solid phase peptide synthesis methodology using Fmoc chemistry.[15, 16] The peptide chain were elongated (assembled) on Wong resin and the synthesis were carried out manually. Coupling were performed with DCC/HOBt. Following the synthesis, the peptides were cleaved from the resin, and side chain protecting groups were removed using TFA standard protocols. These hexapeptides were mixed with the target odorant to prepare the molecularly imprinted peptides then coated on a piezoelectric (PZ) quartz crystal.

Imprinted functional peptide preparation:

(a) The hexapeptide was dissolved in the butyric acid solution to form the covalently interaction between peptide and the template butyric acid.
(b) The hexapeptide - butyric acid complex was coated on the surface of piezoelectric quartz crystal.
(c) The template butyric acid was removed by vacuum from surface of piezoelectric quartz crystal.

Non-imprinted peptide preparation:

(a) The hexapeptide was dissolved in the 75% ethanol solution.
(b) The hexapeptide solution was coated on the surface of piezoelectric quartz crystal.

A quartz crystal microbalance (QCM) was used to quantify interactions between

synthetic peptides and odorants.[17, 18, 19] The PZ crystal was served as a signal transducer to determine the binding affinity of synthetic peptides for odorants (Fig. 1).

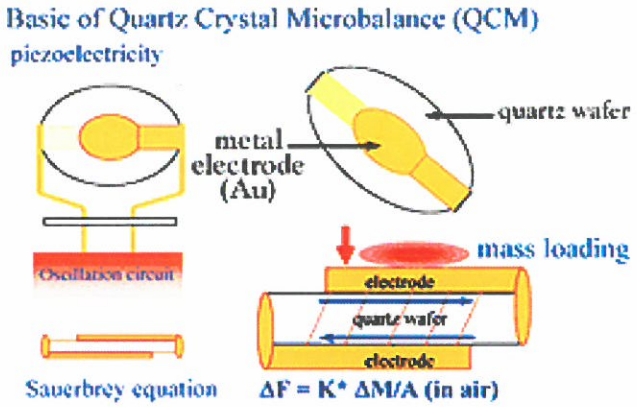

Fig. 1 The Piezoelectric Crystal Served As a Signal Transducer

The sensitivity between peptide and odorant molecule on a piezoelectric quartz crystal was determined. The sensitivity is defined as

$$\textbf{Sensitivity} = \frac{\textbf{The change of frequency } \Delta F \textbf{ [Hz]}}{\textbf{Coating amount of peptide } \Delta M \textbf{ [}\mu g\textbf{]}}$$

3. Results and Discussion

The conformations of peptides are considered as one of the key factors for absorption effect of peptides on odorant molecules. The molecular imprinting process is applied to enhance the binding ability of peptide with the target odorant (Fig. 2). An imprinted functional peptide can form a specific configuration with a complementary target odorant and enhance the binding ability with the target odorant.

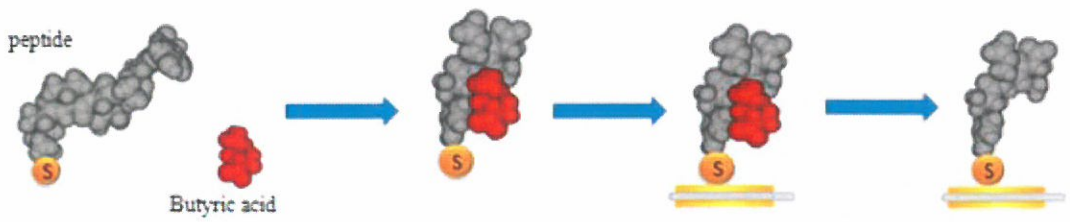

Fig. 2 Schematic Diagram Representation of Peptide Molecular Imprinting Process

Based on our previous study result using the positional scanning synthetic peptide combinatorial libraries (PS-SPCL) approach examined the binding affinity of the 114 hexapeptide libraries with odorant butyric acid, the top two amino acid candidates at six positions for hexapeptide C X_1 X_2 X_3 X_4 X_5 X_6 were identified (Table 1).

