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Quantitative study of stereospecific binding of monoclonal antibody to *anti*-benzo(a)pyrene diol epoxide-N^2-dG adducts by capillary electrophoresis immunoassay

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ABSTRACT

The stereospecific binding of monoclonal antibody (mAb) 8E11 to *anti*-benzo(a)pyrene diol epoxide (BPDE)-dG adducts in single nucleoside, long oligonucleotide, and genomic DNA were quantitatively evaluated using noncompetitive and competitive capillary electrophoresis (CE) immunoassays. Two single-stranded TMR-BPDE-90mers containing a single *anti*-BPDE-dG adduct with defined stereochemistry and a fluorescent label at 5′-end were used as fluorescent probes for competitive CE immunoassay. To quantitatively evaluate the binding affinity through competitive CE immunoassays, a series of equations were derived according to the binding stoichiometry. The binding of mAb 8E11 to *trans*-(+)-*anti*-BPDE-dG displays strongest affinity (K_b: 3.57×10^8 M^{-1}) among all four investigated *anti*-BPDE-dG mononucleoside adducts, and the *cis*-(−)-*anti*-BPDE-dG displays lowest affinity (K_b: 1.14×10^7 M^{-1}). The binding of monoclonal antibody (mAb) 8E11 to BPDE-dG adducts in long DNA (90mer) preferentially forms the complex with a stoichiometry of 1:1, and that mAb 8E11 displays a slightly higher affinity with *trans*-(+)-*anti*-BPDE-90mers (K_b: $6.36 \pm 0.54 \times 10^8$ M^{-1}) than *trans*-(−)-*anti*-BPDE-90mers (K_b: $4.52 \pm 0.52 \times 10^8$ M^{-1}). The mAb 8E11 also displays high affinity with BPDE-dG adducts in genomic DNA (K_b: 3.74×10^8 M^{-1}), indicating its promising applications for sensitive immuno-detection of BPDE-DNA adducts in genomic DNA.

1. Introduction

Specific binding of antibody to antigen or hapten dictates the applications of immunoassays [1]. Qualitative and quantitative study of such binding is essential not only to the understanding of the molecular basis for immune functions, and also important to the method development of immunoassays. Various techniques have been developed for binding study, such as enzyme-linked immunosorbent assay (ELISA) [2–4], surface plasmon resonance (SPR) [5–7], gel electrophoresis mobility shift assay (EMSA) [8,9], and affinity chromatography [10,11]. Most immunoassays heavily rely on the adsorption of antigen or antibody on solid/liquid surface, e.g. ELISA, however, such adsorption can significantly alter the binding activity of the reactant (e.g. partially denaturing antibodies), and cause a decrease in binding capacity of the antigen-antibody [12]. Moreover, solid phase based immunoassays only provide limited binding information. For example, it is hard for ELISA to distinguish the complexes with different binding stoichiometry through the involved sequential washing.

Capillary electrophoresis (CE) immunoassay is a solution based affinity technique, and can provide accurate aqueous solution related binding information on affinity and stoichiometry [13,14]. In typical CE immunoassays, the initial concentration of antigen/hapten or antibody is known, and the bound and unbound species can be measured. Moreover, due to high efficiency of the CE separation, antigen–antibody complexes with different binding stoichiometry may be separated from each other. In addition, with combined laser-induced fluorescence detection (LIF), the CE immunoassay has demonstrated a number of advantages, e.g. high sensitivity, rapid separation, minute amount of analyte consumed, and ease-of-automation [15,16].

Both noncompetitive and competitive immunoassays have been employed in CE-LIF immunoassay [17,18]. In typical noncompetitive CE-LIF immunoassays, a known amount of fluorophore-labeled antibody (Ab*) is mixed with antigen (Ag) to form detectable noncovalent Ab*–Ag immunocomplex. The formed Ab*–Ag complex and unbound Ab* can be separated by highly efficient CE and detected by coupled highly sensitive LIF. Both Ab*–Ag complex and free Ab* can be measured, and both the measured signals can be used for accurate quantification of target antigen. Noncom-

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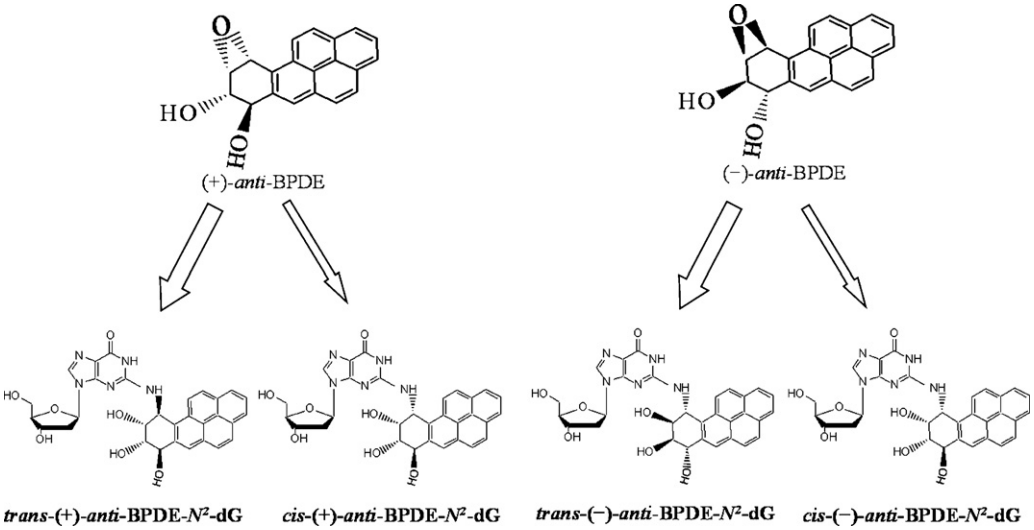

