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Molecularly imprinted polymer (MIP) as micro-reactor for dipeptide formation

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Abstract: The study explored the development of molecularly imprinted polymer (MIP) as micro-reactor for the formation of amide bond between aspartic acid and phenylalanine. The MIP was synthesized using l-α-aspartame as template and 2-methoxy-4-vinylphenol as functional monomer with divinylbenzene as host monomer. The MIP was prepared by thermochemically initiated bulk polymerization. The MIP made, after template removal, was employed to aid the dipeptide synthesis from the amino acid precursors with thionyl chloride and methanol. FTIR characterization of MIP and its complexes revealed presence of spectral peaks differentiating each complexes from other MIP complexes. TGA data suggest low substrate to MIP ratio implying low product yield. SEM showed porous and rough solid MIP surface. This was supplemented by N_2 adsorption/desorption analyses for porosity by BJH method and surface area measurement by BET that gave an ave. pore radius of 19.298Å, categorizing the MIP as microporous, and an ave. surface area of 404 m^3/g, respectively. The formation of dipeptide product was indicated by presence of m/z 295.1196 in MS, amide bond of product complex in FTIR, and amide proton peak in Nuclear Magnetic Resonance (NMR).

Key Words: MIP, aspartame, dipeptide formation, MIP synthesis

1. INTRODUCTION

Molecularly Imprinted Polymer (MIP) is a versatile material that has been used as biosensors, sorbent assays, membranes, solid phase extraction, chromatographic separation, and catalysts (Hwang and Lee, 2001; Stevenson, 1999; Sellergren, 2001). Due to its three-dimensional macromolecular nature, MIPs offer advantages similar to real enzymes such as high cooperativity of the functional groups allowing induced fit, allosteric effect, and steric strain for incoming substrate (Wulff G., 2002). The preparation of MIP enzyme-mimics requires synthetic polymeric substances that allow stability against heat, chemicals, and solvents. Moreover, the MIP design can be tailored for specific properties to which the desired processes are achieved (Yan & Ho, 2006). A cursory survey of literature revealed limited studies on MIP and peptide synthesis. It is the objective of this research to study a new method of

synthesizing a dipeptide with the aid of Molecularly Imprinted Polymer (MIP).

2. METHODOLOGY

2.1 Synthesis of MIP

The synthesis of MIP was done by thermopolymerizing the self-assembled template-functional monomer along with the host monomer and porogen. The L-α-aspartyl-L-phenylalanine methylester (0.111 mmol) template molecule was mixed with 3 equiv 2-methoxy-4-vinylphenol in 20mL tetrahydrofuran under sonic bath. Divinylbenze (2.9mL), was added followed by 1,1'-Azobis(cyclohexanecarbonitrile) (ABCN, 0.1g). The mixture was then sonicated under nitrogen and heated to 65°C for 40 h to afford a bulk MIP-aspartame complex. MIP was obtained by removing the template using Soxhlet process with water as solvent at 90°C.

2.1 Synthesis of dipeptide

Aqueous solution of aspartic acid (Asp) (0.1mmol) and phenylalanine (Phe) (0.1mmol) in THF solvent (2ml) was prepared and allowed to bind in MIP. After filtration and drying, thionyl chloride was added to facilitate the formation of amide. The reaction mixture was then soaked in methanol for the formation of the methylester and was settled for another hour before drying in desiccator for 12 h. The MIP:Product was then extracted with Water:Toluene solvent. The dipeptide product was collected from the aqueous layer while the MIP, composed of mostly styrene-like structure, was left in toluene (organic layer).

3. RESULTS AND DISCUSSION

Visual, and mechanical inspections showed that the resulting MIP:Template is hard, homogenous, crystalline, and yellow translucent solid. Template removal was easily carried out in water at 90 °C. This condition ensures minimal damage to the MIP and promotes easy template removal by degrading the template to its hydrolysis products. The resulting mass spectra of the aqueous extract of free MIP confirmed the removal of the template as indicated by the absence of detectable peaks due to the template and its hydrolysis products.

The SEM image of MIP showed bulk material with irregular shapes and rough texture (Figure 1a) characteristic of porous material (Jakubiak-Marcinkowska, et al., 2012). A closer look of the MIP grain showed a uniformly porous surface (Figure 1b). Its porosity was confirmed by N_2 adsorption/desorption analyses revealing ave. surface area of 404 m^3/g (BET method) and ave. pore radius of 19.298 Å (BJH method). This data agrees with the expected results due to effect of porogen component.

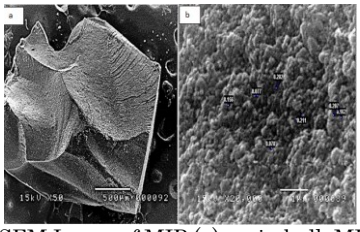

Figure 1. SEM Image of MIP (a) grain bulk MIP; and (b) magnified image (20,000x) showing the porous surface

The porous MIP allows adsorption of Asp and Phe precursors through H-bonding interaction bringing the two precursors in close proximity with each other. The addition of thionyl chloride facilitates the formation of amide bond and the acyl chloride functional groups. The reaction mixture was then soaked in methanol for the formation of the methyl ester. Confirmation of the presence of peptide bond was done using QTOF mass spectral analysis with the presence of intense base peak of quasi molecular-ion $[M+H]^+$ at m/z 295 expected for the product (HRMS calcd for $C_{14}H_{19}N_2O_5^+$ = 295.1293, found: 295.1196).

The IR spectra showed the characteristic Amide I stretching (1670cm^{-1}) that is not present in the NIP confirming desirable formation of amide bonds exclusively by using the MIP. Extraction of the product(s) from the MIP, however, afforded very low yield (~1% via TGA). The ^1H NMR spectra of the crude synthesized product(s) showed presence of peptide bond formation as indicated by the highly deshielded amide proton peak at 8.53 ppm that could not be possible with simple amino acids with amine (0.5-5ppm). The low yield can be attributed to competing non-specific binding of precursors limiting

the entry of two substrates in a cavity. Moreover, low yield can also be due to the nanoporous (< 20 Å) morphology of the MIP resulting to slow substrate diffusion leading to low site accessibility due to capillary forces (Sellergren, 2001).

4. CONCLUSIONS

The synthesis of MIP afforded bulk porous material where precursors can be adsorbed to facilitate proximity of reactants within the MIP matrix. The precursors were demonstrated to cause amide formation. The quantity of yield is low but implied success on showing the proof of concept. Further adjustment is recommended to improve the MIP porosity to promote the binding of substrates and to increase the yield of products.

5. ACKNOWLEDGMENTS

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