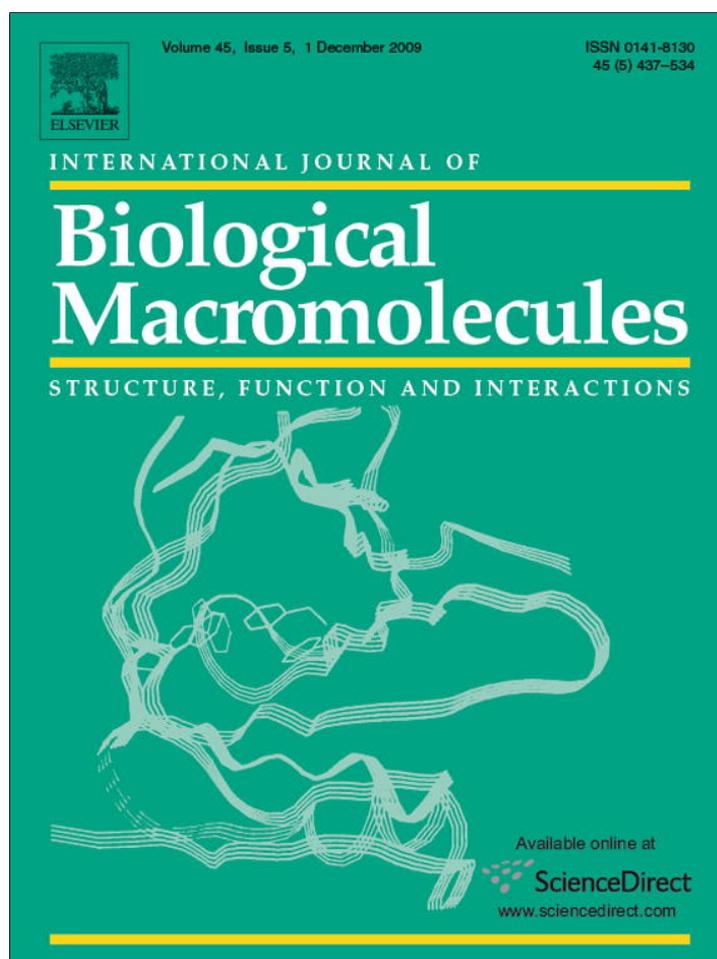


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Short communication

Insight into the denaturation transition of DNA

Boualem Hammouda

Center for Neutron Research, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899-6102, United States

ARTICLE INFO

Article history:

Received 10 June 2009

Received in revised form 27 August 2009

Accepted 2 September 2009

Available online 19 September 2009

Keywords:

Denaturation transition

UV absorption spectroscopy

DNA in mixed solvents

ABSTRACT

DNA undergoes a helix-to-coil transition (also called denaturation transition) upon heating. This transition can also be facilitated by using solvent mixtures (for example water–alcohol). An increase in the hydrophobic tail of the second solvent molecule first decreases then increases the melting temperature appreciably. Measurement on 4% DNA in a series of water–alcohol mixtures shows that the helix-to-coil melting transition is driven by the solvent ability to cross the hydrophobic sugar-rich region. DNA is behaving like a cylindrical micelle.

Published by Elsevier B.V.

1. Introduction

DNA is the basic blueprint for the synthesis of proteins in living cells. It is made out of backbone phosphate groups connected to sugars to which amine bases are attached [1]. The phosphate groups are hydrophilic, the sugars (and the methylene groups connected to them) contain mostly hydrophobic groups and the amine bases contain mostly hydrophilic groups. A DNA helix is formed to shield the hydrophobic groups from contact with water. Water hydrates the phosphate groups on the outside and the amine bases on the inside of the helix. The helix phase is formed through stacking of the amine bases and hydrogen bonding between them. The DNA helix structure has been the subject of extensive studies [2–7].

The DNA helical structure is known to melt into an open coil structure when temperature is increased. This helix-to-coil transition (also referred to as the denaturation transition) is investigated here. DNA in its native form dissolves in pure water and in pure ethylene glycol [2,3]. These are two solvents known to dissolve DNA in their pure form. DNA also dissolves in mixtures of water and other solvents such as alcohols or glycols. Series of solvent mixtures are used here in order to get some insight into the nature of the helix-to-coil transition in DNA.

UV absorption spectrometry is the prime tool for measuring the helix-to-coil transition temperature in DNA [4,5]. A Cary 50 UV absorption spectrophotometer was used along with temperature control. Slow heating rate was used. The 260 nm line is a good monitor of amine base stacking and therefore changes appreciably upon melting of the helix. The strength of this line follows a

sigmoid shape when plotted vs. temperature because absorbance increases upon melting of the helix. The inflection point of this “s” shaped curve determines the helix-to-coil melting temperature.

The melting transition of DNA in ethylene glycol (HO–CH₂CH₂–OH) is much lower than that in water [7]. This is due to the fact that ethylene glycol is less effective (than water) at forming a “hydration” layer around the phosphate groups. Ethylene glycol is more effective at diffusing across the hydrophobic sugar-rich region (because of its hydrophobic –CH₂CH₂– group) and disturbing the amine base stacking and hydrogen bonding. Water has difficulty crossing the hydrophobic sugar-rich region till higher temperatures are reached.

