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DETEKSI DNA TERMETILASI DAN *SINGLE NUCLEOTIDE POLYMORPHISM* BERBASIS TEKNOLOGI MONOLITH UNTUK DETEKSI DINI KANKER SECARA CEPAT DAN AKURAT

Tahun ke 1 dari rencana 2 tahun

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ABSTRAK

DNA termetilasi berkaitan erat dengan penyakit genetik, tumor, maupun berbagai jenis kanker. Begitu juga halnya dengan pola *single nucleotide polymorphism* (SNP) yang mempunyai korelasi terhadap penyakit menular, kanker, dan lain-lain. Oleh karena itu, menjadi suatu hal yang sangat penting untuk mengembangkan teknik pemisahan dan deteksi DNA termetilasi dan SNP secara cepat dan akurat sehingga dampak negatif yang dapat ditimbulkan dikemudian hari (adanya penyakit tertentu ataupun gejala karsinogenesis) bisa dihindari sedini mungkin. Material berpori kontinyu lapis tunggal yang disebut dengan "*monolith*" telah berkembang dengan cepat pada bebera dekade terakhir ini and memegang peranan yang sangat penting dalam tenik pemisahan kinerja tinggi dan penelitian-penelitian yang berkaitan dengan *genomics, proteomics,metallomics* dan *bioelementomics*. Dalam penelitian ini dilaporkan tentang pembuatan kolom monolithic berbasis polymer organik yang dapat diaplikasikan tidak hanya untuk tujuan analisis tetapi juga dapat diaplikasikan untuk tujuan preparative. Monolith yang mempunyai gugus epoxy disintesis secara *in situ* kopolimerisasi dari glycidyl methacrylate (GMA) yang direaksikan dengan ethylene dimethacrylate (EDMA) menggunakan porogen sistem tersier di dalam *silicosteel tubing*. Monolith ini kemudian dimodifikasi lebih jauh dengan mereaksikan gugus penukar anion lemah terhadap gugus epoxy monolith. Selanjutya, dengan menggabungkan monolith ini pada HPLC, pemisahan dan deteksi DNA termetilasi dengan perbedaan 1 gugus metil dan SNP telah sukses dilakukan.

Kata kunci: DNA termetilasi, Single Nucleotide Polymorphism, HPLC, Monolith, Pemisahan

ABSTRACT

Methylated DNA and single nucleotide polymorphism (SNP) in the human genome are considerably correlated with cancer development and progression. Therefore, the accurate, sensitive, and rapid analytical methods for the detection of Methylated DNA and SNP are absolutely required for early detection of cancer. A single piece continuous of porous material called as "monolith" has been rapidly developed for several decades and nowadays holds an impressively strong position in separation science and other researchs dealing with proteomics, genomics, metallomics and bio-elementomics. This experiment is dealing with the preparation of organic polymer-based monolithic columns suitable not only for analytical purposes but also for potentially preparative application. Epoxy-containing monolithic matrix was synthesized by in situ copolymerization of glycidyl methacrylate (GMA) with ethylene dimethacrylate (EDMA) in the presence of a ternary porogen inside a silicosteel tubing. This monolith was further modified by attaching weak anion exchange moiety via covalent bonding into expoxy group of the monolith. By coupling the monolith with HPLC system, separation and detection of methylated DNA by difference of 1 methyl and also SNP was successfully attempted.

Keywords: Methylated DNA, Single Nucleotide Polymorphism, HPLC, Monolith, Separation

RINGKASAN

Methacrylate-based monolith dibuat menggunakan monomer fungsional glycidyl methacrylate (GMA), dan ethylene glycol dimethacrylate (EDMA) sebagai cross-linker. Adapun porogen yang digunakan adalah system tersier yang terdiri dari 1-propanol : 1,4-butanediol : air dengan ration 7:4:1 (v/v). Komposisi total monomer %T = 40 dan cross-linker %C = 25. In situ kopolimerisasi diprosess dalam silicosteel tubing (i.d 1 mm x 10 cm length) pada 60^{o}C selama 24 jam. Selanjutnya dilakukan postpolimerisasi terhadap poly-(GMA-co-EDMA) yang terbentuk dan terikat secara kovalen didalam silicosteel tubing menggunakan diethylamine 1M. Uji permeabilitas dilakukan dengan cara mengalirkan larutan yang terdiri dari fase gerak (A): 20 mM 20 Tris-HCl pH 8, dan fase gerak (B): 1 M NaCl dengan komposisi 50/50 ke dalam monolithic column menggunakan pompa HPLC dengan kecepatan 1 mL/min. Dari uji ini didapatkan hasil yaitu pressure drop sebesar 10 MPa. Rendahnya prossure drop ini mengindikasikan bahwa monolith ini mempunyai permeabilitas yang baik, dan didominasi oleh karakter mesopore dan flow-through pore. Melalui uji morfologi menggunakan scanning electron microscope (SEM) diperoleh hasil bahwa diameter globules monolith ini sebesar 2-5 ɜm, sedangkan ukuran flow-through path bervariasi hingga 12 ɜm. Hasil ini membuktikan bahwa monolith ini mempunyai

