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Immuno-capillary electrophoresis for the characterization of a monoclonal antibody against DNA

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Abstract

A monoclonal antibody against DNA established from a mouse strain that spontaneously develops systemic lupus erythematosus was characterized by migration shift immuno-capillary electrophoresis. The minimal size for DNA binding antibody was >16 bases and the interaction with a double-stranded 32-mer oligonucleotide was almost one order of magnitude stronger than the interaction with a single-stranded oligonucleotide. The binding was highly dependent on the ionic strength conditions with an increase in binding with a decrease in ionic strength. The estimate of the dissociation constant for the antibody binding of a single-stranded 32-mer oligonucleotide was 0.62 μM at pH 7.90. This value was in good agreement with the value of 0.44 μM measured by an independent method using biosensor (surface plasmon resonance) technology.

Keywords: Immunoelectrophoresis; Sytemic lupus erythematosus; DNA; Monoclonal antibodies

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown etiology in which the morbidity is associated with immune complex deposits in kidney, brain, and skin [1]. A variety of antibodies against nuclear constituents are present in SLE patients. In particular, circulating antibodies against double-stranded DNA (dsDNA) are a serological hallmark of the disease and anti-nuclear antibodies are part of the criteria for the diagnosis of SLE [1]. Pathological antibodies against DNA are also found in mouse models of SLE such as the NZW×NZB F_1 females [2,3]. The level of anti-dsDNA antibodies in patients and mice correlates with the risk of developing nephritis, but the pathogenesis and possible importance of the affinity of the antibodies for disease initiation and progression are not well understood [4,5]. A part of the problem is the difficulty of measuring the avidity of antibodies against DNA. Often patients are assessed by measuring specific antibody levels in sera by immunosorbent assay methods in which affinity determination of antibodies is not entirely reliable chiefly because of the influence of the solid-phase on non-specific interactions [6–8].

Antigen–antibody interactions and DNA binding proteins may alternatively be characterized quantitatively by electrophoretic mobility shift assays in gels [6,9–11]. Preequilibration of samples followed by nondenaturing gel electrophoresis may give estimates of complexes of different stoichiometry and/or conformation and of binding constants [6,10,11]. This method is only reliable if the ligand binding is tight so that the complexes do not dissociate while entering the gel or during electrophoresis [6,10]. For

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less tight binding, gel electrophoresis in the presence of ligand may be employed. The ensuing migration shifts as a function of ligand concentration allow for binding constant estimation under a limited range of ionic strength and pH conditions [9,12–14]. A general limitation in any of these methods is that the resolving power of native gel electrophoresis is not sufficient to give a precise characterization of complex formation that only lead to minor migration shifts. In contrast, capillary electrophoresis (CE) gives highly efficient separations that often are achieved under non-denaturing conditions and thus are compatible with the study of molecular function such as ligand binding. Accordingly, affinity CE has been used for a large number of binding studies including ligand affinity screening, binding site mapping, binding constant determination, and characterization of structural requirements for ligand binding [15–23]. DNA-binding peptides and proteins have been studied using affinity CE and also binding of antibodies to antigens (immuno-CE) have been explored in CE-based migration shift assays [26]. The parameter involved in CE-based binding studies is migration changes of complexed as compared to free, unbound molecules and this results in migration time shifts of peaks or, for more tightly bound ligands, in area changes of non-ligated acceptor molecules [26].

Another new method for biomolecular interaction analysis is biosensor analysis technology where a plasmon resonance signal from the surface of a biospecific chip changes when the mass increases upon ligand binding. In this way kinetic data may be estimated as has been shown for several different monoclonal antibodies [27–29]. Interactions of DNA with a nuclear repressor protein have also been studied by a biosensor method using immobilization of biotinylated DNA on a streptavidin chip [30].

In the present study the first objective has been to explore the possibility of measuring equilibrium constants electrophoretically for anti-DNA antibodies using as a model system synthetic 16-mer and 32-mer oligonucleotides and a monoclonal mouse antibody (Mab) derived from the NZW×NZB F_1 SLE-mice. The second objective of the study has been to validate the immuno-CE generated estimates of binding constants for this Mab by comparison to results of biosensor experiments based on anti-DNA Mab reacting with streptavidin-immobilized biotinylated oligonucleotides.

The experiments illustrate the characteristics of the binding of this anti-DNA Mab to single-stranded and double-stranded synthetic oligonucleotides with regards to equilibrium binding constant, ionic strength and pH dependency, and oligonucleotide structural requirements.

