

Intrachain Reactions of Supercoiled DNA Simulated by Brownian Dynamics

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ABSTRACT We considered an irreversible biochemical intrachain reaction of supercoiled DNA as a random event that occurs, with certain probability, at the instant of collision between two reactive groups bound to distant DNA sites. Using the Brownian dynamics technique, we modeled this process for a supercoiled DNA molecule of 2.5 kb length in dilute aqueous solution at an NaCl concentration of 0.1 M. We calculated the mean reaction time τ_{Σ} as a function of the intrinsic second-order rate constant k_1 , the reaction radius R , and the contour separation S of the reactive groups. At the diffusion-controlled limit ($k_1 \rightarrow \infty$), the kinetics of reaction are determined by the mean time τ_F of the first collision. The dependence of τ_F on R is close to inversely proportional, implying that the main contribution to the productive collisions is made by bending of the superhelix axis. At sufficiently small k_1 , the mean reaction time can be satisfactorily approximated by $\tau_{\Sigma} = \tau_F^{(app)} + 1/k_1 c_L$, where c_L is the local concentration of one reactive group around the other, and $\tau_F^{(app)}$ is an adjustable parameter, which we called the apparent time of the first collision. The value of $\tau_F^{(app)}$ depends on R very weakly and is approximately equal to the mean time of the first collision caused by mutual reptation of two DNA strands forming the superhelix. The quasi-one-dimensional reptation process provides the majority of productive collisions at small k_1 values.

INTRODUCTION

Many biochemical reactions, such as initiation of transcription and site-specific recombination, require a close spatial contact between two reactive groups bound to distant DNA sites (see, for example, Rippe et al., 1995; Stark et al., 1992). Such a contact, or collision, is a random event caused by thermal fluctuations of the DNA conformation. However, one single contact is, in general, not sufficient for the chemical transformation: the act of reaction is preceded by many “unsuccessful” collisions. In this study we investigate the question of how the reaction kinetics depend on the probability α that a single collision leads to the chemical transformation.

Consider a supercoiled DNA molecule in a dilute solution. We assume that the activity of the reactive groups is “switched on” at the initial time instant $t = 0$, with the DNA chain being in statistical equilibrium. The quantity of interest is the reaction time τ_{Σ} , i.e., the time corresponding to the final, “successful,” collision.

If the successive recurrence times, i.e., the time intervals between collisions, were not correlated, the mean reaction time would be given by the simple formula (see, for example, Klenin and Langowski, 2001b)

$$\tau_{\Sigma} = \tau_F + \langle n \rangle \tau_R, \quad (1)$$

where τ_F is the mean time of the first collision, τ_R the mean recurrence time, and $\langle n \rangle$ the average number of the “unsuccessful” collisions preceding the reaction: $\langle n \rangle + 1 = 1/\alpha$.

For many simple polymer systems, Eq. 1 proved to be a good approximation (Friedman and O’Shaughnessy, 1994), but it is not valid for supercoiled DNA. This fact can be qualitatively understood by the following considerations.

In a supercoiled DNA there are two possible mechanisms of collisions between the reactive groups, as illustrated in Fig. 1. One can distinguish the collisions by relative reptation of the two strands forming the superhelix and the collisions by bending of the superhelix axis (Marko and Siggia, 1995). These two processes are characterized by their own values of τ_F and τ_R . Assuming that for each type of collision, taken separately, the recurrence times are not correlated, we can write

$$\tau_{\Sigma}^{(r)} = \tau_F^{(r)} + \langle n \rangle \tau_R^{(r)}, \quad (2a)$$

$$\tau_{\Sigma}^{(b)} = \tau_F^{(b)} + \langle n \rangle \tau_R^{(b)}. \quad (2b)$$

