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Molecularly Imprinted Nanocavity-Based Fluorescence Polarization Assay Platform for Cortisol Sensing

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Abstract

We prepared core-shell-type molecularly imprinted polymer particles (MIP-NPs) for cortisol using cortisol-21-monomethacrylate as a template molecule, itaconic acid as an additional functional monomer, styrene as a comonomer and divinylbenzene as a crosslinker, and established a fluorescence polarization-based sensing nano-platform for the competitive binding assay of cortisol using dansyl-labeled cortisol (dansyl-cortisol). Before the preparation of MIP-NPs, the binding behavior of bulk MIP prepared by conventional radical polymerization was preliminarily characterized. NIP prepared with methacrylic acid instead of cortisol-21-monomethacrylate showed less binding activity than the MIP, revealing that the molecular imprinting process enhanced the affinity toward cortisol. Since the imprinting effect was confirmed in this system, the fluorescence polarization-based sensing nano-platform for cortisol was constructed using MIP-NPs with dansyl-cortisol, where the binding event of cortisol was transduced into the fluorescence anisotropy change of dansyl-cortisol from the bound-state to the free-state, on the basis of the concentration-dependent competitive replacement of dansyl-cortisol by cortisol added on MIP-NPs. The complex of MIP-NPs with dansyl-cortisol was more effectively formed than that of the reference polymer particles (R-MIP-NPs) prepared without itaconic acid, suggesting that the itaconic acid and cortisol-21-monomethacrylate-derived methacrylic acid residues can work cooperatively. Highly sensitive cortisol detection was achieved by the proposed molecularly imprinted nanocavity-based fluorescence polarization assay for cortisol sensing with dansyl-cortisol, and the apparent limit of detection was estimated to be ca. 80 nM.

1. Introduction

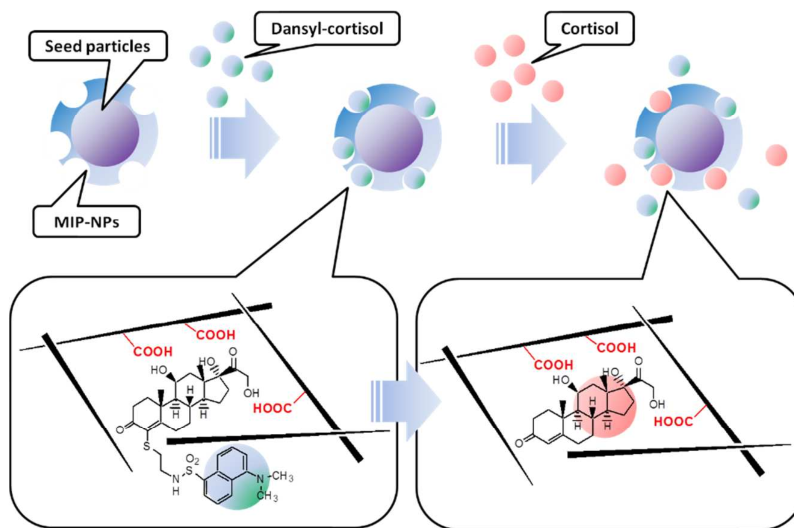
Molecular imprinting has been used to construct tailor-made specific binding cavities for versatile target molecules in synthetic polymer matrices.[1–12] Molecularly imprinted polymers (MIPs) can be prepared by radical copolymerization of a template molecule covalently or non-covalently conjugated by functional monomers with a cross-linking agent and comonomers and then the removal of the template molecule from the resulting polymer. This results in the formation of tailor-made molecular recognition cavities complementary in size and shape toward the target molecule and possessing the interaction sites capable of the specific binding of the template molecule. An important feature of this technique is that their morphology is easy to control from bulk to thin film as well as particles, allowing MIPs to combine any kinds of analytical methods and sensing devices.[13–18] Nanometer-sized MIP particles (MIP-NPs) are particularly under the spotlight due to their size effects of the wide surface area and the kinetic advantage.[19–23]

