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Accelerated Publications

Cyclization of Globular DNA. Implications for DNA−DNA Interactions in Vivo[†]

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ABSTRACT: The rate of cyclization of λ DNA varies over more than 6 orders of magnitude, from 3.2×10^{-7} s^{-1} to 2 s^{-1}, in a Tris−EDTA buffer as a function of spermidine concentration. This variation is strictly correlated with the conformation of the chain. The highest rates are obtained when the chain is collapsed into a dense globular state. The effective concentration of the chain ends in the reaction is then 87 000-fold greater than in the random coil state. These results show that DNA globularity must be taken into account to understand biological processes involving intramolecular DNA−DNA interactions.

Long DNA molecules are in globular states in cells and viruses (*1*). These globular states are characterized by an overall DNA conformation which is much denser than in the random coil state. A central issue in molecular biology is to understand the consequences of the globularity of DNA on such fundamental processes as chromosome condensation, DNA replication and recombination, and gene expression. This is a complex problem, as witnessed by the large number of components participating in the compaction process, and by the diversity of globular states found in vivo (in eukaryotic cells, for instance, the density of the globular states is regulated in a subtle manner during the cell cycle: it is minimal during interphase and maximal during mitosis).

Many years ago, Lerman discovered that in very dilute solutions, phage DNA molecules can collapse from a random coil state to a compact globular state (*2*). The globular states of DNA obtained in vitro are of great biological interest since they can serve as simple models to investigate the properties of the more complex globular states of DNA that prevail in vivo. Physically, DNA collapse is due to the presence of net-attractive intramolecular interactions between the segments of the molecule. Agents promoting DNA collapse are called condensing agents, and include multivalent cations such as the polyamines spermidine (3+) or spermine (4+) (reviewed in *3*). The attractive forces induced by condensing agents can also act at the intermolecular level, giving rise to a multimolecular aggregation of long or short DNA chains. DNA condensation is a generic term that refers to the formation of a DNA-rich state of high density, and therefore applies both to aggregation of many molecules and to the compaction of a single molecule (*4*).

There exist numerous biological processes involving DNA−DNA interactions. Previous studies have shown that DNA condensation can greatly affect such processes. The rate of DNA renaturation, for instance, and of DNA strand-exchange without protein is greatly accelerated by DNA condensation (*5*, *6*). DNA condensation also favors an extensive catenation of circular DNA chains by DNA topoisomerases (*7*). In these examples, the condensing agent operates by a multimolecular aggregation of the DNA molecules. We show here by studying the cyclization of the DNA of bacteriophage λ that intramolecular DNA−DNA

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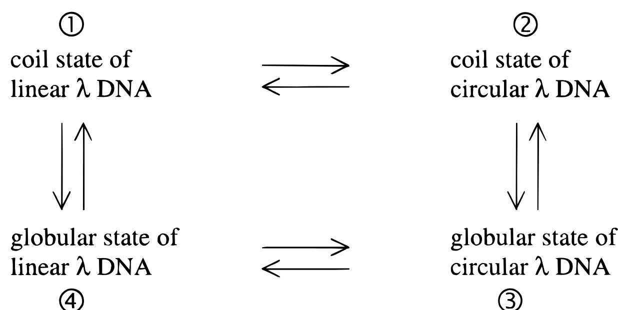
interactions can also be drastically affected by the globularity of DNA.

The DNA of bacteriophage λ is a 50 kb long linear double-stranded molecule, terminated by two 12 base long complementary single strands. The cohesive ends can anneal to generate a circular form of the molecule known as the Hershey circle (*8*). The cyclization of λ DNA is observed in vivo: the DNA is linear in the bacteriophage's head and becomes circular within a few minutes after the injection into its host (*9*). Taking into account linear and circular states, one can define four conformations for a single λ DNA chain. There are two coil states, linear ① and circular ②, and two globular states, linear ④ and circular ③:



In a now classic work, Wang and Davidson studied the interconversion between the linear and circular forms of λ DNA in the presence of sodium chloride, and found that the rate of the reaction was too slow to account for the rapidity of the reaction in vivo (*10*−*12*). Under these conditions, λ DNA is in a random coil conformation, and, accordingly, their work was focused on the interconversion between the states ① and ② of λ DNA.

