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Fluctuations in superhelical DNA

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ABSTRACT

The effect of superhelicity on the base-pair opening probability and on the probability of occurrence of cruciform states in palindromic regions is theoretically treated. The calculations show that below the superhelix density value of $-\sigma=0.05$ superhelicity does not appreciably affect the characteristics of DNA secondary structure fluctuations. In the range of physiological superhelix densities σ ($-\sigma=0.05$-0.09) the base-pair opening probability markedly increases. However, within this range of σ the base-pairs are opened only transiently and permanently open regions are not formed. Permanently opened regions appear at higher negative superhelix densities ($-\sigma \geqslant 0.10$). At the values of $-\sigma$ higher than 0.06 a cruciform structure in the palindromic region centred in position 3965 proves to be the most probable fluctuational disturbance in the ϕx174 duplex DNA.

Different experimental approaches used for probing the fluctuations in superhelical DNA have been analysed. The results suggest that most direct quantitative information can be derived from data on the nicking of closed DNA by single strand-specific endonucleases. Such data (Wang,1974) accord with the results of theoretical calculations. Calculations show that, due to base-pair opening, the total free energy of superhelical DNA should depend parabolically on σ only up to some critical value of $\sigma=\sigma_c$. If negative superhelicity exceeds this critical value, which under physiological conditions proves to be $-\sigma=0.085$, the free energy should increase linearly with $-\sigma$. The biological role of supercoiling is discussed in the light of obtained results.

INTRODUCTION

As a rule DNA functions in the cell in the form of a closed circular duplex. The double helical DNA accurately extracted from many cells and virus particles proves to be not only closed circular but at the same time twisted into a left-handed (negative) superhelix. This supercoiling arises from a deficit of the number of revolutions of one strand of the double helix

round the other, compared to linear DNA under the same external conditions (ionic strength, temperature, etc.). Until recently the biological significance of this negative superhelicity remained questionable. All doubts vanished after the discovery in a variety of organisms of a new class of enzymes, topoisomerases, which change the DNA superhelix density (for review see (1)). Among them of particular interest is the DNA gyrase. This enzyme induces negative superhelical turns in closed duplex DNA and is shown to be necessary for DNA replication and recombination (2,3).

In this connection the question of physical consequences of DNA supercoiling becomes of great interest. There is a lot of experimental evidence to the effect that the double helix in negatively supercoiled DNA is somewhat weakened (4-14). That the superhelicity should have some destabilizing effect on the DNA secondary structure immediately follows from the available estimates of the free energy of superhelix formation (15-19). To calculate the probabilities of different fluctuational violations of the DNA double helical structure one needs a thorought statistical-mechanical treatment of the equilibrium between various conformational states of DNA and its perturbation by supercoiling.

For the case of linear DNA a practically adequate theoretical description of the equilibrium between the two main conformational states of DNA base-pairs, the closed and opened ones, has been achieved over the past years. The theory was crucially tested by a direct comparison of calculated and measured differential melting profiles for the DNAs whose complete nucleotides sequences were available (20,21). Originally developed to explain the helix-coil equilibrium within the melting range, i.e. at elevated temperatures, the theory has proved to be applicable to the calculations of base-pair opening probability outside the melting range. This applicability was shown by a quantitative comparison of the results of such calculations with the results of probing the DNA and synthetic polynucleotides with formaldehyde (22,23). These findings have made it possible to carry out reliable calculations of fluctuational violations of the DNA double helix for practically any conditions.

The calculations have shown that under physiological conditions linear DNA has so stable helical structure that virtually only individual base pairs may be opened with the low probability of about 10^{-5} (22).