Here the upper indexes (r) and (b) refer to the collisions by reptation and by bending, respectively. The mean reaction time can be roughly estimated as

$$\tau_{\Sigma} = \min(\tau_{\Sigma}^{(r)}, \tau_{\Sigma}^{(b)}). \quad (3)$$

The plots of $\tau_{\Sigma}^{(r)}$ and $\tau_{\Sigma}^{(b)}$ versus $\langle n \rangle$ are straight lines. If these lines do not cross at $\langle n \rangle > 0$, then the plot of τ_{Σ} versus $\langle n \rangle$ is also a straight line, and Eq. 3 reduces to Eq. 1, but in many practical cases they do cross! Monte Carlo simulations (Vologodskii and Cozzarelli, 1996) imply that the dominating contribution to the local concentration c_L of one reactive group around the other is provided by the reptation mechanism. Because the recurrence time is inversely proportional to the local concentration (see, for example, Klenin and Langowski, 2001b), we have $\tau_R^{(r)} \ll \tau_R^{(b)}$. The ratio of the first collision times, $\tau_F^{(r)}$ and $\tau_F^{(b)}$, may vary considerably, but according to preliminary theoretical estimations one would expect that $\tau_F^{(r)} \gg \tau_F^{(b)}$, if the DNA length L and

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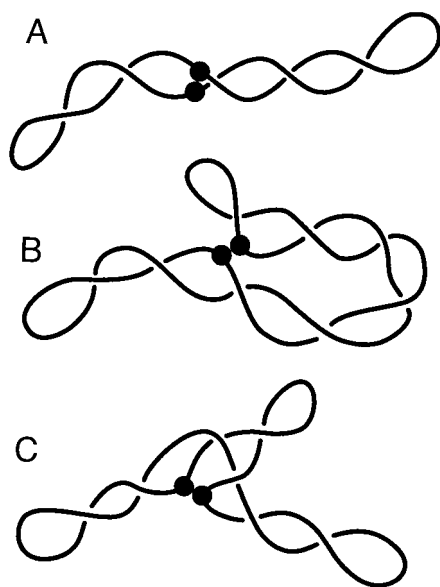


FIGURE 1 Various types of intrachain collisions of supercoiled DNA: (A) collision by mutual reptation of the DNA strands; (B) collision by bending of the superhelix axis; (C) collision that cannot be ascribed definitely to one of the two types.

the contour separation S between the reactive groups are sufficiently large (Marko and Siggia, 1995; Klenin and Langowski, 2001b). This case is illustrated in Fig. 2. As a consequence, for small $\langle n \rangle$, the productive collisions are provided by the bending mechanism and the reaction time τ_{Σ} is given by Eq. 2b. For large $\langle n \rangle$, on the contrary, the productive collisions are due to the reptation, and τ_{Σ} is given by Eq. 2a.

This is, of course, only a simplified qualitative illustration of the real processes. For example, certain collisions cannot be ascribed definitely to one of the two types (Fig. 1 C). Equations 2a and 2b are, probably, only very rough approximations. In this work we model the intrachain reactions quantitatively by a Brownian dynamics (BD) technique.

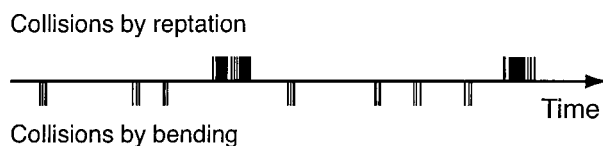


FIGURE 2 Time pattern of intrachain collisions for long supercoiled DNA with large contour separation of the reactive groups. The two types of collisions are shown separately. When the reactive groups are close to each other in the space, a batch of multiple collisions takes place. The mean time of the first collision, τ_F , is determined by the mean time interval τ_B between the batches, so that $2\tau_F = \tau_B$. The mean recurrence time is equal to $\tau_R = \tau_B/n_B$, where n_B is the average number of collisions in a batch. The collisions by reptation are characterized by large τ_B and very large n_B . For the collisions by bending, both τ_B and n_B are relatively small. As a result, $\tau_F^{(r)} \gg \tau_F^{(b)}$ and $\tau_R^{(r)} \ll \tau_R^{(b)}$, where the upper indexes (r) and (b) refer to the collisions by reptation and by bending, respectively.