2. Experimental

2.1. Chemicals

All chemicals were analytical grade from Sigma (St. Louis, MO, USA) except otherwise stated. HPLC grade water was from Merck (Darmstadt, Germany). Streptavidin was from Zymed Laboratories (San Francisco, CA, USA). Synthetic single-stranded 5′-biotinylated random sequence 16- and 32-mer oligonucleotides and non-biotinylated complementary sequence oligonucleotides were produced in-house and by Pharmacia Biotech (Roosendaal, Netherlands). The biotinylated sequences were: Biotin-AACAGCTATGACCATG and Biotin-AACAGCTATGACCATGCCGACTGCATATCCGT. M_r of the sense biotin-ssDNA 32-mer is 10 241, of the antisense biotinylated ssDNA it is 9894 and thus the calculated M_r of the biotin-dsDNA is 20 135. Purity of oligonucleotides was ascertained by CE and their molecular masses were verified by mass spectrometry on a Voyager Elite MALDI instrument (Perspective Biosystems) with delayed ion extraction using 3-hydroxypicolinic acid as a matrix [31] for the analysis of 10–50 pmole of each oligonucleotide. Masses were within 2 units of the expected values. Equimolar 32-mer sense and antisense DNAs were annealed by boiling in 0.15 *M* NaCl for 10 min on a waterbath followed by slow cooling over 1 h to room temperature.

2.2. Anti-DNA monoclonal antibodies

An anti-DNA monoclonal antibody (Mab) was established from a mouse of the NZW×NZB F_1 strain that spontaneously develops SLE. After fusion of spleen cells with the myeloma cell line P3X63Ag8.653 a hybridoma (clone 35I9) that was

strongly positive for anti-DNA antibody activity in indirect immunofluorescence on *Crithidia luciliae* [32] was selected and cultured. The IgG of culture supernatants was purified on Protein G Sepharose (Pharmacia) using acidic elution (0.1 *M* glycine–HCl, pH 2.7) and immediate neutralization (1/20 volume 1 *M* Tris) followed by dialysis at 4°C against Tris-buffered saline (5 m*M* Tris–HCl, pH 7.4, 150 m*M* NaCl). The antibody was of the IgG_{2a}, κ-light chain isotype (mouse monoclonal antibody isotyping kit, Amersham, Buckinghamshire, UK) and was kept at −20°C. Concentrated stock solutions of the Mab preparation were made by filtration at 4000 g_{av} through Ultrafree-MC M_r 30 000 cut-off centrifuge filters (Millipore, Malvern, MA, USA) chasing with 3–4 volumes electrophoresis buffer to a protein concentration of 15 mg/ml. Total protein concentration was estimated using a BCA assay using bovine serum albumin as a standard (Pierce, Rockford, IL, USA).

2.3. CE experiments

A Beckman P/ACE 2050 instrument with sample cooling at 10°C, 27 cm long (20 cm effective length) uncoated fused capillaries (Beckman), and UV-detection at 200 nm was employed. The capillary internal diameter was 50 μm. Sample injection was by timed pressure (1 s injection corresponds to an injected sample volume of approximately 2 nl). Electrophoresis buffer was 0.10 *M* sodium phosphate (45.75 ml 0.2 *M* $Na_2HPO_4 \cdot H_20$ + 4.25 ml 0.2 *M* $NaH_2PO_4 \cdot H_20$ diluted with H_20 to 100 ml and filtered through a 0.22 μm pore size filter before use), pH 8.13 or pH 7.90 unless otherwise specified. Electrophoresis took place at 10 or 8.5 kV with the anode at the injection end of the capillary. The sample mixture for the CE experiments unless otherwise stated was 20 μl Mab stock solution in electrophoresis buffer diluted 1/15 in water (corresponding to 1 mg/ml total IgG) + 8 μl 0.1 mg/ml tyrosine phosphate (Sigma) in water. Different concentrations (0.03–2.19 μM dsDNA and 0.06–1.56 μM ssDNA) of biotinylated oligonucleotides were added to the inlet electrophoresis buffer vial from stock solutions of 0.50 or 0.25 mg/ml in water or 150 m*M* NaCl (dsDNA) in volumes not exceeding 1/10 of the volume of the inlet electrophoresis buffer vial. Between electrophoretic runs the capillary was rinsed for 30 s with water and 30 s with 0.1 *M* NaOH.

2.4. Data handling in CE experiments

Data were handled by the System Gold Software (Beckman Instruments). Inverse detection times ($1/t$) of the Mab peaks in each of the CE experiments were adjusted by subtracting the inverse detection time of a non-interacting marker molecule (tyrosine phosphate) to equalize with regard to variations in electroosmotic flow [23]. Mobility shifts (induced by the presence of oligonucleotide in the buffer) were then expressed as the difference [$\Delta(1/t)$] between the adjusted mean detection time of the experiments from the control value obtained with no ligand added. Curves of $\Delta(1/t)$ as a function of the concentration of oligonucleotide were then drawn to verify the saturability of the system. The dissociation constant (K_D) was estimated by plotting the experimental data according to the Eadie–Hofstee (or Woolf–Hofstee) equation [33,34]: $\Delta(1/t) = \Delta(1/t)_{max} - K_D[\Delta(1/t)/c]$ where $\Delta(1/t)$ as a function of $\Delta(1/t)$/[DNA] yields $-K_D$ as the slope of the best-fit straight line. Results are reported as the means ± S.D. of triplicate experiments.

