Thermodynamic Instability in DNA

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Abstract. The exact Hill's treatment of the Ising model describing DNA has been developed to make it applicable to the study of environment-coupled instabilities in DNA. Thermodynamic properties of DNA close to a co-operative order-disorder melting point have been investigated in terms of the developed Ising model. Assuming a weak coupling between the environment and Watson-Crick hydrogen bonds in DNA, it has been shown that the partition function can be broken down into a product of an environmental part, random coil part, and a helix part, the last one being dependent on $w_{\mathrm{k}}^{\beta}/\mathrm{k}T$ and $w_{\alpha\beta}/\mathrm{k}T$ only, where w_{k}^{β} and $w_{\alpha\beta}$ are DNA energy melting parameters. If the energy parameters depend on the volume V only, then the specific heat at a constant volume $C_{V,\beta}$ tends to approach very large values along the melting curve; however as may be deduced, the Ising-DNA model is unstable in the immediate neighbourhood of its melting point and undergoes denaturation. A suitable experimental measure for the stability of the native double-helical structure of DNA was formulated. Equations were constructed which permit the prediction of the typical thermodynamic behaviour of helix-coil transition under weak interactions with the environment. Instability in DNA has been shown to occur very close to the melting curve only, and $C_{V,\beta}>0$ (thermal stability) and the isothermal compressibility $\beta_e>0$, $\beta_\alpha>0$ (mechanical stability) — are all positive definite quantities, may be expected to parallel each other much from the melting point.

Key words: DNA — Ising model — Thermodynamic stability

Introduction

Recent success in the determination of relationships between genetic maps and nucleotide sequences for several viruses has resulted in a deepened interest in establishing a more exact relationship between the sequence and the thermodynamic stability of DNA (Lyubchenko et al. 1978; Gabbarro-Arpa et al. 1979; Azbel 1980a,b). A sensitive experimental approach to this problem is the analysis of high-resolution melting curves obtained by monitoring melting process

associated changes in UV absorbance (Ansevin et al. 1976; Vizard 1976; Gotoh et al. 1976; Yen and Blake 1981).

The stability of DNA is usually examined in terms of the response to changes in pH, ionic strength, temperature, and to various auxiliary chemical agents. The major feature of a detailed model of the solvent melting of DNA is that melting must proceed via the opening of many "gaps" rather than "unzipping" through the entire DNA molecule (this and the "all-or-none" model: all base pairs are coil or helix). The conditions of the stability of native DNA have now been quite adequately characterized: melting of the helical structure is observed in a large variety of non-aqueous solvents. For example, from Azbel's (1980a,b) high-resolution melting curves it follows that the conformational state of DNA correlates with its energy level at any temperature point within the range of melting, and that it exhibits discrete order-disorder regions, since melting results in formation of one or more phase boundaries that require more energy than normally needed to dissociate a given base pair. (The concept of phase boundary, the interphase between helical and coil regions, is an important factor in the overall energy consideration). In the DNA system, the helix-coil transition, in which the macromolecular configuration change also occurs, is relatively sharp, so that it is useful to consider the melting process in terms of a co-operative phenomenon occuring within a narrow temperature, pH, or solvent composition range. In a rough approximation, it is therefore possible to summarize the available data on the stability of DNA in terms of the conventional Watson—Crick hydrogen—bonding model of DNA stability having in mind that solvent medium (chemical agents including carcinogens) may radically affect the stability of hydrogen bonds, and that in a molecule of the structural complexity of DNA, other stabilizing and destabilizing factors must certainly be present (Sturtevant et al. 1958).

While the theory on helix-coil transition, which is of interest in association with the theoretical study of co-operative phase transitions in general, has been an active field of study over the past two decades, chemical instability of DNA in terms of a more exact relationship between the sequence and the thermodynamic stability has been the subject of relatively recent studies only. Nevertheless, there is still a justifiably widespread interest in this field. However, in order to understand DNA instabilities found experimentally in reactive DNA-chemical agent systems it is necessary to estimate the importance of general thermodynamic properties of the genetic material. The aim of this paper is to throw light on the physical basis of the solvent effect (e. g. generally denaturant) in the DNA stability, and to derive thermodynamic conditions under which DNA becomes instable. This problem has its own theoretical and mutagenic significance since the DNA denaturation process is widely assumed to lie at the heart of many of the most fundamental processes in living systems.

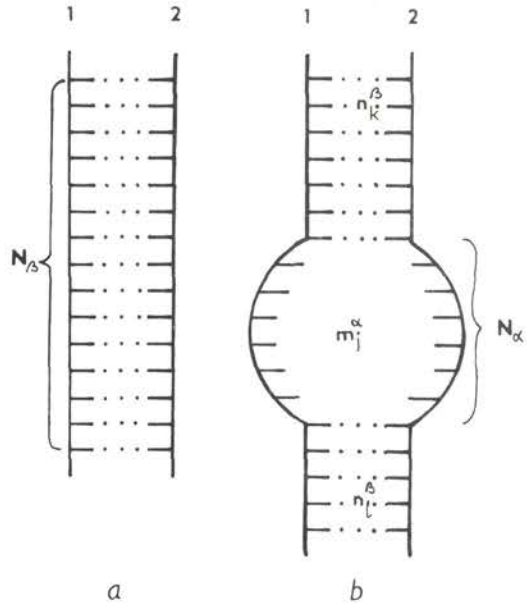

Fig. 1. (a) schematic representation of polynucleotide DNA — chains with hydrogen bonds (β) formed between nucleotide pairs in the straight portions (a) but with no hydrogen bonds formed in the "loop" portions (b).

Description of the model and underlying assumptions

The stability of the Watson—Crick model of DNA is attributed to the presence of highly specific pairs of hydrogen bonds between the purine and pyrimidine bases attached to the phosphosugar chain skeleton. If other secondary bonding is the source of stability, our principal considerations remain valid.

The co-operative nature of the instability of DNA is due to the necessity of having a minimum sequence of broken bonds contiguous with another one before sufficient flexibility is introduced into the structure to permit contraction. From a priori considerations hydrogen bonds which are broken singly in a very long linear array, would be believed to be isolated from each other at small extents of reaction. This is a result of the entropy of mixing which maximizes the probability of a dispersed configuration. However, it does further permis us, under certain assumptions, a somewhat speculative analysis of the thermodynamics of DNA stability. It is accepted that the DNA molecule is approximated as a chain of base pairs, each of which exists in either of two states: the ordered state (β), or randomly coiled configurations (α) usual to chain polymers. As shown in Fig. 1 a sequence of ordered base pairs β forms a region of double helix, while a sequence of random residues α constitutes a random coil chain part. This

structure involves the formation of a random-coil chain loops. If special properties of chain ends are neglected and the states of the whole chain and their energies are enumerated, then for the system whose states are discrete, stated in the traditional helix-coil theory which is of obvious importance for the full understanding of the deformation and stability of DNA, the canonical ensemble partition funkction is

$$Q = \sum_{\{r\}} \exp(-\gamma E_r),\ \gamma \equiv 1/\mathrm{k}T, \tag{1}$$

where the index $\{r\}$ enumerates all accessible states characterized by the corresponding free energy E_r, k is the Boltzmann constant; and T is absolute temperature.

