Journal of Biomolecular Structure & Dynamics, ISSN 0739-1102
Volume 20, Issue Number 2, (2002)

Modeling of DNA Condensation and Decondensation Caused by Ligand Binding

Dmitri Y. Lando*
Vladimir B. Teif

Institute of Bioorganic Chemistry
Belarus National Academy of Sciences
Kuprevich St. 5/2
220141 Minsk, Belarus

http://www.jbsdonline.com

Abstract

A theoretical method for computer modeling of DNA condensation caused by ligand binding is developed. In the method, starting (*s*) and condensed (*c*) states are characterized by different free energies for ligand free DNA (F_s and F_c respectively), ligand binding constants (K_s and K_c) and stoichiometry dependent parameters (c_{sm} and c_{cm} – maximum relative concentration of bound ligands (per base pair) for starting and condensed state respectively). The method allows computation of the dependence of the degree of condensation (the fraction of condensed DNA molecules) on ligand concentration. Calculations demonstrate that condensation transition occurs under an increase in ligand concentration if $F_s < F_c$ (i.e. $S_{sc} = exp\ [-(F_c - F_s) / (RT)]$, the equilibrium constant of the *s-c* transition, is low ($S_{sc} << 1$)) and $K_s < K_c$. It was also found that condensation is followed by decondensation at high ligand concentration if the condensed DNA state provides the number of sites for ligand binding less than the starting state ($c_{sm} > c_{cm}$). A similar condensation-decondensation effect was found in recent experimental studies. We propose its simple explanation.

Introduction

DNA molecules are stored *in vivo* in the compact (condensed) state within small volumes of cell nucleus and viral capsids. Condensation is required for efficient packing and protection of the genome. However, to allow transcription, DNA must be decondensed in part. Therefore transitions between different degrees of DNA compactization are involved in many biological processes such as transcription (1-3), gene silencing (2, 3) and viral transfection (4, 5). *In vitro* experiments show that DNA condensation dramatically accelerates complementary recognition (6) and cyclization of DNA (7), and suppresses the double helix melting (8).

DNA condensation may be induced *in vitro* by "crowding agents" such as uncharged flexible polymers or small globular proteins (9, 10), or by adding multivalent counterions, e.g. multivalent metal ions, polyamines or cationic lipids (10, 11). The condensation effect is important for modeling of DNA packing *in vivo*; it is used in gene therapy (12, 13) and is investigated for the use in nanoelectronics (14, 15).

As it is known for a long time, addition of critical (c_{ocr}) or overcritical ligand concentration to DNA solution gives rise to an abrupt DNA transition to the condensed state (10, 11). Besides, during the past few years, it was revealed that when the concentration of ligands grows far beyond c_{ocr} to some new critical value, c_{od}, condensed DNA undergoes an inverse transition to the starting state (7, 16-19). In the framework of electrostatic approaches, DNA condensation at $c_o = c_{ocr}$ corresponds to a decrease in the DNA linear charge density (11, 20), while the DNA decondensation at $c_o = c_{od}$ corresponds to the full charge neutralization or charge reversal (21, 22).

We propose another approach for calculation of DNA condensation (23). It is sup-

Phone: 375-17-2648263
Fax: 375-17-2648647
Email: Lando@iboch.bas-net.by

posed that penalty of the condensed state in ligand free DNA is compensated by stronger ligand binding to this state. Instead of detailed consideration of the electrostatic effects we calculate the free energy difference for ligand complexes with the starting and condensed state of a DNA molecule omitting consideration of mechanisms underlying the difference. The possibility of a change in stoichiometry in DNA-ligand complexes during condensation is taken into account because stoichiometry effects strongly influence DNA conformational transitions (26, 27). Calculations show that DNA condensation at medium ligand concentrations is followed by decondensation at higher concentrations, if the condensed state is characterized by ligand binding constant greater, and the number of binding sites less than the starting state.

Model

Let a DNA molecule of L units (base pairs) can be either in the starting (s) or condensed (c) state. Partially condensed molecules are absent. Such a situation is often observed in single molecule microscopy experiments (24, 25). In the absence of ligands, the starting and condensed states are characterized by different free energies (F_s and F_c respectively). The equilibrium constant for s → c transition in free DNA (without ligands) is given by Eq. [1]:

