Trends in Analytical Chemistry 79 (2016) 247–256

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

A molecular biology approach to protein coupling at a biosensor interface

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ARTICLE INFO

Keywords:
Immobilisation
Peptide tag
Fusion protein
Synthetic biology
Molecular biology
Biosensor

ABSTRACT

Amino acid residues on the outside of proteins are discussed as potential sites for chemical coupling of proteins to sensor surfaces. This strategy is compared with the use of peptide tags, added to proteins, with an affinity for a particular surface material or chemical structure. Using molecular biology to extend the amino acid protein sequence, in order to include an immobilisation component, is also shown to be suitable for fusion to binding proteins, that can act as the immobilisation partner, so that a compendium of immobilisation strategies is seen to emerge from this common approach of protein engineering.

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1. Introduction

The innovation model for biosensors is both application and technology driven. Eg, gene chip/DNA probe technology emerged from the human genome project and was translated to other areas of research and then to medical diagnostics. The original amperometric enzyme electrode concept and the glucose biosensor has achieved a dominant biosensor market, driven by a population where diabetes is increasing. Similarly, other particular assay systems have gained prominence; for example, luciferase, which was discovered in 1962, began to emerge in molecular biology in the 90s, and is now a core 'reagent' in the development of many new sensor

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http://dx.doi.org/10.1016/j.trac.2016.01.024
0165-9936/

diagnostics. Surface plasmon resonance discovered in 1968 was first incorporated in an immunoassay in 1983. BIAcore first commercialized the technology in 1991 to provide sensing platforms for drug discovery. The diversity of applications emerging from such biomechanistic technologies gives some insight into how we might exploit signal generation in/from cell components. However, identification of further signaling systems are only one component required to increase the portfolio of diagnostic information that could be obtained and we are beginning to be able to engineer access to the diversity and selectivity of bio-inspired molecular apparatus with its capacity for analyte recognition, by merging the properties of transduction materials with the capacity of molecular biology.

An important challenge in this mission is the interface between the transducer and biomolecule. Traditional immobilisation methods are broadly categorised into adsorption-based methods [1], entrapment inside a membrane [2], or in a gel/polymer matrix [3], or via cross-linking reagents [4]. These methods do not take the orientation of the biomolecule into account, and regularly end up with the biorecognition molecule showing reduced functionality and poor communication with the underlying transducer [5].

Whereas the historical approach to immobilisation has been to use these traditional chemical methodologies, understanding how biology can be better interfaced with electronic or optical nano- and microsystems, in a platform that can be evolved and reconfigured for detection and diagnosis, is now expanding the biosensor portfolio. This review looks at the evolution of peptide molecular biology immobilisation techniques and considers developments that could further impact the biosenor field. The review begins with reactive amino acids introduced at the surface of a protein target and the strategies that have allowed chemical coupling with various transducer materials (section 2). This method is close to the historical chemical methodologies. In contrast, inspiration from high affinity protein binding partners in nature offer ways of providing some generic immobilisation methods (section 3) that can be applied to many different systems by fusion of one of the binding partners to the biorecognition element. This also begins to show how our understanding of peptide-protein binding can lead to biorecognition proteins with a fused peptide tag for binding to the transducer surface – all achieved with molecular biology. Peptides and their direct affinity for materials used as transduction surfaces in biosensors therefore expand this concept, with examples of sequences that might be fused to a biorecognition protein (section 4).

2. Selected amino acid coupling

2.1. Cysteine coupling

Due to the low prevalence of cysteine, it can be a natural target for insertion or exchange into a protein's natural sequence. In terms of immobilisation, the choice of cysteine is favoured by easy thiol group chemical activity. Depending on its positioning in the protein, it can therefore be an effective method of controlling protein orientation on the surface. Beissenhirtz et al. [6] for example, introduced one or two additional cysteine residues in the enzyme, superoxide dismutase, and observed that binding to unmodified gold electrodes could then be achieved, together with direct electron transfer to the electrode, with higher sensitivity towards superoxide radicals compared to conventional cytochrome c.