Cortisol is known to be a stress biomarker, *i.e.* the cortisol concentration level increases after stress is felt. Therefore, the detection of cortisol has been attracted an attention for diagnosis of mental diseases.[24–27] We previously reported core-shell-type MIP-NPs possessing specific binding cavities for cortisol in their shell layer using cortisol-21-monomethacrylate as the template molecule and itaconic acid as the functional monomer.[28] Dansyl-labeled cortisol (dansyl-cortisol) as a fluorescence-labeled target molecule was also synthesized to investigate the cortisol recognition capabilities of the MIP-NPs, where fluorescence spectral shift occurred after the complexation of MIP-NPs and dansyl-cortisol.[28]

Fluorescence polarization methods have been commonly used for examining binding behavior of fluorescent compounds.[29] A fluorescent molecule possesses a certain fluorescence transition dipole moment depending upon its molecular structure. When polarized incident light is irradiated, only the fluorescent molecules with the same directed transition dipole moment are excited and emitted light is polarized. Meantime, the fluorescent molecules are rotated by Brownian motion, so the direction of transition dipole moment is changed constantly, and as a result, the emitted light depolarizes. The rotation rate depends on the molecular size, and when the fluorophore is complexed with a large molecule, the degree of fluorescence depolarization decreases due to their slower rotation. This phenomenon enables us to analyze intermolecular interaction behavior without bound/free separation, *i.e.* fluorescence anisotropy (r) can be used as an index of molecular recognition activity.[30–43] To date, several fluorescence polarization-based assays have been reported using MIPs as artificial receptors; Hunt et al. reported the competitive fluorescence polarization assay of 2,4-dichlorophenoxyacetic acid as a target molecule using MIP and 7-carboxy-4-methylcoumarin as a fluorescence competitor molecule.[44] Ton et al. reported the direct fluorescence polarization assay of fluoroquinolones as a fluorescent target molecule using MIP.[45]

In this study, we demonstrate a molecularly imprinted nanocavity-based fluorescence polarization assay platform for cortisol sensing using competitive binding of dansyl-cortisol and cortisol against the cortisol-imprinted cavities in the MIP-NPs We previously reported that dansyl-cortisol was able to interact with MIP-NPs, which was confirmed by fluorescence spectral change. Based on the results, herein the complex of MIP-NPs and dansyl-cortisol was being used as

a sensing nano-platform for the sensitive detection of cortisol. In the presence of free cortisol, dansyl-cortisol bound to the MIP-NPs may be replaced by free cortisol depending upon the cortisol concentrations, which results in the increase of free dansyl-cortisol in the incubation solution. In accordance with the ratio of free to bound dansyl-cortisol concentration, the fluorescence anisotropy changes. Utilizing the competitive binding manner of cortisol and dansyl-cortisol, we established the fluorescence polarization-based assay using the complex of MIP-NPs and dansyl-cortisol as a sensing nano-platform. Furthermore, fluorescence polarization-based assays are more important from the viewpoint of general versatility than other sensing systems using expensive devices as well as expensive sensor chips such as surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) sensors.[46,47]



Scheme 1. Fluorescence polarization-based cortisol sensing using competitive binding of dansyl-cortisol and cortisol against the cortisol-imprinted cavities in MIP-NPs.

2. Experimental section

2-1. Materials

Cortisol (≥98%), 2-(Boc-amino)ethanethiol (97%), dansyl chloride (≥99%), and progesterone (≥99%) were obtained from Sigma-Aldrich Co. LLC. (USA). Methacryloyl chloride, triethylamine (Et_3N), sodium hydroxide (NaOH), 1 M hydrochloric acid (HCl), 0.5 M HCl methanolic solution, styrene, divinylbenzene (DVB) (55% mixture of isomers), itaconic acid, 2, 2'-azobis(4-methoxy-2,4-dimethylvaleronitrile) (V-70), 2,2'-azobis(2-methylpropionamidine) dihydrochloride (V-50), testosterone, tetrahydrofuran (THF), ethyl acetate, hexane, methanol, and acetonitrile were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sodium hydrogen carbonate, citric acid, sodium chloride, sodium sulfate, hydrogen peroxide (30%), and chloroform were purchased from Nacalai Tesque Inc. (Kyoto, Japan).

Styrene and DVB were used after distillation under reduced pressure to remove the inhibitors. THF (for organic synthesis and polymerization) and chloroform and hexane (for incubation) were used after distillation to remove the stabilizer and moisture. Water was purified with a Milli-Q (Merck Millipore Corp., Germany).

