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Thermodynamic basis for antibody binding to Z-DNA: Comparison of a monoclonal antibody and its recombinant derivatives

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Abstract

Antibody engineering represents a promising area in biotechnology. Recombinant antibodies can be easily manipulated generating new ligand and effector activities that can be used as prototype magic bullets. On the other hand, an extensive knowledge of recombinant antibody binding and stability features are essential for an efficient substitution. In this study, we compared the stability and protein binding properties of two recombinant antibody fragments with their parental monoclonal antibody. The recombinant fragments were a monomeric scFv and a dimeric one, harboring human IgG1 CH2–CH3 domains. We have used fluorescence titration quenching to determine the thermodynamics of the interaction between an anti-Z-DNA monoclonal antibody and its recombinant antibody fragments with Z-DNA. All the antibody fragments seemed to bind DNA similarly, in peculiar two-affinity states. Enthalpy–entropy compensation was observed for both affinity states, but a marked entropy difference was observed for the monomeric scFv antibody fragment, mainly for the high affinity binding. In addition, we compared the stability of the dimeric antibody fragment and found differences favoring the monoclonal antibody. These differences seem to derive from the heterologous expression system used.

Keywords: Enthalpy–entropy compensation; Anti-DNA; Protein engineering; Recombinant antibody; *Pichia pastoris*

1. Introduction

Immunoglobulins (Ig) are highly specific receptors of the immune system composed of two heavy and two light chains, specialized for binding to a virtually infinite repertoire of antigen (Ag) molecules. Antigen–antibody binding is basically driven by surface and electrostatic complementarities between the epitope, in the antigen, and the paratope, in the immunoglobulin counterpart [1]. Most of the energy involved in the complex formation comes from enthalpic contributions relative to the side-chain interactions and a hydrophobic effect resulting from the release of immobilized water molecules [2].

Anti-nucleic acid antibodies are an important class among these specialized molecules, specially associated with the diagnosis of autoimmune diseases, such as systemic lupus eritematosus (SLE), and also used as biochemical reagents [3] in structural and functional studies of nucleic acids [4,5]. Although structural data have yielded information about combining site architecture and contact residues, they have not improved the knowledge of the dynamics involved in the antibody–DNA complex formation [6,7]. A better comprehension of the energetics of the formation of these complexes will also shed light on the rules that govern the binding of proteins to DNA, a multivalent antigen. Regarding this last point, it also can be a valuable tool to the comprehension of antigen valency and antibody avidity in the mechanisms related to B cell activation [8].

In this study, we used Z-DNA as the antigen. It appears in vivo as an alternative left-handed DNA structure dependent on local highly negative super-coiling. Its presence has long been associated with transcriptionally active chromosome domains in vivo [9–11]. Interest in Z-DNA forming chromosomal

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regions has recently been resurrected with new reports on Z-DNA roles in processes related to transcription [12–14] and pathologies [15,16]. The monoclonal antibody Z22 (mAb Z22) recognizes several polynucleotides in the Z conformation [17,18]. Z22 contains a VH10 derived heavy chain responsible for most of the affinity to DNA and a VL that contributes to antibody selectivity to the Z-DNA conformation [19–21]. Its epitope was extensively mapped [17,22,23]. Although well characterized, the biophysical properties of mAb Z22 in solution and the mechanism of anti-Z-DNA binding still demand further investigations.

In a previous study, we reported that Z22 antibody fragments produced in bacteria retain all the immunochemical characteristics of the parental mAb [24]. The same results were obtained using *Pichia pastoris* expression systems to produce two Z22 recombinant products: a monovalent fragment scFv (single chain Fv) and a divalent one, FvFc, which harbors an Fv fused directly to a human IgG1 Fc [25]. In this study, we compare these yeast produced antibody fragments and the parental Z22 mAb using spectroscopic studies. These data allowed us to determine the dissociation constant for Z-DNA binding. From these thermodynamic data, we discuss the role of the entropy and enthalpy effects during the nucleic acid–antibody interaction and recombinant antibodies' stability in the presence of a denaturing agent. These results impart new insights for understanding DNA/antibody interaction and the independence of antigen binding site among recombinant and native antibodies.

2. Materials and methods

2.1. Materials

The polynucleotide poly (dG-dC)•poly(dG-dC) was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). The herring sperm DNA, goat anti-human IgG (Fc specific), alkaline phosphatase conjugated rabbit anti-goat IgG (Fc specific) and the *p*-nitrophenyl phosphate substrate (pNPP) were purchased from Sigma Chemical Co (Saint Louis, MO). The rabbit IgG and the alkaline phosphatase conjugated rabbit anti-mouse IgG (Fc specific) were purchased from Pierce (Rockford, IL). The alkaline phosphatase conjugated goat anti-rabbit IgG (Fc specific) was purchased from Promega (Madison, WI) or Pierce. Herring sperm DNA was dissolved at 10 mg/mL in TE buffer (10 mM Tris–HCl pH 8.0/1 mM EDTA) and used as B-DNA, or boiled for 10 min and quickly chilled on ice for preparing ssDNA. Z-DNA was prepared using a poly (dG-dC)•poly(dG-dC) as described [26]. The antigen was biotinylated by adding *Escherichia coli* Klenow fragment, dGTP and biotin-dCTP, as recommended by the manufacturer (Invitrogen, Carlsbad, CA). The enzyme was inactivated at 75 °C for 20 min, and the DNA was precipitated with ethanol and resuspended in TE buffer. The biotinylated polynucleotide was brominated as reported previously [26].