A quite different situation may be found in the case of negatively supercoiled closed duplex DNA. To study this case theoretically one has to introduce into the ordinary helix--coil equilibrium calculation scheme the supercoiling free energy as a function of superhelix density. Since the latter function has been evaluated (17,18), a theoretical study of fluctuational violation of the double helix for the case of closed circular DNA has become possible. Note that Hsieh and Wang (16) were the first to treat the problem theoretically. The main advantage of our sofisticated statistical-mechanical approach over their simple thermodynamic analysis consists in the possibility to calculate the probability of occurrence of different conformational states for specific DNA sequence. The results of such a study are presented in this paper.

THEORY AND CALCULATION METHODS

To perform calculations of fluctuations in supercoiled DNA the original model used in the case of linear molecules (22,23) has had to be modified. This original model is known to be based on an assumption that each base-pair may fall into any of two possible states: helical (closed) or disrupted (opened). The free energy of transition between these states depends on the particular base-pair type (AT or GC). Besides, the formation of a new open region inside the helical one requires additional energy F_s. The free energy of transition of a given base-pair from the closed to the open state depends, under fixed ionic conditions, on temperature alone. It is not true in the case of closed circular DNA. Indeed, an open base-pair in a closed circular DNA molecule changes the energy of superhelix formation. As a result, to calculate the probabilities of base-pair fluctuational openings in closed duplex DNA, one needs the energy of superhelix formation G as a function of two variables: the titratable superhelix density σ, and the fraction of open base-pairs θ ($\theta=m/N$ where m is the number of open

pairs and N is the total number of base-pairs in DNA). Unfortunately, the $G(\sigma,\vartheta)$ function cannot be directly determined from experimental data due to the lack of data on independent variation of the two variables, σ and ϑ. In fact, one can only measure experimentally the value of $G(\sigma,\langle\vartheta\rangle)$ where $\langle\vartheta\rangle$ is the mean fraction of open base-pairs corresponding to the given value of σ. So, to determine the $G(\sigma,\vartheta)$ function from the available experimental data we need some additional assumptions. In accordance with (16,24) we assume that the opening of any ten base-pairs entails the same change in the energy of superhelix formation as a decrease by unity in the number of titratable superhelical turns (this assumption and its limitations are discussed at length in (25)). It means that the $G(\sigma,\vartheta)$ function satisfies the equality:

$$\frac{\partial G}{\partial \sigma} = \frac{\partial G}{\partial \vartheta} \qquad (1)$$

The general solution of equation (1) is $G(\sigma,\vartheta)=f(\sigma+\vartheta)$ where f is an arbitrary function. Both, the experimental data (9) and the theoretical calculations (see below), show that in the region of small absolute values of σ the equilibrium fraction of open base-pairs $\langle\vartheta\rangle$ in superhelical DNA is negligible compared with σ. It means that in experiments on the equilibrium closing of nicked circular DNA the $G(\sigma,0)$ value is in fact measured. The results of these experiments (17,18) are known to satisfy the equation:

$$G(\sigma,0) = 10\ RT\ N\ \sigma^2 \qquad (2)$$

So, finally we obtain the following generalization of empirical equation (2):

$$G(\sigma,\vartheta) = 10\ RTN(\sigma + \vartheta)^2 \qquad (3)$$

It is on this equation that our present study is based. We shall once again dwell on the problem of its validity in the Discussion.

When the energy of superhelix formation is introduced into the partition function for the helix-coil equilibrium in the form of equation (3) the factors $\exp(-10N(\sigma + m/N)^2)$ appear in addition to the ordinary terms. From the formal point of view these factors are characterized by an unusual nonlinear (para-

bolic)term for the number of opened base-pairs m at the exponent. It reflects the dependence of the state of a given base--pair on the states of all the other pairs along the molecule. Special methods for calculation of the DNA partition function have been elaborated to allow for these new factors. In detail these methods are presented in (25). The most effective and practically rigorous method which we have used to obtain the results of present paper is as follow.