2.5. Surface plasmon resonance (SPR) measurements

Amine coupling kits, carboxymethylated dextran sensor chips (CM5, certified grade), P20 detergent, and the BIAcore biosensor instrument were from Pharmacia Biotech (Uppsala, Sweden). Data recording, analysis, and reporting were performed by the BIAevaluation version 2.1 software (Pharmacia Biotech). Streptavidin was immobilized onto activated chips using the amine coupling kit as described by the manufacturer. Experiments using biotinylated dsDNA failed because material was lost during the regeneration of the chip. Biotinylated 32-mer ssDNA corresponding to 231 resonance units (RU) was bound to the streptavidin-derivatized surface. Response curves at different concentrations of anti-DNA Mab diluted from a stock solution of 10 μM (1.53 mg/ml) in run buffer (10 m*M* 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES),

pH 7.4, 150 m*M* NaCl, 3.4 m*M* EDTA, 0.05% (v/v) P20 detergent) in the range 50–500 n*M* was recorded (30 μl of Mab at 10 μl/min). Temperature was kept at 25°C. The dissociation curve was then recorded with empty run buffer at 10 μl/min for 3 min. Only experiments in which the same RU level (±5 RU) was reached between runs were used for the calculations. Control experiments were carried out to ensure that the regeneration procedure using 5 m*M* NaOH for 30 s at 10 μl/min did not elute any biotin-ssDNA from the chip and that there was no appreciable binding of Mab either to the blocked carboxymethylated dextran or to the streptavidin-derivatized dextran.

2.6. Data handling in SPR experiments

Mass transport contributions to the response curve were evaluated by the BIAevaluation 2.1 software program and found to be negligible at the level (cf. above) of biotin-ssDNA attached to the sensor chip. After adjusting the baseline experimental curves, dissociation and association rates were calculated by nonlinear curve fitting (BIAevaluation 2.1 software). As CE experiments indicated that a fraction of the Mab preparation was inactive in DNA-binding, half the total Mab concentration was assumed active and thus the concentration of Mab binding sites equalled the total Mab concentration. K_D's corresponding to three different Mab concentrations were then estimated as the ratio between the experimentally derived dissociation and association rate constants.

3. Results and discussion

Using 0.1 *M* phosphate, pH 7.90 as the electrophoresis buffer, the purified anti-DNA Mab was detected as a broad peak just after the electroosmotic flow (Fig. 1A). Under the same conditions the 32-mer ss- and dsDNA had migration times in excess of 7 min (not shown). In the presence of 25 μg/ml (1.24 μ*M*) biotin-dsDNA added to the electrophoresis buffer, the migration time of a reactive Mab fraction (arrow, Fig. 1B) was prolonged indicating that complexation of Mab with DNA occurs during the electrophoresis experiment. The marker molecule (T) and small impurities were not affected by the

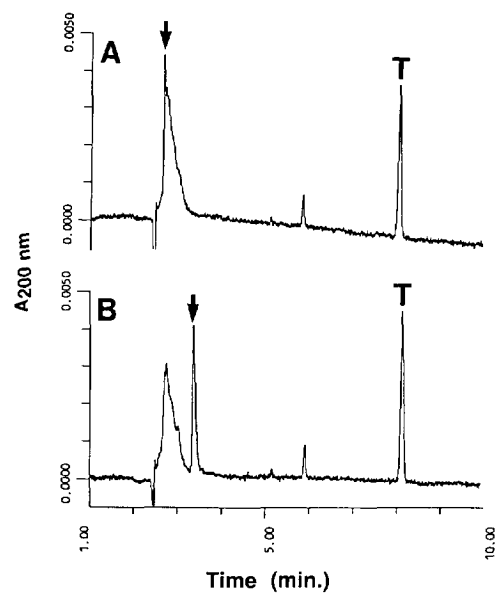

Fig. 1. Immuno-CE demonstration of anti-DNA Mab reaction with dsDNA. Monoclonal antibody (arrow), 0.7 mg/ml with 0.03 mg/ml tyrosine phosphate (T) added as a marker was injected for 2 s using pressure and then analyzed at 10 kV using 0.10 *M* phosphate, pH 7.90 as the electrophoresis buffer. Conditions otherwise correspond to those given in Section 2. In (B), 25 μg/ml (1.24 μ*M*) double-stranded biotinylated 32-mer oligonucleotide was added to the electrophoresis buffer.

presence of ligand in the electrophoresis buffer (Fig. 1B). The variably sized reactive Mab fraction may be due to Mabs which were denatured during the protein G affinity chromatography purification procedure. The reactive Mab fractions, however, showed homogeneous migration shifts of symmetrical peaks in the 0.10 *M* phosphate at pH 7.90 and 8.13 (Figs. 1 and 3). No DNA concentration dependent changes in peak shape or area were observed. This indicates that the binding interactions were sufficiently fast to allow an equilibrium to exist between free Mab and Mab engaged in complexes with oligonucleotide during the time of the electrophoresis experiment [35]. The interaction is, therefore, characterized by fast association and dissociation rates.

