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A possible purification method of DNAs' fragments from humic matters in soil extracts using novel stimulus responsive polymer adsorbent

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Dedicated to Professor Terumichi Nakagawa on the occasion of his retirement and 63rd birthday.

Abstract

Novel stimulus responsive, uniformly sized polymer-based adsorbent was prepared for a possible purification method of DNAs from humic matters in soil extracts. The prepared polymer adsorbent has a pair of anion exchangeable and cation exchangeable polymeric selectors, which are reversibly responded by the changes of column temperature as well as pH of mobile phase. At pH 5, DNAs and humic matters were completely adsorbed on the polymer adsorbent at room temperature, while up to 90% of the adsorbed DNAs were released and recovered at 70 °C with no release of adsorbed humic matters. Cleaning up of the polymer adsorbent could be performed by washing the adsorbed humic matters with alkaline mobile phase (pH 9) to recover those and realize repeatable use of the adsorbent.

Keywords: Purification of DNA; Stimulus responsive adsorbents; Polymer stationary phases

1. Introduction

In order to evaluate environmental soil condition, evaluation of diversity and complexity of DNAs from microorganisms has been utilized as one of effective methods [1,2]. However, the separation of DNAs and humic matters, which involve humic acids and another various similar poly-anionic compounds, in soil extracts is rather difficult due to their complexities and becomes a big problem because the contaminated humic matters should suppress PCR process of the extracted DNAs. Size exclusion method using hydroxyapatite and centrifugation have been utilized for this separation so far [3,4], but these methods are relatively complicated and recovery of DNAs was presumably limited.

In fact, DNAs as well as the humic matters can be easily caught using ionic interaction as well as

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hydrophobic interaction because those compounds are either polymeric or oligomeric substances having large numbers of cation exchangeable groups (poly-anions). Therefore, main problem encountered so far is how we can recover only DNAs after complete adsorption of both of the substances for isolation from other inorganic and organic impurities in soil extracts. In addition, the humic matters should be also recovered from the adsorbent for repeatable use of the adsorbent and also usage of the humic matters involves other interesting purposes [5].

We have developed novel stimulus responsible polymer-based adsorbent for purification of DNAs by the surface modification technique that affords polymeric surface functionality [6]. This adsorbent has anion exchangeable polymeric selectors as well as cation exchangeable polymeric selectors, which might be responded through a change of pH of the mobile phase and/or temperature of the column due to change in interactions between both of ion exchangeable polymeric selectors. These stimulus responsive characteristics are effective for the recognition of complicated environmental mixtures.

2. Experimental

2.1. Materials

The adsorbent and reference adsorbents were prepared through a two-step swelling and polymerization method, which afforded uniformly sized macroporous particle nicely. Poly-styrene seed particle utilized as a shape template in the two-step swelling and polymerization method was prepared by the previously reported polymerization method [7].

The purified seed polymer particle (3.7×10^{-2} g/ml in water), 1.0 ml was swollen by the micro emulsion of dibutylphthalate (0.38 ml) followed by a micro emulsion of cyclohexanol (5 ml) as porogenic solvent and glycerol dimethacrylate (GDMA) (4 ml) as cross-linking agent. Then, the swollen particle was started to be polymerized at 50 °C and after 4 h, methacrylic acid (MAA, 0.5 ml) was directly added into the polymerization medium with potassium peroxodisulfate (PPS) followed by subsequent addition of the other monomer, vinyl-pyridine (0.5 ml) with PPS. Additional 20 h polymerization took place after the addition of the monomers.

As the reference adsorbents, two kinds of modified GDMA adsorbents were also prepared through the similar procedure but by the addition of either MAA or vinyl pyridine (VPD). Therefore, these adsorbents involve either cation exchangeable polymeric selector or anion exchangeable polymeric selector. In addition, unmodified adsorbent of GDMA (base adsorbent) and traditional adsorbent with both of MAA and vinyl-pyridine by a simple co-polymerization technique were also prepared as another reference adsorbents. The prepared adsorbents were packed into bio-inert PEEK column (4.6 mm ID × 30 mm) by slurry methods to evaluate their properties.

Elemental analysis data proved that added MAA was combined onto the base particle almost quantitatively, while 70–80% of added VPD was combined onto the base adsorbent. Although, high introduction rates onto the base polymer particles were observed for both ion exchange monomers, the ion exchange capacity was not able to be determined with good reproducibility. This might be because the ion exchange group introduced was grafted polymer, which might prevent the normal titration method.

In this study, we utilized a model of DNAs and the humic matters to simplify the test study, which were commercially available DNAs from calf thymus (Sigma, 1% in water) and humic acid (Wako, saturated in water), respectively. We changed column temperature as well as pH of the mobile phase to study the adsorption properties of the prepared adsorbents for DNAs and humic acid, where injection volume was 10 μl for each aqueous solution.

For every experiment, we tested at least three times to validate the reproducibility. The reproducibility observed was excellent.

2.2. Results and discussions

On the un-modified GDMA adsorbent (base adsorbent), 85% of the humic acid and 70–80% of

the DNAs were adsorbed at pH 5, while 25% of the humic acid and 35% of the DNAs were adsorbed on the base adsorbent at pH 9. In these cases, no temperature effect was observed through the temperature range from 10 to 70 °C. These findings can be explained based on typical adsorption phenomena of acidic substances under reversed phase condition with the relatively hydrophobic base adsorbent.

