Biomaterials 32 (2011) 3008–3020

Contents lists available at ScienceDirect

Biomaterials

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

Review

Challenges for the effective molecular imprinting of proteins

Ellen Verheyen [1], Joris P. Schillemans [1], Martin van Wijk, Marie-Astrid Demeniex, Wim E. Hennink, Cornelus F. van Nostrum*

Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, P.O. Box 80.082, 3508 TB Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 16 December 2010
Accepted 4 January 2011
Available online 1 February 2011

Keywords:
Molecular imprinting
Protein imprinting
Crosslinked polymers
Functional monomers
Template removal
Protein–polymer interaction

ABSTRACT

Molecular imprinting is a technique that is used to create artificial receptors by the formation of a polymer network around a template molecule. This technique has proven to be particularly effective for molecules with low molecular weight (<1500 Da), and during the past five years the number of research articles on the imprinting of larger (bio)templates is increasing considerably. However, expanding the methodology toward imprinted materials for selective recognition of proteins, DNA, viruses and bacteria appears to be extremely challenging. This paper presents a critical analysis of data presented by several authors and our own experiments, showing that the molecular imprinting of proteins still faces some fundamental challenges. The main topics of concern are proper monomer selection, washing method/template removal, quantification of the rebinding and reproducibility. Use of charged monomers can lead to strong electrostatic interactions between monomers and template but also to undesired high aspecific binding. Up till now, it has not been convincingly shown that electrostatic interactions lead to better imprinting results. The combination of a detergent (SDS) and AcOH, commonly used for template removal, can lead to experimental artifacts, and should ideally be avoided. In many cases template rebinding is unreliably quantified, results are not evaluated critically and lack statistical analysis. Therefore, it can be argued that presently, in numerous publications the scientific evidence of molecular imprinting of proteins is not convincing.

1. Introduction

Molecular imprinting is a technique used to create artificial receptors by the formation of a polymer network around a template molecule (Fig. 1). In the pre-polymer mixture, several possible interactions, such as hydrophobic interactions, hydrogen bonds, Van der Waals forces and electrostatic interactions determine the spatial arrangement of monomers around the template. This spatial arrangement is then fixed by polymerization of monomers and crosslinker. Removal of the template leaves a chemically and sterically complementary void (imprint) in the polymer network, which is able to rebind the template.

Although the first paper describing the formation of imprints was published in 1931 [1], research on molecular imprinting was scarce until the 1980's. In an excellent and extensive review, Whitcombe et al. illustrated the maturation of the field by the dramatic increase in publications seen over the past 20 years (Fig. 2A) [2]. From this and many other reviews that describe the progress made over the years, it becomes clear that molecular imprinting is a very promising and rapidly evolving technology, with many possible applications such as analytical separations, enzyme-like catalysis, chemical sensors and drug delivery [2–6].

Molecular imprinting has proven to be particularly successful for low molecular weight compounds [7–10]. Although imprinting of larger, more complex molecules such as proteins, DNA, and even whole cells and viruses has also been reported [11–14], the number of research papers using such templates is relatively small (Fig. 2B). Till 2003, less than 10 research papers on imprinting of biomacromolecules were published per year, which reflects the difficulties faced when trying to imprint large and sensitive biomolecules [15,16]. Firstly, for low molecular weight compounds, highly crosslinked gels are used to ensure preservation of the imprint cavity after removal of the template. However, for large template molecules, high crosslink densities seriously hinder mass transfer of the template, leading to slow template removal and rebinding kinetics or, in the worst case, permanent entrapment of the template in the polymer network due to physical immobilization. Additionally, crosslinking of the template to the network can also lead to chemical immobilization [17]. Secondly, due to the solubility properties and sensitive structural nature of biomacromolecules, imprinting can generally only be

* Corresponding author. Tel.: +31 0 30 2536970; fax: +31 30 2517839.
E-mail address: c.f.vannostrum@uu.nl (C.F. van Nostrum).
[1] These authors contributed equally.

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.01.007

Fig. 1. Schematic representation of the principle of molecular imprinting. (A) The template (shown in blue), (functional) monomers (shown in yellow, green and orange) and crosslinker (+) form a pre-polymerization complex. (B) Polymerization of monomers and crosslinker fixes the complex. (C) Removal of the template leaves rebinding cavities. Reprinted with permission from [1].

performed in aqueous environment, which limits the choice of monomers. Moreover, hydrogen bonding interactions strongly contribute to the affinity of molecularly imprinted polymers (MIPs) for low molecular weight compounds in organic, aprotic solvents, but are seriously hampered in water. Thirdly, biomacromolecules are highly complex. Physicochemical properties such as charge or hydrophobicity can strongly vary in different regions of e.g. the protein template, whereas similar regions may be present in other templates. This could lead to high aspecific binding and cross-reactivity of the imprinted polymer.

