A Brief Discussion of the Mechanisms of Substance Recognition and Signal Transformation in Chemical Sensing

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Abstract. With the development of technology, the need for personalized medicine has increased day by day. There are also growing concerns about harmful substances in the environment. Therefore, it is crucial to monitor and detect the specific target in the environment and biological systems in real time and accurately. Chemical sensors have shown wide application prospects as highly sensitive and selective detection tools. This article systematically compares the similarities and differences of various recognition methods in terms of recognition mechanism, sensitivity, and selectivity, and deeply explores the diversity and applicability of signal transduction methods from optical to electrical signals. Overall, these methods have their advantages, and in specific applications, the optimal solution needs to be selected in combination with the characteristics of the target. Moreover, this paper also briefly discussed methods that transform the recognition signals into electrochemical and optical signals. Future research should apply more small-molecule-specific recognition methods to the recognition of larger molecules, finding more specific molecules and universal synthetic methods.

1 Introduction

The origin of the biosensor and chemical sensor can be traced back to the study of pH meters in the 20th century. Along with the breakthroughs in material science and biotechnology, biosensing technology has emerged. Chemical and biological sensing technologies are increasingly important in today's pursuit of personalized medicine and have already had mature applications in blood glucose testing and tumor marker testing [1, 2]. Except for the applications in the medical field, sensing technologies have also been widely applied in food safety, environmental monitoring, and pharmacy. Currently, there are two main types of sensors used for environmental monitoring and medical diagnosis: biosensors and chemical sensors [3]. Between them, biosensors mainly recognize target molecules through natural enzymes, antibodies, or nucleic acids. However, not all the substances have their corresponding antibodies or enzymes, and proteins are easy to denature, then losing their recognition ability, as well as the cost of extraction or synthesis of natural substances being

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high. In contrast, chemical sensors have the advantage of lower cost and wider application conditions. Through literature reading and analysis and comparison, this paper starts with the specific recognition of potassium ions by valinomycin, analyzes the evolution of mechanisms that use a single substance for recognition, and gives a horizontal comparison. Then, it briefly discussed and summarized the conversion of signals into optical signals and electrochemical signals.

2 Discussion on recognition mechanism

This section begins with the natural product valinomycin and then discusses the evolution of the mechanisms that utilize single substances to recognize target molecules. It highlights both the advancements and the limitations of such approaches.

2.1 Recognition of potassium ions by the natural product valinomycin

Valinomycin is a kind of antibiotic produced by streptomyces. It can selectively coordinate potassium ions, enabling their transport across phospholipid bilayers. valinomycin is a dodecacyclic peptide constructed by three groups of D-valine, L-lactic acid, L-valine, and D-hydroxyisovalerate. When it coordinates with a potassium ion, six oxygen atoms of the cyclic peptide form an octahedron; the other six oxygen atoms form hydrogen bonds with hydrogen atoms on nearby peptide bonds, which makes the whole structure rigid.

The sodium ion, which has similar properties to potassium ions, has a great influence on the specificity of valinomycin recognition. When sodium ions interfere, due to the rigidity of valinomycin, six oxygen atoms are not easy to shrink inward to coordinate the smaller sodium ion. The selectivity of potassium ions over sodium ions 104 [4]. However, currently, the highest-yield method for the synthesis of valinomycin is biosynthesis using the ZJUT-IFE-354 strain. The yield can achieve 191.26mg/L but has not been widely applied. The cost of valinomycin is still high [5].

2.2 Traditional recognitive substances

2.2.1 Crown ether

Crown ether is a series of oligomers of ethylene oxide. It can coordinate different metal ions depending on the size of the ring. For instance, 18-crown-6 is a ligand with high selectivity of potassium ions. Normal crown ethers can be modified to have the ability of fluorescence recognition. As shown in Fig. 1, attach a pyrene group to the 15-crown-5. When it coordinates a potassium ion by forming a sandwich, the two pyrene structures are fixed by γ-cyclodextrin on the outside, which makes the whole structure emit characteristic fluorescence at 470nm. The number of (CH2) in the structure has a great influence on the selectivity of potassium. Better selectivity can be obtained when the number of methylene is three. At this time, the binding constant for potassium ions is 3.8×106 while for sodium ions is 1.5×106 [6]. This structure expands the method of applying crown ether, making metal ions to be “binder” between two structures other than conventionally coordinate with a crown ether alone.