2.2. Antibodies

mAb Z22 was obtained as described [17]. The expression and purification of two soluble antibody fragments derived from mAb Z22 in the methylothrophic yeast *Pichia pastoris* was obtained as we previously described [25]. The scFv fragment harbors the V_H and V_L regions (scFv) of the mAb Z22 linked by a hydrophilic $(Gly_4Ser)_3$ peptide and fused to an Ig binding domain of the staphylococcal protein A as a C-terminal tag. The antibody fragment FvFc contains an scFv derived from mAb Z22 fused to an human Fc fragment (hinge–CH2–CH3 IgG1 domains) as a C-terminal tag. In the FvFc fragment, the preserved hinge region allowed the formation of a disulfide bond between monomers of antibody fragment, creating a dimeric and bivalent Ag binding-site.

2.3. Inhibition ELISA

96-well polystyrene microtiter plates (Immulon type I, Dynatec Scientific Laboratories, Inc., El Paso, TX) were pre-coated for 1 h at room temperature with 4 μg/mL streptoavidin in PBS (150 mM NaCl, 10 mM $NaHPO_4$ pH 7.2, 0.002% (w/v) NaN_3) and blocked for 1 h with PBST (PBS/0.05% Tween 20—Sigma) containing 3% BSA (w/v), at room temperature. After washing, the antigen was immobilized by adding 1.5 μg/mL of biotinylated-Z-DNA in PBS. For the inhibition assay, the samples were pre-incubated with varying amounts of related antigens (from 1 to 0.01 μg/mL) in a total volume of 100 μL of PBS in a separated microtiter plate. Remaining bound antibodies were detected as described in Fig. 1. Each sample was tested in duplicate and experiments were repeated at least twice.

2.4. Fluorescence measurements

The fluorescence spectra were obtained using a Jasco FP-777 spectrofluorometer equipped with a thermally controlled cell holder and a quartz cuvette of 1 cm pathlength. The fluorescence emission (λ_{em}) spectra were recorded from 300 to 400 nm, with the path length for fluorescence excitation and emission of 5 mm, at an excitation wavelength (λ_{ex}) of 280 nm. The nucleotide–antibody binding was monitored by changes in the fluorescence emission intensity recorded at 330 nm. In each experiment, the temperature was maintained constant by a Peltier-type temperature controller.

Changes in intrinsic fluorescence of mAb and antibody fragments were used to monitor ligand binding at 20 °C, 25 °C, 30 °C and 35 °C by titration with Z-DNA. All Ag and Ab solutions were prepared in PBS buffer with Milli-Q H_2O. All titrations were performed with varying amounts of Z-DNA added to a fixed protein concentration. It used 30 nM of mAb Z22 and 90 nM of FvFc or scFv fragment. To cover the broad ligand concentration range, stock solutions with concentrations of Z-DNA ranging from 4.79×10^{-8} to 8.72×10^{-7} M were used. A fresh 2-mL solution of mAb Z22 (in PBS pH 7.2) or antibody fragment at the desired concentration was prepared in a 3-mL quartz fluorescent cuvette, and 1 μL aliquots of a concentrated Z-DNA solution were added to the cuvette under constant stirring. At the end of Ag titration, 26 μL of Z-DNA were added, and the final concentration of polynucleotide was 8.83×10^{-9} M for the mAb Z22 assay and 8.04×10^{-9} M for the antibody fragments assay. The equilibrium of the binding reaction was monitored by fluorescence emission at 330 nm, recorded during 10 min after the addition of Z-DNA at intervals of 2 min. Reported values are an average of three independent measurements.

2.5. Guanidinium hydrochloride effect

The chaotropic effect of guanidinium hydrochloride (Gdn–HCl) on the monoclonal antibody and the FvFc fragment was measured at 25 °C. A fresh 2-mL PBS solution containing 30 nM of mAb Z22 or FvFc fragment was prepared in a 3-mL quartz fluorescent cell, and was constantly stirred. These solutions were titrated with 30 μL aliquots of 8 M Gdn–HCl concentrated stock solution in PBS, to a final concentration of 3.5 M. The emission spectra were recorded after 4 min. The obtained values are an average of three independent measurements.

2.6. Analyses of fluorescence titration data and equilibrium dissociation constants

The fluorescence quenching Q after each titration of Z-DNA was determined using the following equation:

$$Q = -(F_{obs} - F_{max})/F_{max} \quad (1)$$

where F_{obs} is the observed fluorescence emission at 330 nm after each addition of Z-DNA and F_{max} is the observed fluorescence emission of the free antibodies, before the addition of Z-DNA. The molar concentration L of Z-DNA bound to all antibody constructions was determined using the following equation:

$$L = (L_o \times F_{obs})/F_{max} \quad (2)$$