Since the terms which correspond to the real equilibrium mean number of opened pairs <m> make the main contribution into the partition function, the free energy of transition of any base-pairs from closed to open state will change due to superhelicity by this value:

$$\delta G = 20\ RT(\sigma + {}^{\langle m \rangle}/N) \qquad (4)$$

Provided δG is known one can calculate the DNA partition function by a standard method and obtain the value of <m> from it. The real equilibrium values of δG and <m> correspond to self-consistent quantities when the value of <m> substituted into equation (4) coincides with one obtained from the partition function. The results obtained by this method accord very well with the results of calculation by another, absolutely rigorous but time-consuming, algorithm (see (25)).

Along with the open states superhelicity should favour a formation of hairpin and cruciform structures in palindromic sequences of DNA (9,16). These structures will diminish the effective superhelix density value in exactly the same manner as the former ones. To allow for the hairpin and cruciform occurrence a special algorithm has been elaborated (25). In our calculations presented in this paper it was assumed that a hairpin should have no less than three bases in its loop. The energy of each helix end wherever it occured was assumed to be $F_s/2$ (i.e. about 3 kcal/mol). This energy corresponds to a statistical weight value lying somewhere between the experimental estimates of the weighting factors of hairpin loops (26-28). Our results show little or no dependence on the particular value of this parameter.

Along with the novel elements mentioned above and discussed in detail elsewhere (25), our model includes all the fea-

tures of the convenient helix-coil transition model (see, e.g. (20) and references therein). In our study we have used a standard set of theoretical parameters: melting enthalpy U_{AT} = 8 kcal/mol; cooperatively factor $\exp(-F_s/RT)=5\times10^{-5}$; loop-weighting factor $\alpha=1.5$; $T_{AT}=64.8^{\circ}C$: $T_{GC}-T_{AT}=41.4^{\circ}$. The last quantities correspond to the particular ionic conditions 0.1 M Na^+) chosen by us.

RESULTS OF CALCULATIONS

The theory outlined in the previous section makes it possible to calculate the probabilities of different conformations of supercoiled DNA under fixed external conditions for an arbitrary sequence of nucleotides and any titratable superhelix density. We have performed our calculations for the parameter values corresponding to 0.1 M Na^+ and $37^{\circ}C$ and the øx174 DNA sequence of nucleotides determined by Sanger et al.(29). In the course of our calculations we have varied the negative titratable superhelix density value - σ from zero up to 0.12. Our conditions differ from the standard conditions (0.2 M NaCl,37°) chosen by Bauer (1) for which he has tabulated the superhelix densities of all closed duplex DNAs studies so far. Fortunately, this difference in sodium concentration leads to utterly negligible changes in our results so that they are valid for the standard conditions as well.

The results of our calculations are presented in Table I. In the first column the titratable superhelix density values are listed. The second one presents the mean probability of a base-pair opening. The third and fourth columns present the opening probabilities for the base-pairs situated in the centre of the AT-richest region (position 1397 of the sequence øx174 DNA) and the GC-richest region (position 888). These data vividly demonstrate not only an increase in the overall opening probability but also a great enhancement of discrimination between different DNA regions with increasing superhelix density. Besides, the mean number of base-pairs in a transiently opened region also sharply increases (see the fifth column of Table I). The correlation length defined in (22) quantitatively characterizes the long-range influence of the state (open or closed) of a given base-pair on the opening probability of anotner one.

Table I Results of theoretical calculations of the main fluctuations characteristics in superhelical DNA[+]

