Nanoengineered Encapsulation of Organic Microcrystal as Novel Biolabels

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ABSTRACT

Immunoassays have been widely used as the standard technology for quantitative detection of analyte concentrations in the fields of medicine, environmental science, and food science. Immunoassays such as enzyme-linked immunosorbent assays (ELISA), fluorescence immunoassays (FIA), electrochemical immunoassay (EIA), immunoagglutination assays (IAA) and radioimmunoassays (RIA) are the most commonly used laboratory techniques. A novel class of biolabel system based on encapsulated organic microcrystals was introduced. The biolablels were constructed by encapsulating organic microcrsytals within ultrathin polyelectrolyte multilayers followed by conjugating with biomolecules. This biolabel system can achieve a high molar ratio of signal-generating molecules to biomolecules which provides a new strategy for signal amplification in bioassay. Various applications of organic microcrystal biolabels especially on the utilization of electrochemical-active microcrystal biolabels for bioassays will be introduced. Moreover, different strategies to improve the performance of the organic microcrystal biolabels will be discussed.

Keywords: Encapsulation, Layer-by-Layer, Biolabel, Organic microcrystals, Bioassays

1 INTRODUCTION

A novel class of biolabel system based on encapsulated organic microcrystals was introduced. Nanoengineered encapsulation of hydrophobic organic microcrystals by polyelectrolyte multilayers (known as Layer-by-Layer (LbL) technique) and there use as a biolabel system was first described by Trau [1]. In brief, organic microcrystals were rendered water dispersible by treatment with an ionic amphiphilic substance followed by encapsulation by alternating deposition of cationic and anionic polyelectrolyte film of ~2 nm thickness [2] followed by conjugation with biomolecules (Fig. 1). The advantages of the nanoengineered polyelectrolyte multilayer capsule are: a) Nanoengineered functional surface design, b) full control of permeability, c) rendering hydrophobic microcrystals water dispersible, d) creating an interface for conjugation with various biomolecules.

The biofunctionalization of the encapsulated organic microcrystals was achieved by conjugating biomolecules such as antibodies, avidin or DNA onto the capsule surfaces. The significant of organic microcrystal biolabels compared with other particulate label systems is the dissolution property of the organic microcrystals upon exposure to a desired solvent which leads to the release of a large number of signal-generating molecules (~ 10^7 to 10^9 molecules per microcrystal); termed as the "Supernova Effect". Moreover, a high molar ratio of signal-generating molecules to biomolecules can be achieved. Our biolabel technology provides a new strategy for signal amplification over the traditional enzymatic amplification system.

We demonstrated various applications of nanoengineered organic microcrystal biolabels by utilizing organic microcrystals with different properties such as mediator microcrystals for silver enhancement [3] or electrochemical immunoassay [4]; fluorogenic precursor microcrystals for fluorescence immunoassay [1,6] and silole microcrystals for aggregation-induction emission based bioassay [5]. Moreover, improvement by decreasing the size of the organic crystals to submicrometer; encapsulating microcrystals with different materials; lowering unspecific binding by surface modification with blocking agents and the relationship between biomolecule surface coverage and molar ratio of signal-generating molecules to biomolecules (S/P ratio) will be discussed.

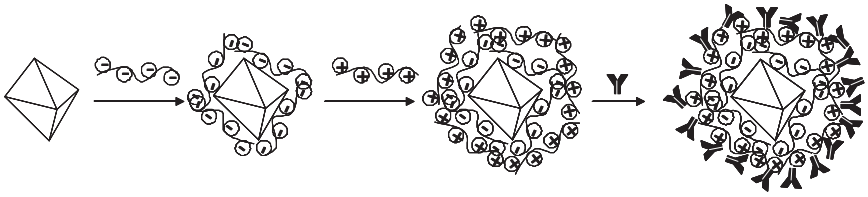

Fig. 1 Schematic diagram illustrating the nanoengineered LbL encapsulation of micro- or nanocrstals with nm thin layers of polyelectrolytes and antibodies.