There are several well-known methods for evaluating complicated partition functions, e. g., the matrix method and the method of steepest descent; but one method, the method of the maximum term is however particularly well adopted to this chain problem, and we shall confine our atention to it.

Hill's partition function for the one-dimensional Ising model of DNA

It is the purpose of the present section to give a brief review of the theory of Hill (1959) and to point out certain of its special consequences related to DNA. Here we shall consider the case of duplex DNA with two identical strands. Hence, the equations presented here may be regarded as a special case of general equations given in section I of Hill's paper.

Figure 1 (*a*) is a schematic representation of two polynucleotide chains, 1 and 2, with hydrogen bonds formed between base pairs in the straight portions but with no hydrogen bonds formed in the "loop" portions. The number of bases in each DNA chain must be identical in the helix and in the coil regions. In each chain, a base is in the state β if it is hydrogen bonded to a base in the other chain and it is in the state α if it is not hydrogen bonded. We put $j_{\alpha 1} j_{\alpha 2} \equiv j_\alpha$, and $j_{\beta 1} j_{\beta 2} \equiv j_\beta$, where j_α and j_β represent intrinsic partition functions for two kinds of bases, including only those contributions which differ in their α and β states.

According to the procedure described by Hill, the canonical ensemble partition function Q for a very long DNA molecule in the absence of solvent, containing a total of N bases and N_α α — bases (e. g. $N_\beta = N - N_\alpha$ β — bases) in each chain and $N_{\alpha\beta}$ phase boundaries between α and β groups, may be written in the form

$$Q(N_\alpha, N_\beta, N_{\alpha\beta}, V, T) = \sum_{N_\alpha} \sum_{N_{\alpha\beta}} j_\alpha^{N_\alpha}\, j_\beta^{N-N_\alpha}\, z^{N_{\alpha\beta}}\, t_\alpha^{\max}\, t_\beta^{\max} \tag{2}$$

Here

$$z = \exp(-\gamma w_{\alpha\beta}) \tag{3}$$

where $w_{\alpha\beta}$ is the free energy for the nearest neighbour $\alpha\beta$ interaction at each of the boundaries; the contribution of both chains is included in $w_{\alpha\beta}$. $N_{\alpha\beta}$ is the number of beginnings of sequences of coil bases. $t_\alpha^{\max}$ and $t_\beta^{\max}$ are the largest terms in the summations

$$\sum_{\{m\}} t_\alpha \sum_{\{n\}} t_\beta \tag{4}$$

where

$$t_\alpha = \Omega_\alpha \prod_i x_i^{m_i}, \qquad t_\beta = \Omega_\beta \prod_k y_k^{n_k} \tag{4'}$$

as determined by the maximum term method. The quantities appearing in these summations have following meaning. Here, Ω_β means the number of ways to divide $N - N_\alpha - (N_{\alpha\beta}/2)$ β base pairs, up into $(N_{\alpha\beta}/2)$ groups if there are n_k β groups each with $k(k \geq 0)$ of these β units (a total of $k+1$ base pairs in a group):

$$\Omega_\beta = (N_{\alpha\beta}/2)! \Big/ \prod_k n_k! \tag{5}$$

subject to the restrictions

$$\sum_k n_k = N_{\alpha\beta}/2$$

$$\sum_k k n_k = N - N_\alpha - (N_{\alpha\beta}/2) \tag{6}$$

Once the sequence of helix and coil bases has been specified, the number of denaturated base pairs is determined. y_k is the Boltzmann factor

$$y_k = \exp(-\gamma w_k^\beta) \tag{7}$$

where w_k^β is the free energy of each n_k group when native. The principal contributions to w_k^β are dipole-dipole electrostatic interactions. Briefly, a group of $k+1$ base pairs of β units is assumed to have a free energy excess w_k^β of that of $k+1$ pairs of single β units. Thus, the factor y_k measures the contribution of the partition function of n_k group of bonded base pairs relative to that of n_k free bases.

Similarly, the number of ways of distributing the excess (over $N_{\alpha\beta}/2$) of α bases is

$$\Omega_\alpha = (N_{\alpha\beta}/2)! \Big/ \prod_i m_i! \tag{8}$$

with restrictions

$$\sum_i m_i = N_{\alpha\beta}/2$$

$$\sum_i i m_i = N_\alpha - (N_{\alpha\beta}/2) \tag{9}$$

The nonhydrogen-bonded regions (loops) are represented in Fig. 1(*b*) by horizontal clusters of one or more α bases in each chain. It is assumed that a loop containing $i+1$ α bases in each chain has a free energy excess w_i^α of that of $i+1$ single α bases in each chain. The principal contributions to w_i^α appearing in

$$x_i = \exp(-\gamma w_i^\alpha) \tag{10}$$

are electrostatic and entropic contributions. We note, that by definition, $y_o = x_o = 1$, and the of $\{m\}$ and $\{n\}$ sums in Eqs. (4) are over all sets of m' s and n' s satisfying Eqs. (6) and (9).

$t_\alpha^{\max}$ and $t_\beta^{\max}$ were determined independently for given N_α and $N_{\alpha\beta}$. For $lnt_\alpha^{\max}$ we can write

$$lnt_\alpha^{\max} = -[N_\alpha - (N_{\alpha\beta}/2)]\, lnp + (N_{\alpha\beta}/2)\, ln\, \Sigma_\alpha \tag{11}$$

where

$$p = \exp(-\sigma_\alpha), \qquad \sum_\alpha = \sum_i x_i p^i \tag{12}$$

Here, σ_α is the undetermined Lagrange's multiplier, and

$$\Sigma'_\alpha/\Sigma_\alpha = \bar{i} = \frac{N_\alpha - (N_{\alpha\beta}/2)}{(N_{\alpha\beta}/2)} \tag{13}$$

$$\Sigma'_\alpha = \sum_i i x_i p = p(\partial\Sigma_\alpha/\partial p) \tag{14}$$

Equation (13) determines p as a function of N_α and $N_{\alpha\beta}$. Similarly, for the most probable distribution it holds

$$lnt_\beta^{\max} = -[(N - N_\alpha - (N_{\alpha\beta}/2)]\, lnq + (N_{\alpha\beta}/2)\, ln\, \Sigma_\beta \tag{15}$$

where

$$q = \exp(-\sigma_\beta), \Sigma_\beta = \sum_k y_k q^k \tag{16}$$

Further

$$\Sigma'_\beta/\Sigma_\beta = \bar{k} = \frac{N - N_\alpha - (N_{\alpha\beta}/2)}{(N_{\alpha\beta}/2)} \tag{17}$$

$$\Sigma'_\beta = \sum_k k y_k q^k = q(\partial \Sigma_\beta / \partial q) \tag{18}$$

Equation (17) determines q as a function of N_α and $N_{\alpha\beta}$.

