Letter to the Editor

Antibody Production for a Rapid Fluorescence Polarization Immunoassay of Estrone*

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Estrone has been identified as a potential endocrine-disrupting chemical (EDC)[1]. Estrone is usually quantified by gas chromatography-mass spectrometry (GC-MS), GC-MS/MS, high performance liquid chromatography (HPLC), HPLC-MS, and HPLC-MS/MS, etc.[2-3]. Meanwhile, several immunoassays based on radioimmunoassay, enzyme linked immunosorbent assay (ELISA) or chemiluminescence immunoassay (CLIA) for determination of estrone in real samples have been developed[2,4]. Although these methods are sensitive, they need multistage separation and are thus time-consuming and laborious. A very promising way for the simplification of immunoassays for routine applications is a shift from heterogeneous methods (with separation) to homogeneous assays (without separation)[5]. Fluorescence polarization immunoassay (FPIA) is one of the homogeneous techniques that meets the requirements of a simple, reliable, fast, and cost-effective analysis[6]. Therefore, the present study is focused on the development of FPIA in order to analyze estrone based on antibody production.

2-(13-methyl-17-oxo-7,8,9,11,12,13,14,15,16,17 -decahydro-6H-cyclopenta[a]phenanthren-3-yloxy) acetic acid (hapten) was synthesized with estrone and bromoacetic acid (Figure 1), and then coupled to BSA for the use as an immunogen. Fluorescein-labeled estrone was synthesized and used as the fluorescein tracer as previously described[7]. The separated tracer at R_f=0.8 on the thin layer chramotography (GF254) was used for the development of FPIA. Two New Zealand white rabbits were immunized with the immunogen, and polyclonal antibody against estrone was obtained after the fifth immunization, which was then used for developing the FPIA.

The titer of antibody 1 and antibody 2 was 1/400 and 1/150 dilution, respectively. However, the negative serum showed no binding to the tracer, indicating that the obtained antibody can specifically bind to the analyte. The obtained antibody and tracer demonstrated their binding activity. The antibody titer in usual ELISA format is often at ten thousands order[2,8]. However, the titer is 1/400 for antibody 1 and 1/150 for antibody 2, which are much lower than that in ELISA, showing that FPIA processes a different dynamics from that of heterologous ELISA.

In order to choose an antibody with a better sensitivity for further investigation, two preliminary calibration curves were plotted based on antibodies 1 and 2. The response span (difference of maximal and minimal FP signal, δmP), medium inhibition concentration (IC_{50}) and limit of detection (LOD) of the calibration curves for two antibodies were used for the performance comparison. Antibody 1 demonstrated a lower IC_{50} (754.3 ng/mL), a higher response span (δmP=120) and a higher titer (1/400) as well as a lower LOD (7.5 ng/mL), indicating that antibody 1 can offer a better sensitivity than antibody 2 for the analysis of estrone. Thus, antibody 1 was selected for the development of FPIA for detection of estrone in the following experiments. The tracer levels (0.5, 1, 2, and 4 nmol/L) were compared in order to obtain a high sensitivity, and 1.0 nmol/L tracer exhibited the highest δmP (184.6) and the lowest IC_{50} (641.2 ng/mL), suggesting that 1.0 nmol/L was the optimal tracer concentration for the following experiments.

An optimized typical calibration curve for

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estrone is presented in Figure 2. The whole reaction time of the developed FPIA was 5 min. The IC_{50} was 193.9 ng/mL, the LOD (IC_{10}) was 5.0 ng/mL, and the dynamic range (IC_{20}-IC_{80}) was 19.8-1487.7 ng/mL.

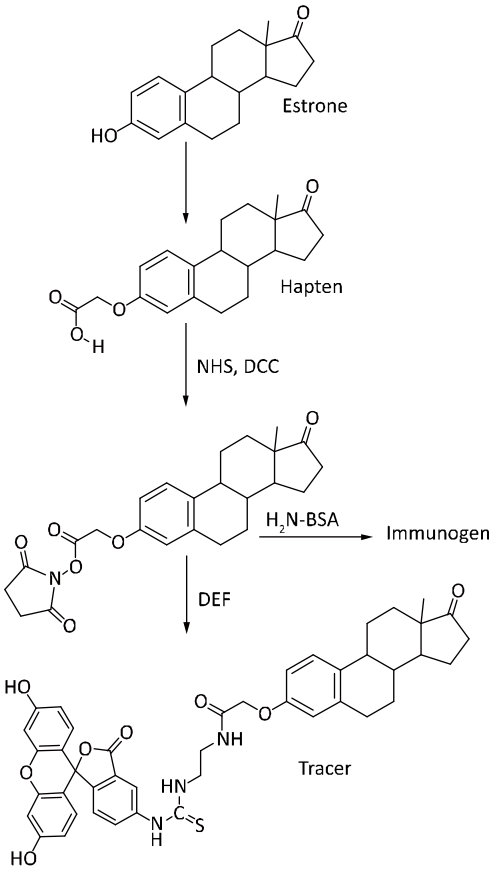


Figure 1. Synthesis of fluorescein-labeled estrone, estrone hapten, active ester intermediate of hapten, and tracer.