Superhelix density - σ	Mean probability of base-pair opening	Probability of opening of a base-pair located in the most AT-rich region	Probability of opening of a base-pair located in the most GC-rich region	Mean number of base-pairs in opened region	Mean correlation length (in base-pairs)	Probability of cruciform occurence
0	1.3×10^{-5}	2.2×10^{-5}	4.0×10^{-6}	1.1	6.9	2.3×10^{-15}
0.02	2.2×10^{-5}	3.9×10^{-5}	6.5×10^{-6}	1.2	8.6	7.4×10^{-12}
0.04	3.9×10^{-5}	7.6×10^{-5}	1.1×10^{-5}	1.3	11.3	3.9×10^{-8}
0.06	8.7×10^{-5}	2.2×10^{-4}	2.0×10^{-5}	1.6	17.7	6.5×10^{-4}
0.08	1.0×10^{-3}	5.2×10^{-2}	4.0×10^{-5}	6.8	35.3	0.59
0.10	1.6×10^{-2}	0.76	4.9×10^{-5}	33.1	65.8	0.97
0.12	3.5×10^{-2}	0.93	5.2×10^{-5}	46.2	81.9	0.99

[+]Calculations were performed for nucleotide sequence of DNA ϕx174 and for theoretical parameters corresponding to conditions: 37°, 0.1 M Na^+.

This value is given in the sixth column of Table I.

But a truly dramatic effect supernelicity has on the probability of occurrence of hairpin and cruciform structures in large palindromes. There are two large palindromes in ϕx174 DNA which may form hairpins containing 8 base-pairs in their stems, centred in positions 3008 and 3965. The probability of occurrence of a cruciform structure in the latter is presented in the last column of Table I. The cruciform, hairpin, opened and helical states of a palindromic sequence compete with each other in superhelical DNA in a very complex way. As a result the probability of occurrence of cruciform and hairpin states in all palindromic regions of ϕx174 DNA other than the two mentioned above remains negligible at any value of superhelix density at least up to the value $-\sigma=0.14$.

COMPARISON WITH EXPERIMENT

Turning to the experimental situation note first of all

that our theory may form the basis for a quantitative interpretation of the numerous data by Lebowitz with co-workers (4,12-14) on the comparison of modification rates and depths in supercoiled and unsupercoiled DNAs using different chemical agents. These studies require thorough theoretical treatment which should take into account a whole set of elementary chemical reactions with monomers, as in the case of linear DNA interaction with formaldehyde (22). Without such a thorough theoretical study any quantitative interpretation of the experimental data of Lebowitz et al.(4,12-14) with respect to the particular DNA states they disclose seems to us virtually impossible. Indeed, a theoretical study of DNA interaction with formaldehyde has demonstrated a great complexity of the process of reversible chemical modification of DNA (see (22)). The very first steps of the modification may change the DNA structure, and the following process may reflect this altered structure rather than the original one. The spectacular data on supercoiled DNA binding with unwinding proteins (such as the product of gene 32 of bacteriophage T4)(5,10,11) are unlikely to give more direct information about the DNA structure before binding than the data on chemical modifications.

The most suitable agent for direct probing of fluctuational opening in DNA would be one whose very first reaction step with DNA could be registered. Closed superhelical DNA offers a unique opportunity for the realization of such an approach. Indeed, a single strand - specific endonuclease will convert superhelical DNA, after an introduction of the first nick, into a relaxed circular form which is easily distinguished from the original superhelical form by a number of techniques. So, the rate constant of the reduction of superhelical form in the presence of single-strand-specific endonuclease should be directly proportional to the mean probability of fluctuational opening of a base-pair. Of course, in reality the situation may be complicated by some dependence of the endonuclease digestion rate constant on the opened region's length and the nucleotide sequence.

The effect of superhelicity on the rate of DNA conversion from the covalently closed to the open circular form under the