Hill's partition function for the maximum term

We shall further approximate the Hill partition function (Eq. 2) using the canonical ensemble and the maximum term method. The system — DNA is characterized thermodynamically by the total number of N — bases of which N_α are helix, and temperature, T. Our task is to find the configurational degeneracy with the nearest neighbouring interaction energy $w_{\alpha\beta}$. Suppose that there are altogether $g(N_\alpha, N, N_{\alpha\beta})$ configurations with exactly $N_{\alpha\beta}$ phase boundaries $\alpha\beta$. That is, suppose there are $g(N_\alpha, N, N_{\alpha\beta})$ different ways in which N_α base pairs can be distributed on N sites giving $N_{\alpha\beta}$ phase boundaries of $\alpha\beta$ interaction. The contribution of these configurations to $Q(N_\alpha, N, N_{\alpha\beta}, T)$ is $g(N_\alpha, N, N_{\alpha\beta}) \cdot \exp(-\gamma N_{\alpha\beta} w_{\alpha\beta})$ and the full expression for $Q(N_\alpha, N, N_{\alpha\beta}, V, T)$ is

$$Q(N_\alpha, N, N_{\alpha\beta}, T) = j_\alpha^{N_\alpha} j_\beta^{N-N_\alpha} \sum_{N_{\alpha\beta}} g(N_\alpha, N, N_{\alpha\beta})\, t_\alpha^{\max} t_\beta^{\max} \exp(-\gamma N_{\alpha\beta} w_{\alpha\beta}) \tag{19}$$

where the sum is over all possible values of $N_{\alpha\beta}$ for given N_α and N. Having related $Q(N_\alpha, N, N_{\alpha\beta}, T)$ formally to $g(N_\alpha, N, N_{\alpha\beta})$, our next problem is to find an explicit expression for $g(N_\alpha, N, N_{\alpha\beta})$. We might note at the outset that for the total number of configurations with given N_α and N we must have:

$$g(N_\alpha, N, N_{\alpha\beta}) = \frac{N!}{N_\alpha!(N-N_\alpha)!} \tag{20}$$

In view of this relation, it is clear that Eq. (18) reduces, as it should, to equation

$$Q(N_\alpha, N, N_{\alpha\beta}, T) = j_\alpha^{N_\alpha} j_\beta^{N-N_\alpha} \frac{N!}{N_\alpha!(N-N_\alpha)!}\, t_\alpha^{\max} t_\beta^{\max} \tag{21}$$

when $w_{\alpha\beta} = 0$.

Since we shall be using only the maximum-term in the sum in Eq. (19), N_α, N, $N_{\alpha\beta}$ may all be regarded as very large numbers.

Analyzing this problem in detail for the configurational factor $g(N_\alpha, N, N_{\alpha\beta})$ we obtain

$$g(N_\alpha, N, N_{\alpha\beta}) = \frac{(N_\alpha - 1)!}{[(N_{\alpha\beta}/2) - 1]!\,[(N - (N_{\alpha\beta}/2)]!} \times$$

$$\times \frac{(N - N_\alpha - 1)!}{[(N_{\alpha\beta}/2) - 1]![N - N_\alpha - (N_{\alpha\beta}/2)]!} \tag{22}$$

If we drop unity compared with large numbers N_α, N, and if this formula for $g(N_\alpha, N, N_{\alpha\beta})$ is now inserted into Eq. (19) we obtain for $Q(N_\alpha, N, N_{\alpha\beta}, T)$

$$Q(N_\alpha, N, N_{\alpha\beta}, T) = j_\alpha^{N_\alpha} j_\beta^{N - N_\alpha} \sum_{N_{\alpha\beta}} \frac{N_\alpha!}{(N_{\alpha\beta}/2)!\,[N_\alpha - (N_{\alpha\beta}/2)]!} \times$$
$$\times \frac{(N - N_\alpha)!}{(N_{\alpha\beta}/2)![N - N_\alpha - (N_{\alpha\beta}/2)]!}\, t_\alpha^{\max} t_\beta^{\max} \exp(-\gamma N_{\alpha\beta} w_{\alpha\beta}) \tag{23}$$

The sum is however difficult, so let us use the maximum-term method. It says that, under appropriate conditions, the logarithm of a summation is essentially equal to the logarithm of the maximum term in the summation, i. e., we replace $lnQ(N_\alpha, N, N_{\alpha\beta}, T)$ by ln (maximum term in Q).

$$lnQ(N_\alpha, N, N_{\alpha\beta}, T) = N_\alpha ln j_\alpha + (N - N_\alpha) ln j_\beta + ln t_\alpha^{\max} + ln t_\beta^{\max} + ln g(N_\alpha, N, N_{\alpha\beta}) - \gamma N_{\alpha\beta} w_{\alpha\beta} \tag{24}$$

Then from the condition

$$\left(\frac{\partial lnQ}{\partial N_{\alpha\beta}}\right)_{N_\alpha, N, T} = 0 = \left(\frac{\partial ln g}{\partial N_{\alpha\beta}}\right)_{N_\alpha, N, T} + \left(\frac{\partial ln t_\alpha^{\max}}{\partial N_{\alpha\beta}}\right)_{N_\alpha, N, T} + \left(\frac{\partial ln t_\beta^{\max}}{\partial N_{\alpha\beta}}\right)_{N_\beta, N, T} - \gamma w_{\alpha\beta} \tag{25}$$

and with respect to the Eqs. (11), (15), (22), and also (13), (14), (17), (18), we find

$$\frac{[N_\alpha - (N_{\alpha\beta}^*/2)][N - N_\alpha - (N_{\alpha\beta}^*/2)]}{(N_{\alpha\beta}^*/2)^2} = \frac{1}{4z^2 p\,\Sigma_\alpha q\,\Sigma_\beta} \tag{26}$$

and when denoting equilibrium values as

$$N_\alpha^* = N - (N_{\alpha\beta}^*/2),\ N_\beta^* = N - N_\alpha - (N_{\alpha\beta}^*/2) \tag{27}$$

where $N_{\alpha\beta}^*$ is the value of $N_{\alpha\beta}$ giving the maximum term in the sum in Eq. (23), then

$$\frac{N_{\alpha\alpha}^* N_{\beta\beta}^*}{(N_{\alpha\beta}^*)^2} = \frac{1}{(2z)^2\, p\,\Sigma_\alpha q\,\Sigma_\beta} \tag{28}$$