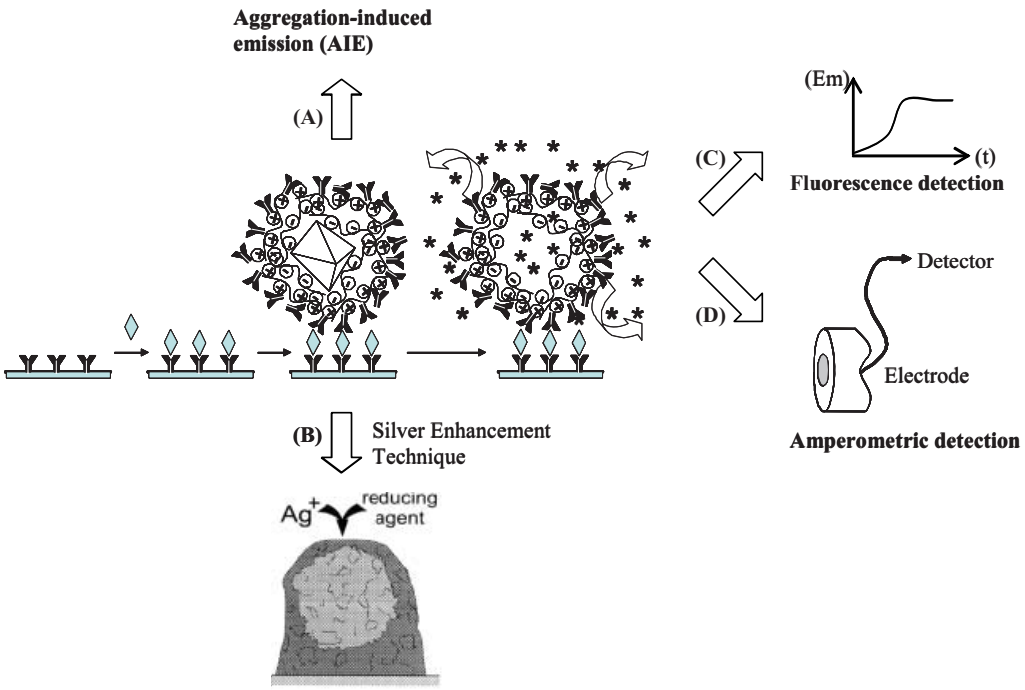

Fig. 2 Different strategies of utilizing organic microcrystal biolabels in bioassays: (A) Aggregation-induced emission (AIE); (B) Silver enhancement technique; (C) Fluorescence detection and (D) Amperometric detection

2 ORGANIC MICROCRYSTALS

The basic principle of encapsulated organic microcrystals based bioassays is the detection of signal generating molecules resulted from the dissolution of the core organic microcrystal upon exposure to a desired solvent. The preparation and utilizing of organic microcrystals with different chemical or physical properties for bioassay applications will be discussed. Figure 2 illustrated the strategies of utilizing different organic microcrystals with different properties for bioassay applications.

2.1 Fluorogenic precursor microcrystals

Fluorogenic precursor microcrystals was the first model for the development of encapsulated organic microcrystals based bioassay [1]. Biolabels were constructed by milling and suspending a fluorogenic hydrophobic precursor; fluorescein diacetate (FDA), with an anionic surfactant; sodium dodecyl sulfate (SDS). The resulted FDA microcrystals were encapsulated by alternate LbL deposition of a positively-charged polyelectrolyte; poly(allylamine hydrochloride) (PAH) and a negatively-charged polyelectrolyte; poly(sodium 4-styrenesulfonate) (PSS) until a desired number of layers was achieved. Finally, the encapsulated microcrystals were biofunctionalized by conjugating anti-mouse IgG antibodies onto the outermost capsule surface by adsorption process.

The concept of utilizing FDA microcrystals as biolabels is because it can easily convert to a highly fluorescent molecule, fluorescein, upon dissolution with a (1:1) mixture of dimethyl sulfonate (DMSO) and sodium hydroxide. Moreover, the dissolution step allows a large amount of fluorescein molecules release from the capsule and the self-quenching problem of the fluorescein molecules can be prevented. Depends on the particle diameter, a high molar ratio of fluorescein molecules to protein conjugate of ~10^8 can be achieved. The FDA microcrystal biolabel was applied to perform a solid phase immunoassay for the detection of mouse IgG. The FDA microcrystal biolabels showed a 70-2000 fold higher sensitivity when compared with the conventional FITC biolabels.

2.2 Electrochemical active microcrystals

Electrochemical active compounds e.g. ferrocene and ferroceneacetic acid can undergoes a reversible one-electron redox reaction. This property allows the development of microcrystal based electrochemical bioassays from ferrocene microcrystals [4]. Encapsulated electrochemical active microcrystal biolabels were prepared as the same procedures as described above. The molar ratio of ferrocene molecules to antibodies conjugate is found to be ~10^4 to 10^5. The ferrocene microcrystal biolabels was applied to a solid phase immunoassay for mouse IgG detection. The ferrocene microcrystals were dissolution by addition of DMSO. An amperometric detection method at +250 mV was used for quantitative analysis of the ferrocene released out from the capsule.

Besides direct electrochemical immunoassays, encapsulated electrochemical active microcrystal biolabels can be use as a non-metallic label system for silver enhancement technique [3]. Encapsulated ferroceneacetic acid microcrystals were conjugated with biotins by covalent bond to the amine groups which presence on the capsule outermost PAH layer. The ferroceneacetic acid microcrystal biolabeds were used to perform a solid phase immunoaasay for detection of neutravidin. After the bioassay, a silver (I) ion solution was added and the ferroceneacetic acid microcrystals which act as a seed for the reduction of silver ion into metallic silver. Consecutively, a reducing agent was added leading to a further silver growth. This approach allows a semi-quantitative detection of the silver deposited by naked eyes. On the other hand, a quantitative analysis of the silver deposit can be performed by potentiometric stripping analysis.