The possibility that a cyclization reaction could take place between states ④ and ③ of λ DNA has been in the literature for some time (*13*−*15*). (1) Schellman and co-workers (*13*) have studied by electron microscopy the collapse of λ DNA in the presence of spermidine (in a medium containing 1 mM NaCl, 1 mM sodium cacodylate, and spermidine at a final concentration of about 0.1 mM). The DNA globule has a toroidal structure. When the collapse is reversed (through the addition of NaCl), λ DNA remains linear, indicating that no cyclization had occurred in the globular state. (2) Louie and Serwer (*14*) have observed that 90% of λ DNA becomes circular within 15 min in the presence of poly(ethylene glycol) and sodium chloride at 55 °C. These compounds promote the collapse of the chain at room temperature (*2*), but whether the collapse takes place at 55 °C is not known. In addition, it is not clear whether the reactions have been efficiently quenched. (3) Murphy and Zimmerman (*15*) have observed a rapid cyclization of λ DNA in the presence of *E. coli* extracts. The half time for this reaction is less than 2 min; only about 1% of the input DNA becomes circular. Whether DNA collapse occurs in this system is not known. An interpretation of the experiments described in (*14*) and (*15*) is therefore difficult.

We have studied here the cyclization of λ DNA in the presence of spermidine (final concentration 0.1−100 mM), and found that very high cyclization rates can be obtained when the chain is globular. A main factor contributing to the feasibility of such experiments is the use of very low DNA concentrations (typically 50 ng/mL). These low concentrations are required to slow the aggregation process which accompanies DNA collapse.

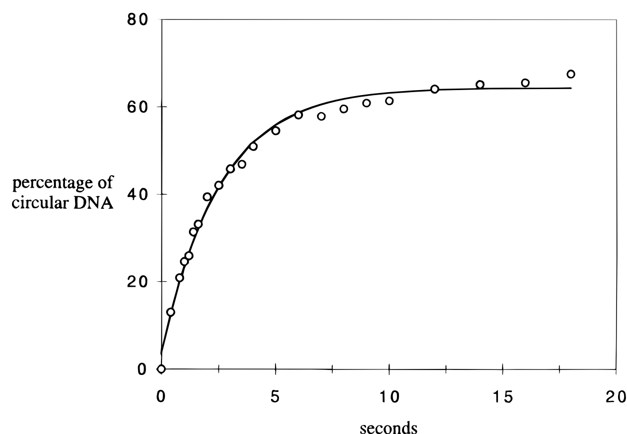


FIGURE 1: Cyclization of λ DNA in TE + 1.5 mM spermidine at 1 °C. Each point is a mean of three experiments. The solid line represents a monoexponential fit. The rate constant determined from this experiment is $k_1 = 0.4 \pm 0.05\ s^{-1}$.

EXPERIMENTAL PROCEDURES

Cyclization Experiments. Cyclization experiments were performed as described in (*16*). The reaction was initiated by mixing equal amounts of ^{32}P end-labeled λ DNA (λcI857S7, 48.5 kb)[1] in a TE buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA) and spermidine at the appropriate concentration. The products of the reaction were separated by field inversion gel electrophoresis and analyzed using a Phosphor Imager (Molecular Dynamics). Since the rate of the reaction varies greatly as a function of spermidine, specific protocols were followed depending on the reaction rate. (1) When the rate of cyclization was slow (smaller than $10^{-3}\ s^{-1}$), silicone oil was layered over the solution to prevent evaporation. (2) When the rate of cyclization became high (greater than $10^{-3}\ s^{-1}$), the reaction was terminated by the addition of tRNA (final concentration 10 mg/mL). The addition of free polynucleotides reverses the collapse of the chain (either in its linear or in its circular form) with a characteristic time of about 5 ms (*17*). Figure 1 shows that the addition of tRNA leads to a fast quench of the reaction. For cyclization rates greater than $0.1\ s^{-1}$, the experiments were performed by two experimentalists, the beginning and the end of the reaction being indicated by a metronome. For the highest cyclization rates (greater than $1\ s^{-1}$), the initial part of the relaxation process is too fast to be studied by hand mixing (*16*). We have tried to use a quench-flow equipment (Bio-Logic and Hi-Tech) to follow the reaction, but the shear generated by these machines leads to an extensive degradation of the DNA chains (data not shown).