Adsorption properties on the surface modified adsorbents with either MAA or VPD are summarized in Tables 1 and 2. As expected, on the adsorbent modified with only anion exchangeable selector VPD, both of the DNAs and the humic acid were completely adsorbed at pH 5 and 9 through 10–50 °C and even higher temperature (70 °C). These are understandable because the DNAs and the humic acid are poly-anions and hydrophobic interaction might supplement the adsorption as well. The observed adsorption properties are suitable for only removal of both of the substances form the impurities through complete catch, but we have to recover DNA and humic acid from the adsorbent; therefore, this adsorbent is not suitable for our purpose.

On the other hand, the modified adsorbent with cation exchangeable selector MAA afforded even moderate adsorption value for the humic acid, while much lower adsorption value was observed for the DNAs. Interestingly, at pH 9, no DNAs were adsorbed on the adsorbent, but only 20% of the DNAs were adsorbed at pH 5, which does not make any sense even by the change of pH of the mobile phase.

Table 1
Adsorption properties at pH 5

	10 °C	20 °C	30 °C	40 °C	50 °C
Humic acid (%)					
MAA	75	70	70	65	65
VPD	100	100	100	100	100
DNA (%)					
MAA	25	20	20	20	20
VPD	100	100	100	100	100

Mobile phase: water, 10 mM citric acid; 20 mM phosphate buffer.

Table 2
Adsorption properties at pH 9

	10 °C	20 °C	30 °C	40 °C	50 °C
Humic acid (%)					
MAA	55	65	65	65	65
VPD	100	100	100	100	100
DNA (%)					
MAA	0	0	0	0	0
VPD	100	100	100	100	100

Mobile phase: 20 mM carbonate buffer.

It has been reported that anion exchangeable polymer and cation exchangeable polymer have interactions between the polymer chains through ionic interaction and hydrogen bonding at lower temperature to result in relatively hydrophobic characteristics by the cancel of both of the ion exchangeable hydrophilic groups, but at elevated temperature, these interactions break to show each ionic characteristics of both of ion exchange groups again to become relatively hydrophilic characteristic [8].

Fig. 1 depicts that adsorption properties of the absorbent having both of ion exchangeable polymeric selectors namely MAA–VPD. At pH 5, both of the DNAs and the humic acid were completely adsorbed on MAA–VPD at room temperature, but at elevated temperature only DNA was found to be released and up to 90% of adsorbed DNAs were recovered at 70 °C.

On the other hand, at pH 9, humic acid was found to be almost completely released at 50 °C. This is a quite interesting phenomenon because the other adsorbent prepared by a traditional copolymerization method using a mixture of GDMA with MAA and VPD afforded 70% adsorption of humic acid under the same condition. These facts prove that MAA–VPD should involve structurally different selectors from the adsorbent prepared by the traditional copolymerization method [6].

In conclusion, newly invented adsorbent MAA–VPD shows complete catches of DNAs and humic acid at pH 5 (room temperature), and release of only DNAs at 70 °C. In addition, the adsorbed

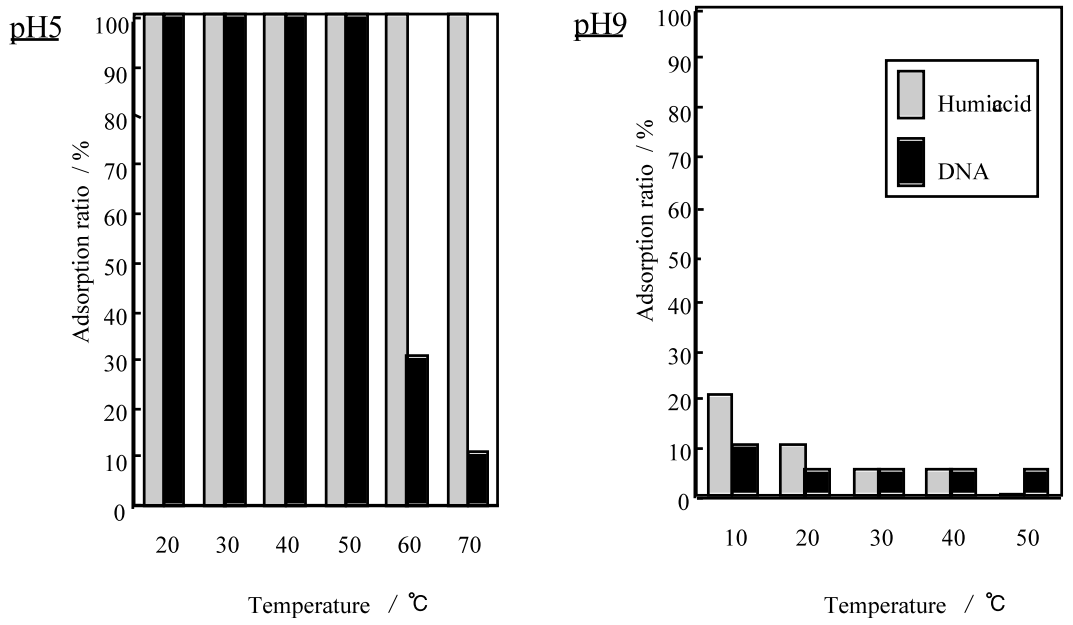

Fig. 1. Adsorption properties on MAA–VPD.

humic acid can be also recovered at 50 °C (pH 9) to make cleaning up of the adsorbent. At this moment, only test has been performed and detailed adsorption mechanism is not clear. Thus further studies using real substances as well as another purification method for naturally occurring substances from environment are in progress.

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