This technique was already successfully used for calculations of the first collision time τ_F (Jian et al., 1998; Huang et al., 2001; Klenin and Langowski, 2001a). The BD method, although very time-consuming, makes it possible to take into account the real three-dimensional organization of the DNA molecule, in contrast to the simple quasi-one-dimensional models proposed recently for simulation of the internal reptation (Marko, 1997; Sessions et al., 1997; Klenin and Langowski, 2001b). Because of limitations in computational time, we were able to simulate only a relatively short plasmid (2.5 kb), and the ratio $\tau_F^{(r)}/\tau_F^{(b)}$ was not particularly large. However, the main conclusions of the above simplified considerations were confirmed.

In particular, we paid much attention to the dependence of the reaction time τ_{Σ} on the reaction radius R . The bending motion of the DNA axis is essentially a three-dimensional process, and for that reason the first collision time $\tau_F^{(b)}$ is inversely proportional to the reaction radius R (Podtelezhnikov and Vologodskii, 1997). However, reptation is a quasi-one-dimensional process, and the first collision time $\tau_F^{(r)}$ is independent of R . The reaction time τ_{Σ} at large $\langle n \rangle$ is defined by the reptation only (Eq. 2a). Hence, the straight lines given by Eq. 2a for different reaction radii R should cross at the point $\langle n \rangle = 0$. Practically, these lines can be obtained as the asymptotes of the dependence of τ_{Σ} on $\langle n \rangle$ for $\langle n \rangle \rightarrow \infty$. Our BD simulations show that, although the asymptotes do not cross precisely at $\langle n \rangle = 0$, their value at this point is a very weak function of R . We do not consider the distribution function of the reaction time here because this question is discussed in detail elsewhere (Klenin and Langowski, 2001a,b).

METHODS

The full description of our BD algorithm was published earlier (Klenin et al., 1998). Here are the principal points. The DNA molecule is modeled by a closed chain of beads connected through straight elastic segments; a local reference frame is attached to each segment. The energy of the system is given by harmonic potentials with respect to 1) the angles between consecutive segments; 2) the twist angles between consecutive reference frames; and 3) the deviations from the equilibrium segment length. Electrostatic interactions are taken into account through a Debye-Hückel potential. The linear charge density of DNA is renormalized as described by Stigter (1977). Each step in a BD trajectory is performed according to the algorithm of Ermak and McCammon (1978), with second-order corrections. The hydrodynamic interactions between the beads are given by the Rotne-Prager tensor (Rotne and Prager, 1969).

The following set of parameters was used. The molecule length was $L = 850$ nm (2.5 kb), the hydrodynamic radius of DNA $r_h = 1.2$ nm (Hagerman and Zimm, 1981), the superhelical density $\sigma = -0.05$ (linking number deficiency $\Delta Lk = -12$), the persistence length $L_p = 50$ nm, the torsion rigidity $C = 2.5 \times 10^{-19}$ erg cm, the NaCl concentration $I = 0.1$ M, the temperature $T = 293$ K. The elastic stretch modulus was "softened" to the value $\delta_s = 63$ pN to provide reasonable computational time. The equilibrium segment length was $l_0 = 10$ nm, the BD time step $\delta t = 1.9$ ns. The parameters correspond to a bead radius $r_b = 2.3$ nm. The contour separation S of the reactive groups and the reaction radius R were varied.