$$S_{sc} = exp\ [-\ (F_c\text{-}F_s)\ /\ (RT)] \qquad [1]$$

Condensation transition is caused by stronger ligand binding to the condensed state. Let the molar concentration of free ligands is denoted as c_o. Ligand binding constants with both states (K_s and K_c) and c_o determine the relative concentration (per base pair) of ligands bound to the starting [$c_s = k_s / (n_s \cdot L)$] and to the condensed [$c_c = k_c / (n_c \cdot L)$] state. k_s, k_c is the number of ligands bound to n_s DNA molecules in the starting state, and n_c condensed DNA molecules, respectively. A ligand bound to the starting (s) or condensed (c) state covers m_s or m_c base pairs (or nucleotides of one strand) respectively. Another ligand can not be bound to the same site. c_{sm} and c_{cm} are the maximum (saturation) relative concentrations for starting and condensed state, respectively ($c_s < c_{sm}$ and $c_c < c_{cm}$ for any c_o value; $c_s \approx c_{sm}$ if $K_s \cdot c_o >> 1$, and $c_c \approx c_{cm}$ if $K_c \cdot c_o >> 1$). c_{sm} and c_{cm} can be also considered as the relative (per base pair) number of binding sites for a given state (s or c). Let us introduce parameters r_s and r_c for the case $c_{im} \neq m_i^{-1}$ where $i = s$ or $i = c$:

$$c_{sm} = (r_s \cdot m_s)^{-1}; \qquad r_s = (c_{sm} \cdot m_s)^{-1} \qquad [2]$$

and

$$c_{cm} = (r_c \cdot m_c)^{-1}; \qquad r_c = (c_{cm} \cdot m_c)^{-1} \qquad [3]$$

Parameters r_s and r_c characterize the arrangement of ligands relative to DNA strands in the starting and condensed states (see (26, 27) for details). Three examples of various types of ligand interaction and corresponding r_s and r_c values are shown in Figure 1.

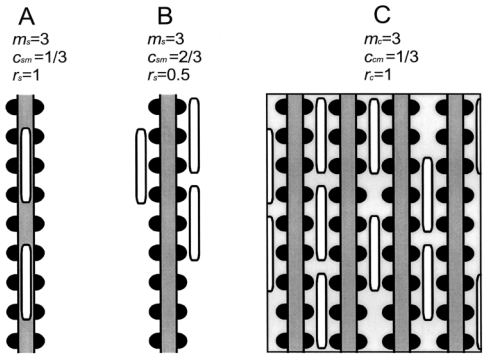

Figure 1: Different modes of ligand binding to DNA and corresponding m_i, c_{im} and r_i values. Figure 1A and 1B correspond to the starting state ($i = s$), and Figure 1C corresponds to the condensed state ($i = c$).

A – starting DNA state. Every base pair can be free or bound to one ligand that covers three base pairs (m_s = 3 bp). It is obvious that c_{sm}, the maximum relative concentration of bound ligands (per base pair), is equal to 1/3. Therefore $r_s = (c_{sm} \cdot m_s)^{-1}$ is equal to 1.

B – starting DNA state. Every nucleotide can be free or bound to one ligand that covers three nucleotides of one strand, which belong to three neighboring base pairs (m_s = 3 bp). Ligands can bind to different strands independently. Therefore every base pair can be either free or bound to one or two ligands. c_{sm} = 2/3 and r_s = 0.5.

C – condensed DNA state. Every ligand covers 6 nucleotides of two remote regions of a DNA molecule (three nucleotides of every strand (m_c = 3)). c_{cm} = 1/3 and r_c = 1.

In Figure 1A, every ligand simultaneously interacts with two strands of the starting state of a given region of the double helix. Any ligand covers m_s = 3 base pairs and another ligand cannot bind to the same base pairs. It is obvious that c_{sm}, the maximum relative concentration of bound ligands (per base pair), is equal to 1/3. Therefore $r_s = (c_{sm} \cdot m_s)^{-1}$ is equal to 1.

In Figure 1B, every ligand interacts with one strand of the starting state of the double helix. A ligand covers m_s = 3 nucleotides of one strand and prohibits binding to them of other ligands. Thus every base pair can bind one ligand, two ligands or be free. The bound ligand does not give rise to any restriction for binding to the

opposite strand. c_{sm}, maximum relative concentration of bound ligands (per base pair), is equal to 2/3 and $r_s = 0.5$

In Figure 1C, every ligand interacts with two remote regions of the condensed double helix. A ligand covers $2 \cdot m_c$ nucleotides (m_c nucleotides of every of the two remote regions). This type of binding is characterized by $m_c = 3$, $c_{cm} = 1/3$, $r_c = 1$ as well as a type depicted in Figure 1A. However, as shown in the figure, it corresponds to another structure of DNA-ligand complex.

In the next part of the paper, equations are derived for calculation of the binding curves for both states (dependences $c_s\,(c_o)$, $c_c\,(c_o)$) and of the condensation curve (fraction of condensed molecules, $\vartheta\,(c_o) = n_c / (n_s + n_c)$) for given S_{sc}, K_s, K_c, m_s, m_c, c_{sm}, c_{cm}.