action of single strand-specific endonucleases was most thoroughly studied by Wang (9). He used two enzymes - from Neurospora crassa and from Mung bean. Both showed a significant difference in the initial nicking rate for the native closed duplex PM2 DNA as compared with the single-stranded circles of native fd DNA. A quantitative analysis of these data leads to the following estimates for the opening probability in the case of native PM2 DNA: 8×10^{-3} in the case of Neurospora enzyme and 2×10^{-3} in the case of Mung bean enzyme (9). Our calculations give the value of 7×10^{-3} for the corresponding superhelix density ($-\sigma=0.09$ under standard conditions (1)). The Mung bean enzyme seems to be less suitable for probing the mean opening probability because, in contrast with the Neurospora enzyme, it is markedly site-specific (see ref.9). Probably the Mung bean enzyme is more sensitive to size and/or sequence of opened regions. The fact that we used in our calculations the sequence of ϕx174 DNA while the experimental data were obtained for PM2 DNA, cannot be of great significance in the case of such averaged quantity as the mean opening probability, provided that these two DNAs have close GC-contents. Both endonucleases show nearly the same dependence of the initial nicking rate on the DNA superhelix density and this dependence accords with the theoretical curve for the opening probability (see Fig.1).

It should be emphasized that in our calculations we did not use any adjustable parameters. So a rather good agreement between some of our results and the most direct experimental data now available should be considered as strong evidence of the general validity of the theoretical model on which the calculations are based.

DISCUSSION

The physical state of negatively twisted DNA. With respect to the influence on DNA structure three ranges of the superhelix density may be distinguished. At small superhelix density values that extend to the value of $-\sigma=0.05$ the characteristics of secondary structure fluctuations remain virtually unaffected by variations in the superhelix density. Within a narrow range from $-\sigma=0.05$–0.06 to $-\sigma=0.08$–0.09 the situation undergoes dra-

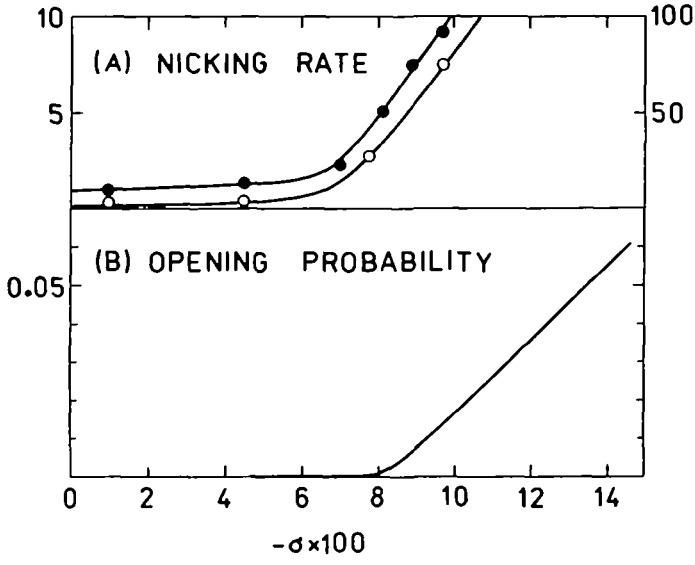

Fig. 1 (A) Experimental dependence of initial nicking rate on superhelix density for single strand-specific endonucleases from Neurospora crassa (•) and Mung bean (o). The original data of Wang (9) have been corrected in accordance with recent indications (1).
(B) Theoretical dependence of the mean base-pair opening probability in DNA on superhelix density. Calculations have been performed for theoretical parameters corresponding to 37° and 0.1 M Na^+. Experimental data were obtained for the same temperature and close ionic conditions.

matic changes. The mean base-pair opening probability increases more than tenfold within this interval. But the main change consists in a great variability of the opening probability for different DNA sites at $-\sigma=0.06$, as compared with $-\sigma=0.06$. It could be seen from the data of Table I. More vividly it is illustrated by a denaturation map, i.e. the dependence of the base-pair opening probability on its position in the sequence, presented in Fig.2. The probability of occurrence of cruciform structures is also shown in the same figure. Along with the appearance of characteristic denaturation maps the mean number of bases in opened regions and the correlation length also increase. It is an indication of a sharp increase in the probability of transient formation of prolonged opened regions. Within the same range of $-\sigma=0.06$–0.08 the probability of formation of