The directly computed quantity was the mean time τ_n of the $(n + 1)$ th collision as a function of n (in other words, τ_n is the mean time of a

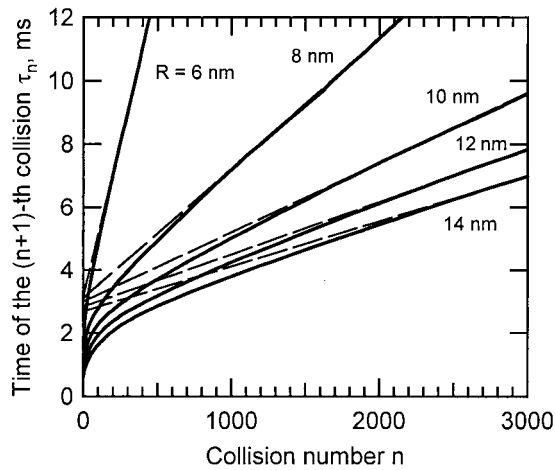


FIGURE 3 Mean time τ_n of the $(n + 1)$ th collision as a function of n for various reaction radii R (solid lines). The contour separation between the reactive groups is $S = 100$ nm. The asymptotes are shown by the dashed lines.

successful collision preceded by n unsuccessful ones). Note that $\tau_0 = \tau_F$. During the simulations, the end-to-end distance d of each subchain of the length S was monitored as a function of time. The time instant when d became less than R corresponded to a collision. The time intervals over which d was less than R were not excluded from the registered collision time, because their contribution is negligible. The collision times were recorded in the following simplified way. The total simulation time (1.9 s) was subdivided into time intervals of $\Delta t = 0.095$ ms (50,000 BD steps). For each pair of colliding beads and for each given value of R , the number of collisions n_i during the i th time interval was recorded. In this way, the length of the record file was kept in reasonable limits (20 Mb), which would be impossible if the time of each individual collision were recorded separately. In the subsequent calculations, we assumed that all the n_i collisions corresponding to the i th time interval occurred at the middle point of this interval. It is obvious that the resulting error in the calculation of τ_n cannot exceed $\Delta t/2$. More rigorous estimations, based on the known form of the distribution of the first collision time (Klenin and Langowski, 2001a,b), showed that the error is, in fact, < 0.005 ms. For any arbitrary (not very large) n , the record file could be used to calculate τ_n as the time of the $(n + 1)$ th collision, averaged over all possible starting points (beginnings of the intervals) and over all the pairs of colliding beads with the given contour separation S .

The local concentration c_L as a function of R for various S was calculated by the Monte Carlo method (Vologodskii et al., 1992; Klenin et al., 1998; Klenin and Langowski, 2000), which in this case is more efficient than the Brownian dynamics. The parameters of the Monte Carlo model were the same as above, with the only exception that the segment length was fixed and equal to 5 nm. The local concentration was obtained as $c_L(R) = \rho(R)/4\pi R^2$, where $\rho(R)$ is the distribution function of the end-to-end distance R for a subchain of a given length S . Overall, our simulations took ≈ 1 year CPU time on the HP-S2000 installation of the German Cancer Research Center.

RESULTS AND DISCUSSION

All our calculations were performed for supercoiled DNA of 850 nm length (2.5 kb) in aqueous solution at a NaCl concentration of 0.1 M. Fig. 3 presents the mean time τ_n of the $(n + 1)$ th collision as a function of n for various reaction

radii R and for the contour separation between the reactive groups $S = 100$ nm. The asymptotes for the large n , shown by the dashed lines, have the form

$$\tau_n^{(\text{asympt})} = \tau_F^{(\text{app})} + n\tau_R, \quad (4)$$

where the apparent mean time of the first collision $\tau_F^{(\text{app})}$ and the recurrence time τ_R are adjustable parameters. The reaction time τ_Σ can be found by averaging τ_n over n :

$$\tau_\Sigma = \langle \tau_n \rangle = \sum_{n=0}^{\infty} \alpha(1 - \alpha)^n \tau_n, \quad (5)$$

where α is the probability of the reaction at a single collision. The asymptotic behavior of τ_Σ as a function of $\langle n \rangle = 1/\alpha - 1$ is given by the average of Eq. 4:

$$\tau_\Sigma^{(\text{asympt})} = \tau_F^{(\text{app})} + \langle n \rangle \tau_R. \quad (6)$$

