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Hysteretic behaviour of a Z-DNA-antibody complex

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The amount of complex observed between Z-DNA in the supercoiled DNA from plasmid pFP332 (with $d(C\text{-}G)_{16}$ cloned into pUC8) with the radiolabelled monoclonal antibody Z-D11 (with very high affinity for left-handed Z-DNA) depends on the temporal order of addition of the ligands. If the antibody is bound first a 20–30-fold higher cloroquine concentration is necessary to dissociate half of the complex compared to the case where the suprahelical density is changed first and the complex formed afterwards. This hysteretic behavior is observed for weeks and presents a kind of molecular memory system, which is based on the topological and conformational properties of DNA and the high-affinity protein binding to Z-DNA.

1. Introduction

DNA shows a considerable polymorphism which depends on the sequence, i.e., the chemical structure of these molecules, and the external conditions, i.e., the chemical activity of different small and/or large molecules in solution [1]. One of the best studied examples is the change between right- and left-handed double-helical DNA, which was first observed in the late sixties in Göttingen in high salt solution for a simple DNA, poly(dG-dC) · poly(dG-dC) [2,3]. The final X-ray structure determination of oligo(dC-dG) in single crystals then revealed the structural details of the left-handed Z-DNA at atomic resolution [4,5]. A review summarizes much of the early work on Z-DNA [6].

The change between right- and left-handed conformations has also been observed in covalent closed circular DNA molecules, such as plasmids (e.g., refs. 7 and 8). Such DNA has a unique molecular property, namely, the ability to store elastic energy within a single macromolecule. This can be observed, for example, in the adoption of supercoiled conformations in solution. In contrast to right-handed DNA, the left-handed Z-DNA conformation provides a very immunogenic stimulus, allowing one to obtain poly- or monoclonal antibodies in relatively high yield [9–12]. For physico-chemical experiments, monoclonal antibodies are the preferred reagents for studying the conformation of Z-DNA in the presence of a vast excess of right-handed DNA. This is due to the ease of obtaining reproducibly and in large amounts well defined antibodies. Such a monoclonal antibody, Z-D11, has recently been used to measure the kinetics of formation or the disappearance of a short stretch of Z-DNA within a supercoiled plasmid molecule after changing the torsional stress altering the concentration of a small ligand, chloroquine [11].

In the course of these studies it was observed that a considerable higher chloroquine concentration than expected has to be applied to dissociate the DNA-antibody complex. This stabilisation of the complex lasts for very long times and is reminiscent of some observations with allosteric en-

Dedicated to Professor Manfred Eigen on the occasion of his 60th birthday.

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zymes with slow conformational changes, the so-called 'hysteretic enzymes' [13,14].

The phenomenon of a hysteretic DNA involving Z-DNA in supercoiled plasmids and the binding of highly specific monoclonal antibodies is an interesting model system which is able to 'remember' the change of some external conditions, such as that of the chloroquine or salt concentration, for a very long time. Since such a property of DNA is rather unexpected, the experiments reported here might serve as a starting point for more detailed investigations of this kind of hysteresis effect which appears to be observable in a relatively small molecular system. While the storage of genetic information in DNA in the base sequence, i.e., the primary chemical structure, is a basis for a molecular understanding of biology, the possibility of an erasable memory based on conformational changes of the double helix cannot be excluded a priori.

The possible importance of hysteresis phenomena in nucleic acids has been stressed previously [15].

2. Materials and methods

2.1. Plasmid DNA

The plasmid pLP32 was kindly provided by Drs. L. Peck and J. Wang [8] and is pRB322 with $(dC\text{-}dG)_{16}$ inserted in the filled-in *Bam*HI site. pFP332 is a derivative, where this alternating $(dC\text{-}dG)_{16}$ was cut from pLP32 with *Bam*HI endonuclease and ligated into the *Bam*HI site of the small vector plasmid pUC8 [16]. The cccDNA was prepared as described previously [11].

2.2. Monoclonal antibody binding

Purification, radiolabelling with $Na^{125}I$ and determination of the concentration of the monoclonal antibody Z-D11 in the DNA-antibody complex were performed as described by using the filter-binding assay, where the DNA and thus the complex are bound to the ion-exchange membrane NA45 (Schleicher & Schüll) [10,11].