This has the form of a chemical equilibrium quocient, for the "reaction"

$$2(\alpha\beta) \rightleftharpoons (\alpha\alpha) + (\beta\beta) \tag{29}$$

The "equilibrium constant" $(2z)^{-2}(p\,\Sigma_\alpha)^{-1}(q\,\Sigma_\beta)^{-1}$ is consistent with the partition functions:

$$j_{\alpha\alpha} = (p\,\Sigma_\alpha)^{-1},\ j_{\beta\beta} = (q\,\Sigma_\beta)^{-1},\ j_{\alpha\beta} = (2z)^{-2}$$

That is,

$$j_{\alpha\alpha}j_{\beta\beta}/j_{\alpha\beta}^2=(2z)^{-2}(p\,\Sigma_\alpha)^{-1}(q\,\Sigma_\beta)^{-1}= \tag{30}$$

$$=\frac{[\exp(\gamma w_{\alpha\beta})/2]^2}{\sum_i \exp[-\gamma(w_i+(i+1)\sigma'_\alpha)]\sum_k \exp[-\gamma(w_k+(k+1)\sigma'_\beta)]}$$

where $\sigma'_\alpha=\sigma_\alpha/\gamma$, and $\sigma'_\beta=\sigma_\beta/\gamma$.

Three types of parameters occur in Eq. (30).

One is the $z(T)$ parameter also known as the co-operative factor (frequently designated as $\sigma(T)$) representing interaction between the helical and coil regions. A co-operativity free energy $w_{\alpha\beta}$ is associated to every pair which is helical, but whose next neighbour is in the coil state. This corresponds to the fact that an extra amount of free energy is needed to denaturate the first base pair in a region. $w_{\alpha\beta}$ is assumed to have the same value for all base pairs. $z(T)$ is defined here as a tendency for bases in the same state to group together, or to "aggregate" in linear sequences. Another parameter appearing in definition of t_β (Eq. (4)) is y_k, which here has the meaning of an equilibrium constant for n_k pairs. The factor y_k (>0 since $w_k^\beta<0$) contains the decrease in statistical weight owing to the restriction of freedom of motion, but it is enhanced by the Boltzmann factor resulting from the n_k group hydrogen bond energy. However, an abnormally large decrease in statistical weight is assumed to be caused by the formation of the first hydrogen bond ($n_k=1$) after several unbonded bases ($m_i>1$) since such a hydrogen bond decreases the freedom of the bounded and restricts the freedom of the bonding base pair itself. Since the same Boltzmann factor is involved, this contribution to the partition function is frequently written as product $y_k z$, where z is less than a unity. From $z(T)<1$, follows that $w_{\alpha\beta}<0$. The negative $w_{\alpha\beta}$ means that the phase boundaries attract each other. Generally, in the case when z is a unity ($w_{\alpha\beta}=0$) there is no interaction between states of successive bases. An infinite helix-coil phase boundary ($w_{\alpha\beta}=\infty$) corresponds in our treatment to z equaling zero. The third parameter, x_i, is defined by the w_i^α interaction parameter, determining the stability of the random-coil conformations. It would appear a very crude approximation to break w_i^α down into interactions between the nearest neighboring bases. It is quite evident that the stability of the random coil structures of DNA which with excluded volume are not mutually easily penetrable depends on our understanding of the factors that are also important for the helix stability. The sum of all these interaction factors would then determine the helicity and stability of a particular region of the sequence. The exact contributions of these counteracting effects on the stabilities of the particular regions of the random coil structures occurring in Eq. (30) are difficult to assess at present.

When we define η, the fraction of broken hydrogen bonds as $\eta=N^*_{\alpha\beta}/N$, and $\Theta=N^*_{\alpha\beta}/2N$ and use Eq. (28) we find

$$\frac{(\eta-\Theta)(1-\eta-\Theta)}{\Theta^2}=(2z)^{-2}(p\,\Sigma_\alpha)(q\,\Sigma_\beta) \tag{31}$$

Equation (31) is a quadratic function in Θ, and gives $N^*_{\alpha\beta}$ as a function of N, N_α, and T

$$\Theta=\frac{N^*_{\alpha\beta}}{2N}=\frac{2\Theta(1-\Theta)}{\lambda+1} \tag{32}$$

where

$$\lambda=[1-4\eta(1-\eta)(1-(2z)^{-2})(p\Sigma_\alpha)^{-1}(q\Sigma_\beta)^{-1}]^{1/2} \tag{33}$$

These equations document the importance of molecular states of mixed character (i.e., partly helix and partly random coil conformations) in the region of DNA melting. Now, instead of the partition function (23), we have

$$Q(N_\alpha, N, N^*_{\alpha\beta}, T)=j_\alpha^{N_\alpha}j_\beta^{N-N_\alpha}\frac{N_\alpha!}{[(N^*_{\alpha\beta}/2)!(N_\alpha-(N^*_{\alpha\beta}/2)]}t_\alpha^{*\max}\times$$

$$\times\frac{(N-N_\alpha)!}{(N^*_{\alpha\beta}/2)![N-N_\alpha-(N^*_{\alpha\beta}/2)]!}t_\beta^{*\max}\exp(-\gamma N^*_{\alpha\beta}w_{\alpha\beta}) \tag{34}$$

A major problem of the above analysis is the validity of the parameters and of the theoretical model for temperatures below the helix-coil transition. Two considerations are relevant. First, what is the influence of temperature on the thermodynamic w's parameters? Second, can the physical process of the opening of large groups of base pairs be extrapolated to the opening of one base pair ($m_1=1$). Calorimetric measurements of several DNA sequences can answer these questions. Recent advances in methods of DNA synthesis should make better model systems available allowing to examine base-pair opening using melting curve and calorimetric analysis. DNA oligomers containing one to five A.T pairs flanked by defined lengths of G.C pairs provide an ideal system to examine the opening of internal A.T loops of different lengths. It has previously been noted (Lukashin et al. 1976. Wartell 1982) that a base-pair change has negligible effects beyond a few base pairs. This observation, however, assumes that the nearest-neighbor model describes accurately DNA co-operativity. If longer-range interactions are significant, then the influence of base-pair changes on the thermal stability of the surrounding region could be stronger. The correlation length may be said to increase considerably as the temperature or ambient environment shifts the base-pair opening equilibrium (29) to the helix-coil transition regions. In this regime, base-pair changes can affect base-pair opening in adjacent regions.

Effect of the solvent

Almost all theories, except for e. g. the approach of Gibbs and DiMarzio (1959), of

order-disorder phenomena are based on the implicit hypothesis that in constructing a partition function, the configurational partition function can be written without taking into account the strong coupling between DNA and the environment. A realistic treatment must allow for the possibility of interactions of molecules of the environment with N—H and C = O groups of helix and random coil regions via hydrogen bonds. It is possible to check on this hydrogen bond interactions for regions far away from the melting point since many properties (i. e., thermal expansion, heat capacity, elastic constants, torsional stiffness of the coil, effective torsional stiffness of the duplex etc.) would then essentially depend on the contribution of the environment.