	X_1	X_2	X_3	X_4	X_5	X_6
1st Amino Acid Candidate	H	T	L	P	G	Y
2nd Amino Acid Candidate	T	V	W	V	Y	

Table 1 The Best Amino Acid Candidates in Hexapeptide

According to the peptide libraries screening result, all possible combination of the top two amino acids of hexapeptide C X_1 X_2 X_3 X_4 X_5 X_6 were synthesized and tested [thus: Position 1 - Histidine (H) and Threonine (T), Position 2 - Threonine (T) and Valine (V), Position 3 - Leucine (L) and Tryptophan (W), Position 4 - Proline (P) and Valine (V), Position 5 - Glycine (G) and Tyrosine (Y), Position 6 - Tyrosine (Y)]. The Cysteine (C) residue is added in the beginning of the peptides provided a thio (-SH) group to form a chemical adsorption between the peptide and the gold surface of the piezoelectric crystal.[20] This generated 32 (2 x 2 x 2 x 2 x 2 x 1 = 32) individual defined peptides from six positions of hexapeptide. The amino acid sequence of 32 defined hexapeptides, BA1~ BA32, were shown in Table 2. In addition, a non-related peptide XBA-01 was used as a negative control. The binding affinity of the imprinted hexapeptides BA1~ BA32 with butyric acid are shown in Fig 3.

	Sequence		**Sequence**
BA-1	CTTLPGY	BA-17	CHTLPGY
BA-2	CTTLVGY	BA-18	CHTLPYY
BA-3	CTTWPGY	BA-19	CHTLVGY
BA-4	CTTWVGY	BA-20	CHTLVYY
BA-5	CTVLPGY	BA-21	CHTWPGY
BA-6	CTVLVGY	BA-22	CHTWPYY
BA-7	CTVWPGY	BA-23	CHTWVGY
BA-8	CTVWVGY	BA-24	CHTWVYY
BA-9	CTTLPYY	BA-25	CHVLPGY
BA-10	CTTLVYY	BA-26	CHVLPYY

BA-11	CTTWPYY	BA-27	CHVLVGY
BA-12	CTTWVYY	BA-28	CHVLVYY
BA-13	CTVLPYY	BA-29	CHVWPGY
BA-14	CTVLVYY	BA-30	CHVWPYY
BA-15	CTVWPYY	BA-31	CHVWVGY
BA-16	CTVWVYY	BA-32	CHVWVYY
XBA-01	CIGDKVN		