The Calculation Method

The chemical potential of a free ligand in solution is given by the standard expression:

$$\mu = \mu_o + R \cdot T \cdot ln\,(c_o) \qquad [4]$$

where c_o is molar concentration of free ligands, R - universal gas constant per mole; T - temperature (K), μ_o - standard chemical potential.

Let k_s ligands are bound to the starting and k_c ligands to the condensed state of DNA molecules, i.e. $k = k_s + k_c$ ligands are withdrawn from a solution of molar concentration c_o and the free energy of the solution is decreased by $k \cdot \mu$. The total change in the free energy of DNA molecules and ligand solution [$\Delta F\,(k_s, k_c, n_s, n_c, c_o)$] caused by binding of k_s ligands to n_s DNA molecules in the starting state and k_c ligands to n_c condensed molecules is given by Eq. [5]:

$$\Delta F\,(k_s, k_c, n_s, n_c, c_o) = \sum_{i\,=\,s,\,c} (n_i F_i + k_i \Psi_i - k_i \mu) - R \cdot T \cdot ln\,(W) \qquad [5]$$

where F_i is the free energy of a DNA molecule in state i in the absence of absorbed ligands ($i = s$ and $i = c$ for the starting and condensed state respectively); Ψ_i is the free energy of a ligand bound to a DNA molecule in state i; W is the number of distinguishable rearrangements for given k_s, k_c, n_s and n_c. If $L >> m_s, m_c$, then W is determined by Eq. [6]:

$$W = \frac{(n_s + n_c)!}{n_s! \cdot n_c!} \cdot \prod_{i\,=\,s,\,c} \frac{[\,N_i / r_i - (m_i - 1) \cdot k_i\,]!}{k_i! \cdot (N_i / r_i - m_i \cdot k_i)!} \qquad [6]$$

where $N_s = n_s \cdot L$, $N_c = n_c \cdot L$ and L is the number of base pairs in each of $(n_s + n_c)$ DNA molecules. Eq. [6] was originally derived for calculation of melting curves of DNA-ligand complexes (26, 27). However, the equation is also valid for the case of DNA condensation if it is assumed that the sliding of a DNA molecule along its axis is prohibited in the condensed state; such an assumption has been successively used for description of ligand binding to liquid crystalline DNA phase (28).

The equilibrium (the most probable) values of n_i and k_i correspond to the minimum of ΔF. One can find the equilibrium values for n_i and k_i from the Stirling expression, $ln\,(n!) \approx n \cdot [ln\,(n) - 1]$, and the condition of ΔF minimum [7, 8]:

$$\partial(\Delta F) / \partial k_i = 0 \qquad [7]$$

$$\partial(\Delta F) / \partial n_i = 0 \qquad [8]$$

From Eq. [7], one obtains the standard mass action law (Eq. [9]) for the system under consideration (26, 27, 29):

$$K_i = \frac{r_i \cdot c_i \cdot [1 - r_i \cdot (m_i - 1) \cdot c_i]^{m_i - 1}}{c_o \cdot (1 - r_i \cdot m_i \cdot c_i)^{m_i}}, \quad [9]$$

where $K_i = \exp[(\mu_o - \Psi_i) / (R \cdot T)]$ is the ligand binding constant for state i; c_i is the relative concentration (degree of binding) of ligands bound to state i, $c_i = k_i / N_i$. The maximum possible relative concentration of ligands bound to the i'th state is $c_{im} = 1 / (r_i \cdot m_i)$.

From Eq. [8], one obtains Eq. [10] for the degree of condensation of DNA molecules, ϑ:

$$\vartheta = U \cdot S_{sc} / (1 + U \cdot S_{sc}), \quad [10]$$

where $S_{sc} = \exp[-(F_c - F_s) / (R \cdot T)]$ is the equilibrium constant for the transition between the starting and condensed DNA states in the absence of ligands and U is determined by Eq. [11]:

$$U = \prod_{i = s, c} \{[1 - r_i \cdot (m_i - 1) \cdot c_i] / (1 - r_i \cdot m_i \cdot c_i)\}^{(-1)^i \cdot (L / r_i)} \quad [11]$$

For calculation of the condensation curve $[\vartheta(c_o)]$, c_s and c_c are obtained from Eq. [9] for a given c_o. After that c_s and c_c are substituted into Eqs. [10], [11]. Computation of ϑ is carried out for given S_{sc}, r_s, r_c, m_s, m_c, K_s, K_c and variable c_o.

