Chemical Engineering Journal 317 (2017) 356–367

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Preparation of protein imprinted microspheres using amphiphilic ionic liquid as stabilizer and emulsifier *via* miniemulsion polymerization

Nan Zhang *, Xiaoling Hu *, Ping Guan, Chunbao Du, Ji Li, Liwei Qian, Xiaoyan Zhang, Shichao Ding, Bangpeng Li

Department of Applied Chemistry, Key Laboratory of Space Applied Physics and Chemistry of Ministry of Education, School of Nature and Applied Science, Northwestern Polytechnical University, Xi'an 710072, PR China

HIGHLIGHTS

- Preparation of BSA interfacial molecularly imprinted microspheres.
- The amphiphilic ionic liquid [DMM]Cl was employed as emulsifier in miniemulsion polymerization.
- [DMM]Cl has superior stabilization effect on BSA conformation.
- Fast mass transfer rate and superior recognition ability of IMIMs-[DMM]Cl for BSA.

ARTICLE INFO

Article history:
Received 11 October 2016
Received in revised form 10 February 2017
Accepted 11 February 2017
Available online 20 February 2017

Keywords:
Biomacromolecular imprinting
Amphiphilic ionic liquid
Protein stabilizer
Miniemulsion polymerization
Interfacial molecularly imprinted microspheres

ABSTRACT

Design and preparation of functional materials for selective recognition of biomacromolecules such as proteins, DNA, viruses, and bacteria have always been extremely challenging due to their structure sensitivity. Regrettably, the structure stability of biomacromolecules in molecularly imprinted technology was rarely addressed. This paper presents an effective procedure for the facile preparation of bovine serum albumin (BSA) interfacial molecularly imprinted microspheres (IMIMs), using an imidazole-based amphiphilic ionic liquid (IL), which played a dual role of a stabilizer and emulsifier, *via* miniemulsion polymerization at 25 °C. In order to prove the stabilizing effect of IL on BSA, a conventional emulsifier sodium dodecyl sulfate (SDS), was also used as the emulsifier for comparison. Different characterization methods were employed to investigate the stability of BSA structure and track the experimental process. The results reveal that the destruction of structure of BSA by amphiphilic IL, 1-dodecyl-3-methylimidazolium chloride ([DMM]Cl), was much lower than that by SDS. Moreover, good selectivity of IMIMs-[DMM]Cl towards BSA was certified in the batch-wise and selectivity rebinding tests, in which an imprinting factor of 3.32 was obtained. This was superior to IMIMs-SDS and demonstrated to be a promising platform for recognition of protein with good specificity.

1. Introduction

Nowadays, selective separation and purification of biomacromolecules for analytical chemistry [1] and medical diagnosis [2] *via* molecular recognition mechanisms have attracted considerable attention. Molecular recognition is a common biological phenomenon that refers to the formation of a complex between the receptor and the target substrate, through intermolecular interactions between complementary functional groups. Natural receptors include enzymes, antibodies, nucleic acids, active proteins, *etc.*, which possess superior binding selectivity and strong affinity for their targets. Nonetheless, natural receptors have some disadvantages, such as elevated costs, immunogenicity, poor shelf-life, and stability, which limit their practical applications, let alone industrialization. Therefore, much research has been directed towards the development of suitable alternatives.

Ideal alternative candidates for applications of natural receptors are molecularly imprinted polymers (MIPs). Molecularly imprinted polymers are synthetic polymers with tailored recognition sites for given targets [3]. They are conventionally prepared *via* copolymerization of appropriate functional monomers and cross-linkers in the presence of the given template molecules. Then, the removal of the template leaves artificial recognition sites, which are functionally and sterically complementary and have affinity towards

* Corresponding authors.
E-mail addresses: zhangnan930928@126.com (N. Zhang), huxl@nwpu.edu.cn (X. Hu).

http://dx.doi.org/10.1016/j.cej.2017.02.066
1385-8947/

predetermined target molecules. Desired predetermination, superior recognition, and excellent stability make MIPs significantly important in practical applications such as in analytical chemistry [4], as biosensors [5], in medical diagnosis [6] and so on. Unlike straightforward imprinting technology developed against small template molecules, the imprinting of biomacromolecules, such as proteins in aqueous medium, still has limitations, due to restricted mass transfer, conformational flexibility and interruption of hydrogen bonding in template-monomer interactions by water molecules [7–9].

To overcome the aforementioned issues associated with poor mass transfer efficiency, due to restricted mobility of macromolecules through the cross-linked matrix, a so-called surface imprinting technology is employed, as the recognition sites are predominately generated at the substrate surface and not within the pores [10–12]. For simplifying the imprinting process of post-grafting or coating of an imprinted layer onto the surface of a pre-functionalized substrate, a combination of surface imprinting and miniemulsion polymerization techniques is of great value [13]. Tan [14,15] et al. took the lead in using surface imprinting technology *via* miniemulsion polymerization and adopted nano-sized spheres to successfully achieve the imprinting of protein ribonuclease A (RNase A). By making use of the interactions between the surfactants and template protein as well as emulsification properties of proteins [16], it could be ensured that a portion of the protein remained at the lipid-water interface, where water was restricted in the oil-in-water miniemulsion system. Then oil-soluble monomers and cross-linkers could be copolymerized in the presence of the template protein trapped on the micelle surface. After the elution of template protein, one-step interfacial imprinted nanospheres were obtained. Therefore, compared with the conventional imprinting methods, miniemulsion polymerization would be particularly promising in surface imprinting technology. Traditionally, sodium dodecyl sulfate (SDS) is employed as the emulsifier in the combined surface imprinting-miniemulsion polymerization. The emulsifiability of SDS was undoubted, but there was an issue of maintaining the flexible and complex configuration of the protein [17,18] during the imprinting process, when SDS was used. Consequently, suitable alternatives to SDS, which can function as an emulsifier and bring about a stabilization effect on the protein conformational integrity, are of great importance.