Fig. 1. Structure of *anti*-BPDE enantiomers (top) and corresponding four *anti*-BPDE-N^2-dG stereomers (bottom).

petitive immunoassays have several remarkable advantages over competitive immunoassays, including wider dynamic range and lower detection limits [19]. However, noncompetitive immunoassays require appropriate label of the antibody, which may scarify the binding avidity of the antibody. Competitive immunoassays may provide alternative choice. In this case, a fluorescently labeled antigen analog (Ag*) was used instead. This approach is based on the competition of Ag and Ag* for the limited binding sites of Ab. CE-LIF analysis of the mixture presents two types of separated zones corresponding to Ag* and Ag*–Ab. The binding affinity of Ab and unlabeled Ag can be indirectly derived from the signal change of Ag* and Ag*–Ab complex shown in the CE-LIF analysis.

Benzo[a]pyrene, an extensively studied carcinogenic polycyclic aromatic hydrocarbons (PAHs) [20], can be stereoselectively metabolized *in vivo* by cytochrome P450 and epoxide hydrolase to form two stereoisomic benzo[a]pyrene-7,8-diol-9,10-epoxide ((±)-*anti/syn*-BPDE) [21]. (±)-*anti*-BPDE is more mutagenic than the diastereomer (±)-*syn*-BPDE in a series of animal and human cell experiments [22,23]. Even the mutagenesis of two *anti*-BPDE enantiomers in bacterial and mammalian cells may be different [24–28]. The reactive carcinogenic species (±)-*anti*-BPDE can react with DNA, primarily at the exocyclic N^2 amino group of deoxyguanosine (dG) to form a bulky adduct of *anti*-BPDE-N^2-dG with four stereoisomers, including (+)-*trans*, (−)-*trans*, (+)-*cis*, and (−)-*cis* (Fig. 1). The stereochemistry of the four *anti*-BPDE-N^2-dG adducts has been proved to determine their respective mutagenesis and carcinogensis [29–34]. A number of antisera have been produced for developing sensitive and specific immunoassay of BPDE-DNA adducts (Table 1) [35–40]. These antibodies exhibit varied affinity, stereoselectivity, and specificity. The affinity is usually evaluated by the concentration of 50% inhibition (IC_{50}) through competitive ELISA assay, which depends upon the concentration of the antibody and labeled antigen. Concentration-independent binding information often lacks. Among these antibodies, mAb 8E11 has been screened in our laboratory as an important diagnostic monoclonal antibody and often used in the detection of BPDE-DNA adducts [18,35,36,41–43]. However, the relevant information on affinity, stereoselectivity, and specificity has not been clarified yet.

In this work, we developed CE immunoassay methods for quantitative study of the binding of BPDE-dG DNA adducts to mAb 8E11, which is useful for human exposure biomonitoring of carcinogenic benzo(a)pyrene. A series of equation were derived and examined by CE immunoassays for quantitative affinity study. Based on derived equations and CE immunoassays study, the binding affinity, stoichiometry, specificity, and stereoselectivity of mAb 8E11 against BPDE-dGs, BPDE-90mers and BPDE genomic DNA were examined.

2. Theoretical section

2.1. 1:1 noncompetitive binding stoichiometry

A series of equations were derived to calculate binding parameters for IgG antibody P and large antigen A, which was labeled with fluorophore in immunoassays. First, it is assumed that P binds with A at 1:1 stoichiometry. This is true when the antigen is very large and the first binding of the antigen may induce steric barrier to the second binding to the bivalent IgG. The reaction between A and P

Table 1
Antisera developed against BPDE-DNA adduct.

Antibody	Animal	Immunogen	Isotype	Cross-reactivity
mAb 8E11 [35,36]	Balb/cCr mice	BPDE-I-G-BSA	IgG1, Kappa	No
mAb E5 [36,40]	Balb/cCr mice	BPDE-I-G-BSA	unknown	No
mAb 5D11 [35,36]	Balb/cCr mice	BPDE-I-DNA-MBSA	IgG2, Kappa	Yes
mAb 41D3 [39]	Balb/cCr mice	BPDE-I-DNA-MBSA	unknown	Yes
mAb 5D2 [37,38]	Balb/cCr mice	BPDE-I-DNA-MBSA	IgG1, Kappa	Unknown
mAb 1D7 [37]	Balb/cCr mice	BPDE-I-DNA-MBSA	IgG1, Kappa	Unknown
mAb 4C2 [37]	Balb/cCr mice	BPDE-I-DNA-MBSA	IgG1, Kappa	Unknown
mAb TNO [39]	Balb/cCr mice	BPDE-I-DNA-MBSA	Unknown	Unknown
pAb #29 [37]	New Zealand white rabbits	BPDE-I-modified DNA	Unknown	Yes
pAb BP1 [38]	New Zealand white rabbits	BPDE-I-modified DNA	Unknown	Yes
pAb F29, F30, NCI [39]	New Zealand white rabbits	BPDE-I-DNA-MBSA	Unknown	Yes

mAb and pAb refer to monoclonal antibody and polyclonal antibody, respectively.