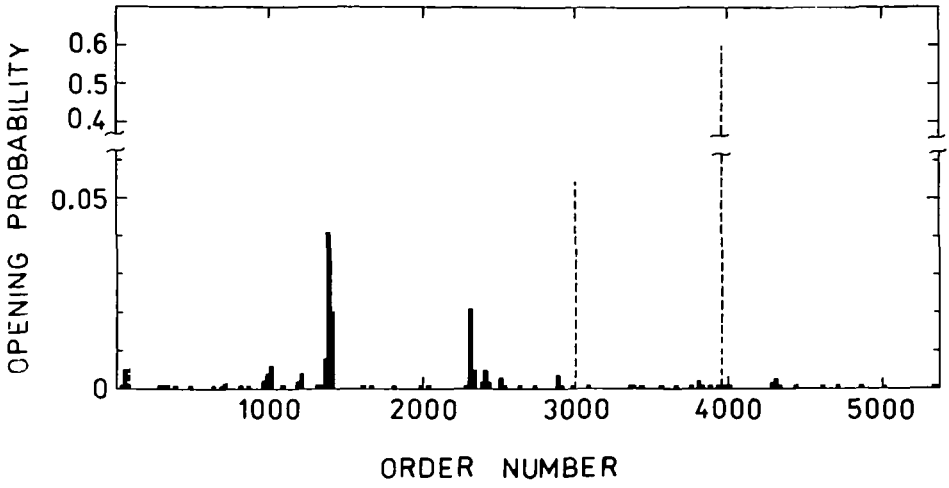

Fig. 2 Theoretical dependence of base-pair opening probability on the base-pair order number along the ϕx174 DNA sequence. Temperature 37°, 0.1 M Na^+, superhelix density $-\sigma$ =0.08. Dotted lines show the probability of occurence of cruciform structures in polyndromic regions.

a hairpin (cruciform) structure in the longest palindromic region becomes greater than the opening probability even for the most AT-rich region (see Table I).

So within the range of superhelix density values $-\sigma$=0.06--0.08 a DNA molecule becomes as it were alive, turning from a stable double helix into a diversely fluctuating entity. It should be borne in mind in this connection that the superhelix density values of most naturally occuring closed circular DNAs lie within the range $-\sigma$=0.05-0.09 (1).

Within this most interesting range of σ values stable unwound regions are practically not formed. Indeed, even for the AT-richest regions of ϕx174 DNA the opening probability is as small as 0.05 at $-\sigma$=0.08 and for the largest $-\sigma$ value known so far for native DNA (0.09) this probability reaches the value of 0.5 but only just. In this range of σ values only comparatively long palindromic regions of the double helix should be definitely disturbed. As a rule such regions include only a tiny portion of DNA sequence. In ϕx174 DNA there is only one such re-

gion centred in position 3965. The large probability of occurrence of cruciform state in this site is further enhanced by the fact that the palindromic region is surrounded by AT-rich regions. In Fig.3 the most probable state of this region for the values of $-\sigma=0.08$ is shown. It should be noted that we have confined ourselves to considering only secondary structures. A tertiary structure which is likely occur in the cruciform state (similarly to tRNA) may entail an additional stabilization of such violations of the regular double helix.

In the high superhelix density range, starting from the value of $-\sigma=0.08-0.09$ which we call the critical value of σ, σ_c, an increase of the mean base-pair opening probability, i.e. the fraction of open base-pairs $\langle\phi\rangle$, becomes equal to the increase of negative superhelix density (see Fig.1). Above this value the increase of $-\sigma$ is cancelled by the increase of opening probability. As a result within this third range of titratable superhelix density no qualitative change in the DNA fluctuational