It should be noted that the mean recurrence time τ_R obtained in our simulations is a property of the model only. It is by no means related to the real recurrence time in a real DNA molecule. In particular, the simulated value of τ_R depends strongly on the BD step δt , so that $\tau_R \rightarrow 0$ as $\delta t \rightarrow 0$. However, the product $\langle n \rangle \tau_R$ remains a meaningful parameter of the model. Imagine that each collision is marked as “successful” with the probability α (α is supposed to be small). Then the first successful collision corresponds to the chemical reaction, and the mean time between successful collisions is given by $\langle n \rangle \tau_R$. This parameter can be rewritten more conventionally as (Klenin and Langowski, 2001b)

$$\langle n \rangle \tau_R = 1/k_1 c_L, \quad (7)$$

where k_1 is the intrinsic second-order rate constant and c_L is the local concentration of one reactive group around the other. Fig. 4 shows the mean reaction time τ_Σ as a function of $1/k_1 c_L$ for various reaction radii R and contour separations S . The asymptotes in this representation are defined by the single parameter $\tau_F^{(\text{app})}$ and have the form

$$\tau_\Sigma^{(\text{asympt})} = \tau_F^{(\text{app})} + 1/k_1 c_L. \quad (8)$$

As can be seen from Fig. 4, the dependence of τ_Σ on $1/k_1 c_L$ approaches its asymptotic behavior quickly. For $1/k_1 c_L > 2$ ms, the reaction time τ_Σ can be approximated by Eq. 8 with an accuracy better than 10%. As a consequence, the parameter $\tau_F^{(\text{app})}$ plays a very important role in the kinetics of reaction. Fig. 5 presents the apparent first collision time $\tau_F^{(\text{app})}$ versus R for various S . The dependence of $\tau_F^{(\text{app})}$ on R is very weak. This fact confirms the conception that, at sufficiently large $1/k_1 c_L$, the reaction is governed by the reptation mechanism, which is insensitive to the reaction radius R . If one totally neglects the dependence of $\tau_F^{(\text{app})}$ on R , the parameter $\tau_F^{(\text{app})}$ can be identified with the time $\tau_F^{(r)}$ of the first collision caused by reptation. Note that in our simulations the reaction radius R is larger than the effective

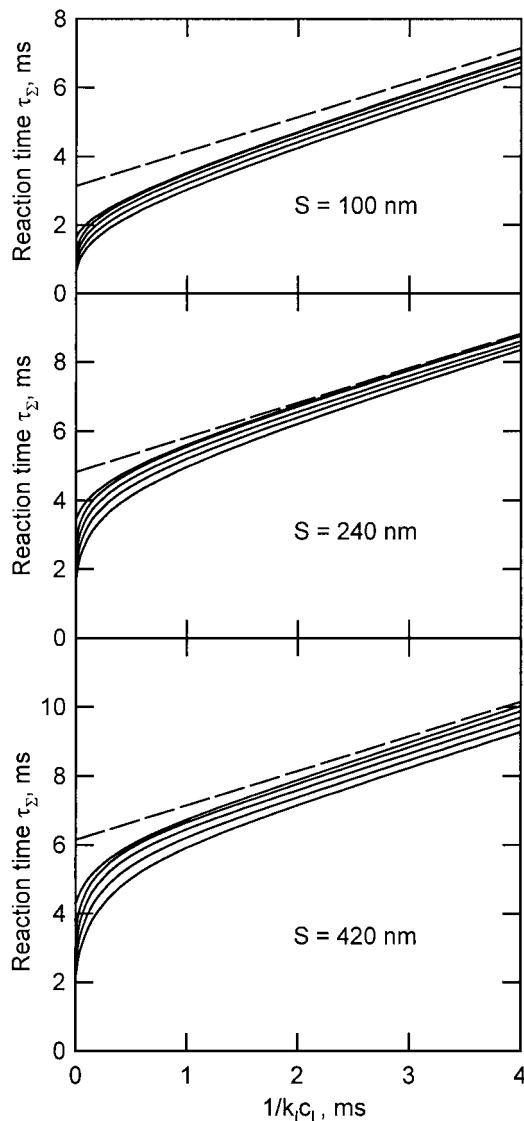


FIGURE 4 Mean reaction time τ_{Σ} versus parameter $1/k_1c_L$ for various reaction radii R and contour separations S of the reactive groups (solid lines). The curves for the reaction radii $R = 6, 8, 10, 12$, and 14 nm follow in order from top to bottom. The asymptotes for $R = 6$ nm are shown by the dashed lines.

diameter of DNA, which at the given ionic strength is equal to 5.6 nm (Stigter, 1977).