A distinct change in the migration shift behavior shown in Figs. 1 and 3 was observed when experiments were performed at a 50% reduced ionic strength (Fig. 2). In this buffer, the magnitude of the observed migration shifts did not depend on DNA

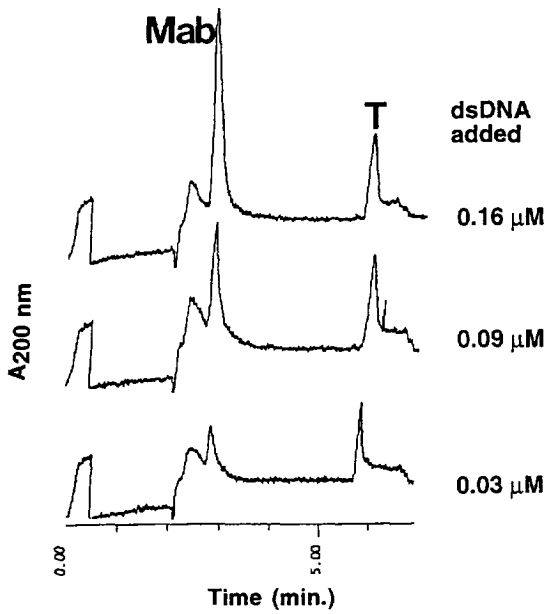

Fig. 2. Immuno-CE of anti-DNA Mab in the presence of various concentrations of 32-mer biotin-dsDNA (A) or 32-mer biotin-ssDNA (B) in the electrophoresis buffer as given in the figure. Sample was a 2 s injection of a mixture of 0.7 mg/ml Mab in water containing 0.03 mg/ml tyrosine phosphate (T) as a marker. CE was performed at 8.5 kV using 0.10 *M* phosphate, pH 8.13 as the electrophoresis buffer.

concentration. Instead, maximally retarded peaks with areas that depended on the dsDNA concentration in the electrophoresis buffer were observed. This suggests that stable complexes which are not in equilibrium with non-bound molecules during electrophoresis are formed at 50 m*M* conditions. Thus, the migration time of the retarded peak is, in this case, a reflection of the mobility of the analyte–ligand complex itself while in the migration shift experiments the observed migration time is indicative of the time spent in complexes during the electrophoresis experiment.

To estimate K_D from migration shifts, immuno-CE experiments were subsequently conducted in the presence of 0.03–2.1 μM of dsDNA and 0.06–1.56 μM ssDNA in 0.1 *M* phosphate, pH 8.13 (Fig. 3). The resulting plots (Fig. 4A and B) showed saturation of the mobility shifts at higher DNA concentrations. After linearization (Fig. 4B), the data yielded K_D's of 0.71 μM and 0.09 μM, for the interaction of the anti-DNA Mab with the 32-mer biotinylated ss- and dsDNA, respectively. Thus, the interaction of this Mab with dsDNA is almost an order of magnitude stronger than the interaction with ssDNA. In this respect the Mab is similar to pathological autoantibodies against DNA associated with SLE in humans [4]. The binding constant estimates are made under the assumption that both ss- and dsDNA only have one binding site for antibody but even if the dsDNA has two binding sites (K_D would then equal 0.18 μM and the occurrence of higher order complexes would be expected) the results still demonstrate a difference in the anti-DNA Mab affinity for ssDNA and dsDNA.

In experiments using the 16-mer biotin-ssDNA oligonucleotide (Fig. 4C), (a 16-mer dsDNA was too unstable (too low a melting temperature) to be used in these CE experiments) it was possible to induce minor migration shifts only at high DNA concentrations (>2.5 μM) and these migration shift data did not yield analyzable curves (data not shown). At the pH 7.90 of the experiments of Fig. 4C, the K_D for the biotin 32-mer ssDNA was calculated to be 0.62 μM which may be compared to the value of 0.71 μM that was obtained at pH 8.13 as shown above (Fig. 4A and B). In similar experiments, the non-biotinylated 32-mer ssDNA was found to have 3–4 times lower affinity (K_D=2.07 μM, data not shown) than the complementary biotinylated ssDNA. Whether this is due to small differences in base composition or to sequence-specific binding and/or conformational differences (e.g. non-B-DNA structures) [36] including conformations that may be induced by biotinylation is not known. None of the oligonucleotides contains purine-rich stretches or adenosine-triplets [36].

Even though the K_D estimates from mobility shifts in electrophoresis are based on equations that assume a much lower analyte concentration than ligand concentration [14,23,37] simple straight-lined relationships were found (Fig. 4B and data not shown) when plotting data according to these equations disregarding the fact that the Mab and DNA concentrations were in the same range. The concentrating effect of analyte zone sharpening during electrophoresis due to stacking effects would make the precise Mab concentration difficult to calculate even though the fraction of reactive antibody was clearly visualized by the experiments.