In a typical experiment the supercoiled plasmid DNA was incubated with the radiolabelled antibody in either the presence or absence of a particular concentration of chloroquine (Sigma). If no chloroquine was present initially a concentrated chloroquine solution was added after leaving time for formation of the complex (15 or 30 min at room temperature), 50 μl of such solutions were applied to the NA45 membrane, which was then washed with buffer containing usually 0.1–0.25 M Na^+. The radioactivity which was still binding to the filter was then determined in a γ-counter.

In most of the experiments the concentration of plasmid molecules (0.3 nM) was chosen to be half of that of the active monoclonal antibodies; in this way saturation of the two strong binding sites for Z-D11 on the Z-DNA of 32 base-pairs in length could be achieved.

2.3. Gel electrophoresis

Aliquots (10 μl) of plasmid DNA (5 nM) were either incubated with chloroquine and then with the iodinated monoclonal antibody (10 nM Z-D11) for 15 min each or vice versa and were applied to the slots of a 1% agarose gel in Tris-phosphate buffer run at about 5 V/cm at room temperature. The gel was stained with ethidium bromide (1 μg/ml), destained with water and photographed to visualize the DNA. After drying of the gel it was exposed on an X-ray film in order to visualize the position of the monoclonal antibody.

3. Results

While studying the formation of Z-DNA in supercoiled DNA with a monoclonal antibody in the presence of different amounts of intercalating molecules, like ethidium or chloroquine, a drastic difference in complex formation is observed, depending on which experimental protocol is used. Depending on the order of addition of the ligands different results are obtained.

In fig. 1 an example of the relative amount of complex between the supercoiled DNA of plasmid pFP332 and the radiolabelled monoclonal antibody Z-D11 is shown. Due to the addition of chloroquine the suprahelical density of the DNA changes as can be deduced from the altered mobil-

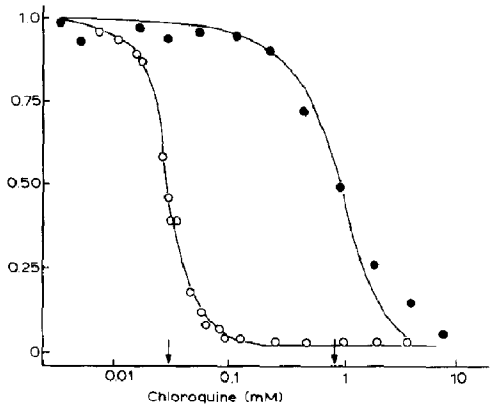

Fig. 1. Binding of the iodinated monoclonal antibody Z-D11 to the supercoiled DNA of plasmid pFP332 with 2750 base-pairs in the presence of different concentrations of chloroquine in Tris-acetate buffer with 0.1 M NaCl. The amount of complex was determined after filtration through an ion-exchange membrane (Schleicher & Schüll, NA45 membrane). (○) Chloroquine was added for 15 min before the antibody was complexed for 15 min. (●) The antibody was bound for 15 min and then chloroquine was added. The arrows indicate CQm, the chloroquine concentration at which half of the antibody-DNA complex is observed.

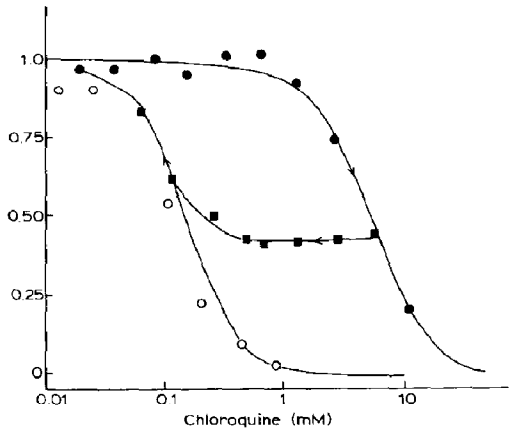

Fig. 3. Amount of complex formation between pFP332-DNA and Z-D11 antibody after exposing the complex to 5 mM chloroquine in phosphate-EDTA buffer (pH 8.0) with 0.2 M NaCl for 30 min. The amount of complex remaining after dilution to the indicated chloroquine concentration with buffer (■) was measured after 5, 60, 120 and 900 min and no significant time dependence observed. The arrows indicate the way taken for this kind of 'learning curve'. The data (○, ●) are those obtained in the same way as in fig. 1, but are shifted due to the higher ionic strength of the solution.