2-2. Synthesis of cortisol-21-monomethacrylate and dansyl-cortisol

Cortisol (907 mg, 2.5 mmol) was dissolved in dry THF (40 mL), and Et_3N (4.2 mL, 30 mmol) was added under nitrogen atmosphere. The reaction solution was cooled at 0 °C in an ice bath. Methacryloyl chloride (1.5 mL, 15 mmol) in dry THF (40 mL) was then carefully titrated and

stirred at 0 °C for 1 h and another 4 h at room temperature. After that, the reaction solution was evaporated, and ethyl acetate was added, followed by washing with saturated sodium hydrogen carbonate aqueous solution, saturated citric acid aqueous solution, and brine. The organic phase was dried over by sodium sulfate, and the solution was filtrated and evaporated. The crude sample was purified by column chromatography (silica gel C-200, ethyl acetate/hexane=1:1 v/v). The solution was evaporated and dried over *in vacuo*, yielding cortisol-21-monomethacrylate as white solid, which was identified by ^{1}H NMR (JNM-LA300, JEOL Ltd., Japan) and MALDI-TOF-MS (Voyager-DE-1000, Applied Biosystems Inc., USA).

^{1}H NMR ($CDCl_3$, 300MHz): δ 0.99 (3H, s, 18-CH_3), 1.45 (3H, s, 19-CH_3), 2.17 (3H, s, methacryloyl-CH_3), 4.49 (1H, s, 11-OH), 4.93 and 5.12 (1H, each, d, J=18Hz, 21-CH_2), 5.67 (2H, s, methacryloyl=CH_2), 6.22 (1H, s, 4-CH), MALDI-TOF-MS m/z: 431.7 $[M+H]^+$

Dansyl-cortisol was synthesized by using a procedure reported elsewhere.[28] In brief, dansyl-cortisol was synthesized from cortisol as a starting material in three steps. The alkene moiety of cortisol was epoxidated and coupled with 2-(Boc-amino)ethanethiol under alkaline conditions. The N-Boc-amine group was then deprotected under acidic conditions. Finally, the free amino group was coupled with dansyl chloride. The crude sample was purified by preparative thin layer chromatography, yielding dansyl-cortisol as a yellow semi-solid, which was identified by ^{1}H NMR (JNM-LA300, JEOL Ltd., Japan).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.94 (3H, s, 18-CH_3), 1.36 (6H, s, N-$(CH_3)_2$), 1.46 (3H, s, 19-CH_3), 3.64 (2H, t, S-CH_2), 4.29 and 4.66 (1H, each, d, J=18Hz, 21-CH_2) , 4.45 (1H, s, 11-OH),

5.13 (1H, broad, NH), 7.12–8.66 (6H, m, dansyl aromatic protons)

2-3. Preparation of MIP for cortisol by bulk polymerization

Cortisol-21-monomethacrylate (215 mg, 0.5 mmol), itaconic acid (262 mg, 2 mmol), DVB (1304 mg, 10 mmol), styrene (209 mg, 2 mmol), and V-70 (86.4 mg, 0.28 mmol) were added to a screw tube and dissolved in dry THF (10 mL). The mixture was polymerized at 30 °C for 72 h under nitrogen atmosphere. After solvent was removed *in vacuo*, the obtained polymer was physically grinded to obtain micro-meter sized particles. The polymer particles were hydrolyzed by 2 M NaOH aqueous solution/methanol (1:1 v/v) (50 mL) at room temperature for 48 h and then stirred in 1 M HCl aqueous solution/methanol (1:1 v/v) (50 mL) and water/methanol (1:1 v/v) (50 mL) for several hours. Finally the polymer particles were washed by Soxhlet-extraction with methanol for 6 h and dried over *in vacuo*, yielding MIP for cortisol. Non-imprinted polymer (NIP) was prepared using methacrylic acid instead of cortisol-21-monomethacrylate (***Table 1***).