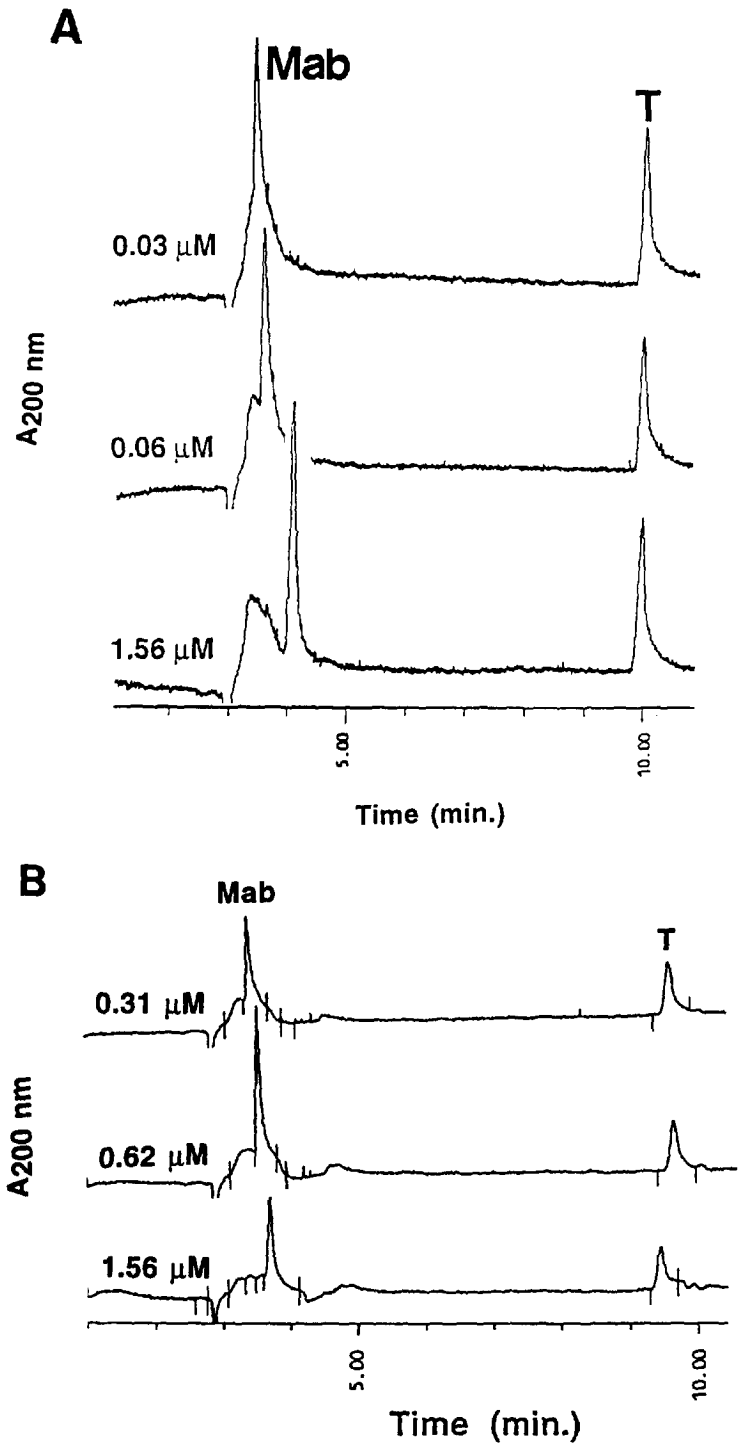

Fig. 3. Ionic strength dependency of anti-DNA Mab binding dsDNA. CE performed in 0.05 *M* phosphate, pH 8.13 in the presence of increasing amounts of dsDNA as given in the figure. Experiments performed at 10 kV after injection for 2 s of a mixture containing 0.5 mg/ml Mab and 0.03 mg/ml tyrosine phosphate (T) in water.