Results and Discussion

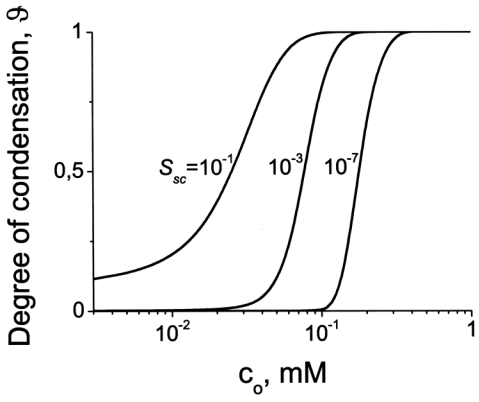

Figure 2: Condensation caused by higher binding constant of ligands to the condensed state. L = 1000 bp, $K_s < K_c$, $r_s = r_c = 1$; $m_s = m_c = 3$; $c_{sm} = c_{cm} = 1/3$; $K_s = 10$ M^{-1}; $K_c = 100$ M^{-1}. $S_{sc} = 10^{-1}, 10^{-3}, 10^{-7}$. S_{sc} values are shown in the figure.

It is obvious that the equilibrium constant of the transition from the starting to the condensed state is very low in the absence of ligands ($S_{sc} << 1$) because the condensed form does not exist without ligands as follows from experimental studies. Therefore to induce condensation transition the binding constant for the condensed state must be higher than that for the starting state ($K_c > K_s$) to compensate free energy penalty of the condensed state. Condensation curves for such a transition are depicted in Figure 2 for the case of equal number of binding sites per base pair for both states ($c_{sm} = c_{cm}$). Curve position relative to the concentration scale is characterized by the concentration of the condensation transition (c_{ocr}). For this concentration, the degree of condensation, $\vartheta(c_{ocr})$, is equal to 0.5. As follows from the figure, the starting state is not stable in the absence of ligands if $S_{sc} = 10^{-1}$. There is an appreciable fraction of condensed molecules for ligand free DNA. If $S_{sc} \leq 10^{-3}$, then the starting state is sufficiently stable in the absence of ligands and the fraction of free condensed DNA molecules is small. For L = 1000 bp, the value of $S_{sc} = 10^{-3}$ corresponds to ~5 kcal difference between F_c and F_s per DNA molecule and to 0.005 kcal difference per base pair (~1% of energy of heat motion (RT)). It means that a very small difference in free energy between the starting and condensed state is sufficient to provide sufficient stability of the starting state in the absence of ligands or for subcritical ligand concentration ($c_o < c_{ocr}$) as well as to provide stable condensed state if $c_o > c_{ocr}$. These results demonstrate that condensation can be caused by very weak interactions. Various approaches give the free energy of DNA condensation in the interval 10^{-2} - 10^{-1} RT per base pair (11).

It must be mentioned that an increase in the binding constant to the starting state (K_s) for a given K_c hinders condensation and increases c_{ocr}. If $K_s \geq K_c$, condensation is impossible in the case of equal stoichiometry of both states ($c_{sm} = c_{cm}$).

Let us consider influence of DNA length on the condensation process. If S_{sc} is not changed with the length of DNA molecules (L) then c_{ocr} strongly decreases with L (Figure 3) because, for a given c_o value, the number of ligands bound to s or c DNA state is equal to $L \cdot c_s$ and $L \cdot c_c$ respectively, where c_s and c_c are independent of L

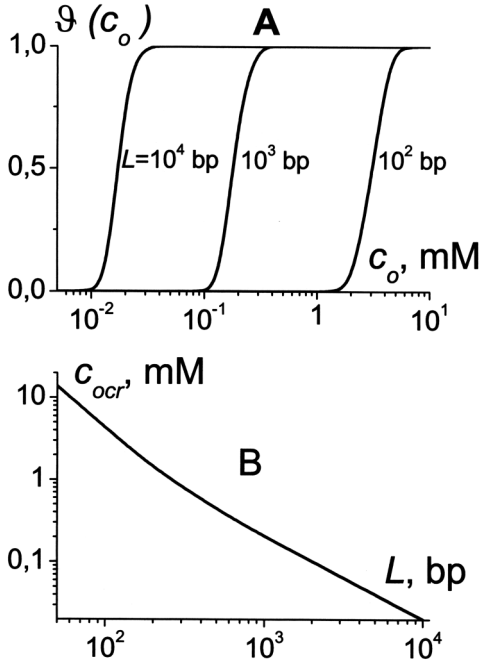

Figure 3:
A) Condensation curves for DNA's of various lengths and constant S_{sc} value ($S_{sc} = 10^{-7}$, $m_s = m_c = 3$ bp, $r_s = r_c = 1$, $K_s = 10$ M^{-1}, $K_c = 100$ M^{-1}). L values are shown in the figure.
B) Dependence of concentration of condensation transition (c_{ocr}) on L for the same parameter values.

as follows from Eq. [9]. Therefore, for longer DNA, lower values of c_o as well as of c_s and c_c are sufficient to compensate a free energy penalty of the condensed state.