Table 1. Composition of MIP and NIP

	MIP	NIP
Cortisol-21-monomethacrylate	215 mg (0.5 mmol)	—
Methacrylic acid	—	43.1 mg (0.5 mmol)
Itaconic acid	262 mg (2 mmol)	262 mg (2 mmol)
DVB	1304 mg (10 mmol)	1304 mg (10 mmol)
Styrene	209 mg (2 mmol)	209 mg (2 mmol)
V-70	86.4 mg (0.28 mmol)	86.4 mg (0.28 mmol)
dry THF	10 mL	10 mL

2-4. Binding experiments of various steroid hormones toward MIP and NIP

MIP (5 mg) or NIP (5 mg) was mixed with cortisol solutions (chloroform/hexane=4:1 v/v, 62.5, 125, 250, 500, and 1000 μM) (1 mL) in a vial and incubated at 25 °C for 48 h in a thermo mixer (Eppendorf AG., Germany). After that, the solutions were filtered by a syringe filter (0.2 μm) (DISMIC-13HP020AN, Advantec Japan, Co., Ltd., Japan), and then 0.5 mL aliquots of the filtrates were dried over *in vacuo*. The residues were taken up by acetonitrile (0.5 mL) and the cortisol in the solutions were quantified by HPLC (Gilson Inc, USA) (eluent: acetonitrile/water=70:30 v/v, flow rate: 1 mL/min, column: Chromolith RP-18e (100 mm × 4.6 mm I.D.) (Merck Millipore Corp., Germany), UV detection: 241 nm). As references, the cortisol solutions (62.5, 125, 250, 500, and 1000 μM) (chloroform/hexane=4:1 v/v) were treated in the same manner without the addition of MIP. Selectivity of MIP and NIP toward cortisol was evaluated by using structural analogues (testosterone and progesterone). Affinity constant (K_a) and maximum amount of cortisol bound (B_{max}) were estimated by a carve fitting software (Origin Pro 7.5J) using the following equation, where B is an amount of cortisol bound to MIP, H is a concentration of free cortisol, G is a parameter, K_a is an affinity constant, and B_{max} is a maximum amount of cortisol bound (asymptotic value).

$$B = \frac{((1 + K_a G + K_a H) - ((1 + K_a G + K_a H)^2 - 4 K_a{}^2 G H)^{\frac{1}{2}}) B_{max}}{2 K_a G} \qquad (1)$$

2-5. Preparation of core-shell-type MIP-NPs for cortisol using emulsifier-free emulsion polymerization

MIP-NPs were prepared by using a procedure reported elsewhere.[28] Briefly, styrene (760 mg, 7.3 mmol), DVB (40 mg, 0.31 mmol), and V-50 (41.3 mg, 2 mol% vs monomer) were added to water (79.2 g) in a round-bottomed flask. The reaction solution was stirred (800 rpm) at 80 °C for 24 h under nitrogen atmosphere to obtain spherical and monodispersed poly(styrene-co-DVB) (P(S-DVB)) seed particles suspended in water.

The P(S-DVB) seed emulsion (20 g), cortisol-21-monomethacrylate (3.9 mg, 9 μmol), itaconic acid (4.7 mg, 36 μmol), DVB (59.5 mg, 457 μmol), styrene (9.5 mg, 91.2 μmol), and V-50 (3.2 mg, 11.8 μmol) were added to a round-bottomed flask. The mixture was stirred (800 rpm) at 80 °C for 24 h under nitrogen atmosphere. After the polymerization, the obtained particles were separated by centrifugation at 18,900 rpm for 20 min. After the supernatant was removed, the polymer particles were hydrolyzed by 2 M NaOH aqueous solution/methanol (1:1 v/v) (50 mL) at room temperature for 24 h. The polymer particles were then neutralized by 1 M HCl aqueous solution/methanol (1:1 v/v) (50 mL) for several hours and washed with water/methanol (1:1 v/v) (50 mL) for several hours (twice). After the polymer particles were separated by centrifugation, the solvent was replaced by the incubation solution (chloroform/hexane=4:1 v/v) to adjust 1 mg/mL in solid content. Reference MIP-NPs (R-MIP-NPs) was also prepared as listed in ***Table 2***.

Table 2. Compositions of core-shell-type MIP-NPs and R-MIP-NPs.

