REACTION OF FORMALDEHYDE WITH POLYNUCLEOTIDES BY MASTER EQUATION APPROACH

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1. Introduction

Formaldehyde has been used as a probe for determining the secondary structure of DNA and also understanding the mechanism of DNA unwinding [1–8]. By assuming the unwinding process is irreversible, the Russian workers [3–6] have obtained the partial differential equation which describe the unwinding process and found an expression for the degree of helicity at time t, $H(t)$, in a closed form as:

$$H(t) = \exp\,[-pv + 2cv)t - pvt^2] \tag{1}$$

where c is the concentration of defects (thus, $c = 1/\bar{N}$, where $\bar{N}$ is the average number of chain segments), v the rate constant for the reaction of the denaturing agent with the base pairs on the end of the helical region, v the number of nucleotide pairs figuring in the despiralization embroyo, p the rate constant for the reaction between base pairs of native DNA and the denaturing agent. They have applied eqn. 1 to u.v. irradiated DNA (i.e., DNA with random defects) and found a close agreement between the theory and experiments. The DNA unwinding induced by formaldehyde, however, is reversible, and hence there are many cases in which eq. 1 will fail to hold (e.g. in the case where the concentration of formaldehyde is low).

In this note, we show that this problem can be easily handled by the master equation approach [9–11]. First, we use a zipper model to show the applicability of the well-defined model to this problem.

2. DNA with multi-random defects

Let us assume that initially all monomeric units (say $N + 1$ units) in DNA are in a helical conformation except the one at the left end which is in equilibrium with a coiled state. Further, let us assume that HCHO reacts only with a monomer in its coiled state [7]. As one adds HCHO into the solution, the unwinding will start from the end successively moving toward the center. This process is depicted by fig.1. In this figure, the reactions along the vertical lines are known to be very fast [7], while those along the horizontal lines which represent the reactions between HCHO and the amino or imino group of DNA are known to be much slower and therefore rate-limiting [7].

Since the two species along the vertical lines are in quasi-equilibrium with each other, by introducing the well-known rapid equilibrium assumption, we find:

$$C^{(0)} \underset{k_\mathrm{b}}{\overset{k_\mathrm{f}'}{\rightleftharpoons}} C^{(1)} \underset{k_\mathrm{b}}{\overset{k_\mathrm{f}}{\rightleftharpoons}} C^{(2)} \underset{k_\mathrm{b}}{\overset{k_\mathrm{f}}{\rightleftharpoons}} \;\ldots$$

$$\underset{k_\mathrm{b}}{\overset{k_\mathrm{f}}{\rightleftharpoons}} C^{(\mathrm{N}-2)} \underset{k_\mathrm{b}'}{\overset{k_\mathrm{f}}{\rightleftharpoons}} C^{(N-1)} \tag{2}$$

where,

$$k_\mathrm{f}' = \frac{k\,K\lambda}{1+s},\; k_\mathrm{f} = \frac{k\,(1+K\lambda)}{1+s}, \text{ and}$$

$$k_\mathrm{b} = \frac{k\,s}{1+s},\; k_\mathrm{b}' = \frac{k\sigma s}{1+\sigma s} \tag{3}$$

In the above equations, $C^{(j)}$ is the concentration of DNA with the j-th reacted site (i.e., the j-th monomer bound to HCHO), s and σ the helix stability and nucleation parameters of Zimm and Bragg [12], respectively, λ the formaldehyde concentration, K the binding constant of HCHO, and k the rate parameter of HCHO.

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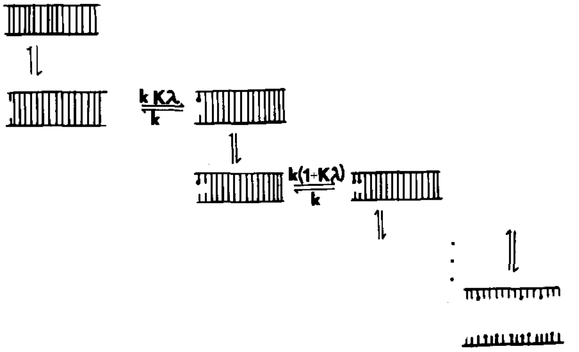

Fig.1. The proposed DNA unwinding mechanism induced by formaldehyde.

The helical content of DNA having $N + 1$ segments at time t, $\theta_N(t)$, can be obtained from $C^{(i)}$ by:

$$\theta_N(t) = \frac{1}{N}\left\{\sum_{i=0}^{N-2} (N-i)\, C^{(1)} - \frac{1}{1+s}\,[1 - C^{(N-1)}]\right\}. \tag{4}$$

An analytic expression of $C^{(i)}$ is given by Chay and Stevens [13].