In this section we shall consider a simple DNA model and we shall show that effects of the environment which have so far been ignored, can rigorously be taken into account without altering the formal appearance of the equations in the above section. In this paper, the constructed partition function (Eq. (34)) will serve as a framework onto which we shall graft the appropriate factors for coupling; so, instead of being concerned with the thermal behavior of an isolated one-dimensional Ising model of DNA usually represented as a "clamped" system of bases only, we wish to consider the mechanical behavior of a coupled DNA model which is a more realistic compressible model of DNA. Now, the variants of the partition function obtained on allowing for interaction are simpler if all the potential groups which are not intramolecularly hydrogen bonded are all allowed to be coupled with equal facility. The same is supposed for the intramolecularly hydrogen bonded bases. Thus, the partition function Q for a DNA chain with N_α coil bases, $N_{e\alpha}$ component molecules of the environment bound to N_α α sites, and $N_{e\beta}$ component of solvent molecules bound $N_\beta = N - N_\alpha$ β sites, can be written in the form

$$Q(N_\alpha, N, N_{e\alpha}, N_e, V, T) = j_\alpha^{N_\alpha} \frac{N_\alpha!}{(N^*_{\alpha\beta}/2)![(N_\alpha - (N^*_{\alpha\beta}/2)]!} q_\alpha^{N_{e\alpha}} \times$$

$$\times \frac{N_\alpha!}{N_{e\alpha}!(N_\alpha - N_{e\alpha})!} t_\alpha^{*\max} j_\beta^{N-N_\alpha} \frac{(N-N_\alpha)!}{(N^*_{\alpha\beta}/2)![N - N_\alpha - (N^*_{\alpha\beta}/2)]!} \times$$

$$\times q_\beta^{N_{e\beta}} \frac{(N-N_\alpha)!}{N_{e\beta}!(N-N_\alpha-N_{e\beta})!} t_\beta^{*\max} \exp(-\gamma N^*_{\alpha\beta} w_{\alpha\beta}) q_e^{N_e - N_{e\alpha} - N_{e\beta}} \tag{35}$$

where the partition function for a bound molecule of the enviroment is $q_\beta(t)$ at helix β site and $q_\alpha(T)$ at a coil α site, and q_e is a molecular partition function for each of the $N_e - N_{e\alpha} - N_{e\beta}$ molecules of the environment which are "free" hydrogen bonded to each other but not to the DNA chain.

The essential feature of our Ising model of the interrupted DNA is the competition between the helical and coil regions of the chain to grow at one anorher's expense. This presumpts the existence of both helical and coil configura-

tions and merely determines the relative probabilities of their occurrence under specified external conditions. These probabilities depend upon the specification of the end-correction. The model we have used completely neglects the role of the environment on the $w_{\alpha\beta}$, w_i^{α} and w_k^{β} free energies, and especially of the environment-end effect and is therefore incomplete in this sense. For the present we need not specify the model of DNA in detail. As far as the values of $w_{\alpha\beta}$, w_i^{α}, w_k^{β} as empirical parameters are concerned this is of no importance, since adjustements made to fit the experiment will automatically adjust them to include the thermodynamic effects of the environment even though this is not specified in the model. If absolute calculations are made based upon some notions of the values of $w_{\alpha\beta}$, w_i^{α}, and w_k^{β} to be anticipated from molecular considerations, then the neglection of the contributions of the environment becomes serious.

Assumption of weak interactions

The possibility of an instability for a compressible solid state lattice near an order--disorder lambda point was first pointed out by Rice (1954; 1967) who presented a very general thermodynamic discussion of the problem. This may serve as a physical basis for the estimation of instabilities in DNA too, since DNA is unstable in the immediate vicinity of its transition point and undergoes melting. Having these idea in mind, we assume a weak coupling between DNA and the evironment; i. e., we have already formulated a partition function for such a case in the factorized form (Eq. (35))

$$Q = Q_{\alpha} Q_{\beta} Q_{c} \tag{36}$$

a crucial feature of our model. Here,

$$Q_{\alpha} = j_{\alpha}^{N_{\alpha}} \frac{N_{\alpha}!}{(N_{\alpha\beta}^{*}/2)![N_{\alpha} - (N_{\alpha\beta}^{*}/2)]!} q_{\alpha}^{N_{c\alpha}} \frac{N_{\alpha}!}{N_{c\alpha}!(N_{\alpha} - N_{c\alpha})!} t_{\alpha}^{*\max} \tag{37}$$

$$Q_{\beta} = j_{\beta}^{N - N_{\alpha}} \frac{(N - N_{\alpha})!}{(N_{\alpha\beta}^{*}/2)![(N - N_{\alpha} - (N_{\alpha\beta}^{*}/2)]!} q_{\beta}^{N_{c\beta}} \frac{(N - N_{\alpha})!}{N_{c\beta}!(N - N_{\alpha} - N_{c\beta})!} t_{\beta}^{*\max} z^{N_{\alpha\beta}^{*}} \tag{37'}$$

$$Q_{c} = q_{c}^{N_{c} - N_{c\alpha} - N_{c\beta}} \tag{38}$$

It is well known, although a striking fact in solid state physics, that substances which undergo co-operative order-disorder transition usually exhibit anomalous variations in volume which extend over the same temperature range as the lambda spikes in the specific heat. Indeed, in most statistical theories the volume of the system is held fixed, and it is assumed that experiments can be carried out at constant volume. Since it is the pressure rather than the volume which is usually subject to experimental control, the mechanical behaviour of DNA below the

denaturation region may be of considerable interest. This conclusion is in agreement with e.g., intrinsic viscosity measurements (Sugalii et al. 1969) influenced by the excluded volume effects on the random coil structures in region of melting point. It should be noted that of all the thermodynamic functions, it is the Helmholtz free energy A that is directly proportional to $lnQ(V, T)$, and that A is the thermodynamic potential the natural independent variables of which are those of the canonical ensemble. The contribution to the properties of DNA which arise from the $Q(N_\alpha, N, N_{c\alpha}, N_c, V, T)$ term (Eq. (35)) can be deduced empirically from experiments performed on DNA considerably below its melting point. We emphasize here the contribution to various properties due to the $Q(N_\alpha, N, N_{c\alpha}, N_c, V, T)$ term which describes the base pairs ordering. In accordance with the above as a result of Eq. (36) equation for the Helmholtz free energy can be written as

$$A(N_\alpha, N, N_{c\alpha}, N_c, V, T) = -kTlnQ_\alpha(N_\alpha, N_{c\alpha}, V, T) - \\ -kTlnQ_\beta(N_\beta, N_{c\beta}, V, T) - kTlnQ_c(N_{c\alpha}, N_c, V, T) \tag{39}$$

As a result of Eq. (35) all the thermodynamic functions were written as a sum of three independent contributions. The contribution to the properties of the system which arise from Q_α and Q_c term can be deduced empirically from DNA denaturation experiments (Lukashin et al. 1976; Wartell 1977).