	MIP-NPs	R-MIP-NPs
Seed emulsion	20 g	20 g
Cortisol-21-monomethacrylate (2.5 wt% vs seed)	3.9 mg (9 μmol)	3.9 mg (9 μmol)
Itaconic acid	4.7 mg (36 μmol)	—
DVB	59.5 mg (457 μmol)	59.5 mg (457 μmol)
Styrene	9.5 mg (91.2 μmol)	9.5 mg (91.2 μmol)
V-50	3.2 mg (11.8 μmol)	3.2 mg (11.8 μmol)

2-6. Detection of intermolecular interactions between MIP-NPs and dansyl-cortisol by fluorescence polarization method

Fluorescence polarization was measured with a fluorescence spectrophotometer (F-2500, Hitachi, Ltd., Japan) equipped with a depolarization attachment (650-0156, Hitachi, Ltd., Japan), where fluorescence was measured with four patterns of the polarization plates for excitation light and fluorescence light, *i.e.* 0°–0° (I_{hh}), 0°–90° (I_{hv}), 90°–90° (I_{vv}), and 90°–0° (I_{vh}). All measurements were carried out using 365 nm as an excitation wavelength and 450 nm as an emission wavelength (excitation slit: 10 nm, fluorescence slit: 10 nm) at 25 °C. Dansyl-cortisol (10 μM in chloroform/hexane 4/1 v/v, 3 mL) was transferred to a quartz cell (10×10×40 mm), and then a 100 μL aliquot of the MIP-NPs suspension (solid content: 1 mg/mL) was titrated (final concentrations: 32.3–250 μg/mL) every 20 min. An affinity constant (K_a) between MIP-NPs and dansyl-cortisol was estimated by a curve fitting software (Origin Pro 7.5J) using the following equations *4* and *6*.

The fluorescence polarization value (P) is generally expressed with the following equation.[48,49]

$$P(\lambda) = \frac{\left(I_{\mathrm{vv}}(\lambda) - GI_{\mathrm{vh}}(\lambda)\right)}{\left(I_{\mathrm{vv}}(\lambda) + GI_{\mathrm{vh}}(\lambda)\right)} \quad \left(G = \frac{I_{\mathrm{hv}}}{I_{\mathrm{hh}}}\right) \tag{2}$$

Here, I_{vv} is an intensity of the parallel components, I_{vh} is that of the perpendicular components, and G is a correction value (v: vertical, h: horizontal). It is well-known, on the other hand, that P filled the following equation (*Perrin-Weber* plot),[50,51] where V means an effective volume of the fluorescent substance (molecular mass times molar volume), η means a viscosity constant of the solvent, T means an absolute temperature, τ means a lifetime of the excited state of the fluorescent substance, R means the gas constant, and P_0 means a value of P at 0 K.

$$\frac{1}{P} - \frac{1}{3} = \left(\frac{1}{P_0} - \frac{1}{3}\right)\left(1 + \frac{RT}{\eta V}\tau\right) \tag{3}$$

If the temperature and viscosity of the solution are constant, P only depends on V. The measurements in this work were carried out at 25 °C with the same solvent, resulting in constant viscosity for the solution. When the fluorescent molecules were adsorbed to the particles, V increased, giving higher P, and the opposite was vice versa. Fluorescence anisotropy (r) is expressed by:

$$r = \frac{\left(I_{\mathrm{vv}} - GI_{\mathrm{vh}}\right)}{\left(I_{\mathrm{vv}} + 2GI_{\mathrm{vh}}\right)} \qquad \left(G = \frac{I_{\mathrm{hv}}}{I_{\mathrm{hh}}}\right) \tag{4}$$

This is generally completed by the following relational expression between a fluorescence polarization value (P) and a fluorescence anisotropy value (r):

$$r = \frac{2P}{(3-P)} \tag{5}$$

In addition, an affinity constant (K_a) was calculated by the following model formula using anisotropy (r), where r_∞ is a saturated r (asymptotic value) and r_0 is an initial r. G is a parameter, and H is a concentration of binding cavities when MIP-NPs were titrated, as it is assumed that the amount of binding cavities was the same as the amount of cortisol-21-monomethacrylate added in the polymerization step.

$$\Delta r = \frac{((1+K_a G+K_a H)-((1+K_a G+K_a H)^2-4K_a{}^2 GH)^{\frac{1}{2}})(r_\infty - r_0)}{2K_a G} \tag{6}$$

2-7. Fluorescence polarization-based assay for cortisol by competitively using complex of MIP-NPs and dansyl-cortisol.