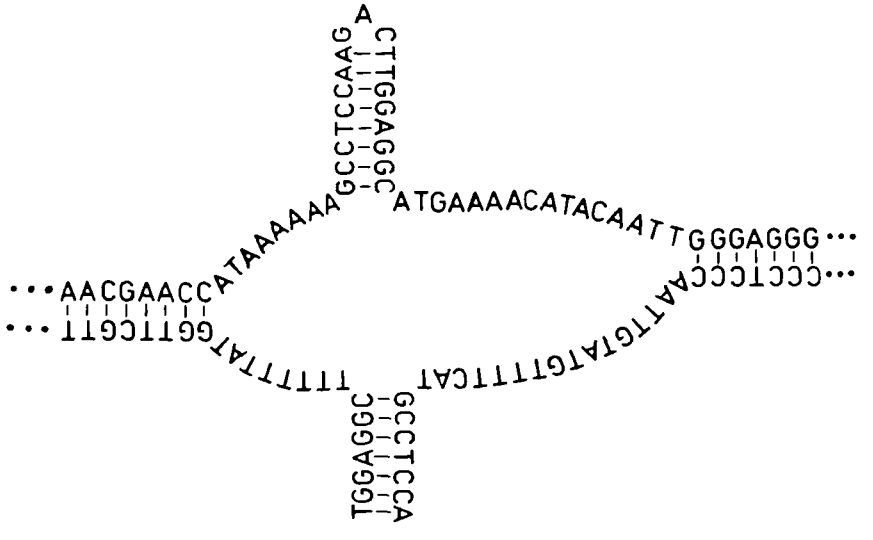

Fig.3 Secondary structure of fluctuation in superhelical øx174 DNA for which the theory predicts the highest probability at $-\sigma \geq 0.06$.

pattern is observed. At the same time marked quantitative changes occur, as seen from Table I and Fig.4.

Equation (3) immediately leads to the conclusion that in this range of σ values the free energy of superhelix formation remains constant. Nevertheless, the total free energy of supercoiled DNA continues to increase with an increase in the negative titratable superhelix density, now as a result of the base-pair opening process. But in contrast with the parabolic dependence on σ which is characteristic of the energy of superhelix formation (see equation (2)), the free energy of base-pair opening linearly depends on σ. So in the vicinity of the value of $\sigma=\sigma_c$ the dependence of free energy of supercoiled DNA on σ changes from parabolic to linear. Our results show that this change should occur abruptly, within a very narrow range of σ values. Indeed, Table I and Fig.1 show that even a small change of the $-\sigma$ value below the $-\sigma_c$ value leads to a drop in the fraction of open base-pairs. As a result, equation (3) converts to equation (2). So it is not surprising that the available experimental data should fit the equation (2). Indeed, Bauer and

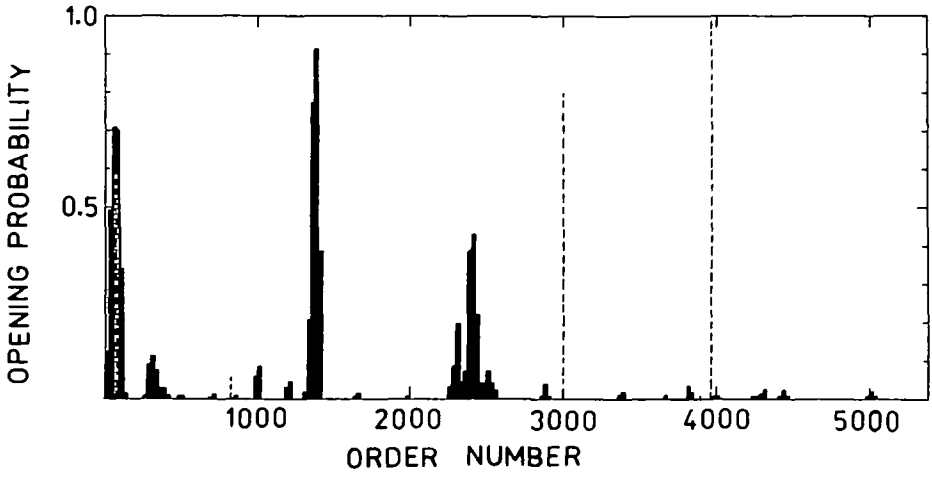

Fig.4 Theoretical dependence of base-pair opening probability on the base-pair order number along the ϕx174 DNA sequence. Temperature 37°, 0.1 M Na^+, superhelix density $-\sigma$ =0.12. Dotted lines show the probability of occurence of cruciform structures in polyndromic regions.