Here, the contribution to various properties due to Q_β term which describes the base pair ordering or helix form of DNA is emphasized. It is assumed that Q_β is a function of undimensional parameter $\xi \equiv (w_k^\beta + w_{\alpha\beta})\gamma$, where w_k^β is the free energy of some k member block of β units with k defined by Eq. (17), and it is assumed to be a function of the volume V only. This, together with Eq. (35), is what we call the assumption of a weak DNA — environment interaction. It should be noted that it includes cases of strong interactions, since the effect of the volume can be great, the only requirement being that ξ depends on V. Direct interaction between the bases and the environment vibrations may be of importance especially in one dimension. Indeed, this would be expected on purely physical grounds, for in one dimension a very direct correlation between the base orientation may exist and the average distance between the pair of the nearest neighbours. The quantity will, however, be a function of the spacing between base pairs.

Assumping a of weak interaction as formulated above, we note that the pressure p and the energy of the system: DNA — environment, by applying of the standard statistical mechanical equations, will be given by logarithmic differentiations with respect to V and T, respectively. Thus,

$$p = kT(\partial lnQ_\alpha/\partial V)_{N_\alpha, N_{c\alpha}, T} + (\partial lnQ_\beta/\partial \xi)_{N_\beta, N_{c\beta}, T}(d(w_k^\beta + w_{\alpha\beta})/dV) + \\ + kT(\partial lnQ_c/\partial V)_{N_{c\alpha}, N_c, T} = p_\alpha + (Q'_\alpha/Q_\beta)(d(w_k^\beta + w_{\alpha\beta})/dV) + p_c \tag{40}$$

The expressions for the configurational internal energy E_β at a constant volume can be written (Huang 1963) as

$$E_\beta = -(w_k^\beta + w_{\alpha\beta})(\partial lnQ_\beta/\partial\xi)_{N_\beta, N_{e\beta}, V} \tag{41}$$

Then, for (40) we have

$$p = p_\alpha + p_e - (E_\beta/(w_k^\beta + w_{\alpha\beta})(d(w_k^\beta + w_{\alpha\beta})/dV) \tag{42}$$

and for the total internal energy of the system E:

$$E = kT^2(\partial lnQ_\alpha/\partial T)_{N_\alpha, N_{e\alpha}, V} - (Q'_\beta/Q_\beta)(w_k^\beta + w_{\alpha\beta}) + kT^2(\partial lnQ_e/\partial T)_{N_{e\alpha}, N_e, V} = \\ = E_\alpha + E_\beta + E_e \tag{43}$$

where the prime indicates differentiation with respect to ξ. Now, from Eq. (43) we have

$$C_V = C_{V,\alpha} - (Q'_\beta/Q_\beta)^2(w_k^\beta + w_{\alpha\beta})^2/kT^2 + C_{V,e} \\ = C_{V,\alpha} + C_{V,\beta} + C_{V,e} \tag{44}$$

where the specific heat at a constant volume $C_{V,\beta}$ is defined as

$$C_{V,\beta} = k\xi^2(\partial lnQ_\beta/\partial\xi^2)N_\beta, N_{e\beta}, V \tag{45}$$

Now, by appropriate use of Eqs. (42), (43) and (44)

$$(\partial p/\partial V)_{N, N_e, T} = (\partial p_\alpha/\partial V)_{N_\alpha, N_{e\alpha}, T} - (kT)^{-1}(Q'_\beta/Q_\beta)^2(d(w_k^\beta + w_{\alpha\beta})/dV)^2 + \\ + (kT)^{-1}(Q''_\beta/Q_\beta)(d(w_k^\beta + w_{\alpha\beta})/dV)^2 + (Q'_\beta/Q_\beta)(d^2(w_k^\beta + w_{\alpha\beta})/dV^2) + \\ + (\partial p_e/\partial V)_{N_{e\alpha}, N_e, T} = (\partial p_\alpha/\partial V)_{N_\alpha, N_{e\alpha}, T} + (T/(w_k^\beta + w_{\alpha\beta})^2 . (d(w_k^\beta + \\ + w_{\alpha\beta})/dV)^2 C_{V,\beta} - (E_\alpha/(w_k^\beta + w_{\alpha\beta}) . (d^2(w_k^\beta + w_{\alpha\beta})/dV^2) + (\partial p_e/\partial V)_{N_{e\alpha}, N_e, T} \tag{46}$$

Analogously for $(\partial p/\partial T)_{N, N_e, V}$ we obtain

$$(\partial p/\partial T)_{N, N_e, V} = (\partial p_\alpha/\partial T)_{N_\alpha, N_{e\alpha}, V} + (Q'_\beta/Q_\beta)^2(w_k^\beta + w_{\alpha\beta})/kT^2) \times \\ \times (d(w_k^\beta + w_{\alpha\beta})/dV) - (Q''_\beta/Q_\beta)(w_k^\beta + w_{\alpha\beta})/kT^2)(d(w_k^\beta + w_{\alpha\beta})/dV) + \\ + (\partial p_e/\partial T)_{N_{e\alpha}, N_e, T} = (\partial p_\alpha/\partial T)_{N_\alpha, N_{e\alpha}, V} - C_{V,\alpha}/(w_k^\beta + w_{\alpha\beta})(d(w_k^\beta + w_{\alpha\beta})/dV) + \\ + (\partial p_e/\partial T)_{N_{e\alpha}, N_e, V} \tag{47}$$

Instability in DNA

The aim of this paragraph is to formulate stability conditions under which the DNA-environment system becomes unstable. At a given temperature T and $N = \text{const.}$, $N_e = \text{const.}$, the system DNA-environment is stable, at least locally, if

the Helmholtz free energy satisfies the condition $(\partial^2 A/\partial V^2)_{N,N_e,T} \geq 0$. For the model system considered above, this stability condition requires that before the opening of base pairs ($N_\alpha = 0$)

$$-(\partial p_\beta/\partial V)_{N_\beta, N_{e\beta}, T} - (\partial p_e/\partial V)_{N_{e\beta}, N_e, T} \geq 0 \tag{48}$$

and after the opening of base—pair and loop formation

$$-(\partial p_\alpha/\partial V)_{N_\alpha, N_{e\alpha}, T} - (\partial p_\beta/\partial V)_{N_\beta, N_e, T} - (\partial p_e/\partial V)_{N_{e\alpha}, N_e, T} \geq 0 \tag{48'}$$

where $(\partial p_\beta/\partial V)_{N_\beta, N_{e\beta}, T}$ is defined by Eq. (47). Since $(\partial p_\alpha/\partial V)_{N, N_e, T}$ is related to β_α, the isothermal compressibility of the disordered DNA regions — loops, by