Table 2 The Amino Acid Sequence of 32 Defined Hexapeptides

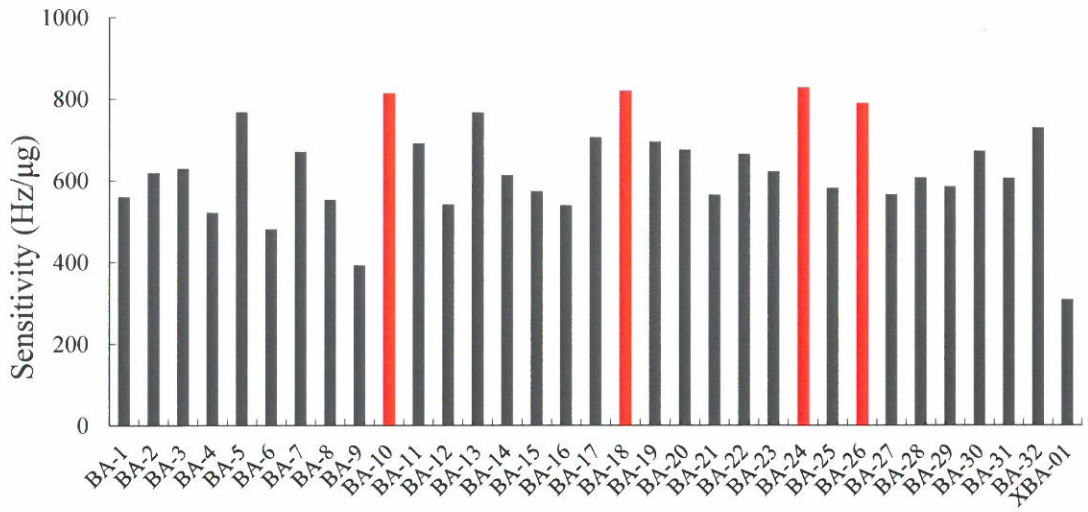

Fig. 3 The Binding Affinity of The Imprinted Hexapeptides with Butyric Acid

The binding affinity results of 32 defined hexapeptides indicate the sensitivity of most of the imprinted peptides to butyric acid were above 500 Hz/μg that are significantly higher than the non-related peptide XBA-01 to butyric acid (309 Hz/μg). Of the 32 defined hexapeptides, 4 imprinted defined peptides exhibiting the binding sensitivity to butyric acid greater than 790 Hz/μg were selected for further studies (BA-10, sensitivity = 814 Hz/μg; BA-18, sensitivity = 820 Hz/μg; BA-24, sensitivity = 829 Hz/μg; BA-26, sensitivity = 790 Hz/μg). Further investigation the effect of molecular imprinting, the binding affinity of odorant butyric acid to these four defined imprinting and non-imprinting peptides were examined. The binding result demonstrated that the imprinting functional peptides have higher affinity to odorant butyric acid than the non-imprinted peptides for the four most potent defined peptides. The sensitivity of imprinted peptides are 1.3~1.9 folds higher than that of non-imprinted peptides in the four potent defined peptides BA-10, BA-18, BA-24 and BA-26 (Fig. 4). The sensitivity of the peptide arise from the molecular imprinting process showed that the imprinted peptide can create a complementary recognition

site which can enhance the binding ability of functional peptide with the target odorant.

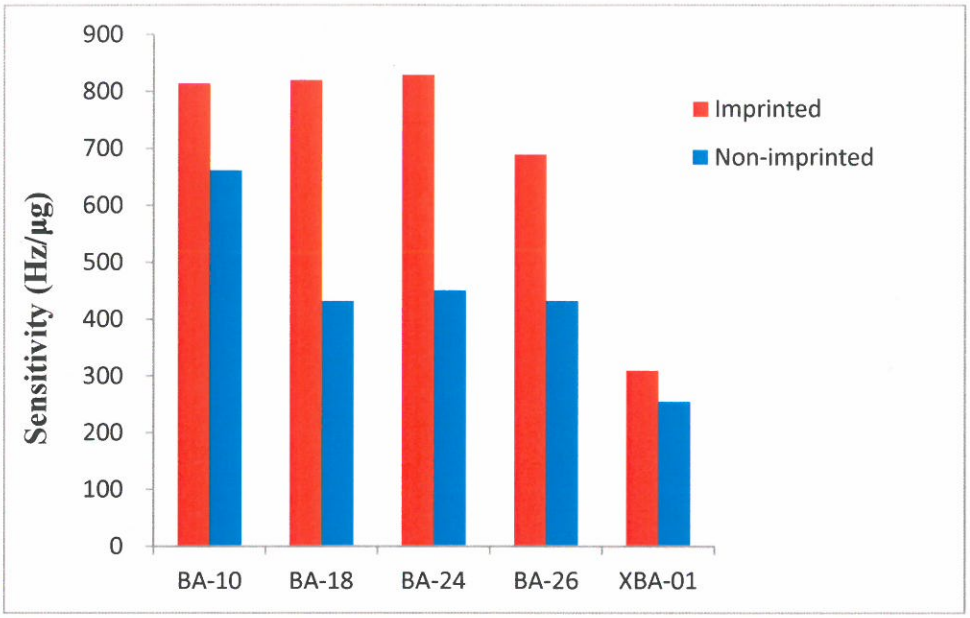

Fig. 4 Binding Affinity of Butyric Acid for the Imprinted and Non-imprinted Peptides

Further assessment of the binding selectivity and specificity of imprinting functional peptides to the target odorant, the binding affinity of different odorant molecules including acids, amines, alcohols, ketones, esters, ethers, alkyl halides, and alkanes functional groups with the four butyric acid imprinted peptides BA-10, BA-18, BA-24 and BA-26 were studied. Evidently, the binding affinity of target odorant butyric acid to the four potent imprinted peptides are higher sensitive than that of other odorants.

The sensitivities of butyric acid imprinted peptide BA-10, BA-18, BA-24 and BA-26 to different odorant molecules were shown in Fig. 5 - 8. The results showed that the molecular imprinting process rationally generated peptides with the desires distinction selectivity. The imprinted peptide BA-10, BA-18, BA-24 and BA-26 differentiated target odorant butyric acid (sensitivity = 790~829 Hz/μg) from other amines (sensitivity = 135~626 Hz/μg), alcohols (sensitivity = 35~325 Hz/μg), ketones (sensitivity = 0~53 Hz/μg), esters (sensitivity = 0~110 Hz/μg), ethers (sensitivity = 0~337 Hz/μg), alkyl halide (sensitivity = 6~133 Hz/μg), alkanes (sensitivity = 0~100 Hz/μg) functional group odorants. The binding affinity of imprinted peptide to homologous series of acids, such acetic acid, propionic acid, butyric acid, and pentanoic acid were also studied. The imprinted peptide BA-10, BA-18, BA-24 and BA-26 can differentiate target odorant butyric acid (sensitivity = 790~829 Hz/μg)