Vinograd (15) who were the first to study the free energy of supercoiled DNA used SV40 DNA which has a comparatively low $-\sigma$ value (about 0.06, under standard conditions (1)). The only study where some deviation from the parabolic dependence of the free energy on σ could be expected is that of Hsieh and Wang (16) who investigated PM2 DNA. This DNA is known to have a high negative superhelix density (about 0.09, under standard conditions). It should be noted, however, that the experiments (16) were carried out at a very high ionic strength (3 M CsCl). Our results cannot be valid for these conditions. Indeed, first, the DNA secondary structure is known to be highly stabilized under these conditions. Secondly, Hsieh and Wang (16) found the free energy of superhelix formation to equal about half the value for the same σ under standard conditions (this difference was briefly discussed in (17)). Both these factors increase the value of $-\sigma_c$ as one passes from standard conditions to 3 M CsCl. Although the value of $-\sigma$ also increases (see, e.g.(1)), it can by no means cancel the above increase in $-\sigma_c$. So most probably the $-\sigma$ value for PM2 DNA in concentrated CsCl solutions is lower than the $-\sigma_c$ value under the same conditions. A complete quantitative analysis of the situation for the case of concentrated CsCl solutions is complicated by the unresolved contradiction between the data of Bauer and Vinograd (15), on the one hand, and those of Hsieh and Wang (16), on the other. In any case, the data (16) are by no means inconsistent with our theory.

The functional role of natural supercoiling. Numerous data show that supercoiling has pronounced effect on DNA functioning both *in vivo* and *in vitro* (1-3,9,30-33). The simplest explanation of the biological role of superhelicity is that it decreases the energy which is required to locally unwind the DNA by unwinding proteins (9,32,33). Our results make it clear why for naturally occuring DNAs the negative superhelix density does not exceed the value of $-\sigma=0.09$ (1). Indeed, above the critical $-\sigma$ value which proved to be $-\sigma_c=0.085$, the decrease in the energy of superhelix formation from the opening of a base-pair vanishes (see equation (4)). So any increase of the negative titratable superhelix density above the value of $-\sigma=0.085$

is useless in the sense that the unwinding proteins would not any further be encouraged to open helical regions.

The results of the present theoretical study show that in the range of physiological superhelix density the fluctuational motility of the double helix should sharply increase. Most probably it is this effect that is responsible for most of the extraordinary properties of the negatively supercoiled DNA when it interacts with various chemical agents, particularly with some mutagens and carcinogens, and with some proteins (such as single strand-specific endonucleases and some DNA-unwinding proteins). For the latter superhelicity should be of particular importance. Indeed, as a rule proteins interact with large DNA regions. At the same time our results show a great enhancement of the effect of supercoiling on the opening probability as the region to be opened becomes longer.

The theory also predicts some specific long-range effects in superhelical DNA. For example, the opening of some region (e.g. as a result of its binding with an unwinding protein) may influence the opening probability of a region located far from the first one. This long-range interaction is quantitatively characterized by the correlation length shown in Table I. Note that the Table presents the mean value. Its concrete value may markedly depend on the nucleotide sequence between the two regions.

Finally, the fluctuational violations of the double helix may play an important role in the process of genetic recombination. In this connection it should be noted that some models of recombination postulate the formation of cruciform structures at intermediate stages (34,35). Our calculations show that the probability of formation of such structures may be caused by supercoiling to increase from a completely negligible value of 10^{-15} up to unity.

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