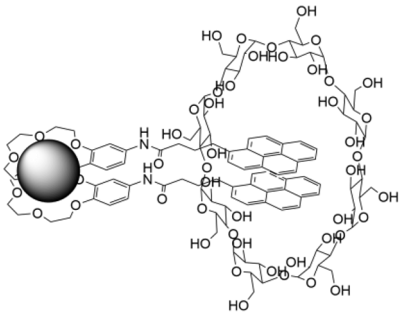

Fig. 1. The possible structure of the γ-CyD combining with the 'sandwich-like' structure [6].

Fig. 2 is a fluorescent recognition unit based on crown ether, in which the most important structure is squarylium dye. It changes the size of the conjugated system through whether nitrogen atoms are coordinating potassium ions; then, the fluorescence of the dye is quenched in the presence of potassium ions to quantitively measure the potassium ion concentration. This sensor has a limit of detection between 1nM and 1μM, and the response time is 2 minutes. But with the existence of nitrogen atoms, this molecule is sensitive to pH, which means it cannot work under acidic conditions [7].

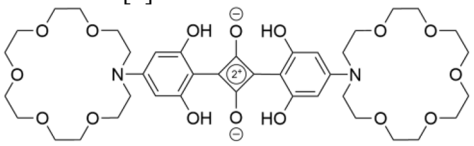

Fig. 2. The structure of potassium ion recognition material based on squarylium dye [7].

2.2.2 Cryptand

Compared with the crown ether, the cryptand is equivalent to adding another dimension to the two-dimensional plane, making the whole a three-dimensional molecule. Due to the additional chain increasing the rigidity of the cryptand, the cryptand possesses better specific recognition ability. Many sensors that use cryptands as molecule recognition elements use triazacryptands, whose cavity sizes are validated to exclude large cation interferences (e.g., Ca, Rb) and have good selectivity for potassium ions and little influence on sodium ions. He, H., et al. developed the sensor based on crypt-222 shown in Fig. 3. The basic principle is that the nitrogen atom is originally located in the conjugated system as a PET donor. When it is not coordinating, the fluorescence is quenched by electron transfer. When coordinating with a potassium ion, the transfer is suppressed, increasing the fluorescence intensity with the increase of potassium ion concentration. The nitrogen atom can also be directly connected to polymer chains to form sensors, whose response time is often less than 1 minute [8].

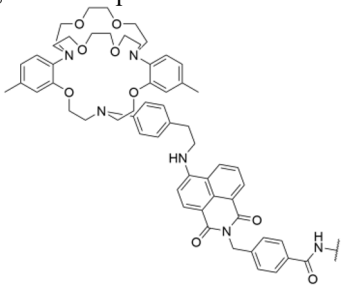

Fig. 3. Potassium ion fluorescence sensor based on crypt-222 [8].

2.2.3 Calixarene

A calixarene is a macrocycle or cyclic oligomer based on methylene-linked phenols. It has a better scalability than the two methods discussed formerly. It can identify complex compounds in addition to easy ions. Its expansion dimensions include the upper and lower rims of its structure, the cavity size (by controlling the number of phenol), lateral chains, and functional groups of upper and lower rims. Connect the sugar-like group to the upper rim and connect to magnetite ore with silicon-oxygen groups like Fig. 4. This can recognize dichromate through hydrogen bonds formed by sugar groups and dichromate [9].