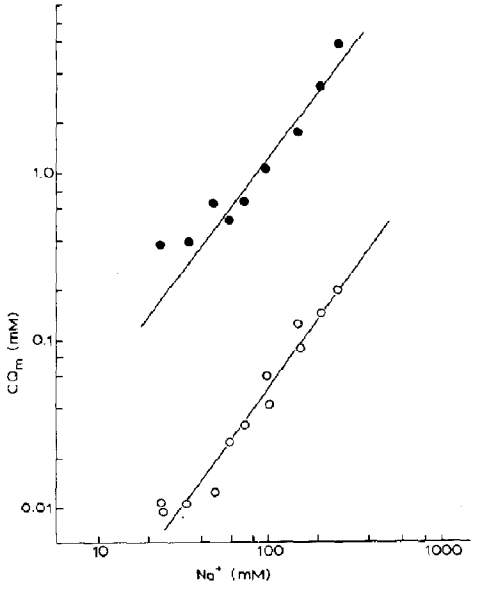

Fig. 2. Dependence of the chloroquine concentration, CQm, at which 50% of the complex is formed with the monoclonal antibody Z-D11, on the salt concentration for supercoiled DNA of plasmids, such as pFP332, with $(dC\text{-}dG)_{16}$ cloned into the polylinker. (○) The supercoil density was changed by the addition of chloroquine before addition of the labelled antibody. (●) Reverse order of addition, first the antibody and then chloroquine.

ity of the topoisomers, when they are subjected to electrophoresis in the presence of chloroquine. Under the conditions of these experiments a chloroquine concentration of 30 μM removes about four superhelical turns (data not shown). At this chloroquine concentration (CQm^0) about half of the plasmid DNA has lost its Z-DNA and therefore does not bind the antibody molecule. If, on the other hand, the plasmid DNA is first incubated with a stoichiometric amount of antibody a much higher concentration of chloroquine ($CQm^* = 0.8$ mM) is necessary to dissociate the complex. A very rough estimate indicates that this amount of chloroquine is enough to change the suprahelical density σ by about 0.1, thereby introducing positive superhelical turns into the DNA. The time dependence of the process after mixing was followed for up to several weeks without