Three concentrations of dansyl-cortisol solutions (chloroform/hexane=4:1 v/v) were prepared (2, 5, and 10 μM). Each solution (3 mL) was transferred to a quartz cell (10×10×40 mm) to measure fluorescence. The MIP-NPs emulsion (solid content: 1 mg/mL, 500 μL, final concentration: 143 μg/mL) was then added to the fluorescence cell by stirring for 20 min at 25 °C. After that, 0.1, 1, 10, 100, and 1000 μM cortisol solution in chloroform/hexane=4:1, v/v (100 μL) was added (final concentrations: 0.00278–27.8 μM) to the fluorescence cell every 20 min, and fluorescence intensity with polarization plates was measured at 450 nm (excitation wavelength: 365 nm). As a selectivity test, progesterone was adopted as a reference.

3. Results and discussion

3-1. MIP for cortisol prepared by bulk polymerization

MIP was prepared by radical polymerization using V-70 as an initiator at low temperature (30 °C) to stabilize the interaction on the basis of hydrogen bonding between itaconic acid and cortisol-21-monomethacrylate during the polymerization. After the polymerization, cortisol was removed by alkaline hydrolysis, yielding the imprinted cavities toward cortisol.

The binding behaviors of cortisol were examined for MIP and NIP. From the binding isotherms (***Figure 1***), apparent affinity constants (K_a) of MIP and NIP were estimated to be 1.3×10^4 M^{-1} and 7.8×10^3 M^{-1}, where the K_a value for MIP was 1.7 times higher than that of NIP, revealing that the imprinting effect was confirmed, which was driven by the presence of the template molecule during the polymerization.

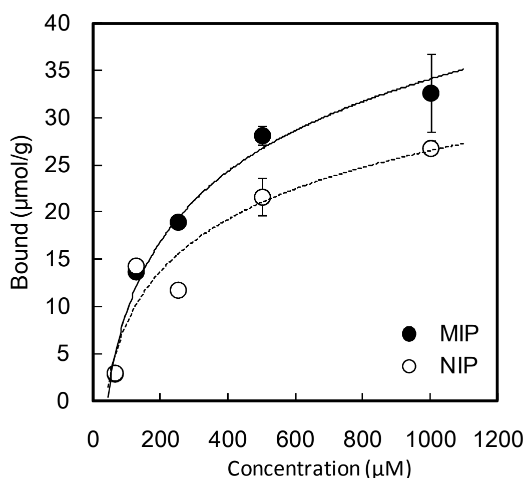


Figure 1. Binding isotherms of cortisol for MIP and NIP in chloroform/hexane (4:1 v/v).

Selectivity tests were carried out for MIP using cortisol and its analogues: testosterone and progesterone (***Figure 2***). The binding activity for cortisol was shown to be highest among them, suggesting that MIP can recognize the difference in the partial structures around the 17 position on the steroid skeleton, namely, β-hydroxy-ketone, hydroxy, and methyl-ketone groups.

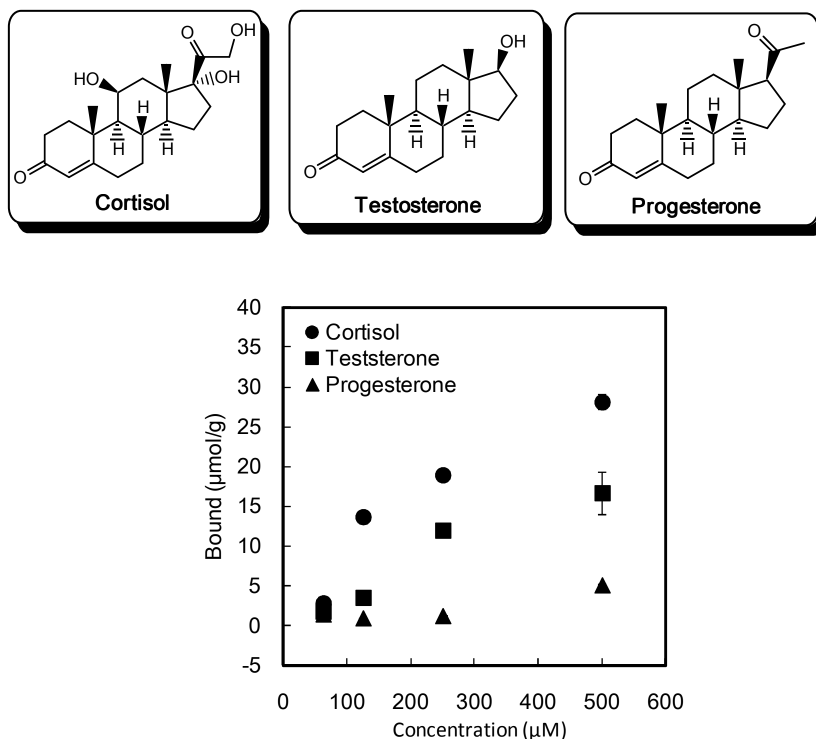


Figure 2. Selectivity test for MIP using cortisol, testosterone, and progesterone in chloroform/hexane (4:1 v/v).