from odorant acetic acid (sensitivity = 321~491 Hz/μg), propionic acid (sensitivity = 327~662 Hz/μg), and pentanoic acid (sensitivity = 417~527 Hz/μg). These results suggested these imprinted peptides were capable of differentiating structurally similar acids. Sensitivity of common interference compound, water, to imprinted peptide BA-10 (sensitivity = 426 Hz/μg), BA-18 (sensitivity = 298 Hz/μg), BA-24 (sensitivity = 366 Hz/μg) and BA-26 (sensitivity = 156 Hz/μg) are lower than sensitivity of target odorant butyric acid. As a comparison, sensitivities of butyric acid imprinted non-related peptide XBA-01 to different odorant molecules was also examined (Fig. 9). On the contrary, the butyric acid imprinted non-related peptide XBA-01 cannot distinguish target odorant butyric acid from other odorant molecules. From the selectivity results of these four potent imprinted peptides, it reveals that imprinted peptides usually contain specific recognition sites that can recognize the target odorant molecule and hence exhibit a high specificity for the target odorant molecule.

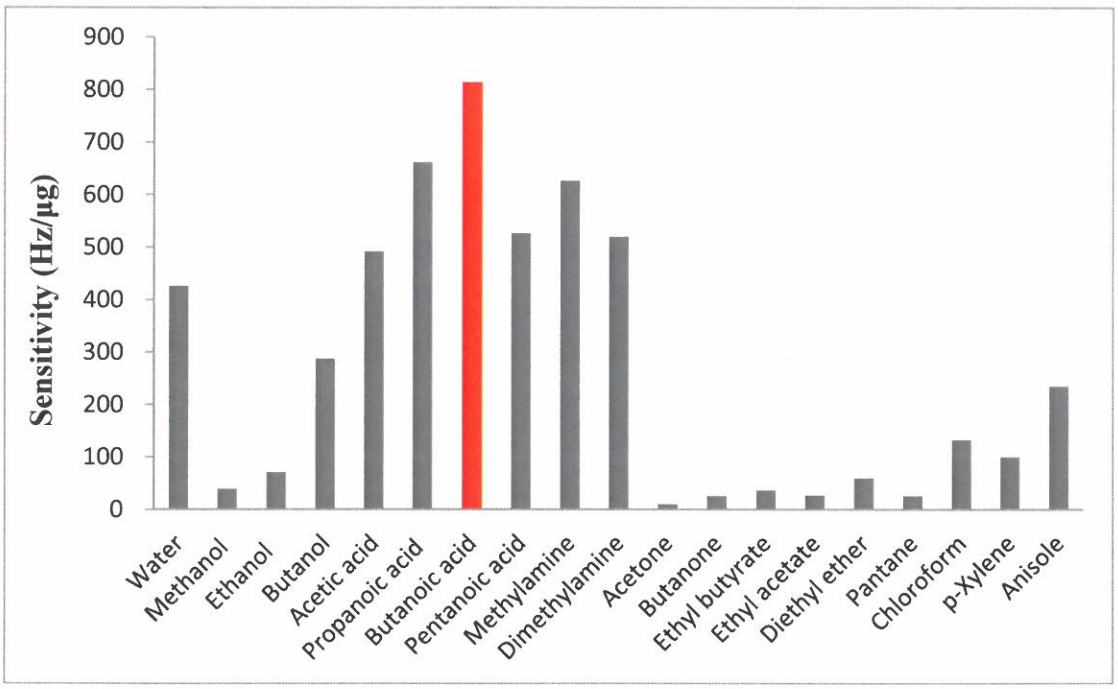

Fig. 5 Sensitivities of Butyric Acid Imprinted Peptide BA-10 to Different Odorant Molecules