2. Experimental results

Series of UV absorption measurements have been performed on 4% DNA in mixtures of water and a second solvent. Solvent mixtures were used because DNA does not dissolve in (most of) the pure second solvents considered here. Salmon DNA (purchased from Sigma–Aldrich) with molecular weight of 1.3×10^6 g/mole and containing a number fraction of GC pairs of 41.2% was used. Owing to the relatively high DNA concentration used here (4% mass fraction), and in order to avoid signal saturation, only thin (50 m thick) samples were measured.

The first series of measurements used water (75% mass fraction) and the following second solvents (25% mass fraction): methanol CH₃OH, ethylene glycol HOCH₂CH₂OH and glycerol HOCH₂CH(OH)CH₂OH. This series can be represented by the formula H[CH(OH)]_nH where $n = 1, 2$ or 3 for methanol, ethylene glycol and glycerol respectively (n represents the number of carbons in the second solvent molecule). In this series the number of hydropho-

E-mail address: hammouda@nist.gov.

bic $-CH-$ groups increases along with the number of hydrophilic $-OH$ groups. Fig. 1 shows that the measured melting temperature increases for this series (Series I). Increasing n makes the second solvent molecule more hydrophilic (dominant effect) thereby keeping this molecule in the phosphates hydration region. This second solvent molecule has more difficulty crossing the hydrophobic sugar-rich region because of the increased number of $-OH$ groups that it contains.

The next series of measurements used water (75% mass fraction) and the following alcohols (25% mass fraction) as second solvent: methanol, ethanol, 1-propanol, butanol, pentanol, hexanol and 1-octanol respectively. The alcohol molecules can be represented by the formula $H[CH_2]_nOH$ where n increases from 1 to 3 for the short alcohols (Series II in Fig. 1) and $n=3, 4, 5, 6,$ and 8 for the long alcohols (Series III in Fig. 1). In these series only the fraction of hydrophobic $-CH_2-$ groups is increased. It is interesting to note from Fig. 1 that the melting temperature decreases for the short alcohol series (Series I) then increases for the long alcohol series (Series III) even-though only the hydrophobic tail of the alcohol molecule is increased. In the Series II case, the short alcohols “help” water cross the hydrophobic sugar-rich region. These short alcohols are of the same size as the sugars themselves. In the Series III case, the long tail alcohol molecules are so hydrophobic that they get “stuck” in the hydrophobic sugar-rich region thereby “plugging” the water passageway across to the amine bases. Water has more difficulty diffusing inside the helical structure and therefore disturbs the amine stacking less. Note the change in trend (T_m decreases for $n < 3$ but increases for $n > 3$) and the break in slope at $n=3$ (for 1-propanol). This trend is the most significant contribution of our findings.

3. Discussion

Stability of the helix structure (or its melting) is controlled by the solvent molecules' ability to cross the hydrophobic sugar-rich region. Hydrophobic and hydrophilic interactions are also found in micellar systems. Micelles form when a surfactant interface separates hydrophobic groups (on the inside) from hydrophilic groups (on the outside). DNA forms helical structures in order to shield

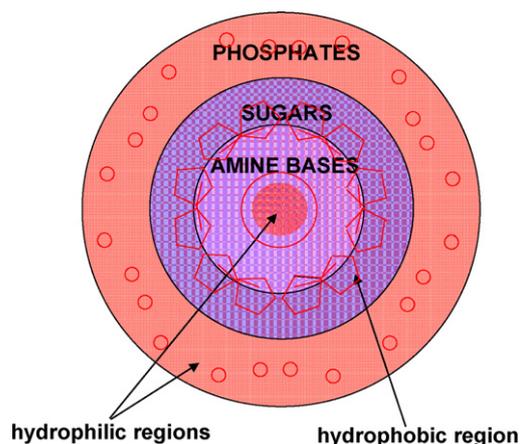


Fig. 2. Schematic representation of the top view of the cylindrical micelle model for the DNA helical structure containing hydrophilic regions around the phosphate groups on the outside and between the amine groups on the inside. The sugar groups form a partly hydrophobic region.

partly hydrophobic sugars inside and hide them from contact with water. The DNA helix behaves similarly to a cylindrical micelle with competition between hydrophobic and hydrophilic interactions. Fig. 2 is a schematic representation.

In Series I samples, the number of $CH(OH)$ groups is increased. Because of the strong hydrophilic interactions of the OH groups with the phosphate groups, the $CH(OH)$ groups act as co-surfactants and help strengthen the micelle nature of the DNA helix. It tightens the helical structure thereby raising the melting temperature.

In Series II samples, when the alcohol molecule gets larger, it becomes more effective at creating pathways for water to cross the hydrophobic sugar-rich region better (up to $n=3$). This helps decrease the melting temperature in Series II. In Series III samples, when the alcohol molecule gets even larger ($n > 3$), it becomes more hydrophobic and therefore tends to increasingly “plug” the sugar-rich region. This increases the melting temperature. The size of an alcohol molecule with 3 carbons is comparable to the size of the sugar groups in DNA.