3-2. Preparation of core-shell-type MIP-NPs using emulsifier-free emulsion polymerization

To develop MIP-based cortisol assay, spherical MIP-NPs could be advantageous, since nanoparticles possess wide surface area, high dispersibility, high accessibility, fast kinetics for binding, and easy handling for optical sensing, enabling us to construct highly sensitive, feasible,

and affordable sensing systems. Here we prepared MIP-NPs by two-step emulsifier-free emulsion polymerization using the P(S-DVB) seed particles as the core particles. The MIP shell-layer for cortisol was prepared by seeded emulsion copolymerization of cortisol-21-monomethacrylate, itaconic acid, styrene, and DVB, followed by the hydrolysis of the ester linkage on cortisol-21-monomethacrylate residue, resulting in the construction of cortisol recognition cavities bearing carboxy groups as the interaction sites with cortisol. FT-IR spectra were measured for P(S-DVB) seed particles and MIP-NPs and the peak (ca. 1720 cm^{-1}) derived from C=O bond was observed only from the MIP-NPs, confirming that the MIP layer was successfully formed by the seed polymerization (***Figure S1*** in Supporting information). The obtained core-shell-type MIP-NPs were ca. 200 nm, larger than the seed particles (ca. 155 nm), confirming that the approximately 20-nm-thick MIP shell-layer was constructed on the P(S-DVB) seed particles.[28]

3-3. Evaluation of the interaction between MIP-NPs and dansyl-cortisol by the fluorescence polarization method

As the titration of MIP-NPs (final concentrations: 32.3–250 µg/mL) to the dansyl-cortisol solution (10 µM) in the chloroform/hexane mixture (4:1 v/v), the fluorescence polarization value (P) increased (***Figure S2*** in Supporting Information), meaning that the rotation of the transition dipole moment of dansyl-cortisol was restricted due to the complexation with MIP-NPs. Accordingly, fluorescence anisotropy (r) was also increased by adding MIP-NPs, and the change in the r value was remarkably larger than that of R-MIP-NPs that were prepared in the absence of

itaconic acid. This suggests that itaconic acid located within the cortisol-imprinted cavities could work as hydrogen donor to form hydrogen bonding with hydroxy groups and/or ketone groups on cortisol, facilitating the cortisol binding to the cortisol-imprinted cavities. An estimated K_a value of dansyl-cortisol for MIP-NPs was 1.0×10^6 M^{-1} from the titration curve (***Figure 3***).

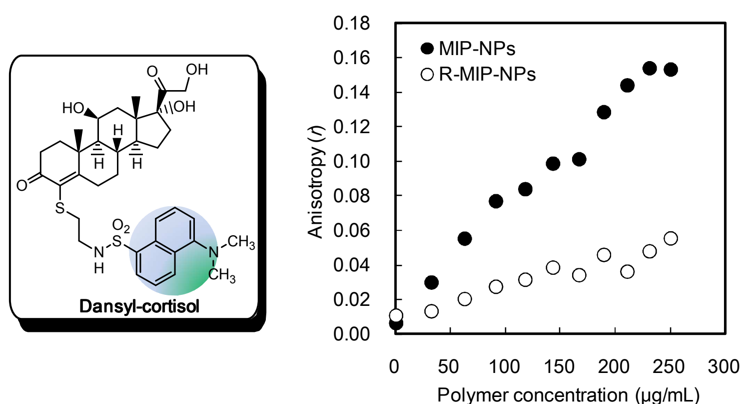


Figure 3. Effect of MIP-NPs and R-MIP-NPs concentrations on the fluorescence anisotropy (r) of dansyl-cortisol in 10 μM in chloroform/hexane (4:1 v/v).