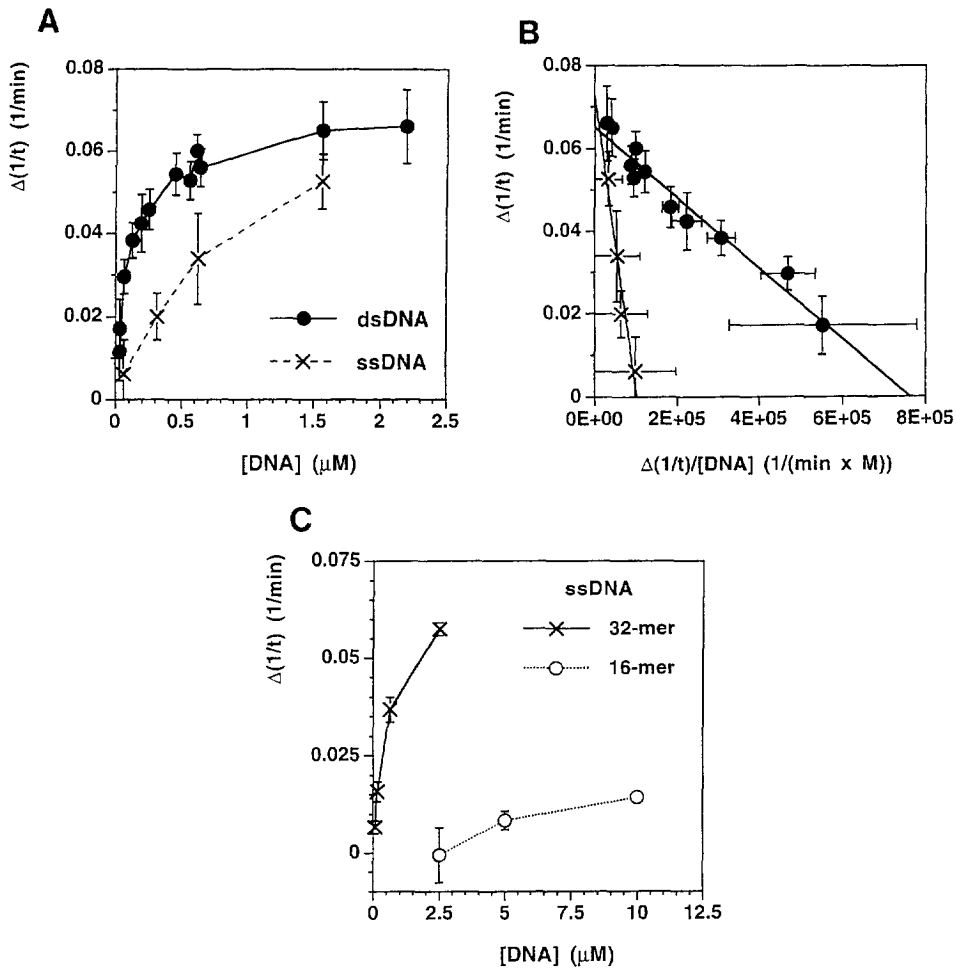

Fig. 4. Immuno-CE derived binding curves for the interaction of anti-DNA Mab with: (A) and (B), 32-mer biotin-ssDNA (×) and 32-mer biotin-dsDNA (●); (C), 32-mer biotin-ssDNA (×) and 16-mer biotin-ssDNA (○). Data depicted in (A) are differences in inverse corrected migration times ($\Delta(1/t)$) as a function of the concentration of DNA present in the electrophoresis buffer. Data are from experiments corresponding to the ones shown in Fig. 3. (B) is the linearization of the data of (A) using the Eadie–Hofstee (or Woolf–Hofstee) equation (cf. Section 2.4. Dissociation constants (K_D) were estimated from the slope of the best-fit straight lines ($r^2 \geq 0.90$ for both). For the interaction of Mab with dsDNA, a K_D of 0.09 μM was calculated while for the ssDNA interaction the K_D was 0.71 μM. Data in (C) are from migration shift experiments conducted at 10 kV in 0.1 M phosphate, pH 7.90. Data points are the mean±S.D. of triplicate experiments.

The theory for the electrophoretic derivation of K_D [38] links mobility shifts induced by ligands to equilibrium binding constants. Since mobility is proportional to the inverse of migration time (t), the use of migration time shifts (Δt) instead of inverse migration time shifts [$\Delta(1/t)$] as pointed out by Shimura and Kasai [23] will lead to errors especially when the shifts are not very small. Throughout this study we have therefore used inverse migration times. Also, instead of the double reciprocal plot often used [9,14,17,39], an Eadie–Hofstee transformation for linearization has been employed as this plot is more sensitive than e.g. the double reciprocal plot to deviations from linearity and less sensitive to variations in low values [40,41].

In an attempt to verify the electrophoretically derived K_D data by an independent method, the anti-DNA Mab interaction with DNA was also characterized by biosensor measurements using the 32-mer ssDNA immobilized through its biotin-moie-

ty to a streptavidin-coated chip. The method is based on analyte-induced (concentration dependent) changes in the refractive index (SPR) at the biospecific sensor chip surface. In the experiments (Fig. 5) association rate constants in the range of 1.0–$1.3\times10^5\ M^{-1}s^{-1}$ were obtained with χ^2 values below 1.1. A model based on a parallel dissociation of two complexes was chosen for the fitting routine of the dissociation phase because experimental data from the dissociation phase of the response curve did not fit a simple single site dissociation model. Of the two dissociation rate constants that were generated by the curve fitting program, only the rate constant (k_1) corresponding to rapidly dissociating complexes (dissociation of monomeric complexes, i.e., one DNA molecule bound to one antibody molecule) was used for the estimation of K_D. The values of k_1 in the three experiments (Fig. 5) were from 0.04–0.06 s^{-1} based on the data modelling (χ^2 values below 0.025, offset values from −0.237 to −0.0257). On the basis of these values a K_D for the interaction of anti-DNA Mab with immobilized biotin-ssDNA was estimated to be 0.44 $\mu M\pm0.06\ \mu M$. This value which is based on experiments performed at pH 7.4 is in good agreement with the immuno-CE derived values at pH 7.90 and 8.13 of 0. 62 μM and 0.71 μM, respectively.