As a contrast, the first collision time τ_F (equal to τ_{Σ} at $1/k_1c_L = 0$) strongly depends on R . As shown in Fig. 6, this dependence is close to inversely proportional: $\tau_F \sim 1/R$. This fact implies that the collisions by bending play an important role in this case. The contribution of the bending motion can indirectly be characterized by the difference $(\tau_F^{(app)} - \tau_F)$. As follows from Figs. 5 and 6, this difference increases with the reaction radius R and the separation S . It should be noted that our τ_F values are consistent with the results of the BD simulations performed recently by Huang

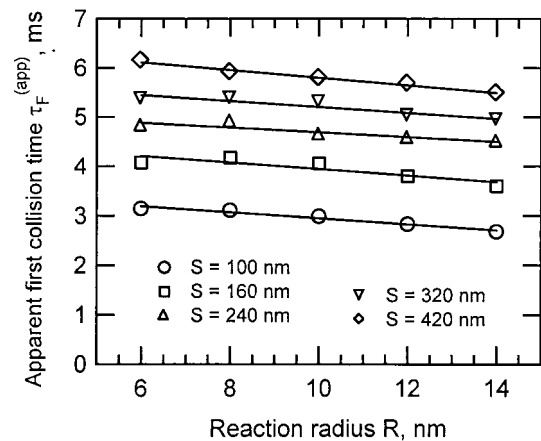


FIGURE 5 Apparent first collision time $\tau_F^{(app)}$ versus reaction radius R for various contour separations S of the reactive groups.

et al. (2001), who used slightly different parameters of the system.

The intrinsic rate constant k_1 is an essentially empirical parameter, whereas the local concentration c_L can be easily estimated by numerical simulations. The local concentration c_L is shown in Fig. 7 as a function of R for various S . Thus, our data make it possible to find the reaction time τ_{Σ} if the constant k_1 and the reaction radius R are known. Unfortunately, one cannot arbitrarily choose these parameters in a real experiment. However, valuable information can be extracted from an experimental dependence of the reaction time τ_{Σ} on the viscosity of the medium, η , as the latter can be varied to some extent. Suppose that the parameter $1/k_1c_L$ is large enough so that the reaction time τ_{Σ} can be approximated by Eq. 8. The first term, $\tau_F^{(app)}$, in the right side of Eq. 8 repre-

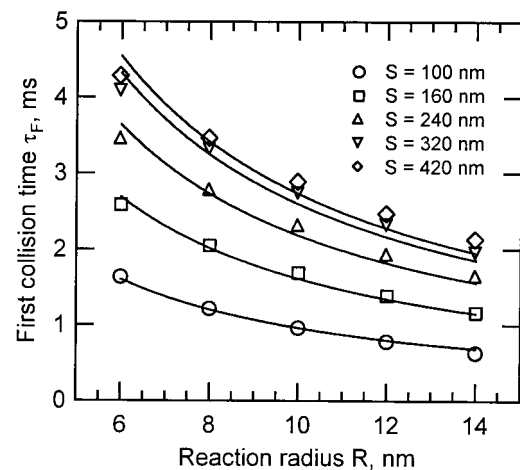


FIGURE 6 First collision time $\tau_F^{(app)}$ as a function of the reaction radius R for various contour separations S of the reactive groups. The interpolation curves are the best fits of the form $\tau_F \sim R^{-1}$.

