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Letter to the Editor

Comment on "Preparation of surface molecularly imprinted polymeric microspheres and their recognition property for basic protein lysozyme": Molecularly imprinted polymer or cation exchanger?

In a recent paper, Gao et al. [1] reported the synthesis of surface-imprinted polymer microspheres with a core–shell structure for lysozyme recognition. The core beads were prepared via copolymerization of N-vinylpyrrolidone (NVP) and 2-hydroxyethyl methacrylate (HEMA) in an inverse suspension system with N,N-methylenebisacrylamide (MBA) as a cross-linker, followed by the reaction with methacryloyl chloride to introduce polymerizable double bonds on the surface. Lysozyme imprinting was achieved through radical-induced graft copolymerization of methacrylic acid (MAA) and MBA on the surface of the as-prepared HEMA/NVP microspheres dispersed in an aqueous medium. MAA was chosen as a functional monomer due to its strong electrostatic interactions and hydrogen bonding with the basic lysozyme template. After template removal, the resulting molecularly imprinted polymer (MIP) microspheres demonstrated satisfactory imprinting effect. The MIPs showed very high binding affinity for lysozyme and the binding capacity reached up to 216 mg/g, while the corresponding non-imprinted polymers (NIPs) gave a binding of ~40 mg/g. Furthermore, the specific recognition selectivity for lysozyme was confirmed with bovine hemoglobin as a contrast protein in the batch rebinding tests. Both the MIPs and NIPs showed hemoglobin binding of ~30 mg/g. In this comment, however, we question the real imprinting effect of such lysozyme-imprinted microspheres.

We notice that the template rebinding results are inconsistent with the polymerization recipe. As described in Section 2.4, lysozyme (0.14 g), MAA (3.5 g), MBA (0.3 g), and the core microspheres (1.0 g) were mixed in the buffer to synthesize the surface lysozyme-imprinted microspheres. We assume that each lysozyme molecule in the mixture was responsible for creating one templated site in the resulting imprinted shells grafted on the core beads and the weight gain due to grafted imprinted shells could be neglected. Therefore, the lysozyme rebinding capacity of the imprinted microspheres resulting from the molecular imprinting effect would not exceed 140 mg/g. In fact, as observed in a lot of studies, the yield in effective imprints relative to the amount of imprint molecule used is rather low, especially when non-covalent imprinting method is adopted [2]. Generally, the difference in the template rebinding capacity between a MIP and the NIP can be defined as specific template rebinding capacity of the MIPs due to the created imprinting sites. Based on the rebinding results reported in the work, the specific rebinding capacity is calculated to be ~176 mg/g. This value is already much higher that the above theoretic limit 140 mg/g, not to mention the fact that most of the added template molecules should be embedded in the gels generated surrounding the core–shell imprinted microspheres. No doubt some significant nonspecific binding other than the imprinting effect should contribute to such too high specific rebinding.

Furthermore, Gao et al. chose an inappropriate contrast protein, hemoglobin with an isoelectric point of pH 6.8, in the rebinding tests at pH 9.0 to verify the binding selectivity of the MIPs. On this condition, both MIP and NIP microspheres carrying carboxylic groups are negatively charged as the hemoglobin molecules, and beyond doubt, showed greatly lower rebinding to hemoglobin than to the positively charged lysozyme with an isoelectric point of pH 11.1. Considering the charge features of the MIPs and the template, anyway, it seems more reasonable to choose basic proteins, e.g. ribonuclease A and cytochrome c, as reference proteins to test the binding selectivity of the MIP microspheres.

On the other hand, neutral acrylamide as well as N-isopropylacrylamide has been widely utilized as a functional monomer for protein imprinting due to the relatively weak multiple hydrogen bondings between the amide groups in the polyacrylamide chains and the protein template [3–6]. Several studies [7–10] have reported the introduction of rationally small amount of charged monomers, such as negative charged MAA or positive charged N,N-dimethylaminoethyl methacrylate, into the prepolymerization recipes to strengthen the interactions and hence to enhance the molecular imprinting effect of the resulting MIPs in terms of specific rebinding capacity and selectivity. The charged monomers added usually accounts for less than 1 wt% of the total monomers. Both Kofinas et al. [9] and Cheng et al. [10] have confirmed that the MIPs will lose the imprinting efficacy because of excessive nonspecific binding when superfluous acidic or basic monomer is incorporated in the prepolymerization mixture. The joint use of two oppositely charged functional monomers may tolerate some more added amounts. Therefore, the polyampholyte hydrogels containing such two monomers with appropriate ratio have been adopted by several groups for the imprinting of different proteins [11–14]. Till now, however, there has been rarely any report on protein imprinting only using charged functional monomer. Obviously, Gao et al. had failed to address these issues in their work and it seems unreasonable to synthesize lysozyme-imprinted microspheres with sole MAA as functional monomer, as they did.

Combining all of the above aspects, we speculate that the lysozyme-imprinted microspheres reported are probably cation exchangers, and not MIPs bearing lysozyme imprints as the authors expected. The observed large difference in template rebinding capacity between the MIPs and the NIPs may be attributed to the different shell thickness and hence different carboxyl amounts grafted to the core spheres. To exclude this possibility, the authors might as well measure the ion exchange capacity of the imprinted and non-imprinted microspheres, respectively. Also, they are suggested to employ a wide range of non-template proteins as references in rebinding experiments to confirm the recognition specificity of the MIP microspheres. In addition, they did not show

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the SEM images of the MIP and NIP microspheres except for those of the HEMA/NVP supports. Direct evidences would be expected for the core–shell structure, since it was meaningless to compare the binding capacity in weight without the dimensional data of the MIP and NIP.

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