An estimation by SPR measurements of the K_D for the interaction of the anti-DNA Mab with dsDNA did not succeed, because the regeneration of the chip (elution of bound Mab) between measurements detached biotinylated dsDNA (not shown) and thus changed the binding characteristics of the chip. This particular problem is not encountered in CE where neither ligand nor analyte is reused. A general drawback of the biosensor approach not found in CE is that a measurable signal requires analytes to be at least of $M_r=2000$–$10\,000$ [27,28,42]. Additionally, mass transfer effects [27,28] may complicate interpretation of response curves in SPR experiments. However the capability of directly measuring interaction kinetics is unique for SPR. Only in selected cases will this be possible based on analysis of peak shape changes in CE [21].

4. Conclusions

The present study illustrates the ability of immuno-CE to characterize ligand structural requirements, heterogeneity, and ionic strength dependency of binding, and binding constants for anti-DNA antibodies.

The CE-based estimation of binding constants for the anti-DNA Mab interaction with ssDNA was in good agreement with the results of biosensor mea-

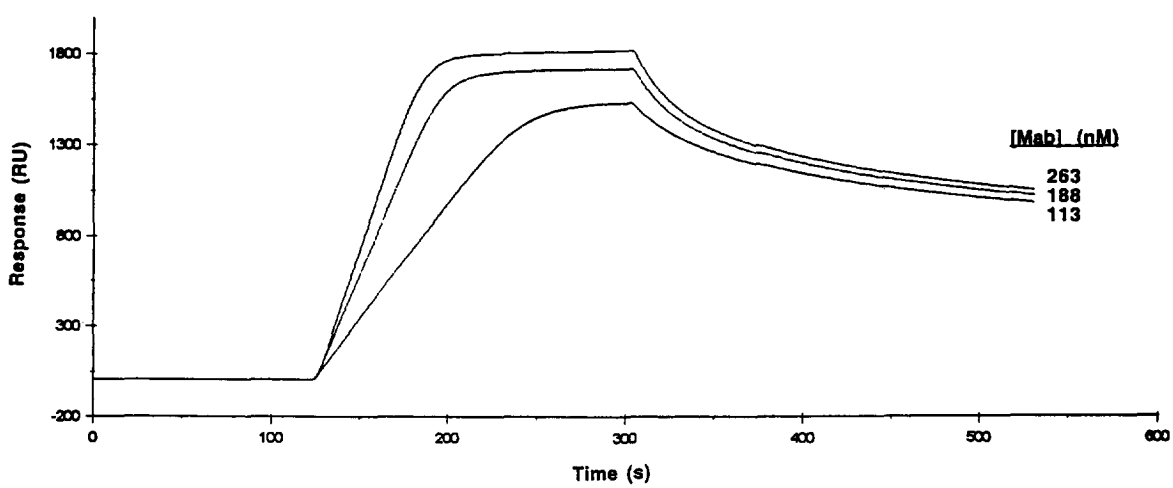

Fig. 5. Overlay plot of response curves from SPR measurement of anti-DNA Mab interaction with streptavidin-immobilized 32-mer biotin-ssDNA. The curves represent the resonance responses (RU) as a function of time at 113, 188, and 263 nM Mab as indicated on the figure.

surements. The small difference in the values is easily accounted for by the differences in pH and composition of the buffers used in the two experimental systems. By CE it was shown that the binding of the anti-DNA Mab to DNA is highly dependent on the ionic strength conditions and that the binding is pH dependent with a decrease in the K_D when decreasing the pH from 8.13 to 7.90. It was also demonstrated that biotinylated ssDNA had a 3–4 times increased affinity for the Mab as compared to the non-biotinylated ssDNA. Further, the CE experiments suggested that a minimal oligonucleotide size for binding is >16 bases and indicated that the binding to dsDNA is stronger than the binding to ssDNA such as is the case for pathological SLE-associated anti-DNA autoantibodies in humans. Finally, in CE it was possible to directly visualize the fraction of non-reactive Mab in the migration shift experiments. The limitations of the biosensor method were illustrated by the difficulties in obtaining data for the Mab interaction with dsDNA due to a gradual loss of dsDNA from the chip during regeneration procedures.

For CE-based analyses of patient samples containing polyclonal anti-DNA antibodies it will be preferable to use the DNA as the analyte and e.g. serum dilutions as ligand addition to the electrophoresis buffer. However, when using uncoated fused-silica capillaries a severe variability in migration times was observed even when using the purified monoclonal antibody preparation of this study at a relatively high ionic strength (i.e., 0.1 *M* sodium phosphate). The use of capillaries with a hydrophilic, neutral coating for the purpose is now under investigation.