However, in real systems F_s and F_c as well as their difference must be proportional to DNA length (L). It means that

$$S_{sc} = S_o{}^L \qquad [12]$$

where S_o is the statistical weight of a base pair in a condensed DNA molecule; the statistical weight of a base pair for DNA molecule in the starting state is equal to 1. In this case, c_{ocr} is independent of L if $L \geq 100$ bp (Figure 4A). There is a linear dependence of the concentration interval (Δc_o) of condensation transition on $1/L$ (Figure 4B) where

$$\Delta c_o = 1 \,/\, \vartheta(c_{ocr})'_{co} \qquad [13]$$

Condensation can also arise in the case of equal binding constants ($K_c = K_s$) if there is higher number of binding sites for the condensed state ($c_{sm} < c_{cm}$). The corresponding curve is exhibited in Figure 5 (curve 1). In this case, condensation occurs due to lower free energy of the condensed state caused by higher number of bound ligands (per base pair, $c_s < c_c$) for any given molar concentration of free ligands (c_o). Besides, the condensed state is characterized by higher entropy for the case of $c_{sm} < c_{cm}$ because there are more rearrangements for a given number of ligands in the case of larger number of binding sites in the condensed molecule.

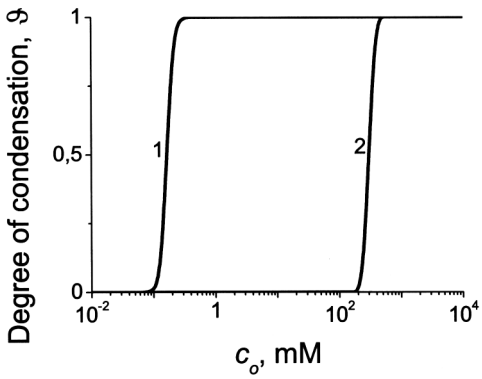

Figure 5: Condensation caused by higher number of binding sites for the condensed state; $c_{sm} < c_{cm}$, $L = 1000$ bp, $S_{sc} = 10^{-7}$.
1 – equal binding constants for the starting and condensed state. $K_s = K_c = 100$ M^{-1}; $m_s = m_c = 3$ bp; $c_{sm} = 1/3$, $c_{cm} = 2/3$; $r_s = 1$, $r_c = 0.5$.
2 - equal full free energies of direct ligand binding for fully saturated starting and condensed states ($K_s{}^{c_{sm}} = K_c{}^{c_{cm}}$). $m_s = m_c = 3$ bp; $r_s = 1$, $r_c = 0.5$; $c_{sm} = 1/3$, $c_{cm} = 2/3$; $K_s = 100$ M^{-1}, $K_c = 10$ M^{-1}.

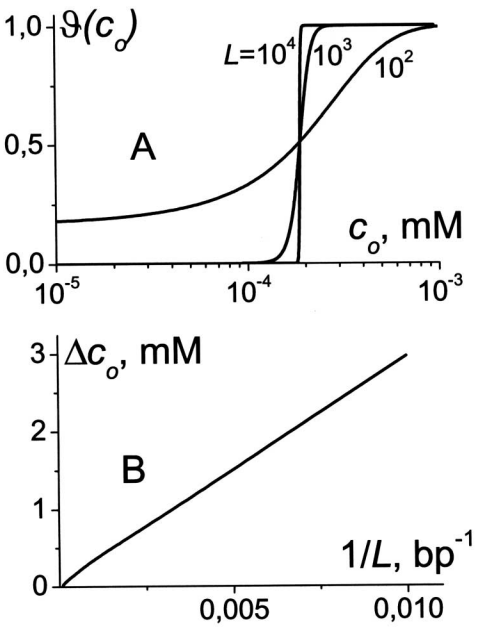

Figure 4: Condensation curves (A), and the dependence of the concentration interval of the condensation transition (Δc_o) on $1/L$ for DNA's of various length (B). $S_{sc} = S_o{}^L$, $S_o = 0.9840$, $m_s = m_c = 3$ bp, $r_s = r_c = 1$, $K_s = 10$ M^{-1}, $K_c = 100$ M^{-1}. $S_{sc} = 0.2$ for $L = 10^2$ bp; $S_{sc} = 10^{-7}$ for $L = 10^3$ bp; $S_{sc} = 10^{-70}$ for $L = 10^4$ bp. In this case, the difference in the free energy between the starting and condensed state is proportional to the DNA length (L). L values are shown in the figure.