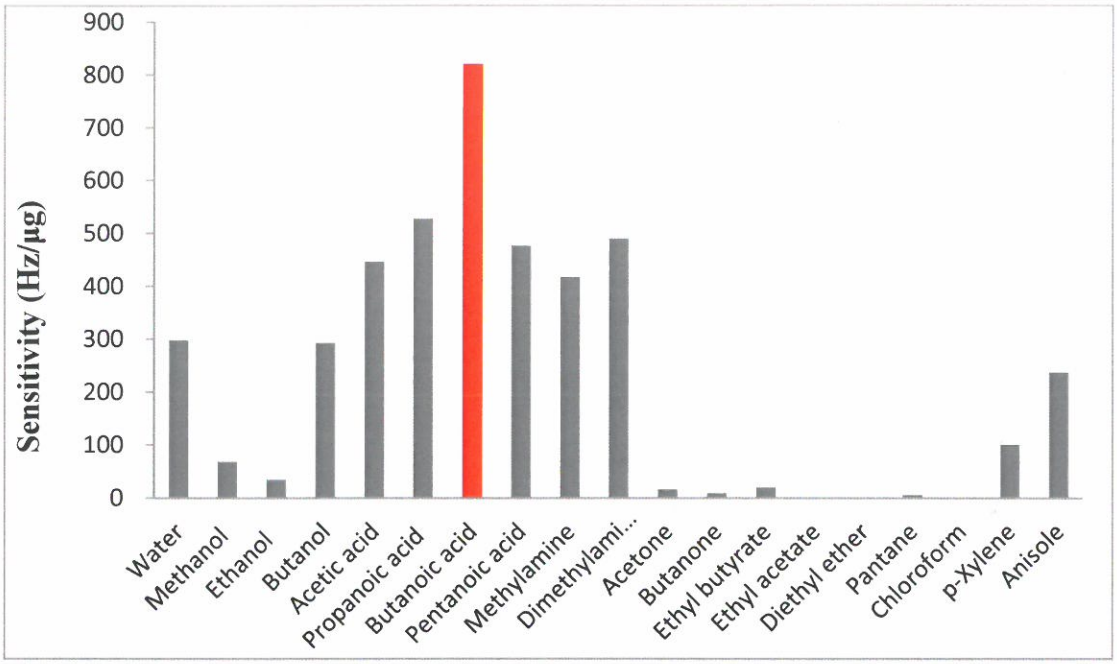

Fig. 6 Sensitivities of Butyric Acid Imprinted Peptide BA-18 to Different Odorant Molecules

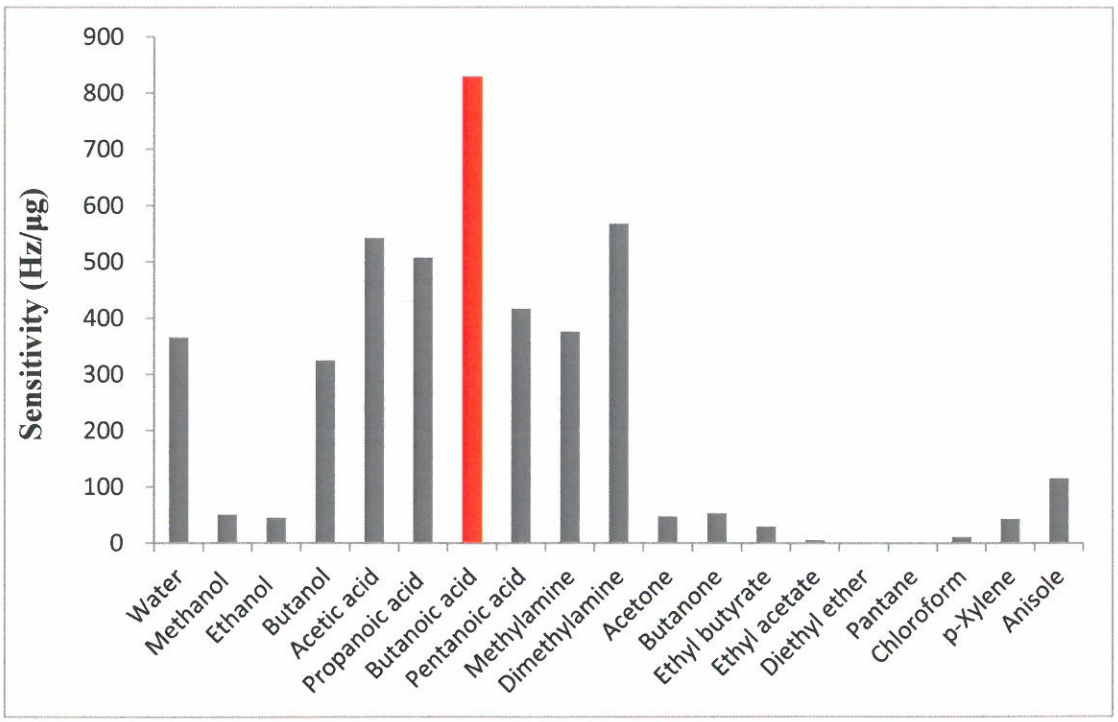

Fig. 7 Sensitivities of Butyric Acid Imprinted Peptide BA-24 to Different Odorant Molecules