2.3 Silole microcrystals

Siloles are organic molecules that are having an aggregation-induced emission feature. In brief, siloles are nonemissive when they are in a soluble state, while the silole molecules in poor solvent will clutter into aggregates which can boost up the photoluminescence quantum yields by two orders of magnitude. This property allows the application of silole microcrystals as a direct biolabel system without requirement of dissolution of the core microcrystals [5]. Silole microcrystals, hexaphenylsilole [$Ph_2Si(CPh)_4$; HPS], were encapsulated by LbL technique and conjugated with antibodies as described previously. This microcrystal biolabels have a molar ratio of silole molecules to antibodies conjugate of ~10^3. The silole biolabels showed a 40 to 140 folds increase in sensitivity for the detection of mouse IgG when compared with immunoassay using direct FITC-labeled antibodies.

3 OPTIMIZATION OF ORGANIC MICROCRYSTAL BIOLABELS

The basic requirements for an ideal biolabel system are high sensitivity, low limit of detection, low non-specific binding, high signal amplification and preserved biomolecule functionality. A number of attempts have been made to improve the performance of organic microcrystal biolabels for bioassay.

3.1 Encapsulation materials and microcrystal sizes

The first step for construction of microcrystal biolabels is milling of an organic crystalline compound with a surfactant to render water dispersible. Different surfactant and amphiphilic polymer systems for dispersing microcrystals and creating microcrystal biolabels have been studied [7]. The results shown that instead of render the microcrystal surface with SDS followed by LbL encapsulation and antibodies conjugation, it is possible to render the microcrystal surface with amphiphilic polymer e.g. alkylated poly(ethylene imine) and 1,2-distearoyl-sn-glycero-3-phosphatoethanolamine-N-[amino(poly(ethyle -ne glycol))] (DSPE-PEG(2000)-amine) followed by direct conjugation of antibodies to the outermost amphiphilic polymer surface by adsorption process. This approach can simplify the tedious polyelectrolytes deposition process. Moreover, the study suggested that commonly used polyelectrolytes e.g. PSS and PAH with a higher charge density than DSPE-PEG(2000)-amine might influence the binding affinity of the attached antibodies and therefore the non-specific binding.

The amount of signal-generating molecules to be encapsulated is mainly influence by the size of the crystals. Bigger crystal sizes are having a larger volume to surface area ratio. Therefore, microcrystal biolabels with bigger crystal size are having higher S/P ratio and thus a higher signal amplification can be achieve [7]. However, the limit of detection on the influence of crystal sizes was insignificant.

3.2 Surface coverage of protein conjugates

The most commonly and simply bioconjugation technique is by adsorption. It has the advantage of minimizing protein denaturation during the conjugation process. Microcrystal biolabels with different protein surface coverage can be achieved by incubating the encapsulated microcrystals in a protein solution with different initial concentrations. It was suggested that protein coverage is related to the value of S/P ratio. Microcrystal biolabels having same dimensions with higher protein surface coverage will result in a lower value of S/P ratio. It was suggested that the S/P ratio is a key parameter on the sensitivity of bioassay that higher S/P ratio results in higher sensitivity [1]. However, results showed that microcrystal biolabel system with a higher S/P ratio showed a lower sensitivity when compared with the microcrystal biolabel system with a smaller S/P ratio [4]. An explanation based on the affinity between antigens and the biolabels was proposed. It was believed that a large force is required to maintain the biointeraction between the antigen and the “huge” microcrystal biolabels. Therefore, microcrystal biolabels with the highest protein surface coverage and with the lowest value of S/P ratio gave the highest sensitivity in the bioassay. It was suggested that although the S/P ratio is an important parameter in determining the sensitivity of a bioassay, protein surface coverage on the microcrystals plays an important role in microcrystal based bioassay.

3.3 Specificity

Due to the large surface area of the microcrystals which may results in the increase of non-specific binding, different attempts have been introduced to low the non-specific of microcrystal based bioassays. This can be achieved by using DSPE-PEG(2000)-amine for the coating process which the PEG group can reduce the non-specific binding [6,7]. Other proposed method is to block the uncovered microcrystal surfaces with a blocking agent e.g. bovine serum albumin (BSA) [4]. Beside physical surface

modification method, results also indicate using a smaller size microcrystal biolabels can be minimized nonspecific binding by reducing the chances of microcrystal surface area to react non-specically to the substrate surface [6,7].

4 SUMMARY

We demonstrated the preparation and potential applications of organic microcrystal biolabel in biochemical assay. The significant of the organic microcrystal biolabels compared with other particulate label system is the dissolution property of the organic microcrystal which results in the release of a large number of signal-generating molecules for detection. Our biolabel technology provides a new strategy to perform biochemical assay over the conventional biolabel system.

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