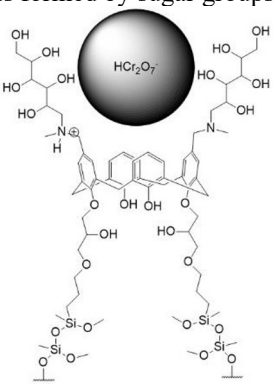

Fig. 4. An example of calixarene derivatives used for anion recognition [9].

2.3 Supramolecular recognition and self-assembly

From the natural product valinomycin to crown ether, cryptand and calixarene, they are all a category of supramolecule recognition. Supramolecule recognition is a summary and extensible recognition mechanism. The intermolecular recognition forces involved are not limited to the electrostatic interaction of coordination interaction between metal ions and coordinating atoms and hydrogen bonds in traditional methods, but also include hydrophobic interactions, π-π stacking, the effects of positive and negative charges on electron-rich and electron-deficient aromatic rings, and some weaker or reversible covalent bonds (e.g., disulfide bonds, acetal, hydrazone and imine).

Common mechanisms of supramolecular recognition are synthetic receptors and dynamic assembly. Traditional crown ether, cryptand, and calixarene are synthetic receptors to recognize molecules. They accomplish recognition through the specific binding of a single receptor molecule to the target molecule. As for dynamic assembly, Kartha, Babu, et al. synthesized a substance called OPVPF. This substance can self-assemble through π-π stacking to form a fibrous structure that can emit fluorescence. The fiber can combine with a trace amount of TNT via hydrogen bonds and charge-benzene ring interaction, which blocks the energy conduction process, leading to fluorescence quenching. This method can detect TNT molecules at the ag level [10].

2.4 Molecularly Imprinted Polymers (MIPs)

The methods mentioned above all need to be designed for different recognition substrates. Although the specificity is good, it is difficult to develop new recognition molecules. Thus, the recognition mechanism of molecular imprinting polymers was born. Its essence is to use the target recognition substance as a template to construct recognition molecules, thereby achieving an effect similar to that of antibodies or enzymes in organisms. At present, there

are three methods for preparing molecular imprinted polymers: synthesis, phase inversion, and soft lithography. Synthesis uses the target molecule as a template, uses small molecules to self-assemble with it in the solution through intermolecular forces, then adds a cross-linking agent to generate a polymer, and finally washes away the template molecule. Phase inversion is to mix the template molecule, functional monomer, and cross-linker in a uniform solvent, then add another phase of solvent, transfer the template molecule through a phase transfer catalyst, and then polymerize the functional monomer and cross-linker in the solution through light or heat. Soft lithography makes the target substance into a form similar to a stamp, then prints on a polymer precursor by a physical method, and finally polymerized by photo-induced polymerization or thermal-induced polymerization to prepare the polymer precursor. These three preparation methods can only guarantee that the cavity size is similar to the target substance. They all face a problem of poor specificity.

Table 1 shows the binding constants, detection limits, and specificity descriptions of the target recognition substances for several single-substance recognition methods mentioned in this article. Due to different solvents and different target substances, specific analysis should be carried out on a case-by-case basis in practical application. It can be found that starting from calixarene, some neutral substances with more complex structures can be identified. Dynamic self-assembled supramolecular detection of TNT has an extremely low detection limit. The binding constant of MIPs to carboxypeptidase A is relatively low.

Table 1. Horizontal comparison of different identification methods

Recognition Methods	Target	Binding Constant (lgK)	Detection Limit	Specificity Description	Data Source
Valinomycin	Potassium ion	6	10^{-6}	10^4 times higher than Na^+	[1][11]
Crown Ether	Potassium ion	2.04	10^{-6}-10^{-9}	Sensitive to ionic radius and charge. Specifically recognize alkali metal ions	[7][12]
Cryptand	Potassium ion	6	10^{-6}	A three-dimensional cavity has stronger selectivity	[13]
Calixarene	Lysine	N/A	$2*10^{-6}$	Functional modifications can be made to improve specificity through π-π interactions and hydrogen bond	[14]
Dynamic self-assembled supramolecule	TNT	N/A	12ag/cm^3	Only reacts to aromatic hydrocarbons containing nitro groups	[10]
MIPs	carboxypeptidase A	K_m=$3.6*10^{-3}$	N/A	Active towards specific carbonate substrates	[15]