Sedimentation Studies. The conformation of λ DNA was studied by ultracentrifugation (*2*). A DNA−spermidine solution (200 μL at 50 ng/mL, ~5 × 10^3 cpm) was layered over a 5 mL sucrose gradient (linear, 5−20%) containing the same spermidine concentration, and centrifuged for 12 min at 50 000 rpm in an SW 50 rotor. About 25 fractions were collected, and each fraction was assayed for radioactivity.

[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; kb, kilobase; Tris, tris(hydroxymethyl)aminomethane.

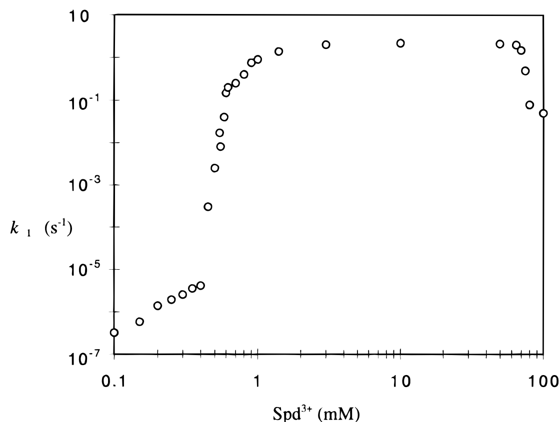


FIGURE 2: Rate of cyclization of λ DNA in TE at room temperature as a function of spermidine concentration. The rate constants are determined to ±20%.

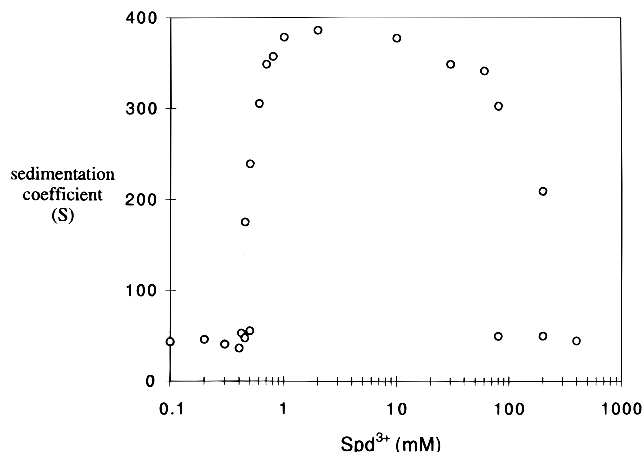


FIGURE 3: Conformation of λ DNA in TE at room temperature as a function of spermidine concentration. The sedimentation coefficients close to 40 S correspond to the coil form of the chain. The sedimentation coefficients of the globules are determined with an accuracy of ±20 S. A coexistence of coil and globules is observed at four spermidine concentrations: 0.45, 0.5, 80, and 200 mM.

RESULTS AND DISCUSSION

We determined the rate of cyclization of λ DNA at room temperature as a function of spermidine concentration. The reaction is always first-order. The cyclization rate constant k_1 varies from 3.2×10^{-7} s^{-1} to 2 s^{-1}, depending in a complex manner on the spermidine concentration (Figure 2). We have also studied by sedimentation the conformation of λ DNA in the same conditions (Figure 3). It can be seen that there is a strict correlation between the conformation of the chain and the rate of cyclization. Five regions can be defined from the sedimentation and cyclization studies.

(1) 0−0.4 mM spermidine. The chain has a sedimentation constant of 40 ± 10 S compatible with a linear random coil state of the molecule (*8*, *18*). The cyclization rate is slow, and increases smoothly from 3.2×10^{-7} s^{-1} at 0.1 mM spermidine to 4.2×10^{-6} s^{-1} at 0.4 mM spermidine.