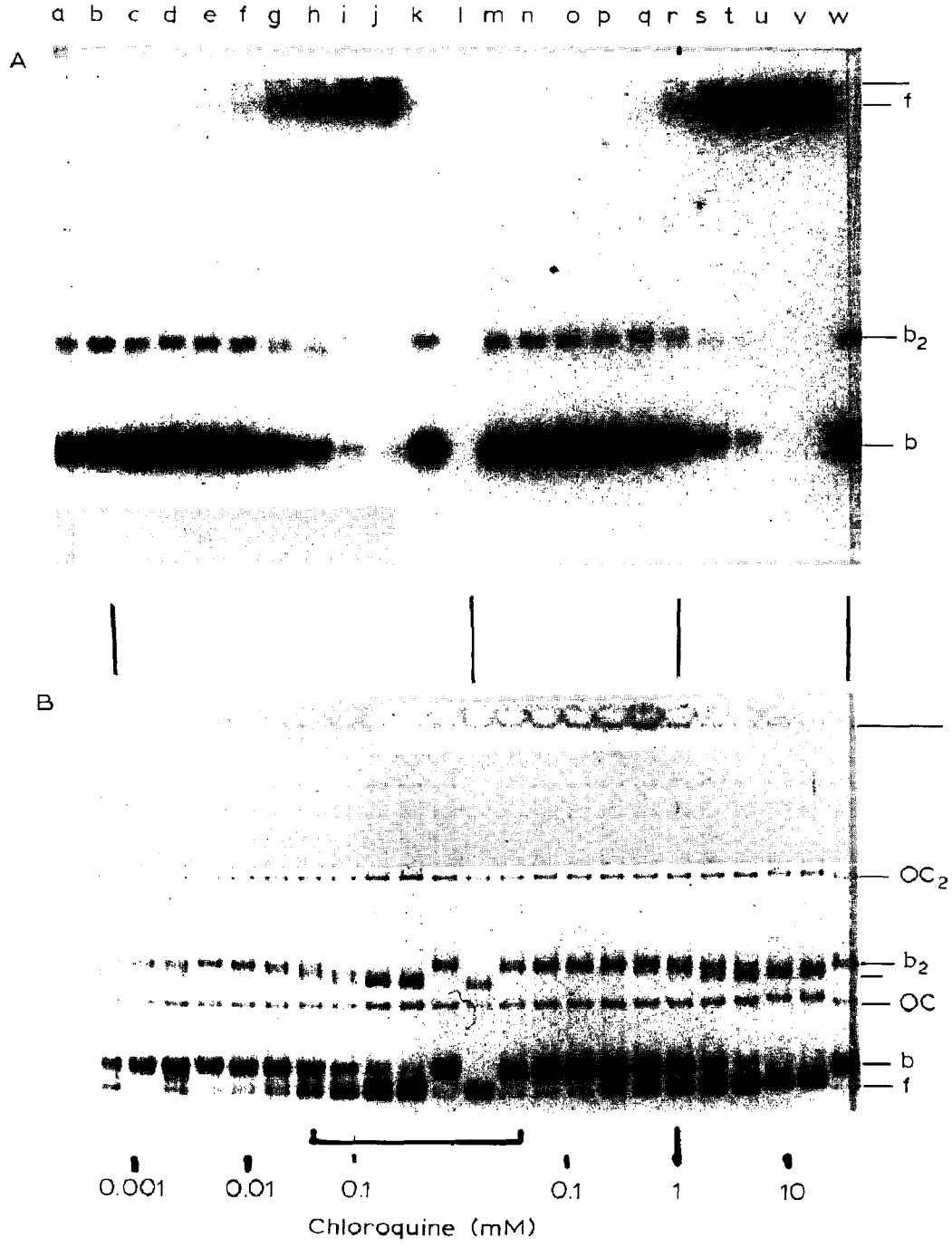

Fig. 4. Agarose gel electrophoresis of pFP332-DNA in the presence of iodinated antibody Z-D11 at different chloroquine concentrations and for different order of mixing. (A) Autoradiograph of the dry gel, showing the position of the labelled antibody; (f) free antibody, (b) antibody bound to monomeric supercoiled DNA and (b2) bound to the dimeric form of the DNA (upper part). (B) Fluorescence of the gel after staining of the DNA with ethidium bromide: (f) free ccc-DNA, (b) ccc-DNA with antibody bound (b2) dimeric form of ccc-DNA with antibody bound, (oc, oc2) open circular form of the monomeric and dimeric plasmid, respectively. Lanes a–j and m–v: DNA incubated with 1 : 2 dilutions of chloroquine in Tris-acetate buffer before (a–j) and after (m–v) addition of the antibody, showing the difference in amount of complex formation upon order of addition. Lanes k and w: complex without chloroquine. Lane l: without antibody.

reaching an equilibrium curve, i.e., where the amount of complex formed was independent of the order of mixing (data not shown). Even after several weeks the system remembers what occurred within a few minutes, whether the antibody or the dye was added first to the supercoiled DNA.

Qualitatively the same observation was made by using ethidium as the effector molecule of the DNA structure. Since the binding of chloroquine is much weaker, the total concentration is practically the same as the free concentration and in this way the analysis is simplified.

The binding of chloroquine molecules depends on the ionic strength. One expects that by decreasing the salt concentration in the buffer, lower chloroquine concentration will be necessary to remove Z-DNA. Fig. 2 shows that this is the case and that within the accuracy of the experiments the logarithmic difference between CQm^{o} and CQm* is nearly constant. For both types of experiments CQm increases with about the 1.4th power of the Na^{+} concentration. (In these experiments the Na^{+} concentration refers to that present when the complex is filtered. In order to reduce the background the filter were washed with buffer containing 0.1–0.2 M NaCl.)

Equivalent results are obtained by changing the salt concentration and leaving that of chloroquine constant. One has to keep in mind that the filter assay based on the binding of DNA to an ion-exchange membrane can only be used below about 0.3 M NaCl. In this way the interplay between two small ligands, salt and dye molecules, can be studied in detail, using the antibody-DNA complex as an indicator.

This simple molecular system shows some kind of memory behaviour indicative of metastable states. A further example of this behaviour is shown in fig. 3, where the DNA-antibody complex was exposed for 30 min to a pulse of 5 mM chloroquine and then diluted to lower concentration. Within the time during which measurements were taken, no change in the amount of complex was observed. Within a factor of about 20 in the chloroquine concentration the system remembers this pulse in the external conditions. Such a behaviour can be considered as a measurement of transient phenomena in solution.

