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Review

Peptide-based biosensors

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ABSTRACT

Peptides have been used as components in biological analysis and fabrication of novel biosensors for a number of reasons, including mature synthesis protocols, diverse structures and as highly selective substrates for enzymes. Bio-conjugation strategies can provide an efficient way to convert interaction information between peptides and analytes into a measurable signal, which can be used for fabrication of novel peptide-based biosensors. Many sensitive fluorophores can respond rapidly to environmental changes and stimuli manifest as a change in spectral characteristics, hence environmentally-sensitive fluorophores have been widely used as signal markers to conjugate to peptides to construct peptide-based molecular sensors. Additionally, nanoparticles, fluorescent polymers, graphene and near infrared dyes are also used as peptide-conjugated signal markers. On the other hand, peptides may play a generalist role in peptide-based biosensors. Peptides have been utilized as bio-recognition elements to bind various analytes including proteins, nucleic acid, bacteria, metal ions, enzymes and antibodies in biosensors. The selectivity of peptides as an enzymatic substrate has thus been utilized to construct enzyme sensors or enzyme-activity sensors. In addition, progress on immobilization and microarray techniques of peptides has facilitated the progress and commercial application of chip-based peptide biosensors in clinical diagnosis.

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1. Introduction

Sensors are integrated systems that receive a certain signal or stimulus (such as bio/chemical substances, actions, processes and

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changes in environment) and respond in a distinct manner. In the biochemical field, sensors are usually defined as a device which includes both a receptor (bio-recognition element) and a transducer, providing specific quantitative or semiquantitative analytical information. The general function of biosensors involves a receptor in the most general sense recognizing an analyte, then a transducer either triggers a measurable signal or catalyzes a reaction related to the analyte concentration to generate a signal [1]. In clinical diagnosis, a sensitive, quick, convenient and versatile molecular biosensor has been desired to simplify the testing process, reduce the cost and shorten testing time [2]. Artificial peptides provide an opportunity to develop the desired molecular biosensor due to their desirable properties such as diversified structure, high affinity to proteins, matured synthesis protocol and modified approach [3,4].

Peptides are formed by natural or synthetic short polymers of amino acids which are linked by peptide bonds with shorter lengths than those of proteins [5]. Peptides have the same building block as proteins, therefore, it is possible for the peptides with a specific sequence to substitute for proteins in biological analysis [6]. Peptides with specific sequences can provide high affinity to particular analytes, and be obtained by screening and optimization of artificial peptide libraries. In addition, peptides have shown further advantages including high stability, standard synthetic protocol, easy modification and large chemical versatility. For example, peptides with short chains of amino acids generally have better chemical and conformational stability than proteins.

Peptides can be prepared with arbitrary sequences according to standard Fmoc and t-Boc solid-phase peptide synthesis (SPPS) protocol [7–9]. The SPPS protocol, typically involves repeated cycles of coupling-wash-deprotection-wash, carried out to couple an Fmoc or t-BOC N-protected amino acid unit to the free N-terminal amine of a peptide attached on a solid-phase Wang-resin. The SPPS protocol also provides an opportunity to attach arbitrarily a wide range of functional molecules at two terminal positions of a peptide sequence or particular amino acid residue with an additional functional group, such as in lysine. Furthermore, peptides thereby modified in a specific manner [10] can also retain their high affinity to the target analyte.

Peptide sequences that are specific enzyme substrates, play a critical role in assays of enzymatic activity and screening of enzymatic inhibitors. Due to these unique properties, peptides are excellent candidates for developing sensitive, fast, and convenient biosensors.