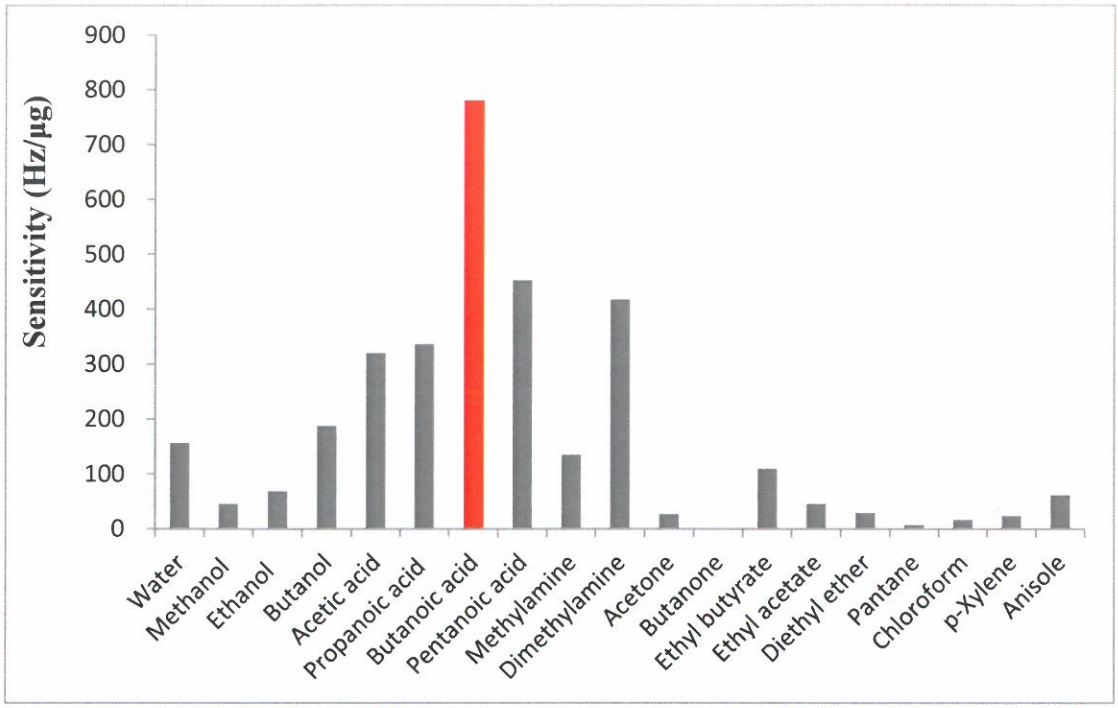

Fig. 8 Sensitivities of Butyric Acid Imprinted Peptide BA-26 to Different Odorant Molecules