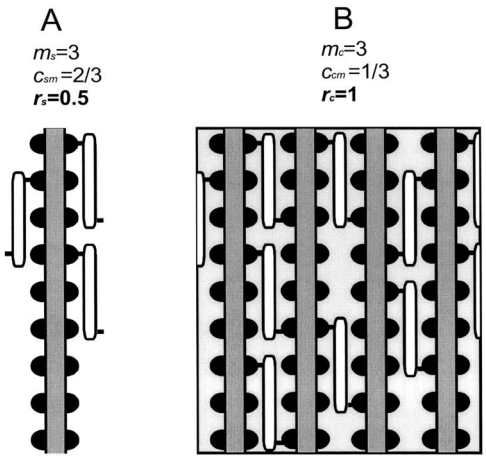

Figure 6: Different modes of ligand binding to the starting and condensed state. The condensed state absorbs less ligands at saturation level ($c_{sm} = 2 \cdot c_{cm}$), but it forms more contacts per ligand increasing the free energy of direct ligand binding by two times ($K_s^2 = K_c = 100$ M^{-1}).
A – starting DNA state. $m_s = 3$ bp, $c_{sm} = 2/3$ (every ligand interacts with one strand and gives one contact with DNA molecule, $K_s = 10$ M^{-1}).
B – condensed DNA state. $m_c = 3$ bp, $c_{cm} = 1/3$ (every ligand forms two contacts with two strands of remote parts of a DNA molecule, $K_c = 100$ M^{-1}).

To study whether this purely entropy effect itself can cause condensation, let us calculate condensation curve for equal free energies of direct ligand binding for fully saturated starting and condensed states. It means that $K_s^{L \cdot c_{sm}} = K_c^{L \cdot c_{cm}}$ or $K_s^{c_{sm}} = K_c^{c_{cm}}$ where $L \cdot c_{sm}$ and $L \cdot c_{cm}$ is the total number of binding sites for the starting and condensed state of DNA molecule. Results of these calculations are shown in Figure 5 (curve 2). As follows from the figure, this purely entropy effect caused by higher number of binding sites gives rise to DNA condensation even if $K_s > K_c$.

However the stoichiometry effects described above (Figure 5) are hardly probable because the condensed form can usually absorb less ligands than the starting one due to spatial restrictions. In real DNA systems, c_{sm} must be greater than c_{cm}. Therefore condensation can arise only if the binding constant is higher for the condensed form ($K_s < K_c$). Such a model of ligand binding is depicted in Figure 6. Every ligand covers three base pairs for both DNA forms ($m_s = m_c = 3$). The starting state can absorb at saturation level ($K_s \cdot c_o >> 1$) double number of ligands in comparison with condensed one ($c_{sm} = 2 \cdot c_{cm}$ because $r_c = 2 \cdot r_s$ and $m_s = m_c$). Every ligand forms a single contact if it is bound to the starting state and two contacts with the condensed state. Therefore $K_s^2 = K_c$ because the free energy of direct binding for two contacts is two times higher. A similar model has been proposed for hexamminecobalt(III)-induced DNA condensation (20, 30), where one ligand forms one contact with starting DNA conformation and two contacts with a condensed one.

As in the previous cases (Figures 2-5), DNA condensation occurs if $c_o > c_{ocr}$ (Figure 7). However, at very high concentration of free ligands ($c_o > c_{od}$), DNA undergoes a transition to the starting state in contrast to models of binding considered in Figures 2-5. Thus, there are two transitions caused by increasing ligand concentration: from the starting to condensed state at medium concentration and from condensed to the starting state at high concentration. A scheme of these transitions is exhibited in Figure 8.

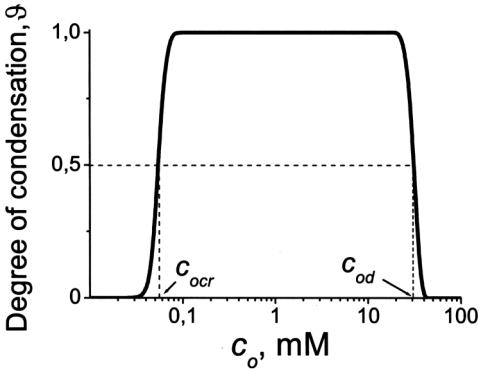

Figure 7: The condensation curve for the model depicted in Figure 6. Condensation does not occur for low ($0 \le c_o < c_{ocr}$) and high ($c_o > c_{od}$) ligand concentrations. $K_s = 10$ M^{-1}, $K_c = 100$ M^{-1}, $S_{12} = 10^{-7}$, $L = 1000$ bp, $m_s = m_c = 3$ bp, $c_{sm} = 2/3$, $c_{cm} = 1/3$.