Peptides generally do not generate a measurable signal directly in response to a binding event, and therefore conjugation with a signal marker is an efficient strategy to convert the information of analyte/binding into a measurable signal. To date, several methods have been utilized to construct peptide-based biosensors via conjugating a peptide with signal markers. These biosensors can be used for detecting various analytes including metallic ions, proteins, proteases, kinases, bacillus species, nucleic acids and antibodies. Environmentally sensitive fluorophores are common signal markers which are widely used when conjugated with peptides. Their fluorescence emission can be affected by changes in the local environment caused by the affinity or interaction between conjugated peptide and analytes. The method of conjugating peptides to environmentally-sensitive fluorophores has been utilized to develop various peptide-conjugated molecular probes/sensors [11], including ion sensors, DNA sensors, redox sensors and protein sensors. This work was reviewed by Choulier [3] and Vazquez [4]. Apart from fluorophores, other materials have also been used as signal markers, such as near-infrared dyes [12], nanoparticles [13], quantum dots [14], graphene [15,16], polydiacetylene (PDA)-liposomes [17], lanthanide chelators [18] and electrochemical markers [19]. Although limited, peptide-based biosensors without signal markers have also been developed. For example, an optical sensor of proteases based on photonic crystals was realized through immobilization of a peptide substrate in the silicon-based pores of the photonic crystal filter [20].

Peptides play various roles in peptide-based biosensors, including acting as the receptor (bio-recognition element), an enzymatic substrate (linker) and framework (scaffold) [21]. The remainder of this review expands on peptide-based biosensors according to these roles that peptides may play in the sensing process.

2. Peptides as recognition elements in biosensors

Since peptides share the same chemical structure with proteins, peptides are an ideal candidate to substitute for protein as the receptor (biorecognition element) in biosensors. Artificial peptides can be obtained through standard solid-phase synthesis protocols to provide a specific sequence or screening a library of peptides. These peptide-based molecular biosensors have been developed for convenient, fast detection of various analytes including proteins, antibodies, DNA, and metallic ions [22].

2.1. Peptide-based protein sensors

Spectral properties of fluorophores are highly dependent on the surrounding environment. Therefore, fluorophores conjugated to peptides have been utilized to develop novel protein molecular sensors to target specific proteins [23,24]. Design strategies including the excimer [25], fluorescent resonance energy transfer (FRET) or probe–quencher pair strategies [26], have been used in protein molecular biosensors, which are summarized in Table 1. An excimer is a short-lived dimeric or heterodimeric molecule formed from two species. The wavelength of an excimer's emission is longer than that of the excited monomer's emission. Therefore, the wavelength shift in emission from transformation between the excimer state and the monomers can be utilized in providing a signal output for biosensors. FRET refers to the phenomenon of energy transfer between two chromophores separated by only a short distance (typically in the range of 1–10 nm) through non-radiative dipole–dipole coupling. The spectral overlap of the donor emission spectrum and the acceptor absorption spectrum, is required for the FRET effect. Therefore, the fluorescence quenching or enhancement caused by the FRET process, can be utilized in the design of signal transducers in biosensors. In comparison to FRET, probe–quencher pair strategies usually depend on a static quenching which occurs when the molecules form a non-fluorescent complex in the ground state.

Fig. 1A illustrates the mechanism of a general protein biosensor based on environment-sensitive fluorophores. The initial process for protein detection is the affinity/recognition between the peptide and analyte. Upon recognition, the binding event induces a change in the spectral properties of the fluorophore. The emission of fluorophores is usually enhanced and blue shifts after affinity/recognition in a polar solvent. Computational studies have showed that the spectral change is caused by the insertion of the peptide-conjugated environment-sensitive fluorophore in the hydrophobic binding groove of the target protein [27]. The environment-sensitive fluorophore strategy has been utilized for detection of cyclin A [27] and HIV-1 specific monoclonal antibodies [28]. Recently, a novel environment-sensitive fluorophore, tetraphenylethylene (TPE), was conjugated with a small peptide sequence referred to as AP2H to form a fluorescent probe for tracking tumor marker in live cancer cells. The binding of the TPE-AP2H probe with the target cancer-related protein LAPTM4B can switch on the fluorescence of TPE due the inhibition of internal rotations within the TPE framework [29]. Environment-sensitive europium chelate was also used to conjugate