In conclusion, UV absorption measurements from 4% DNA in series of mixed solvents showed that the DNA helix-to-coil melting transition is driven by the solvent's ability to cross the hydrophobic sugar-rich region. These hydrophobic interactions soften upon temperature increase or by using solvent mixtures. These mixed solvents act as denaturing agent.

A number of publications have addressed the thermal melting transition. Some relevant ones are mentioned here. The denaturation of DNA is favored by heating or by the addition of denaturing agents such as DESO or DMSO sulfoxides [8]. The addition of such denaturing agents helps lower the melting temperature. This is due to hydrophobic interactions between the ethyl groups of the sulfoxide molecule and the nonpolar groups of DNA. These results agree with the conclusions reported in this paper. The thermal denaturation of a protein (lysozyme) was investigated [9] in the presence of three sulfoxide solvents (DMSO, DESO, and DPSO). The denaturing ability of these solvents was found to decrease with increasing number of carbons in the sulfoxide series. This underscores the importance of hydrophobic interactions in the denaturation of DNA as well as proteins. Moreover, DNA dissolved in 99% glycerol was characterized by a melting transition some $30^\circ C$ higher than the same DNA (at the same concentration) dissolved in a buffered water solution [3]. This finding is in agreement with the Series I samples reported in this paper. The thermal melting transition of DNA in the presence of monohydric alcohols showed an

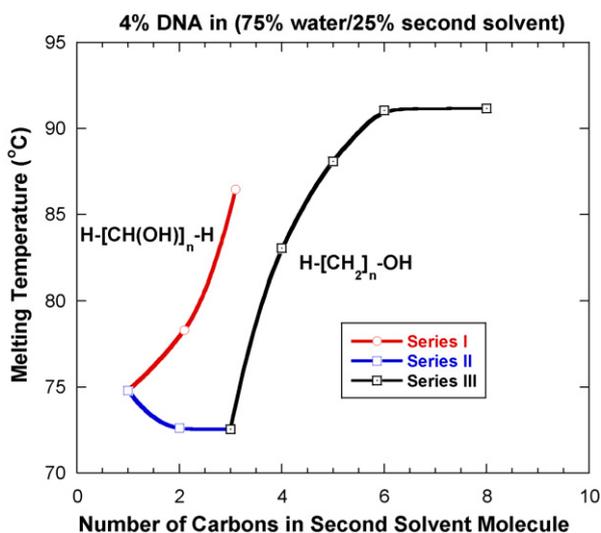


Fig. 1. Variation of the helix-to-coil melting temperature for 4% DNA in a mixture of 75% water and 25% “second” solvent. The abscissa represents the number of carbons in the second solvent molecule. The second solvents for Series I are methanol, ethylene glycol or glycerol. The second solvents for Series II and III are alcohols with increasing hydrocarbon tail. Note the change in trend and in slope at $n=3$ (for 1-propanol). Error bars are smaller than the plotting symbols.

interesting behavior [10]. The melting temperature was seen to decrease then increase with increasing alcohol concentration. This was attributed to an increase in the electrostatic repulsion of the phosphate groups as well as hydrophobic effects in the sugar-rich region.

Acknowledgments

The identification of commercial products does not imply endorsement by the National Institute of Standards and Technology nor does it imply that the products are the best possible for the intended use. This work is based upon activities supported in part by the National Science Foundation under Agreement No. DMR-0454672.

References

- [1] W.M. Becker, L.J. Kleinsmith, J. Hardi, *World of the Cell*, Benjamin/Cummings Publishing, 1999.
- [2] T. Iwataki, S. Kidoaki, T. Sakaue, K. Yoshikawa, S. Abramchuk, *Journal of Chemical Physics* 120 (2004) 4004–4011.
- [3] G. Bonner, A.M. Klibanov, *Biotechnology and Bioengineering* 68 (2000) 339–344.
- [4] S. Arnott, R. Chandrasekaran, D.L. Birsall, A.G.W. Leslie, R.L. Ratliff, *Nature* 283 (1980) 743–745.
- [5] E. Rozners, J. Moulder, *Nuclei Acids Research* 32 (2004) 248–254.
- [6] Y.K. Cheng, B.M. Pettitt, *Progress in Biophysics & Molecular Biology* 58 (1992) 225–257.
- [7] B. Hammouda, D. Worcester, *Biophysical Journal* 91 (2006) 2237–2242.
- [8] M.G. Aznauryan, S.A. Markarian, *Biopolymers* 81 (2006) 1–5.
- [9] A. Torreggiani, M. Di Foggia, I. Manco, A. De Maio, S.A. Markarian, S. Bonora, *Journal of Molecular Structure* 891 (2008) 115–122.
- [10] G. Baldini, H. Fu-Hua, G. Varani, *Il Nuovo Cimento* 6D (1985) 618–630.