permeabilitas yang tinggi serta resistansi aliran yang kecil (low flow resistant). Distribusi porositas diuji menggunakan inverse size exclusion chromatography (ISEC) dan diperoleh hasil bahwa volume fraksi flow-trough pore/macropore (50 – 300 nm) sebesar 34%, sedangkan mesopore (1,5 – 50 nm) sebanyak 63% , dan micropore (< 1.5 nm) yaitu 3%. Dengan jumlah macropore yang memadai maka dihasilkan transfer massa yang efficient sehingga analisis sampel dapat dilakukan dengan cepat, sedang jumlah mesopore yang dominan akan memberikan interaksi yang efektif antara sampel biomolekul. Dari uji awal (preliminary test) dapat diketahui bahwa monolith ini mempunyai kemampuan yang sangat baik dalam pemisahan oligonucleotide dari dT10 – dT30 dan hanya memerlukan waktu selama 12 min untuk memisahkan 21 fragments dengan resolusi yang baik (baseline resolved). Monolith ini juga mampu memisahkan DNA termetilasi dalam waktu yang sangat cepat (<4 min) dengan resolusi yang baik jika terdapat perbedaan 4 metil, 2 metil, dan 1 metil yang terdapat sekuen DNA. Namun demikian masih diperlukan optimasi yang lebih jauh lagi agar monolith ini mampu memisahkan single nucleotide polymorphism (SNP). Pemisahan dan analisis SNP menghasilkan resolusi yang kurang baik. Walaupun demikian, monolith ini mampu mendeteksi split peak secara jelas dari 4 sampel SNP yang digunakan dalam penelitian ini.

SUMMARY

In this experiment, methacrylate-based monolith was prepared using glycidyl methacrylate (GMA) as a functional monomer and ethylene glycol dimethacrylate (EDMA) as a corss-linker. Tertiary solvent composed of 1-propanol : 1,4-butanediol : water with the ratio of 7 : 4 : 1 (v/v) was employed as the pore-forming agent (porogen). The optimized total monomer (%T) and the cross-linker (%C) compotions were 40 and 25, respectively. In situ copolymerization of poly-(GMA-co-EDMA) was performed inside a silicosteel tubing (i.d 1 mm x 10 cm length) at 60^{o}C for 24 h. This monolith was further modified by passing diethylamine 1M through the column to provide weak anion exchange group for separation of biomolecule target (DNA). Permeability test was conducted by flowing the mobile phase (A): 20 mM 20 Tris-HCl pH 8, and the mobilephase (B): 1 M NaCl with the composition of 50/50 through the monolithic column using the HPLC pump at flow rate of 1 mL/min . At optimum composition of monolith, the pressure drop resulted from this test was found to be 10 MPa. The low pressure drop indicated that this monolith possesses good permeability, and predominated by mesopore and flow-through pore characters. From morphology test assessed by scanning electron microscope (SEM), it was found that the globule diameter size of this monolith was 2-5 ȝm, whereas the flow-through path varies up to 12 ȝm. These results revealed the low flow resistant of this monolith due to the excellent permeability. Porosity distribution investigated by inverse size exclusion chromatography (ISEC) showed volume fraction of flow-trough

pore/macropore, mesopore (1,5 – 50 nm), and micropore (< 1.5 nm) of the optimized monolith were 34%, 63%, and 3%, respectively. Adequate amount of the macropore fraction resulted in high efficiency of mass transfer, allowing high throughput sample analysis, whereas predominated amount of mesopore provides effective interaction of the monolith with biomolecule samples. In our preliminary test, we found that this monolith is able to efficiently (baseline resolved) separate oligonucleotide (dT_{10} – dT_{30}), which contains 21 fragments, within 12 min. Further investigation showed that this monolith exhibits excellent ability for separation of methylated DNA. The methylated DNA samples with the length of base of 33 with different of 4 methyl could be separated efficiency (baseline resolved). Similarly, the different of 2 methyl, and 1 methyl in DNA sequences were also baseline resolved. However, we still need more efforts to separate single nucleotide polymorphism (SNP) samples. The resolution of SNP samples is less than 1.5 so that baseline resolved is not achieved. However, split peaks of 4 SNP samples could be clearly detected.

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