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Kinetic analysis of human topoisomerase IIα and β DNA binding by surface plasmon resonance

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Abstract Topoisomerase IIβ binding to DNA has been analysed by surface plasmon resonance for the first time. Three DNA substrates with different secondary structures were studied, a 40 bp oligonucleotide, a four way junction and a 189 bp bent DNA fragment. We also compared the DNA binding kinetics of both human topoisomerase isoforms under identical conditions. Both α and β isoforms exhibited similar binding kinetics, with average equilibrium dissociation constants ranging between 1.4 and 2.9 nM. We therefore conclude that neither isoform has any preference for a specific DNA substrate under the conditions used in these experiments.

Key words: DNA binding; Topoisomerase II; Surface plasmon resonance

1. Introduction

DNA topoisomerase II (EC 5.99.1.3; topo II) catalyses DNA strand passage in an ATP dependent manner enabling relaxation of supercoiled DNA and decatenation of DNA. Binding of topo II to the DNA is the first step in their catalytic cycle. DNA binding does not require magnesium ions [1,2] or ATP [3] but is inhibited by increasing KCl concentration with maximal binding at 50 mM [2–4]. Footprinting studies have shown that eukaryotic topo II protects 25–28 bp of DNA surrounding each cleavage site [4,5]. Mammals have two isoforms of DNA topo II, α and β. During mitosis the α isoform associates with chromatin, whilst the β isoform diffuses away from the chromatin [6,7].

Type II enzymes bind more strongly to supercoiled DNA compared to relaxed DNA or linear B form DNA [8]. Topo II may recognise supercoiled DNA by detecting DNA secondary structure such as the regions of helix–helix juxtaposition or crossovers. *Drosophila* topo II can interact with DNA crossovers independent of the DNA sequence [9]. Human and *Drosophila* topo II have a higher affinity for Z and bent DNA [10–12], however, yeast topo II had no preference for bent DNA [13]. Binding of human (h) topo IIβ to four way junction (4wj) DNA was stronger (K_D 29 nM) than binding to linear DNA (K_D 130 nM) as shown by gel shift assay [14]. The human isoforms have been shown to bind preferably to curved compared to linear DNA [10]. Another study suggests that the α isoform has a preference for AT rich sequences and the β isoform prefers GC rich sequences [15]. Here we present for the first time a surface plasmon resonance (SPR) analysis of the kinetics of topo IIβ DNA binding and compare it to the binding kinetics of topo IIα to the same three substrates under identical SPR conditions.

2. Materials and methods

2.1. Materials and equipment

SPR experiments were performed with a BIAcore™ 2000 biosensor system (Biacore, Uppsala, Sweden) and BIAcore SA streptavidin chips. Human DNA topo IIα and β were purified as described previously [16]. Chemicals were purchased from Sigma (Poole, UK). All oligonucleotides were synthesised and purified by high performance liquid chromatography (from Invitrogen or Sigma).

2.2. DNA substrates

The linear 40 bp substrate contains a single mAMSA cleavage site and allows the binding of one topo II dimer [14]. The 4wj substrate contains the sequence of the 40 bp linear substrate along two adjacent arms, with the cleavage site straddling the point of strand exchange [14]. The bent DNA substrate is a 189 bp AT rich region from the *Drosophila* genome that forms a bend [17].

Formation of the 4wj was achieved by annealing 1 nmol of each oligonucleotide (5′-biotin-CTGGACGCAATCTGACAATGCGCTCATCGTCATCCTCGGCACGCGCCG-3′; 5′-CGGCGCGTGCCGAGGATGACGATGAGTTAGGCGTTAACGCGGCCTA-3′; 5′-TAGGCCGCGTTAACGCCTAATTGCCCGGGAGTACCGGCATTCCT-3′; 5′-AGGAATGCCGGTACTCCCGGGCAACGCATTGTCAGATTGCGT-3′) as described in [14]. This 4wj was immobilised on the BIAcore chip. The 4wj structure on the sensor chip was verified by the reduction of RU (resonance units, one unit is defined as a 10^{-4} degree shift in resonance angle) by NaOH (50 mM) after completion of experiments. NaOH injection separates complementary DNA strands, this caused a 75% reduction in RU since only one of the four oligonucleotides was immobilised on the surface.