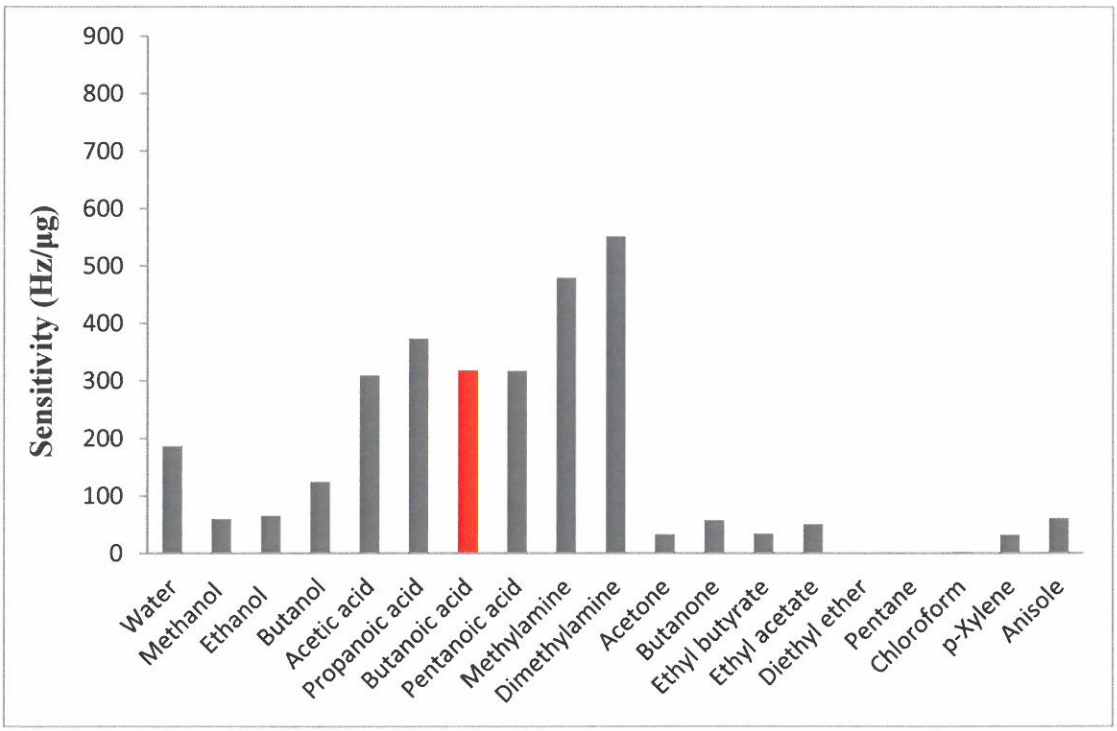

Fig. 9 Sensitivities of Butyric acid Imprinted Non-related Peptide XBA-01 to Different Odorant Molecules

4. Conclusion

We have demonstrated our strategy for using molecular imprinting methodology creating synthetic peptide receptor which has the selective recognition for the target odorant molecule. The utility of synthetic peptide incorporated with molecular imprinting process could correctly identify target odorant butyric acid from different functional odorant molecules and structurally similar acids odorants. Our studies showed that the refinement of molecular imprinting can enhance the binding affinity of peptide for the odorant and improve the selectivity and specificity of peptides. These results indicate that the imprinted peptide creating synthetic recognition sites with a complementary shape offers a novel strategy for the further development of odorant detection in sensor systems. The combination of peptide library and molecular imprinting technology can be used for development of odorant sensing tools in environmental, food, and medical sample analysis. Furthermore, the incorporation of molecular imprinting approach also can greatly enhance the utility of synthetic peptide combinatorial libraries for target drug screening.

5. References

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應用分子印模改善合成胜肽在氣味分子偵測之研究

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摘要

目前從嗅覺受體蛋白篩選到對氣味分子具有活性的胜肽序列，皆為源自於天然的胜肽序列。為探討合成胜肽對氣味分子的選擇性吸附，本研究應用組合化學(combinatory chemistry)的概念及分子印模(molecular imprinting)的策略，設計及製備一系列具有氣體辨識能力的合成胜肽。先期研究已經應用組合胜肽庫(combinatorial peptide library)及分子印模技術，篩選對目標氣味分子丁酸具有高選擇性吸附的六胜肽序列中六個位置最佳的可能候選胺基酸。本研究進一步製備32種最佳化胺基酸組合的六胜肽序列，以合成胜肽(synthetic peptide)為模板分子與氣味分子混合，將模板胜肽聯結於壓電晶體訊號轉換元件(piezoelectric transducer)製成生物晶片，保留被氣味分子印模的特異構形。進而找出對目標氣味分子有最佳靈敏度(sensitivity)的六胜肽序列BA-10、BA-18、BA-24和BA-26。

本研究探討最佳化六胜肽序列BA-10、BA-18、BA-24和BA-26與目標氣味分子丁酸間經印模處理與未經印模處理後的吸附靈敏性效能。同時研究最佳化六胜肽序列經印模處理處理對其它不同種類氣味分子的選擇性(selectivity)測試。本研究結果顯示六胜肽序列經印模處理後可以提高對目標氣味分子丁酸偵測的靈敏性和選擇性。結合組合胜肽庫及分子印模技術可增強氣味檢測的靈敏度和特異性，本研究可應用於氣味分子檢測工具的開發。

關鍵字: 合成胜肽、分子印模、氣味分子