As follows from the figure, the starting state conserves if concentration of ligands added to DNA solution is low ($c_o < c_{ocr}$). Transition to the condensed state occurs after addition of medium ligand concentration ($c_{ocr} < c_o < c_{od}$). For this concentration interval, higher binding constant ($K_c > K_s$) compensates free energy penalty of condensed state inherent in free DNA. Therefore, for medium concentration, the free energy of the condensed state is lower than of the starting state. However for sufficiently high ligand concentration ($c_o > c_{od}$ and $K_s \cdot c_o > 1$, $K_c \cdot c_o >> 1$), higher number of binding sites in the starting state conditions higher number of bound ligands ($c_s > c_c \approx c_{cm}$) compensating the penalty of low binding constant (K_s) because more ligands can be absorbed at starting state at high ligand concentration (energy effect). Besides, there are more distinguishable rearrangements for these ligands among binding sites due to lower value of the stoichiometry parameter r_s in comparison with r_c when $m_s = m_c$ (entropy effect). Thus, the free energy of the starting state is lower at low ligand concentration ($c_o < c_{ocr}$) as well as at high one ($c_o > c_{od}$).

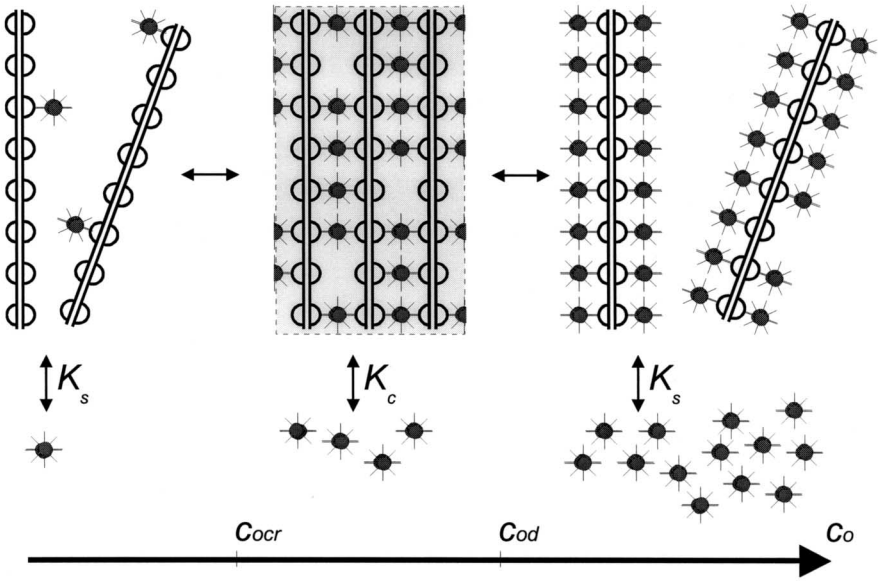

Figure 8: A model of condensation-decondensation effect. $K_s < K_c$, $c_{sm} = 2$ (two sites of ligand binding per base pair for the starting state) and $c_{cm} = 1$ (one site of ligand binding per two nucleotides for the condensed state).
A – The starting state is conserved if the concentration of ligands added to DNA solution is low ($c_o < c_{ocr}$).
B – Transition to the condensed state after addition of medium ligand concentration ($c_{ocr} < c_o < c_{od}$). At this concentration, higher binding constant ($K_c > K_s$) compensates free energy penalty of condensed state inherent in free DNA.
C – Conserving of the starting state after addition of sufficiently high ligand concentration ($c_o > c_{od}$). Higher number of binding sites in starting state compensates the penalty of its low binding constant due to a strong increase in the number of bound ligands and higher number of their rearrangements.

Thus calculations show that DNA condensation at medium ligand concentration is followed by decondensation at high concentration if the condensed state is characterized by greater binding constant ($K_c > K_s$) but less number of binding sites ($c_{cm} < c_{sm}$) in comparison with the starting state.

A similar effect has been revealed in recent experimental studies of DNA condensation caused by polyamine binding (7, 16-19).

It should be mentioned that the effect of "condensation-decondensation" is not unique for DNA condensation but is general for transitions between the states with different DNA-ligand stoichiometry.

A stoichiometry effect similar to the effect considered above was described about 30 years ago by Frank-Kamenestkii and co-authors for the helix-coil transition of DNA-ligand complexes (26, 27). A monotonous increase in DNA melting temperature with ligand concentration was shown for equal stoichiometry of ligand complexes with helical and melted DNA states if the binding constant is higher for the helical state. However, stabilization of the double helix (increase in melting temperature) at low and medium concentration is changed by destabilization at high concentration if the helical state is characterized by lower maximum relative concentration of bound ligands in comparison with the melted state. Strong influence of this stoichiometry effect on DNA stability during protonation and deprotonation has been demonstrated (31).

Acknowledgments

We are grateful to J.-L. Sikorav, E. Raspaud, F. Livolant and J. F. Marko for fruitful discussion and critical remarks on the manuscript. This work was supported by the Fund of Fundamental Investigations of the Republic of Belarus (grants X02R-049 and B02M-091) and by ISTC Foundation (grant A302-2).