The bent DNA was amplified from plasmid pQ189 [17] using two primers (5′-biotin-GAGCTCGAATTCTCCACACA-3′ and 5′-CTATAGGGCGAATTCTTTTC-3′). A 3 bp overhang was introduced at the biotin end of the DNA in order to eliminate possible steric effects during immobilisation on the sensor chip. To achieve this, the exchange reaction of T4 DNA polymerase was utilised.

2.3. Preparation of the SPR sensor chip

The DNA substrate molecules were immobilised onto the streptavidin coated SA sensor chips (BIAcore) by the capture of their biotinylated moiety. Before any DNA immobilisation the sensor chips were activated by three 1 min injections of 50 mM NaOH, followed by 0.5 M NaCl injections. The amount of DNA immobilised on the chip was kept low to minimise mass transport effects. The BIAcore sensor chips contain four flow cells, one was left without DNA as a control for non-specific binding. Three flow cells contained different biotin labelled DNA substrates. The amount of DNA bound was kept low by keeping the RU below 150 as advised by BIAcore (www.bia-

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core.com), so that the chip is not totally saturated with DNA. This helps ensure that binding is specific for the DNA substrates and is not due to charge effects of a chip totally covered in DNA, it also minimises the difference between the control flow cell and the three test cells. The DNA was immobilised at a flow rate of 5 μl/min (25°C), the chip was then washed with 0.5 M NaCl to remove unbound ligand. The 40 bp oligonucleotide was annealed on the chip in two steps; first the biotin labelled strand was immobilised, then the complementary strand was injected, (5′-biotin-CGCAATCTGACAATGCGCT-CATCGTCATCCTCGGCACGCG-3′; 5′-CGCGTGCCGAGGAT-GACGATGAGCGCATTGTCAGAT-3′). The 4wj and bent DNA structures were prepared before immobilisation. The amount of DNA that was immobilised on the sensor chip was 84 RU for linear oligonucleotide, 135 RU for 4wj DNA and 33 RU for bent DNA. In a single experiment binding to three different DNA substrates was monitored.

2.4. SPR conditions for topo II DNA binding

The binding buffer was 50 mM Tris–HCl pH 7.7, 1 mM EDTA, 1 mM EGTA, 100 mM KCl, 1 mM ATP, 0.05% (v/v) Tween p20 and 1% (v/v) Triton X-100. Protein samples were injected using the BIAcore function 'kinject' at a flow rate of 70 μl/min. The protein was injected for 60 s and the dissociation phase was recorded for 180 s, at 25°C. After injection the chip was regenerated by a 30 s pulse of 0.5% (w/v) sodium dodecyl sulphate followed by a 60 s injection of 0.5 M NaCl. The concentration range of protein used was 5–60 nM. When protein becomes bound to DNA on the sensor chip, the refractive index of the medium at the chip surface is affected. This alteration is referred to as a change in RU, which represents an indirect measure of the amount of bound protein.

2.5. Analysis of kinetic data

The association and dissociation data were modelled with BIAevaluation software, version 3.0 (Pharmacia Biosensor AB, Uppsala, Sweden). Following subtraction of data from the control flow cell to remove non-specific binding, all curves were fitted by the numerical integration method as described in the BIA evaluation handbook (www.biacore.com). The residual plots and the χ^2 values were used to assess the appropriateness of the various models for analysing the sensor data. Residual plots are a measure of the difference between the experimental data and the curve calculated or fitted from the model. χ^2 is the standard statistical measure used in SPR experiments to analyse the closeness of the fit between the experimental data and the model. If the data fit the model exactly, χ^2 represents the signal noise. Typically values of χ^2 below 10 represent acceptable use of a binding model as recommended by BIAcore (www.biacore.com). Topo II DNA binding data were produced for various protein concentrations and were fitted simultaneously, assuming the 1:1 Langmuir model.