Fig. 4 shows in a more direct experimental set-up the difference in the amounts of complex formed, depending on the order of addition of chloroquine and antibody. Since the complex has a rather long lifetime, with a rate of dissociation of about $2 \times 10^{-6}\ s^{-1}$ [10], the complex can still be observed after running an electrophoresis in an agarose gel. The chloroquine concentration of the solution loaded onto the gel is indicated at the bottom of the figure. The complex can be observed in two different ways: either as radioactivity moving with the DNA in fig. 4A or as a small change in mobility of the ccc-DNA in fig. 4B. Even after separation from the free antibody, salt and chloroquine, the different order of addition is obvious from these results.

Further experiments will be necessary to determine the maximal time for which this system

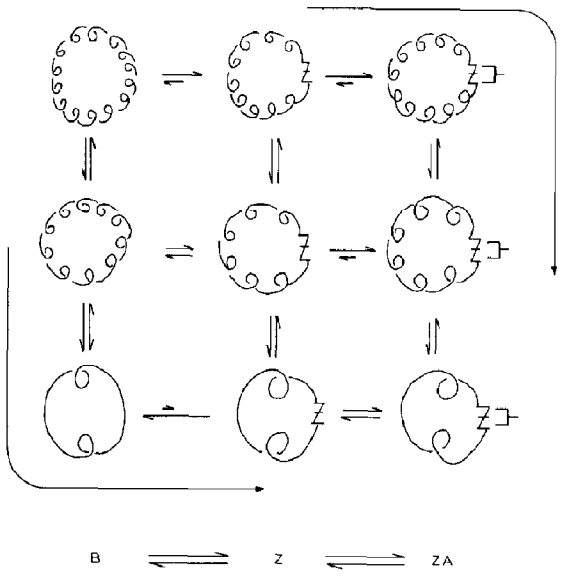

Fig. 5. Schematic presentation of some of the reactions involved in the formation of hysteretic DNA-antibody complexes. The negatively supercoiled DNA is shown in a toroidal conformation; (Z) Z-DNA, (⊐) antibodies. The superhelical density changes from top to bottom due to the intercalation of molecules like ethidium or chloroquine. Between B and Z the superhelical density changes due to the change in handedness of about 1% of the base-pairs.

can remember and what the shortest concentration pulses are which can be recognized by the difference in the amount of complex between the Z-DNA formed in the supercoiled plasmid and the highly specific antibody.

Fig. 5 shows in a schematic way the important reactions involved in this system and where the suprahelical density is changed upon binding of ligands or the formation of Z-DNA [17].

4. Discussion

Covalently closed circular double helical DNA is a rather unusual molecular entity. Due to the topological restrictions imposed such molecules are able to store 'mechanical' energy, which may show up in the formation of supercoiled structures, of unusual DNA conformations, such as left-handed Z-DNA or cruciform structures, or the facilitated binding of small molecules, like ethidium or chloroquine. This energy depends on the square of the suprahelical density and can in this way give rise to non-linear phenomena.

The B-to-Z transition of a short piece of alternating $(dC\text{-}dG)_n$, cloned into a plasmid, is a very cooperative process, which is reflected by a nucleation parameter of the order of 10^{-6}–10^{-7} for the formation of Z-DNA within a right-handed B-DNA [8]. The transition within a supercoiled DNA occurs with relaxation times up to about 1 h under conditions similiar to those used here and the overall rate constants are strongly dependent on the chloroquine concentration [11]. Together with the slow dissociation rate of the monoclonal antibody Z-D11 from $(dC\text{-}dG)_{16}$ in the Z-form (ref. 10 and unpublished results), the steps involved in the change of the amount of complex formed are relatively slow and can be measured with high sensitivity using the filter assay or by electrophoresis. But still these data appear to be insufficient to explain the hysteretic behaviour of this system. The stabilisation of Z-DNA by binding of the antibody is certainly one of the requirements for observing such memory effects. Although a detailed quantitative understanding is still lacking, it appears that the interaction of a protein with a particular structure of DNA in a topologically restricted molecule does provide an interesting model system, where the order-order transition of DNA might provide the basis for hysteresis and memory effects. It remains to be seen whether such phenomena are of biological relevance but the observation that DNA conformations can serve as a transient memory of external conditions – and not only as a permanent memory of the genetic information as reflected in the sequence of the nucleotides – should at least be considered as a possibility in biological systems.

Acknowledgements

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