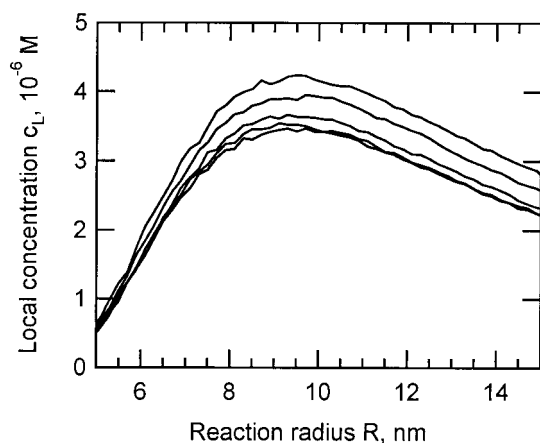


FIGURE 7 Local concentration c_L as a function of the reaction radius R . The curves, given for the contour separations $S = 100, 160, 240, 320$, and 420 nm, follow in order from top to bottom.

sents the contribution of the internal diffusion and, hence, is directly proportional to the viscosity η . On the contrary, the second term, $1/k_1 c_L$, is viscosity-independent. Consequently, if the viscosity is increased by a factor of

η/η_0 , the new value of the reaction time is given by

$$\tau_\Sigma = (\eta/\eta_0)\tau_F^{(app)} + 1/k_1 c_L. \quad (9)$$

The experimental plot of τ_Σ versus η allows, in principle, determination of $\tau_F^{(app)}$ and $1/k_1 c_L$ according to Eq. 9.

In some cases, such as site-specific recombination, the product of reaction depends on the type of the successful collision. The site-specific recombination has two main steps: 1) the formation of a synaptic complex (the process modeled here), and 2) the exchange of DNA strands. Although the overall reaction time is not necessarily limited by the first step, the kinetics of synapsis is crucial for the topology of the product. Suppose that the intrinsic rate constant k_1 is small and the parameter $1/k_1 c_L$ is large. Then the synapse is caused by reptation. As a consequence, the product should have unique and relatively simple topology. For example, if the product consists of two catenated rings, they will be linked only once (Fig. 8 A). In the absence of supercoiling, the rate of synapsis will drop dramatically following the decrease in the local concentration c_L .

In the opposite case, when k_1 is so large, that $1/k_1 c_L$ is close to 0, the majority of the synaptic complexes is formed by bending. Consequently, the recombination sites trap a variable number of supercoils. The products do not have unique topology. If the products are catenanes, they will be, in general, multiply linked (Fig. 8 B). The synapsis rate does not strongly depend on the superhelical density because the mean time $\tau_F^{(b)}$ of the first collision caused by bending is not as sensitive to this parameter as the local concentration c_L .

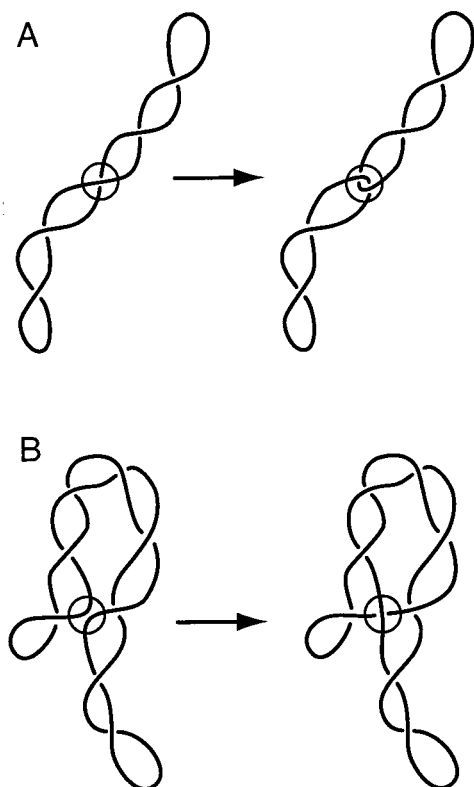


FIGURE 8 Schematic representation of site-specific recombination in the cases of small (A) and large (B) intrinsic second-order rate constant k_1 .

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