References and Footnotes

1. J. A. Armstrong and B. M. Emerson, *Curr. Opin. Genet. & Develop. 8*, 165–172 (1998).
2. J. Wu and M. Grunstein, *TIBS 25*, 619-623 (2000).
3. J. Zlatanova, S. H. Leuba and K. van Holde, *Biophys. J. 74*, 2554–2566 (1998).
4. S. B. Zimmerman and L. D. Murphy, *FEBS Letters 390*, 245-248 (1996).
5. M. Sun, D. Louie and P. Serwer, *Biophys. J. 77*, 1627–1637 (1999).
6. J.-L. Sikorav and G. M. Church, *Mol. Biol. 222*, 1085-1108 (1991).
7. D. Jary, J.-L. Sikorav, *Biochemistry 38*, 3223-3227 (1999).
8. S. V. Mikhailenko, V. G. Sergeyev, A. A. Zinchenko, M. O. Gallyamov, I. V. Yaminsky, and K. Yoshikawa, *Biomacromolecules 1*, 597-603 (2000).

9. R. de Vries, *Biophys. J. 80*, 1186–1194 (2001).
10. V. A. Bloomfield, *Curr. Opin. Struct. Biol. 6*, 334-341 (1996).
11. V. A. Bloomfield, *Biopolymers 44*, 269-282 (1998).
12. Y. Kakizawa, A. Harada and K. Kataoka, *Biomacromolecules 2*, 491-497 (2001).
13. M. Schmutz, D. Durand, A. Debin, Y. Palvadeau, A. Etienne and A. R. Thierry, *PNAS 96*, 12293–12298 (1999).
14. Yu. M. Yevdokimov, V. I. Salyanov, B. V. Mchedlischvili, V. A. Bykov, A. V. Belyaev, S. A. Saunin, F. Spener, M. Palumbo, *Nucleosides, Nucleotides and Nucleic Acids 19*, 1355 (2000).
15. V. B. Teif and D. Y. Lando, *Sensor Technology 2001*, ed. Elwenspoek M. (Kluver Academic Publishers, Dordrecht), 155-160 (2001).
16. E. Raspaud, I. Chaperon, A. Leforestier, and F. Livolant, *Biophys. J. 77*, 1547-1555 (1999).
17. E. Raspaud, M. Olvera de la Cruz, J.-L. Sikorav and F. Livolant, *Biophys. J. 74*, 381–393 (1998).
18. J. Pelta, F. Livolant, and J.-L. Sikorav, *J. Biol. Chem. 271*, 5656–5662 (1996).
19. M. Saminathan, T. Antony, A. Shirahata, L. H. Sigal, T. Thomas and T. J. Thomas, *Biochemistry 38*, 3821-3830 (1999).
20. D. Matulis, I. Rouzina and V. A. Bloomfield, *J. Mol. Biol. 296*, 1053-1063 (2000).
21. T. T. Nguyen, I. Rouzina and B. I. Shklovskii, *J. Chem. Phys. 112*, 2562-2568 (2000).
22. F. J. Solis, M. Olvera de la Cruz, *Europ. Phys. J. E1*, 1-18 (2000).
23. V. B. Teif and D. Y. Lando, *Molecular Biology (Mosk.) 35*, 106–107 (2001).
24. Y. Yamasaki, Y. Teramoto and K. Yoshikawa, *Biophys. J. 80*, 2823-2832 (2001).
25. K. Yoshikawa, M. Takahashi, V. V. Vasilevskaya, and A. R. Khokhlov, *Phys. Rev. Lett. 76*, 3029-3031 (1996).
26. M. D. Frank-Kamenetskii and A. T. Karapetian, *Molecular Biology (Mosk.) 6*, 621-627 (1972).
27. D. Y. Lando, V. I. Krot and M. D. Frank-Kamenetskii, *Molecular Biology (Mosk.) 9*, 856-860 (1975).
28. Yu. D. Nechipurenko, S. A. Strel'tsov and Yu. M. *Evdokimov, Biofizika (Mosk.) 46*, 428-435 (2001).
29. J. D. McGhee and P. H. von Hippel, *J. Mol. Biol. 86*, 469-489 (1974).
30. B. I. Kankia, V. Buckin and V. A. Bloomfield, *Nucl. Acid. Res. 29*, 2795-2801 (2001).
31. D. Y. Lando, S. G. Haroutiunian, A. M. Kul'ba, E. B. Dalian, P. Orioli, S. Mangani and A. A. Akhrem, *J.Biomol. Struct. Dynam. 12*, 355-366 (1994).

Date Received: June 3, 2002

Communicated by the Editor Valery Ivanov