The Langmuir model assumes that the analyte is both monovalent and homogeneous and the ligand is also homogeneous and that binding events are independent. The binding of topo II to DNA follows first order kinetics: $A+B \leftrightarrow AB$ [13,14,18]. The kinetics of the Langmuir model are described in [19]. The fitting parameters provide estimates of both association and dissociation rate constants (k_a and k_d) and from these values the equilibrium constants K_A and K_D were calculated ($K_D = k_d/k_a$ and $K_A = k_a/k_d$).

3. Results and discussion

3.1. Binding of htopo IIβ to bent DNA

The binding of different concentrations of htopo IIβ to bent DNA substrate is shown in Fig. 1. The data were fitted to the 1:1 Langmuir binding model. The association phase fitted well and the χ^2 values were less than 2 (fitted lines not shown). The initial stage of the dissociation phase was rapid followed by a slower dissociation rate, fitting of dissociation data to a single rate constant gave χ^2 values of up to 10. This phenomenon became more apparent as the protein concentration increased and is illustrated in Fig. 1 by showing the fitting curves as black lines; they clearly fit better at the lower protein concentrations. The residuals of the association phase follow a random pattern, whilst the residuals of the dissociation phase show a non-random pattern (data not shown). For experiments using topo IIβ at 10 nM and 15 nM the data clearly fit the Langmuir model but at 20 nM the first 20 s of the dissociation phase do not fit the model well (Fig. 1), accounting for the non-random error observed in the residuals of the dissociation phase. Our analysis of the results was restricted to the lower protein concentrations and to the last 150 s of the dissociation phase. A possible explanation to account for the poor fit of the dissociation data for the first 20–30 s of dissociation would be if the htopo IIβ binding is described by a two state model with a conformational change instead of the 1:1 Langmuir isotherm. The data were fitted using the two state model; they did not fit and the possibility that we were observing a two state reaction was excluded (data not shown).

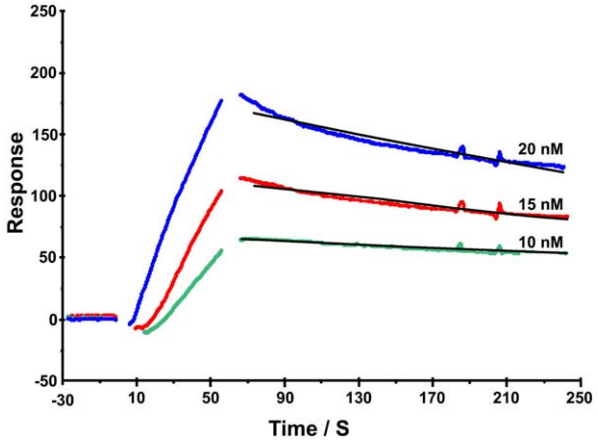

Fig. 1. Binding of bent DNA to human DNA topo IIβ. The binding of htopo IIβ to bent DNA was examined as a function of protein concentration. The response is measured in RU. The association phase was for 60 s and the dissociation phase for 180 s. The fitting curves for the dissociation are indicated as a black line fitted on the experimental curves. The χ^2 of the association phase was 0.120 and of the dissociation phase 2.950.