$$\beta_\alpha = -(V(\partial p_\alpha/\partial V)_{N_\alpha, N_{e\alpha}, T} > 0 \text{ (mechanical stability)} \tag{49}$$

and $(\partial p_e/\partial V)_{N_{e\alpha}, N_e, T}$ is related to β_e, the isothermal compressibility of the environment

$$\beta_e = -V(\partial p_e/\partial V)_{N_{e\alpha}, N_e, T} > 0 \text{ (mechanical stability)} \tag{50}$$

then the stability criterion for our isothermal system in the absence of convection may be written as

$$\begin{aligned} &1/\beta_\alpha + 1/\beta_e - V(T/(w_k^\beta + w_{\alpha\beta})^2 \times (\mathrm{d}(w_k^\beta + w_{\alpha\beta})/\mathrm{d}V)^2 C_{V,\beta} + \\ &+ \mathrm{VE}_\alpha/(w_k^\beta + w_{\alpha\beta}) \times (\mathrm{d}^2(w_k^\beta + w_{\alpha\beta})/\mathrm{d}V^2) \geq 0 \end{aligned} \tag{51}$$

Now, if direct correlation between the base pairs and the average distance between a pair of the nearest neigbours are taken into account as r, then Eq. (51) can be rewritten as

$$\begin{aligned} &1/\beta_\alpha + 1/\beta_e - V(T/(w_k^\beta + w_{\alpha\beta})^2)\left[\frac{\mathrm{d}w_k^\beta}{\mathrm{d}r}\frac{\mathrm{d}r}{\mathrm{d}V} + \frac{\mathrm{d}w_{\alpha\beta}}{\mathrm{d}V}\frac{\mathrm{d}r}{\mathrm{d}V}\right]^2 C_{V,\beta} + \\ &+ VE_\beta/(w_k^\beta + w_{\alpha\beta})\left[\frac{\mathrm{d}}{\mathrm{d}r}\frac{\mathrm{d}w_k^\beta}{\mathrm{d}V}\cdot\frac{\mathrm{d}r}{\mathrm{d}V} + \frac{\mathrm{d}}{\mathrm{d}r}\frac{\mathrm{d}w_{\alpha\beta}}{\mathrm{d}V}\cdot\frac{\mathrm{d}r}{\mathrm{d}V}\right] \geq 0 \end{aligned} \tag{52}$$

Inequalities such as Eq. (51) are generally referred to as thermodynamic stability conditions. We shall not go into further details concerning such thermodynamic stability conditions. This theory has been initiated by Gibbs and is presented in many textbooks. Let us only mention that including Eq. (51), β_α, $\beta_e > 0$ (mechanical stability), $C_{V,\beta} > 0$ (thermal stability). Thus the isothermal compressibility and the specific heat (at a constant volume) have to be positive definite quantities, and the stability condition (51) is dependent on the signs of the coefficients β and $C_{V,\beta}$. This statement follows from the general considerations. An absolute physical requirement for $A(\varrho, \xi)$, where ϱ is the density in the thermodynamic limit, as a function of $\xi \equiv W/\mathrm{k}T$ ($W \equiv w_k^\beta + w_{\alpha\beta}$), is that it became concave. This is

equivalent to the fact that the specific heat is non-negative since is defined by Eq. (45). Fortunately, it is true. Another absolute requirement is that $A(\varrho, \xi)$ is convex as a function of ϱ. This is called thermodynamic stability and is equivalent to the fact that the compressibility is non-negative, since $(\text{compressibility})^{-1} = \partial p/\partial \varrho = \varrho \partial^2 A(\varrho, \xi)/\partial \varrho^2$. Now, $1/\beta_\alpha$ and $1/\beta_e$ shall in general have a finite positive value which is a slowly varying function of temperature, while w's and their derivatives with respect to V will be finite non-zero quantities which are independent of temperature (or free energies if w's are functions of T in the process) and the zero of energy for w's is finite separation. The Ising internal energy E_β will also be finite at all temperatures; however the configurational heat capacity at a constant volume, $C_{V,\beta}$, is known to approach very large values in the vicinity of the melting point. The behavior is the crucial factor. If at the melting point $C_{V,\beta}$ approaches $+\infty$, there must be an instability near that point unless the components of the environment are completely incompressible (in which case, $1/\beta_\alpha = 1/\beta_e = +\infty$). This result depends only on our assumption of a weak coupling in our model of DNA. We call attention to the fast rise of the specific heat near the melting point (Scheffler and Sturdevant 1969; Albergo et al. 1981) and point out that it is not possible to decide by these experiments whether the specific heat of DNA may be infinite at all, e. g., for deoxyadenylic acid and deoxythymidylic acid a heat capacity difference between intact and broken base pairs of no more than 84—167 J (mol bp K)$^{-1}$ was observed calorimetrically. On the other hand, representation of heat capacity as a function of tempereture for systems which undergo double thermal transition obtained from the derived relations (Cabani et al. 1976) are not directly comparable with the experimental results, due to difficulties in assigning a correct and meaningful base line to the latter. The closer one comes to the melting point, the greater fluctuations in energy, and hence in temperature will be. We note further that the fluctuations in temperature will eventually exceed a temperature difference in the melting point, $|T - T_m|$; then the temperature of the sample cannot be actually determined. Under such circumstance the specific heat (either $C_{V,\beta}$ or $C_{p,\beta}$) will be rounded off; however, the fluctuations are the smaller the larger the sample, and in the case of DNA it seems possible, at least in principle, to take a very large sample, so that the rounding off will occur at a very low value of $|T - T_m|$. The increase in fluctuations, the rise and divergence of the thermal capacity seems possible in this case. Data to test this idea are not available.

The instability of a compressible system DNA-environment in the immediate vicinity of its melting point follows directly from Eq. (51). The first two terms are positive, the third one negative; about the fourth term we are not sure whether it is negligible or not; whether it is positive or negative depends on the sign of w's. It is now easy to see how the mechanical instability will cause DNA to undergo a spontaneous melting across the unstable region by making the positive terms in Eq. (51) larger in magnitude than the negative ones. The larger $(\mathrm{d}(w_k^\beta + w_{\alpha\beta})/\mathrm{d}V)^2$

$/(w_k^\beta + w_{\alpha\beta})^2$ and the smaller $1/\beta_\alpha + 1/\beta_e$ (that is, the greater the intrinsic compressibility of the environment and of α loops), the sooner this effect occurs. The parallelism between $(\partial p_\alpha/\partial T)_{N_\alpha, N_e, V}$ and $C_{V,\beta}$ as noted previously in the thermodynamic equations, is brought out and sharpened by Eq. (47). The possibility of hysteresis and discontinuities are associated with this melting transition.