When DNA is heterogeneous in size or the defects in a long DNA are distributed randomly, $\theta_N(t)$ should be averaged over the Poisson distribution of Flory [14]:

$$\phi_N = \frac{1}{\bar{N}}\,(1 - \frac{1}{\bar{N}})^{N-1} \tag{5}$$

The optical density D_t, which is an experimentally observable quantity, is proportional to $\langle N\theta_N\rangle$, and hence the fraction of helicity, $H(t)$, at time t may be related to the optical density by:

$$H(t) = \frac{\langle N\theta_N\rangle_t - \langle N\theta_N\rangle_\infty}{\langle N\theta_N\rangle_0 - \langle N\theta_N\rangle_\infty} = \frac{D_t - D_\infty}{D_0 - D_\infty} \tag{6}$$

Fig.2, which has been calculated from eqns. 4 and 5, shows that $H(t)$ obtained from the zipper model is indeed in agreement with eqn. 1 where p is taken to be zero (the zipper model assumes no coil formation from the middle of the chain). It is interesting to note that although eqn. 1 has been derived for the irreversible case, eqn. 1 is also applicable to the reversible case, where one must substitute v for v $\simeq k_f - k_b$.

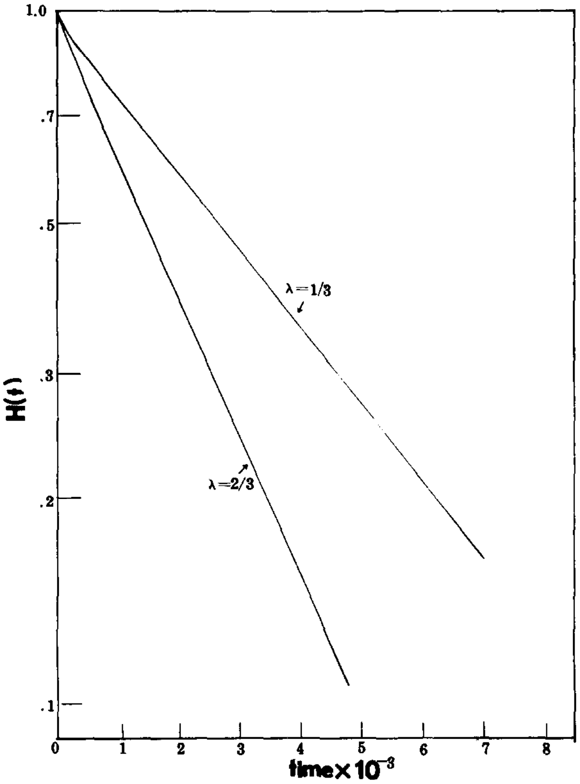

Fig.2. This figure shows the exponential unwinding behavior for polydispersed DNA. The parameters used are $\bar{N} = 86$, $K = 2$, $\sigma = 0$, $k = 9.73$ and $s = 1.68$ for $\lambda = 2/3$, and $k = 9.85$ and $s = 1.35$ for $\lambda = 1/3$.

The above approach demonstrates that the model for the thermally induced helix–coil transition [11,13,15] is directly applicable to the formaldehyde induced unwinding problem. Similarly, the formation of coils from the middle of the chain may be handled by the master equation approach [9–11]. Fig.3 shows ln $H(t)/(\sigma^{1/2}t)$ versus $\sigma^{1/2}t$ calculated from the master equation [11] for infinitely long chains. From this figure, we find that for sufficiently small σ, ln $H(t)/t$ may be approximated by the following equation:

$$-\frac{\ln H(t)}{\sigma^{1/2}t} \simeq \sigma^{1/2}v + p'\,[1 - s(f)]\,\sigma^{1/2}t$$

$$\text{where } s(f) = k_b/k_f, \tag{7}$$

which is a deduced result of eqn. 1.

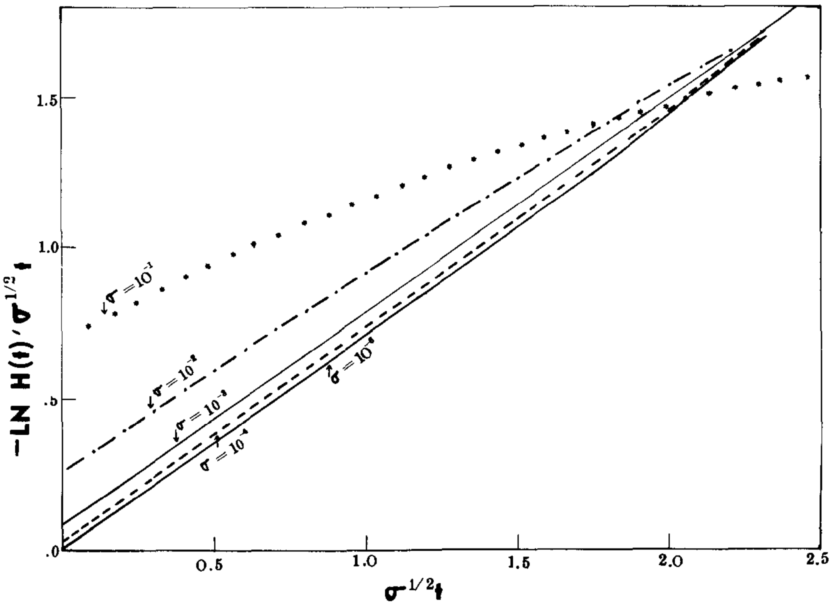

Fig.3. $-\ln H(t)/\sigma^{1/2}t$ versus $\sigma^{1/2}t$ for infinitely long chains ($N=\infty$). The parameters used are $\lambda_H=\lambda_C=1$, $k_b=1$, $s(0)=2.0$, and $s(f)=0.2$. (The definition of the parameters is given in [11]). The quadruplet assumption [11] has been used for the computation.