3.2. Htopo IIβ binds to linear, bent and 4wj DNA with similar affinity

The binding of different concentrations of htopo IIβ to linear, 4wj and bent DNA was analysed. Fig. 2 shows the protein binding (RU) against time (s). The different magnitudes of the RU seen for the three substrates in Fig. 2 reflect the different amounts of each DNA substrate bound on each flow cell. Rate constants of the association k_a (M^{-1} s^{-1}) and the dissociation k_d (s^{-1}) phases were estimated from the data and from these the equilibrium constants K_A and K_D were calculated. The constants of association and dissociation are similar for linear, bent and 4wj DNA and are summarised in Table 1A. The average k_d varied between 2.14 and 3.32 $s^{-1} \times 10^{-3}$ for the three substrates, indicating that between 0.2 and 0.3% of the complexes dissociated per second. The calculated K_D values were 1.73 nM, 1.86 nM and 2.85 nM, respectively. Thus htopo IIβ has no preference for any of the three DNA substrates bound to the sensor chip.

Using electrophoretic mobility shift assay (EMSA) htopo IIβ showed a fourfold preference for 4wj DNA [14] which is not observed by SPR; a preference for 4wj in EMSA may be due to the lengthy gel running process during which time linear DNA may dissociate from the topo II more easily

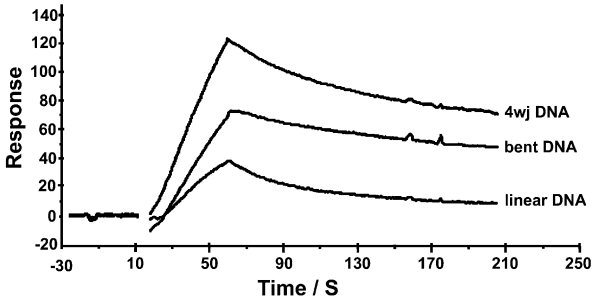

Fig. 2. SPR experiments showing binding of htopo IIβ (6.6 nM) to linear DNA, bent DNA and 4wj. The plot shows the response in RU as a function of time.

than 4wj DNA. SPR measures binding in real time without a lengthy gel running process, also as the DNA is attached to the chip at one end protein can only leave the linear DNA from one side.

3.3. Htopo IIα interaction with linear, bent and 4wj DNA

The binding of increasing concentrations of htopo IIα to linear (data not shown), 4wj and bent DNA (Fig. 3) was analysed. As seen for htopo IIβ the association phase fits very well to the binding model but the dissociation phase showing an initial rapid dissociation followed by a slower dissociation rate. Table 1B shows the k_d and k_a, and the K_A and K_D for binding of htopo IIα to the three DNA substrates; htopo IIα has no preference for any of the three DNA substrates.

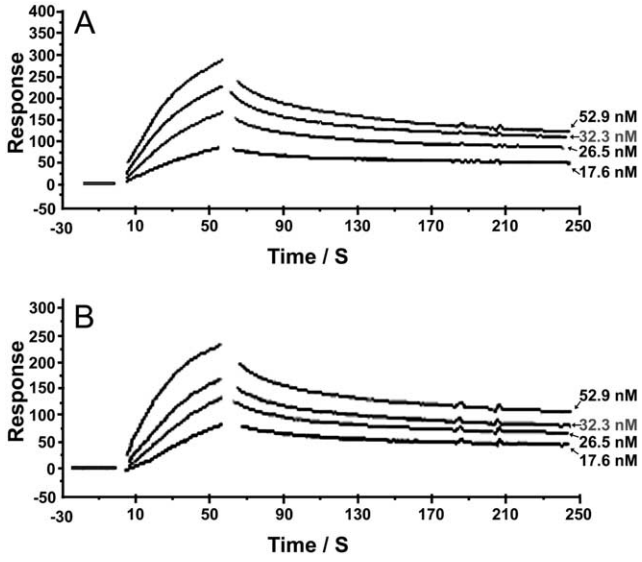

Fig. 3. An SPR experiment showing binding of increasing concentrations of htopo IIα to 4wj DNA (A) or bent DNA (B). The response is measured in RU.