Now, we shall investigate the significance of the fourth term in Eq. (51). Assuming V to be proportional $V = kr^3/3$, where $k > 0$ is the proportionality constant, with a length hydrogen bonds r, one finds

$$\frac{dw_k^\beta}{dV} = \frac{1}{kr^2}\frac{dw_k^\beta}{dr}, \qquad \frac{dw_{\alpha\beta}}{dV} = \frac{1}{kr^2}\frac{dw_{\alpha\beta}}{dr} \tag{53}$$

$$\frac{d^2 w_k^\beta}{dV^2} = -\frac{2}{(2kr)^2 r}\frac{dw_k^\beta}{dr} + \frac{1}{(2kr^2)^2}\frac{d^2 w_k^\beta}{dr^2}$$

$$\frac{d^2 w_{\alpha\beta}}{dV^2} = -\frac{2}{(2kr^2)^2 r}\frac{dw_{\alpha\beta}}{dr} + \frac{1}{(2kr^2)^2}\frac{d^2 w_{\alpha\beta}}{dr^2} \tag{54}$$

From the derived Eqs. (53), (54), without any detailed analysis necessity of considering also the fourth term in Eq. (51) becomes obvious.
At the equilibrium nuclear configuration, the potential energy is a minimum, and

$$\left(\frac{dw_k^\beta}{dr}\right)_{r=r_e} = \left(\frac{dw_{\alpha\beta}}{dr}\right)_{r=r_e} = 0 \tag{55}$$

$$\left(\frac{d^2 w_k^\beta}{dV^2}\right)_{V=V_0} = \frac{1}{(2kr_e^2)^2}\left(\frac{d^2 w_k^\beta}{dr^2}\right)_{r=r_e} = \frac{1}{(2kr_e^2)}\,k_k \tag{56}$$

where k_k is the valence hydrogen bond force constant, and

$$\left(\frac{d^2 w_{\alpha\beta}}{dV^2}\right)_{V=V_0} = \frac{1}{(2kr_e^2)^2}\left(\frac{d^2 w_{\alpha\beta}}{dr^2}\right)_{r=r_e} = \frac{1}{(2kr_e^2)^2}\,k_{\alpha\beta} \tag{57}$$

where $k_{\alpha\beta}$ is the helix-coil boundary interaction force constant.

Under the condition of equilibrium ($dT = dV = 0$) before the base-pair opening ($1/\beta_\alpha = 0$) Eq. (51) can be rewritten in the form

$$1/\beta_e - (VE_\beta/w_k^\beta)(k_k/(2kr_e^2)) > 0,\ k_k > 0,\ w_{\alpha\beta} = 0 \tag{58}$$

Since for the bonding state E_β and w_k^β must be negative, the second term in Eq. (51) is positive and instability shall therefore not occur; it follows from this that the system DNA-environment is thermodynamically stable.

By definition we say that the reference state is a stable one. In our considerations two hydrogen bond potentials may be used: the Lippincott-Schroeder potential and that of Morse (Birshtein et al. 1976).

Let us now consider the compressibility parameter β_α. It can be argued

that in local disordered base structure the corresponding base pairs of DNA must interact and their interaction must depend on the state of the system as a whole. This is of great interest because it is closely related to the process of uncoiling of DNA in translation and transcription of genetic information. Sugalii et. al (1969) pointed out that in the region of the helix-coil transition where partially helical DNA is considered to be a sequence of segments either in randomly coiled regions or in the helical regions, there have place a long-range electrostatic interactions of specific character occur which intensively increases the volume of DNA in solution. been done. These long-range interactions associated with excluded volume repulsive forces could be evoked by the mutual hard penetration of the two approaching during the thermal motion distant parts localized along the DNA chain. The true theory of excluded volume for macromolecules with alternation of helical and coil regions has not yet been developed. However, it was concluded that the influence of the excluded volume effects on mutual penetration of the two coil regions must be extremely large as compared with their volume in solution. This is in agreement with the corresponding conclusion of the classical theory of Flory (1953). On the other hand, according to the majority of computer experiments (Khokhlov 1981) polymeric coils with excluded volume are not mutually impenetrable even in the limit of large monomer units — they easily penetrate inside each other with the overlap volume of the order of the self-volume of a polymeric coil. This is in disagreement with the corresponding conclusions stated above. This indicates that the compressibility parameter of the disordered regions β_c is dependent on the excluded volume effects and may be regarded as a complex quantity.

Conclusions

The presented generalized environment-coupled Ising model of DNA obviously represents some simplification of helix-coil transition problems. Its properties are certainly different from those of real DNA and it is hard to expect more than a semi-quantitative of only a qualitative agreement with experimental data. From the theoretical point of view, it has the advantage to be exactly solvable. Equations were derived which describe the behaviour of the most significant thermodynamic variables associated with the transition process brought about by the environment (solvent or temperature). The introduction of different w_i^{α} and w_k^{β} in the Hill Ising model of DNA for differently sized groups of α and β units allows a very considerable flexibility of application of the ensuing equations, and it represents a generalization over the usual nearest neighbour type assumption. It should also be noted that this scheme is not simply equivalent the higher neighbour (stacking--type) interactions (Wartell and Benight 1982) since it does not include interac-

tions between units in neighbouring groups, except for the boundary. A simple theoretical analysis made by Wartell and Benight (1982) shows that stacking-type interactions extending beyond nearest-neighbour base pairs will make the parameters of the nearetneighbour model appear to change as the average loop size changes. This must be beared in mind also in considering of the transition parameters in our model of DNA.

Inevitably, DNA is subject to perturbation of various kinds. These can be either external excitations arising from a random or a systematic variation of the environmental conditions, or internal fluctuations generated by the system itself and as a result of molecular interactions and random thermal motion of the components. As a result, DNA deviates continuously — although usually weakly — from the macroscopic behaviour described by the equations of the thermodynamic macrovariables. In summary, an order-disorder co-operative transition is to be expected in DNA near the melting point unless some special kind of strong environment (Watson-Crick hydrogen bonds) coupling is invoked. The observable effects of this instability should be large only when (i) the environment is quite compressible (β_c, and β_α are large), and (ii) the Watson—Crick hydrogen bonds are a sensitive function of distance ($(dw_k^\beta/dr)(dr/dV)$, $(dw_{\alpha\beta}/dr)(dr/dV)$ are large). For a real physical or physiological systems, $1/\beta_c$ and $1/\beta_\alpha$ are finite at all temperatures, including melting point, and according to our model, it is therefore finite at all temperatures. The method presented here provides a full picture of all the known phenomenology of DNA, once these thermodynamic parameters are determined.

Also it is a means allowing the comparison of experimental data obtained from different kinds of experiments, as well as a tool to disclose relations between experimental data and transition parameters determining the instability in DNA. The theory of instabilities in DNA is entirely based on the equilibrium thermodynamics and statistical mechanics. In principle our approach may be also extended to non-equilibrium situations in agreement with the statistical meaning of stability coupled with the statistical macroscopic fluctuation theory.

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