However, cysteine coupling can also cause inhibition due to opportunist bond formation with non-ideal orientation. For example, Lin et al. [5] showed that cysteines 14 and 17 of cytochrome c interact with a bare gold electrode inhibiting electron transfer. However, if a C-terminal cysteine tag is introduced on cytochrome c [7], enzyme immobilisation occurs with its binding domain on the opposite side, accessible from solution.

Similarly, when Davis et al. [8] engineered a cysteine residue into cytochrome $P450_{cam}$, where the enzyme's haem group was closest to the surface, the enzyme was still catalytically active, and had higher and more ordered coverage of a gold electrode than the wild type enzyme. The activity was also higher, which was attributed to the cysteine being able to orientate the haem group, buried in the protein matrix, so that it was as close as possible to the electrode.

Loechel et al. [9] also introduced a cysteine mutation of the residue Y442 in the protein trimethylamine dehydrogenase (TMADH) to couple into the protein's electron transfer pathway to facilitate charge transfer between enzyme and an electrode. However, in this instance the necessary attachment point was in a cleft in the protein which was not accessible to couple directly with an electrode. This required a redox wire, poly-$[Fe(5\text{-}NH_2\text{-}phen)_3]^{2+}$, to join and communicate between enzyme and electrode. Thus, surface modification of an amino acid doesn't necessarily yield a direct match between transducer and protein immobilisation and coupling.

2.2. Lysine coupling

Lysine is typically located on the outside of protein because of its polarity and not involved in the catalytic sites. It is often in good abundance, making its amino group an obvious site for further biomolecule interaction and it can be readily and selectively targeted for interaction with activated surfaces (e.g. –COOH). However, its abundance also inhibits individual site directed immobilisation and orientation of a resultant protein is likely to include any of the surface lysines.

Rather than attempting single amino acid attachment, polylysine could be engineered as a protein tag to increase the likely position for site directed activation, and in principle, it can be electropolymerized on a glassy carbon electrode (GCE) surface by potential cycling (Fig. 1), but at rather high potentials where water oxidation is also likely and a part of the surface activation mechanism. Huang et al. [10] have proposed that the polymerisation process involves electrode activation (–C = O, dependent on the water activation effect) of a GCE, followed by coupling to the protonated $–NH_3^+$ of the lysine. This C-N bond formation has also been shown by others and the electrochemical polymerisation method proposed for other aminoacids [11,12]. On the other hand, protonated polylysine itself can be deposited directly on a pre-prepared graphene oxide surface, without electrochemical oxidation, and then provides a good non-specific immobilisation medium for a layer-by-layer type of electrostatic or covalent assembly.

These preparation methods have also been applied in various other types of sensors, not just those involving proteins. Sun et al. [13] introduced a DNA sensor using graphene oxide as a platform to immobilize DNA on the surface of polylysine-modified GCE. Polylysine film supported the stabilization of graphene oxide nanosheet through electrostatic attraction on GCE. Due to the high surface area, the authors proposed that an increased amount of probe DNA can be amide bonded, which results in better performance and detection limit of 1.69×10^{-13} M. Similarly, Wang et al. [14] immobilized probe DNA on gold nanoparticles, adsorbed on polylysine-modified GCE. Positively charged hexaaammineruthenium(III) chloride bound to the negatively charged phosphate back bone of probe DNA on the gold nanoparticle served as a electroactive indicator. Wang demonstrated that this sensor can detect 3.5×10^{-14}M of target DNA. Although these are not polylysine tagged, their outcome suggests that a tagging approach with electrochemical or chemical graphene oxide preparation could also be successful.

Similarly Hua et al. [15] applied a polylysine modified GCE in a glucose sensor, where glucose oxidase was immobilized through self-assembly on graphene oxide which had been polylysine–modified. Direct electron transfer was suggested with good electrocatalytic activity in glucose detection of in the range of 0.25 to 5 mM. In the