Previously a lower K_D of 0.16 nM was reported for htopo IIα binding to linear DNA by SPR [18]. The activation energy

Table 1
Kinetic and association constants of human DNA topoisomerase II DNA binding

	k_a (M^{-1} $s^{-1}\times10^6$)	k_d ($s^{-1}\times10^{-3}$)	K_A ($M^{-1}\times10^8$)	K_D ($M\times10^{-9}$)	Exp. no.
A: Htopo IIβ (nM)					
Linear DNA					
11	1.91 (±0.67)	3.49 (±0.07)	5.47	1.83	3
16.5	2.02 (±0.66)	3.45 (±0.46)	5.85	1.72	3
22	1.83 (±0.45)	3.01 (±0.26)	6.10	1.64	4
Average	1.92 (±0.09)	3.32 (±0.27)	5.7	1.73	10
Bent DNA					
11	1.35 (±0.32)	1.92 (±0.39)	7.03	1.42	3
16.5	1.16 (±0.32)	2.17 (±0.48)	5.34	1.87	3
22	1.10 (±0.26)	2.62 (±0.84)	4.20	2.38	4
Average	1.20 (±0.13)	2.24 (±0.35)	5.36	1.86	10
4wj DNA					
11	0.57 (±0.33)	2.19 (±0.28)	2.60	3.84	3
16.5	1.02 (±0.23)	2.25 (±0.56)	4.53	2.20	2
22	0.66 (±0.20)	1.98 (±0.31)	3.33	3.00	3
Average	0.75 (±0.24)	2.14 (±0.14)	3.50	2.85	8
B: Htopo IIα (nM)					
Linear DNA					
10	3.86 (±1.97)	3.79 (±0.00)	10.2	0.98	2
15	1.60 (±0.37)	2.51 (±1.31)	6.37	1.57	2
17.6	1.55 (±0.98)	3.22 (±0.84)	4.81	2.08	3
Average	2.34 (±1.32)	3.17 (±0.64)	7.38	1.35	7
Bent DNA					
15	2.02 (±0.98)	2.83 (±1.52)	7.14	1.40	3
17.6	1.25 (±0.87)	1.83 (±0.80)	6.83	1.46	4
26.5	0.75 (±0.21)	2.04 (±0.50)	3.68	2.72	4
Average	1.34 (±0.64)	2.23 (±0.53)	6.00	1.66	11
4wj DNA					
15	0.84 (±0.72)	1.65 (±0.09)	5.10	1.96	2
17.6	1.26 (±1.13)	2.18 (±0.09)	5.78	1.73	2
20	0.28 (±0.03)	1.99 (±0.14)	1.41	7.10	2
Average	0.79 (±0.49)	1.94 (±0.27)	4.10	2.40	6

A: The association (k_a) and dissociation (k_d) of htopo IIβ to linear, 4wj and bent DNA as a function of enzyme concentration. B: The association (k_a) and dissociation (k_d) of htopo IIα to linear, 4wj and bent DNA as a function of enzyme concentration. The rate constants described here are the average of at least two independent experiments and the standard deviation is shown in parentheses.

for htopo IIα binding to DNA was estimated to be −13.34 kcal/mol from our data and −11.95 kcal/mol from the results reported previously [18], this 2 kcal/mol difference is not significant. The SPR conditions used in the topo IIα [18] study were different which may account for the different K_D.

4. Conclusions

The Langmuir model has previously been applied to both htopo IIβ [14] and htopo IIα DNA binding properties [18]. The data for the association of htopo II isoforms to DNA substrates, k_a, fitted the binding model well giving low values of χ^2. The dissociation data at low protein concentrations also fitted the binding model well. However, at high protein concentrations some deviation from the Langmuir model was observed, a rapid dissociation was seen at the beginning of the dissociation phase. This agrees with data where htopo IIα showed a similar rapid dissociation at higher protein concentrations (> 20 nM) [18]. The rapid initial dissociation at high protein concentrations is intriguing. Topo II has been shown to be a dimer in solution by both biochemical [20–23] and structural studies [24], it can also form larger, at least tetrameric complexes in vitro [25]. This aggregation increased at higher protein concentrations, whilst dilution of the protein reduced the protein aggregates in a linear fashion [25]. At higher protein concentrations such aggregates may bind to the DNA with one dimer directly interacting with the substrate. We propose the initial rapid dissociation phase seen at high protein concentration is probably the aggregates of topo II rapidly dissociating from the chip, whilst the topo II dimers bound directly to the DNA dissociate more slowly (Fig. 4), this model is supported by the observation that it is concentration dependent and such concentration dependent aggregates have been reported previously [25]. However, an alternative explanation could be that the different dissociation rates derive from two different DNA binding modes to topo II, for example, a rapid phase due to loose binding of a 'T' or transported DNA segment to the ATPase domain of the protein and a slower phase due to tighter binding of a 'G' or gate DNA segment to the central domain of the protein.