Ionic liquids (ILs) have always been considered as miraculous solvents for biological system. Due to their favorable properties such as negligible vapor pressure, good thermal stability, wide liquid regions, low flammability, powerful dissolution ability, high ionic conductivity and designability [19], ILs have been widely used in enzyme catalysis [20] and for protein stability and separation [21]. In our group, Qian et al. [22] researched the stabilizing role of imidazolium-based ionic liquids on protein conformational integrity and suggested that well designed ionic liquids containing chaotropic cations and kosmotropic anions were found to superiorly stabilize biological macromolecules such as enzymes and proteins, in accordance with Hofmeister series [23]. In this regard, chaotropes refer to large-sized and weakly hydrated ions with low charge density that break the H-bonding in bulk water. On the other hand, kosmotropes refer to small-sized and strongly hydrated ions with high charge density that increase the H-bonding in bulk water [24,25]. Therefore, taking into consideration the designability and potential of ionic liquids to stabilize proteins, the aim of this work was to design a kind of amphiphilic ionic liquid consisting of an imidazolium cation as the polar group and a hydrophobic tail along with a kosmotropic anion.

According to Sourav Chakraborty et al. [26], 1-cetyl-3-methylimidazolium has a very low CMC (0.61 mM) and hence has to be used at a concentration above its CMC in miniemulsion, which was adverse for droplet nucleation. Unlike 1-cetyl-3-methylimidazolium, 1-dodecyl-3-methylimidazolium has a suitable CMC value of 9.8 mM and thus a stable miniemulsion could be obtained at a much lower concentration, compared to its CMC value. Therefore, in this work, we aimed to take advantage of an IL surfactant, 1-dodecyl-3-methylimidazolium chloride ([DMM]Cl). This IL consisted of a chaotropic cation (imidazolium cation as the polar group and a hydrophobic tail) and chlorion as a kosmotropic anion, to afford superior biocompatibility and stabilize the protein, based on the Hofmeister series. Compared to the common anionic surfactant SDS that could easily denature the protein but was still always used in many investigations involving proteins [27], this kind of ionic liquid-based cationic surfactant would not denature the template protein bovine serum albumin (BSA) during systematic analysis, circular dichroism (CD) spectroscopy, and synchronous fluorescence spectroscopy. Following that, [DMM]Cl was employed as the emulsifier to prepare interfacial molecularly imprinted microspheres (IMIMs) by ultraviolet (UV) irradiation *via* miniemulsion polymerization at 25 °C, in order to preserve the protein configuration as much as possible. The procedure for preparation is shown in Scheme 1. The obtained IMIMs were fully characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), dynamic light scattering (DLS), and Brunauer–Emmett–Teller (BET) nitrogen adsorption. Subsequently, the adsorption capacity, selectivity, competitive adsorption, and reusability of IMIMs were systematically investigated.

2. Experiments

2.1. Materials

N-methylimidazole, 1-chlorododecane, sodium dodecyl sulfate (SDS), cetyl alcohol (CA), methyl methacrylate (MMA, 99%), ethylene glycol dimethacrylate (EGDMA, 98%), benzoin dimethyl ether (DMPA, 99%), and fluorescein isothiocyanate (FITC) were purchased from Aladdin. Lysozyme (Lys; chicken egg white, M_W 14.4 kDa, pI 10.7), bovine serum albumin (BSA; M_W 69 kDa, pI 4.9), and ovalbumin (OVA; M_W 45 kDa, pI 4.7) were purchased from Sigma-Aldrich. All the chemicals used were at least of analytical grade.

2.2. Synthesis of amphiphilic ionic liquid surfactant

The synthesis of amphiphilic IL surfactant [DMM]Cl was performed according to the procedure reported by Sourav Chakraborty et al. [26], with some modification. Briefly, equimolar quantities of N-methylimidazole and 1-chlorododecane were mixed in a round-bottomed flask fitted with a reflux condenser. Then the mixture was stirred for 72 h under reflux at 70 °C. After the reaction, the crude product, 1-dodecyl-3-methylimidazolium chloride ([DMM]Cl), was thoroughly washed with ethyl acetate several times to remove unreacted reagents and the product was dried in a vacuum oven at 50 °C until constant weight. The structure of [DMM]Cl is shown in Fig. 1.

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.31 (s, 1H), 7.60 (s, 1H), 7.39 (s, 1H), 4.25 (t, J = 7.4 Hz, 2H), 4.06 (s, 3H), 2.03–1.63 (m, 2H), 1.44–1.08 (m, 18H), 0.82 (t, J = 6.8 Hz, 3H).

2.3. Determination of conformational stability of BSA

The conformational stability of BSA was investigated by circular dichroism (CD) and synchronous fluorescence spectroscopy.

CD experiments were conducted using a quartz cuvette with 1 cm path length. The samples were scanned in the wavelength range of 190–260 nm with 1 nm resolution and baseline correction