The competitive binding behavior of cortisol and dansyl-cortisol against MIP-NPs was investigated by measuring the fluorescence anisotropy change with the different concentrations of dansyl-cortisol (2, 5, and 10 μM). The r value decreased by the addition of cortisol, indicating that cortisol was competitively bound to MIP-NPs with dansyl-cortisol, where the r values decreased as the concentration of dansyl-cortisol increased. This may be due to the excess of free dansyl-cortisol that may not interact with MIP-NPs leading to low r values (***Figure 4***).

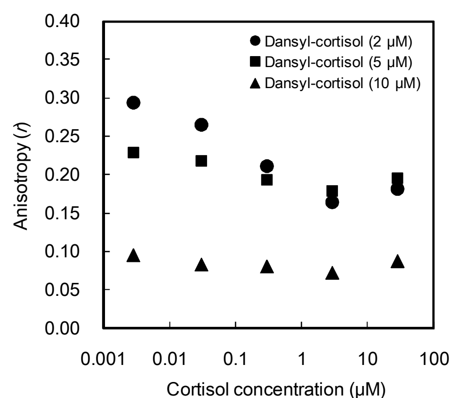


Figure 4. Fluorescence anisotropy (r) of dansyl-cortisol in the presence of MIP-NPs (143 μg/mL) and various concentrations of cortisol in chloroform/hexane (4:1 v/v).

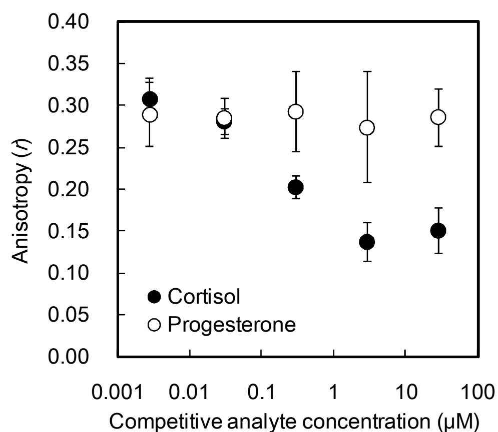


Figure 5. Fluorescence polarization-based competitive binding assay of cortisol and progesterone using MIP-NPs (143 μg/mL) and dansyl-cortisol (2 μM) in chloroform/hexane (4:1 v/v).

As 2 μM dansyl-cortisol showed the largest change in the r values, the fluorescence polarization-based competitive binding assay of cortisol using MIP-NPs (143 μg/mL) and dansyl-cortisol (2 μM) was demonstrated. As shown in ***Figure 5***, the cortisol

concentration-dependent r value change was observed, and a limit of detection appeared to be ca. 80 nM. Furthermore, negligible change in the r value was observed for progesterone, indicating that no competitive binding between dansyl-cortisol and progesterone occurred (***Figure 5***). The results clearly confirmed that highly sensitive and selective molecularly imprinted nanocavity-based fluorescence polarization assay for cortisol was developed by using the complex of MIP-NPs and dansyl-cortisol.

4. Conclusions

MIP for cortisol was prepared with cortisol-21-monomethacrylate as the template molecule, itaconic acid as the additional functional monomer, styrene as the comonomer, and DVB as the crosslinker. After the imprinting effect and the selectivity binding characteristics of the conventional bulk polymer-based MIP were investigated, MIP-NPs were prepared to develop the fluorescence polarization-based competitive binding assay of cortisol using the complex of MIP-NPs and dansyl-cortisol as the cortisol sensing nano-platform. In the competitive assay, the limit of detection was estimated to be ca. 80 nM, and the selectivity was confirmed by the difference in the binding of cortisol and progesterone. Sensitive and selective cortisol sensing was successfully achieved, and considering the rational design of MIP for versatile molecules, we believe that the proposed sensing nano-platform will be able to be expanded across a wide range of target compounds in the fields of life science, environmental science, and other analytical chemistry-associated fields.

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Keywords

Fluorescence polarization method; molecular imprinting; core-shell polymers; emulsifier-free emulsion polymerization; cortisol

Text for Table of Contents

The sensing nano-platform for cortisol detection was developed on the basis of the fluorescence polarization assay involving the competitive binding of dansyl-cortisol and cortisol against molecularly imprinted polymer nanoparticles.

Graphics for Table of Contents