(2) 0.45−1 mM spermidine. This region begins with the onset of the coil−globule transition, indicated by a steep increase of the sedimentation coefficient. The cyclization rate constant increases sharply, from 3×10^{-4} s^{-1} at 0.45 mM, to 0.9 s^{-1} at 1 mM spermidine. In a narrow range of spermidine concentration (0.45 and 0.5 mM), the random coil and the globular states coexist. In this coexistence region, the collapse of the chain takes place on a time scale of 10 min. Similar observations have been made previously (*19*, *20*). The average sedimentation coefficient of the globules increases with increasing spermidine concentration, from 180 S at 0.45 mM to 370 S at 1 mM spermidine. The highest value (370 S) corresponds to an equivalent hydrodynamic sphere of about 70 nm, in good agreement with the value expected for a compact toroidal state of λ DNA (*19*).

(3) 1.5−60 mM spermidine. The sedimentation coefficient decreases weakly (from 380 to 350 S), while the cyclization rate reaches a plateau value of 1.4−2 s^{-1}. Dr. Pörschke has performed a field-jump experiment (*17*) at a concentration of 1.5 mM spermidine to investigate the rate of the coil−globule transition. In this experiment, λ DNA in TE + 1.5 mM spermidine was submitted to an electric field pulse to dissociate the counterions from the DNA chains. This dissociation leads to a transient decondensation of the DNA. The subsequent collapse of the chains is monitored by light scattering. In the presence of 1.5 mM spermidine, the collapse of λ DNA is completed within 2 ms. Since the rate of the collapse of the chain is much faster than the rate of cyclization, this implies that the cyclization process takes place between states ④ and ③ of the chain.

(4) 75−200 mM spermidine. Coils and globules coexist again. The average sedimentation coefficient of the globules decreases from 300 S at 80 mM to 200 S at 200 mM spermidine. The cyclization rate constant decreases from 0.5 s^{-1} at 75 mM to 0.05 s^{-1} at 100 mM spermidine. We have not been able to follow the kinetics of the cyclization reaction above this concentration.

(5) Above 200 mM spermidine. In this region, the chain is again in a random coil state with a sedimentation constant of 40 ± 10 S.

We have further characterized the cyclization reactions taking place between the globular states ④ and ③ (at a spermidine concentration of 1.5 mM), and between the coil states ① and ② [at a sodium chloride concentration of 2 M; the highest rate constants in the presence of NaCl are obtained at this concentration (*10*, *12*)]. The cyclization rate constant at room temperature is equal to $(3.3 \pm 0.3) \times 10^{-5}$ s^{-1} in the presence of NaCl: the rate of cyclization is thus increased by a factor of 40 000 in the globular state. A study of the temperature dependence of the rate of the reaction (to be detailed elsewhere) shows that the activation energy of the cyclization reaction is reduced in the presence of 1.5 mM spermidine, being equal to 35 ± 7 kJ/mol instead of 106 ± 15 kJ/mol in the presence of 2 M NaCl.

To determine the effective concentration of the chain ends in the two reactions (see ref *21* for a general review on effective concentrations), we have performed competition experiments at room temperature, using a 12 base long oligonucleotide having the sequence of the left cohesive end of λ DNA. In the presence of this oligonucleotide, O_L, two reactions can take place: (1) the cyclization reaction, L → C (where L and C are the linear and circular forms of λ DNA, respectively), characterized by the rate constant k_1; (2) the annealing of L and O_L, $L + O_L \rightarrow LO_L$, which yields a linear form of the chain, LO_L, unable to cyclize, characterized by a rate constant k_2.