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References

[1] P.H. Schur, in W.N. Kelley, E.D. Harris, S. Ruddy and C.B. Sledge (Editors), Textbook of Rheumatology, Saunders, Philadelphia, 1993, p. 1017.
[2] S.N. Theofilopoulos, in R.G. Lahita (Editor), Systematic Lupus Erythematosus, Churchill Livingstone, New York, 1992, p. 121.
[3] A.N. Theofilopoulos and F.J. Dixon, Adv. Immunol., 37 (1988) 269.
[4] D. Buskila and Y. Shoenfeld, in R.G. Lahita (Editor), Systemic Lupus Erythematosus, Churchill Livingstone, New York, 1992, p. 205.
[5] J.B. Katz, W. Limpanasithikul and B. Diamond, J. Exp. Med., 180 (1994) 925.
[6] S.Y. Stevens, P.C. Swanson and G.D. Glick, J. Immunol. Methods, 177 (1994) 185.
[7] W.P. Jencks, Proc. Natl. Acad. Sci. USA, 78 (1981) 4046.
[8] S.J. Seligman, J. Immunol. Methods, 168 (1994) 101.
[9] N.H.H. Heegaard and O.J. Bjerrum, Anal. Biochem., 195 (1991) 319.
[10] D.F. Senear and M. Brenowitz, J. Biol. Chem., 226 (1991) 13661.
[11] M.G. Fried, Electrophoresis, 10 (1989) 366.
[12] V. Matousek and V. Horejsí, J. Chromatogr., 245 (1982) 271.
[13] K. Takeo and E.A. Kabat, J. Immunol., 121 (1978) 2305.
[14] T.C. Bøg-Hansen and K. Takeo, Electrophoresis, 1 (1980) 67.
[15] N.H.H. Heegaard, H.D. Mortensen and P. Roepstorff, J. Chromatogr. A, 717 (1995) 83.
[16] N.H.H. Heegaard and F.A. Robey, Anal. Chem., 64 (1992) 2479.
[17] N.H.H. Heegaard and F.A. Robey, J. Immunol. Methods, 166 (1993) 103.
[18] Y.-H. Chu, L.Z. Avila, H.A. Biebuyck and G.M. Whitsides, J. Org. Chem., 58 (1993) 648.
[19] Y.-H. Chu, L.Z. Avila, H.A. Biebuyck and G.M. Whitsides, J. Med. Chem., 35 (1992) 2915.
[20] Y.-H. Chu and G.M. Whitesides, J. Org. Chem., 57 (1992) 3524.
[21] L.Z. Avila, Y.-H. Chu, E.C. Blossey and G.M. Whitesides, J. Med. Chem., 36 (1993) 126.
[22] S. Honda, A. Taga, K. Suzuki, S. Suzuki and K. Kakehi, J. Chromatogr., 597 (1992) 377.
[23] K. Shimura and K. Kasai, Anal. Biochem., 227 (1995) 186.
[24] N.H.H. Heegaard and F.A. Robey, J. Liq. Chromatogr., 16 (1993) 1993.
[25] J. Xian, M.G. Harrington and E.H. Davidson, Proc. Natl. Acad. Sci. USA, 93 (1996) 86.
[26] N.H.H. Heegaard, J. Chromatogr. A, 680 (1994) 405.
[27] R. Karlsson, A. Michaelsson and L. Mattsson, J. Immunol. Methods, 145 (1991) 229.

[28] R. Karlsson, L. Fägerstam, H. Nilshans and B. Persson, Anal. Biochem., 166 (1993) 75.
[29] M. Malmqvist, Curr. Opinion Immunol., 5 (1993) 282.
[30] J.-P. Jost, O. Munch and T. Andersson, Nucl. Acid. Res., 19 (1991) 2788.
[31] F. Kirpekar, E. Nordhoff, K. Kristiansen, P. Roepstorff, A. Lezius, S. Hahner, M. Karas and F. Hillenkamp, Nucleic Acids Res., 22 (1994) 3866.
[32] R. Smeenk, in W.J. Van Venrooij and R.N. Maini (Editors), Manual of biological markers of disease. Section A, Kluwer Academic, Dordrecht, 1993, p. 1.
[33] C.R. Cantor and P.R. Schimmel, Biophysical Chemistry. Part III: The behavior of biological macromolecules, Freeman, New York, 1980.
[34] J.B.S. Haldane, Nature, 179 (1957) 832.
[35] N.H.H. Heegaard, Appl. Theor. Electrophor., 4 (1994) 43.
[36] M. Herrmann, T.H. Winkler, H. Fehr and J.R. Kalden, Eur. J. Immunol., 25 (1995) 1897.
[37] V. Horejsí, J. Chromatogr., 178 (1979) 1.
[38] S. Nakamura and T. Wakeyama, J. Biochem. (Tokyo), 49 (1961) 733.
[39] K. Takeo, in A. Chrambach, M.J. Dunn and B.J. Radola (Editors), Advances in electrophoresis. Vol. I, VCH Verlag, Weinheim, 1987, p. 229.
[40] M. Dixon and E.C. Webb, Enzymes, Longman, London, 1979.
[41] J.E. Dowd and D.S. Riggs, J. Biol. Chem., 240 (1965) 863.
[42] BIAtechnology Handbook, Pharmacia Biosensor, Uppsala, 1994.