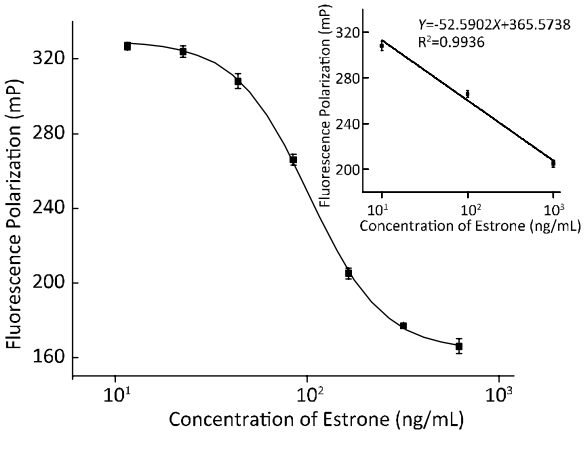


Figure 2. Typical FPIA calibration curve for estrone (mean±SD) for 3 assays.

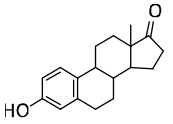
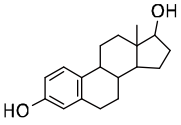
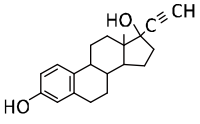
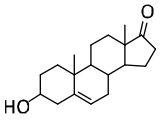
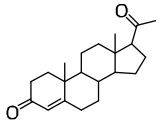
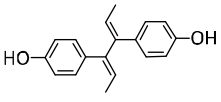
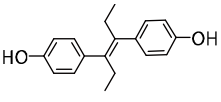
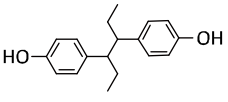
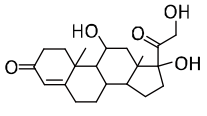
The cross-reactivity (CR) of FPIA was evaluated by testing some structurally related compounds (Table 1). The CR to estradiol and other tested hormones was 15.1% and <0.5%, respectively, showing that the developed FPIA was highly specific to estrone. The structures of estrogens estrone, estradiol and ethinylestradiol are different in ring D, where the substitute groups are carbonyl for estrone, hydroxyl for estradiol and hydroxyl, ethinyl for ethinylestrodiol.

Therefore, it can be assumed that the carbonyl in ring D is identified by the antibody as an epitope characteristic of analyte. When the substitute group in ring D was changed from carbonyl to hydroxyl, the CR decreased by more than 6 folds from 100% (estrone, with carbonyl group) to 15.1% (estradiol, with hydroxyl group). Moreover, adding one more ethinyl group onto the rind D of estradiol, the CR continued to decrease by around 37.7 folds from 15.1% (estradiol) to 0.4% (ethinylestradiol), proving that the opposite position of the coupling site of the hapten was a part of the epitope. However, it is hereby interesting to find out that the distant part of the coupling site of the hapten is related with the antigen determinant and the part nearby the coupling site. The latter could be supported by the CR change from 100% to 0.3%, accompanying the structure change from estrone to dehydroepiandrosterone.

The sensitivity of the developed FPIA was slightly lower than that of previously reported HPLC-MS (LOD, 6 ng/mL) and ELISA (LOD, 0.14 ng/mL) for estrone[2,9]. However, the FPIA is a simple and rapid screening method with an undoubted practical advantage for estrone test in food samples. According to the Announcement No.193/2002 of the Ministry of Agriculture of the Peoples' Republic of China, the usage of estrogens and chemicals with estrogenic activity such as methyltestosterone and androlin has been prohibited in animals reared for food. However, no guideline or maximum residue level (MRL) set officially or organizationally in the world is available to restrict endogenous estrone level in food. Thus, the developed FPIA is a good method for determination of estrone in environment or food. Moreover, the time required for the detection in the proposed FPIA was only 5 min, much shorter than that in ELISA[2,5].

In conclusion, a polyclonal antibody to estrone is successfully produced, and a sensitive and specific FPIA is developed for the analysis of estrone. The developed FPIA shows a greater potential than

Table 1. Cross-reactivity (CR) of Antibody to Related Compounds Determined by FPIA

Compound	Structure	IC_{50} (nmol/mL)	CR (%)
Estrone		0.72	100
Estradiol		4.75	15.1
Ethinylestradiol		180.22	0.4
Dehydroepiandrosterone		261.90	0.3
Progesterone		917.94	0.08
Dienestrol		3 581.89	0.02
Diethylstilbestrol		>50 000	<0.01
Hexestrol		>50 000	<0.01
Hydrocortisone		>50 000	<0.01

conventional analysis methods for the rapid screening of estrone in environment or food.

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