At the beginning of the reaction, the concentration of linear chain is L_0. At the end of the reaction, the concentrations of linear and circular chains are L_∞ and C_∞, respectively. The

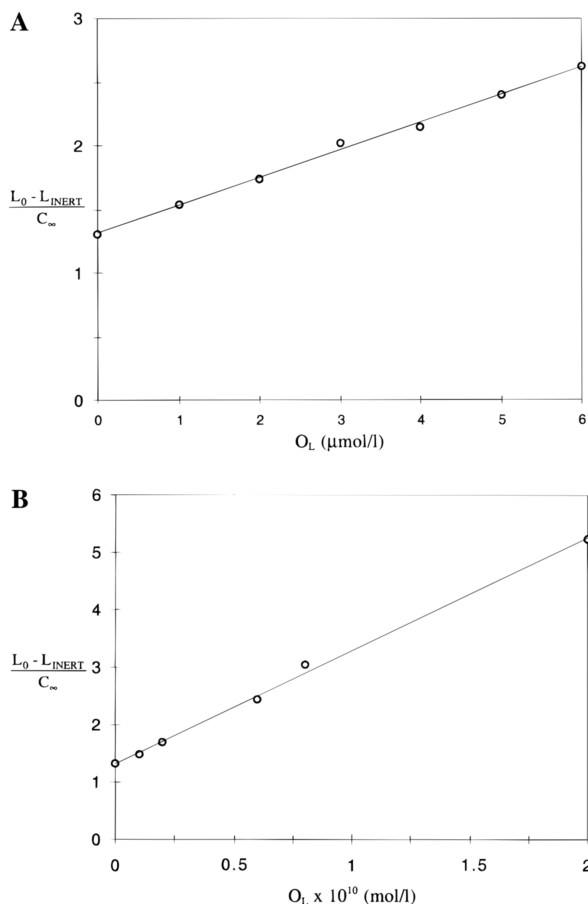


FIGURE 4: Competition experiments. Inhibition of the cyclization reaction at room temperature by increasing amounts of the oligonucleotide O_L. (A) 1.5 mM spermidine. (B) 2 M NaCl.

molecules remaining linear at the end of the reaction (with a concentration L_∞) are made up of the molecule unable to circularize even in the absence of O_L (denoted L_{INERT}, 20–30% of the chains), and of the molecules LO_L. The concentration of circular chains C_∞ is expected to decrease with increasing amounts of the oligonucleotide O_L according to the equation

$$\frac{L_0 - L_{INERT}}{C_\infty} = 1 + \frac{[O_L]}{C_{eff}} \quad (1)$$

where $C_{eff} = k_1/k_2$. This ratio has the dimension of a molarity and defines an effective concentration of the chain ends in the cyclization reaction (*21*). Equation 1 is obtained under the assumption (justified experimentally) that $[O_L] \gg L_0$.

Figure 4 shows the two competition experiments performed in the presence of either spermidine or sodium chloride. In both cases, the reciprocal of C_∞ increases linearly with $[O_L]$, in agreement with the above equation. The corresponding values of C_{eff} are equal to 6.1×10^{-6} M in the presence of spermidine, and to 7×10^{-11} M in the presence of sodium chloride. The effective concentration measured in the coil state is close to the value predicted by Jacobson and Stockmayer for the ring closure probability of a random coil (*11*, *22*). In the globular state, the effective concentration is increased by a factor of 87 000. A detailed interpretation of these two effective concentrations will be presented elsewhere.

The increases in cyclization rates and effective concentrations observed in the globular state have biological implications. First, the cyclization rate becomes fast enough to account for the cyclization reaction observed in vivo. Second, the intramolecular approach of DNA segments is also required in biological processes involving in DNA looping (*23–25*), and DNA cyclization experiments (*26*, *27*) provide a model reaction to study such processes. The effective concentration measured in the globular state is quite high. Indeed, it is much greater than the plateau value of 3×10^{-8} M measured for the effective concentration of small chains (0.3–1 kb long) using a DNA ligase catalyzed cyclization (*26*). The observation that the globularity of DNA can dramatically increase effective concentrations suggests that it could facilitate the formation of DNA loops in vivo, such as the large loops present in the metaphase chromosome (*28*). DNA globularity, along with DNA superhelicity, must be taken into account to understand biological processes involving intramolecular DNA–DNA interactions. Third, beyond the problem of DNA looping, much of our current knowledge on DNA–protein interactions—following the pioneering work of Bourgeois and co-workers on the interaction of the lac repressor with λ DNA (*29*, *30*)—comes from experiments performed on the coil state of the DNA chains. How these interactions are affected by the globular state of the DNA is not known and could be investigated using approaches similar to the one followed here.

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