For finite chains with no defects, one can easily derive the following equation from the differential equation of the Russian workers [3–6]:

$$H(t) = \left(1-\frac{2vt}{N}\right)\exp\left(-pvt-pvt^2\right) \tag{8}$$

In fig.4, we have compared $-\ln H(t)/t$ of the master equation with,

$$-\frac{\ln H(t)}{t} = \frac{-\ln\left\{1-\frac{2[1-s(f)]k_f t}{N}\right\} + \sigma v + p'\sigma[1-s(f)]k_f t}{t} \tag{9}$$

which has been deduced from eqn. 8. One can see a close agreement between the two for various sizes of DNA. Fig.5 shows $-\ln H(t)/t$ of the master equation versus t for finite chains when $\sigma = 0.01$. This also demonstrates the applicability of eqns. 1 and 8 to a reversible case.

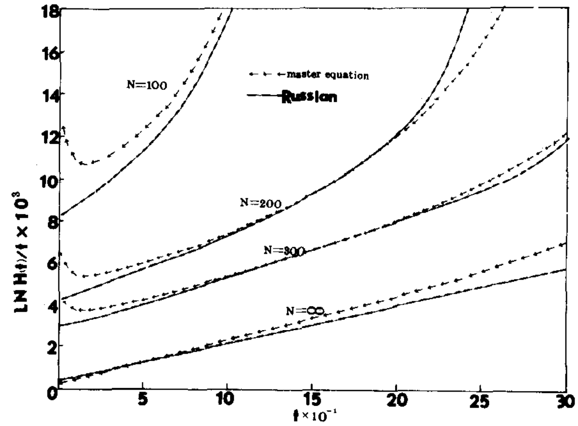

Fig.4. Comparison between $-\ln H(t)/t$ of the master equation and eqn. 9 of Trifonov et al. The parameters used are $k_b=1$, $\lambda_H=\lambda_C=1$, $s(0)=2.0$, $s(f)=0.6$, and $\sigma=10^{-4}$ for the master equation, $k_f=1$, $s(f)=0.6$, $p'=0.5$, and $v=2.5$ for eq. 9. The triplet closure assumption [11] has been used for the master equation approach.

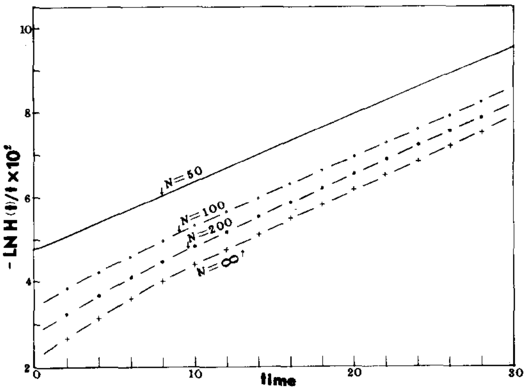

Fig.5. $-\ln H(t)/t$ versus t for finite chains. The parameters used are $k_b=1$, $\lambda_H=\lambda_C=1$, $s(0)=2.0$, $s(f)=0.6$, and $\sigma=0.01$. The triplet assumption [11] has been used for the calculation.

3. Conclusion

If $p = 0$ (i.e., if the coil formation from the middle of the chain is not allowed) eqn 1 predicts $H(t) = \exp(-2k_f t/\bar{N})$. In a reversible case, however, we have shown with the zipper model that this expression should be replaced by

$$H(t) \simeq \exp[-2(k_f - k_b)t/\bar{N}]$$

This is in agreement of the experimental result of Haselkorn and Doty [1] on the unwinding of poly(A·U) by formaldehyde. These investigators have found that the rate of unwinding of unfractionated poly(A·U) is of first order. It is interesting to note that the zipper model predicts the unwinding rate is of zero-th order with the rate constant equal to $2(k_f - k_b)/N$ for monodispersed (fractionated) DNA [11,13,16] and is of first order with the rate constant equal to $2(k_f - k_b)/\bar{N}$ (see eqn. 10) for polydispersed (unfractionated) DNA.

For an infinitely long chain and sufficiently small σ(or p), we have shown that the master equation agrees with eqn. 7 of the Russian workers. For finite chains without initial defects, on the other hand, the master equation agrees with eqn. 9, which came from the differential equation of the Russian workers. We find from figs.3, 4, and 5, the value of υ lies between 2 and 3. Although this agrees with the experimental finding of the Russian workers, υ, the number of nucleotide pairs figuring in the despiralization embroyo, of the Russian workers was set to be unity in the master equation approach.

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