Despite the challenges, after an initial lag in biomacromolecule imprinting relative to the rest of the field (Fig. 2), the number of

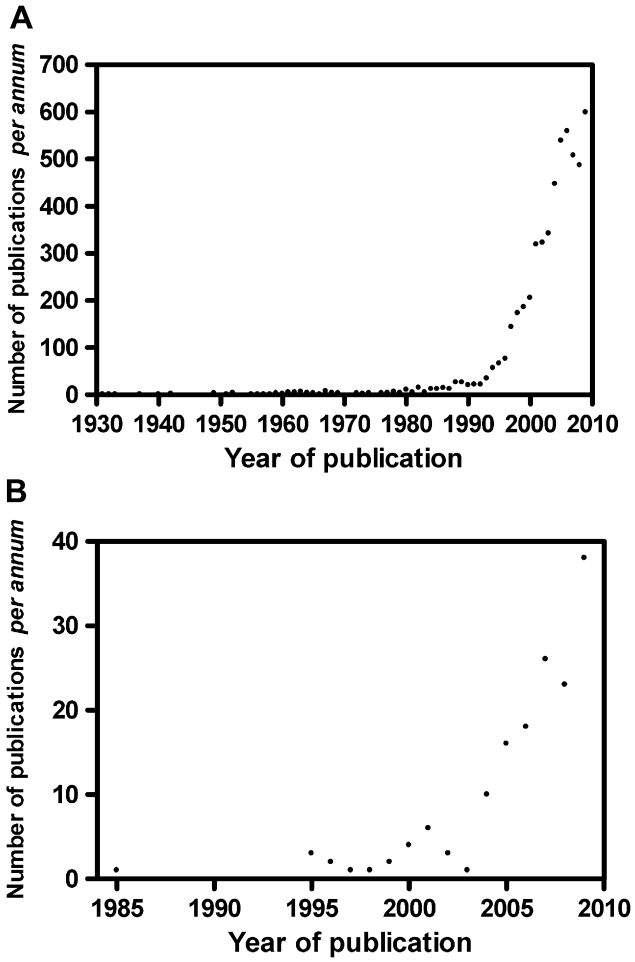

Fig. 2. (A) The number of publications within the field of molecular imprinting science and technology per annum for the period 1931–2009 (adapted from [3] supplemented by data from [16]). (B) Number of research papers on biomacromolecular imprinting per annum for the period 1985–2009.

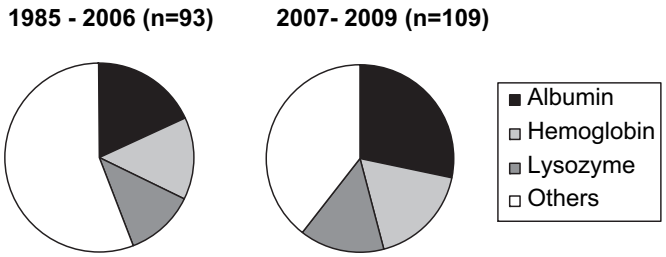

Fig. 3. Relative frequency of the templates used in molecular imprinting of protein over the periods 1985–2006 and 2007–2009.

papers has now begun to increase. Interestingly, Fig. 3 shows that in recent years (2005–2009) the model proteins albumin, hemoglobin, and lysozyme are being used more frequently (54%) than in the period up to 2006 (44%). This is opposed to what can be expected from an emerging research field and illustrates that molecular imprinting of proteins is still in its initial phase of development, where research is mostly focused on proof of concept using well defined, relatively stable and inexpensive model proteins. We believe that especially in this time of increasing research intensity, proof of concept, and setting of standards for future research, it is important to subject the published data to a critical review. Therefore, the aim of this paper is to critically analyze published data and conclusions in relation to our own experimental data. The articles discussed are selected on the basis of an extensive literature study on papers published between 2001 and 2009. We focused on the publications that contained sufficient data to allow proper analysis and recalculations. We would like to emphasize that the points raised in this chapter are only meant to initiate debate and it is not our intention to discredit anyone.

2. Experimental basis

2.1. Materials

Acrylamide (AAm, ultra pure) and *N,N'*-methylene-bisacrylamide (MBA, ultra pure) were purchased from MP Biomedicals, methacrylic acid (MA, 99%), *N,N'*-bis(acryloyl) cystamine, fluorescein isothiocyanate (FITC), lysozyme from hen egg white (96,381 U/mg), cytochrome C from bovine heart (purity >95%), hemoglobin from bovine blood (purity >90%), myoglobin from horse heart (purity >90%) and *N*-octyl β-D-glucopyranoside (OG) from Sigma–Aldrich. Acetic acid (AcOH), acrylic acid (AAc, synthesis grade) and *N,N*-dimethylformamide p.a. were obtained from Merck. *N,N,N',N'*-tetramethylethylenediamine (TEMED), ammonium peroxodisulfate (APS) and sodium dodecylsulfate (SDS) were obtained from Fluka. *N,N*-dimethylaminoethyl-methacrylamide (DMAEMA) was obtained from Polysciences Europe GmbH. The Bio-Rad *DC* protein assay was purchased from Bio-Rad Labs. Dioleoylphosphatidylcholine (DOPC) was purchased from Lipoid GmbH, Triton X100 (TX100) from BDH Laboratory Supplies, and Irgacure 2959 from Ciba Specialty Chemicals. Lipid II, a bacterial membrane-associated peptidoglycan precursor [18], was kindly provided by Dr. E. Breukink (Utrecht University). FITC-labeled lysozyme was synthesized as described before [19]. In detail: 300 mg lysozyme was dissolved in 50 mL borate buffer (100 mM, pH 9). While stirring, 0.28 mL FITC solution (10 mg/mL in DMF, FITC:lysine mol ratio 1:20) was added to the lysozyme solution drop-wise and the resulting mixture was stirred for 1 h at room temperature. Next, the pH was adjusted to 7.2 by adding boric acid and the protein solution was filtered (0.2 μm). Finally, the solution was extensively dialyzed against water (1 week, at 4 °C) to remove unreacted FITC and the FITC-lysozyme was collected after freeze-drying.