3 A brief analysis of signal conversion methods

Most of the previous discussions on traditional recognition mechanisms have been about quantitatively identifying target substances by constructing optical sensors. In addition to adding chromophores to the molecular structure, controlling fluorescence through the PET mechanism, or generating fluorescence by restricting molecular motion, color changes can also be generated by applying the size effect of nanoparticles. For instance, Sun, Wang, et al modified crown ethers and adsorb them on the surface of silver nanoparticles. When potassium ions are added to the solution, a sandwich structure similar to the one in Fig. 1 is created, forcing the two nanoparticles to aggregate, resulting in a macroscopic color change. This change is highly linearly correlated with potassium ion concentration, and the potassium

ion concentration in urine can be quantitatively detected to a certain extent by the naked eye using colorimetry. Chitbankluai, Khwanrudee, et al. also achieved similar results using gold nanoparticles [16].

In addition to using coordination to force nanoparticles to aggregate, similar effects can be achieved by changing the hydrophilic and hydrophobic properties of the nanoparticle surface and then causing nanoparticle aggregation through hydrophobic interaction. Ali, Reham et al. use valinomycin to modify the surface of gold nanoparticles and use BSA to occupy the remaining vacancies to prepare the Val-AuNCs particles. After the coordination of potassium ions, the conformation of valinomycin changes, causing the hydrophobic groups to face outward, increasing the hydrophobicity of the nanoparticles and reducing their solubility in water, so they tend to aggregate. The fluorescence characteristics of aggregated nanoparticles disappear, resulting in fluorescence quenching.

For converting the recognition signal into an electrical signal, the concentration of the target substance can be measured by the potential difference formed by the concentration difference on both sides of the membrane, similar to the principle of a pH meter. Valinomycin and an organic solvent that is immiscible with water together form a liquid membrane. When valinomycin binds to potassium ions, it shuttles across the membrane to the side with a lower potassium ion concentration, forming a membrane potential difference. The selectivity constant for sodium ions is 10000 [17].

Recently, electrochemical sensors based on quantum dots have been developed. Iannazzo, D. et al. bond crown ether to the quantum dots from carbon nanotubes through an amide bond to form GQDs18-c-6 and GQDs15-c-5. Then characterize electrochemical and optical properties. For the electrochemical properties, they apply GQDs18-c-6 on the screen-printed carbon electrode. At pH=7, as the sodium and potassium ions increase in the range of 1 to 1000 mM, the electrode potential increases accordingly. The response time is shorter than 4 seconds. However, due to the poor selectivity of crown ethers, it is unable to distinguish between sodium ions and potassium ions [18].

All these methods have a certain scalability. However, for the concentration potential methods, the preparation of the membrane is difficult, and this method is only useful for charged ions, not for neutral molecules such as glucose. As for the effect of conductivity on fixing the recognition body, it is relatively small, which will lead to insufficient specific recognition ability and is also only effective for charged ions.

4 Conclusion

In terms of recognition mechanism, the most important problem faced by using artificial single substances for recognition is poor specificity or insufficient binding constant. Although the latest recognition method on MIPs can be generally applied to the recognition of various substances, its specificity is not good. In contrast, supramolecule recognition mechanisms have an obvious advantage in specificity. However, there is currently a lack of a supramolecular recognition strategy that can be universally applied to complex and diverse molecules.

In the future, more attention should be paid to incorporating supramolecular recognition mechanisms into the synthesis of molecularly imprinted polymers to increase their binding constants and enhance their specificity. In terms of signal conversion methods, electrochemical methods seem to be a method that is easier to apply widely, while conversion into optical signals requires a more precise design of molecules.

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