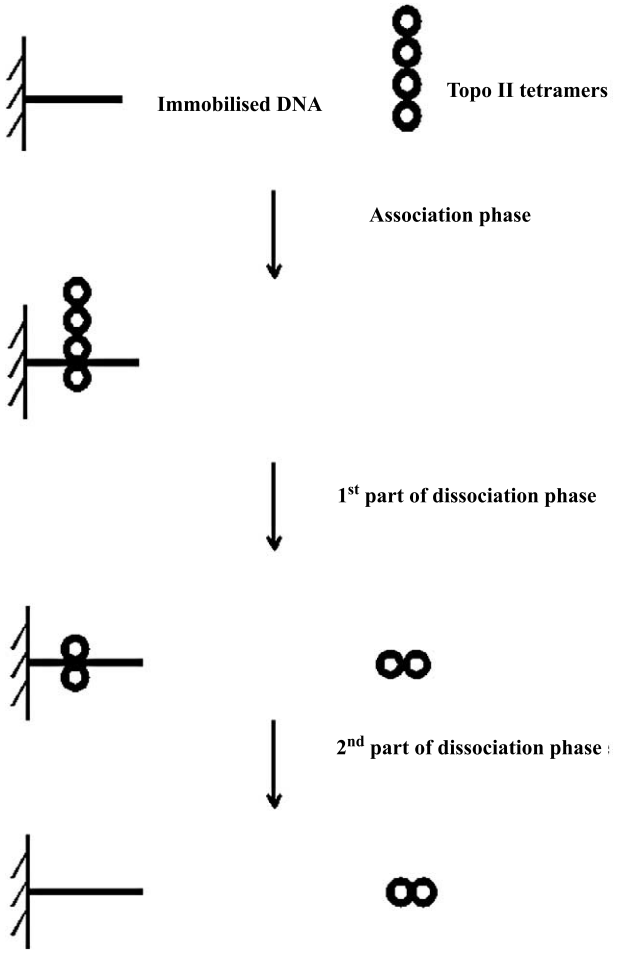

Fig. 4. Schematic diagram of topo II binding to DNA. Topo II in solution occurs as dimers or aggregates (e.g. tetramers shown here). During the association phase topo II dimers and aggregates will bind to the DNA. At the beginning of the dissociation phase the aggregates will dissociate rapidly from the DNA. The topo II dimers that are directly bound to the DNA will dissociate at a much slower rate. This schematic shows the binding of topo II tetramers to DNA, however this does not exclude the presence of dimers or higher order multimers in the reaction.

This is the first report of topo IIβ DNA binding measured by SPR, and the first comparison of the DNA binding by both human isoforms by SPR. Neither isoform showed a significant preference for any of the three DNA substrates with different secondary structures. Both bound with comparable high affinity to the three DNA substrates with K_D values between 1 nM and 3 nM, thus any cellular functional differences between topo IIα and β are not reflected in their intrinsic DNA binding abilities. This affinity is comparable to that measured by SPR for human DNA polymerase β [26]. This reflects the fact that topoisomerases and polymerases do not require specific sites on the DNA in contrast to transcriptional activators or repressors that recognise very specific target sites.

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