Diaminoalkanes with an odd number of carbon atoms induce compaction of a single double-stranded DNA chain

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Abstract **We have performed direct observations on the conformational change of a single double-stranded T4 DNA molecule induced by diaminoalkanes, $NH_3^+(CH_2)_nNH_3^+$ ($n = 1 \sim 6$), by use of fluorescence microscopy. It was found that diamines with three and five methylene groups show a significant effect on the compaction of individual single DNA molecules. On the other hand, diamines with two, four and six methylene groups are not effective in the compaction of DNA. Such a drastic difference in the action of diamines suggests that not only the number of charge but also the length between the amino groups in the diamines plays an essential role in their interactions with DNA.**

Key words: Diaminoalkane; Higher order structure of DNA; Globular DNA; Single molecular observation

1. Introduction

In living cells, DNA is generally packed into a small space. In eukaryotic cells, DNA is tightly bound to histones and compacted into chromosomes. DNA molecules are also compacted in bacteria and in viruses. In spite of recent advance in the knowledge of the primary structure of various DNA molecules, studies on the higher order structure of the tightly packed DNA within the cellular environment seem to remain at a primitive stage. This may be due to the lack of suitable methodology to study the higher order structure of DNA in aqueous solution.

It is well known that polyamines, such as putrescine (1,4-diaminobutane), spermidine and spermine, are present in millimolar concentrations in most tissues and microorganisms [1,2]. Other polyamine derivatives including cadaverine (1,5-diaminopentane) and 1,3-dianinopropane are also found in some living cells [2–5]. Although there are a large number of papers that describe the effects of the polyamines on the higher order structure of DNA, the mechanism of the action of the polyamines on DNA molecules has not been clarified yet. Using electron microscopy, it has been reported that shrunken particles of DNA molecules with various shapes, such as toroid and rod structures, are formed by the cations with charges of +3 or greater, including polyamines [6–12]. However, with electron microscopy, due to rather severe pre-treatments such as drying and staining, the observed structure of DNA may change significantly from its original structure in aqueous environment. Light scattering seems to have been the most useful method to observe the structural change such as the formation of the compacted form of DNA in aqueous environment [9,13]. However, with this method, it is difficult to gain insight into the structural change in individual DNA molecules at dilute concentrations, avoiding multi-molecular aggregation. In addition to this, the applicability of the light scattering is usually limited up to the molecular size with several tens kbp. About a decade ago, it was found that, with fluorescence microscopy, the structure of giant DNA molecules with more than several tens kbp can be visualized by using appropriate dye molecules, although the spacial resolution is rather low with the order of 0.2–0.4 μm [14]. More recently, it has been shown that fluorescence microscopy is a useful tool to monitor the change of the high order structure of DNA induced by various kinds of chemicals, such as intercalators and minor-grobe binders, in aqueous solutions [15–21].

In the present study, we have performed the measurement of fluorescence microscopy to study the structural change in individual T4 DNA molecules induced by divalent polyamines, diaminoalkanes $NH_3^+(CH_2)_nNH_3^+$ (DA_n; $n = 1 \sim 6$) in aqueous solution. We will show that diamines with an odd number of the methylene groups exhibit a significant effect on compaction of single DNA chains.

2. Materials and methods

T4 DNA, 166 kbp with a contour length of 55 μm [16], was purchased from Nippon Gene. The fluorescent dye, 4′,6-diamidino-2-phenylindole (DAPI), and antioxidant, 2-mercaptoethanol (2-ME), were obtained from Wako Pure Chemical Industries, Ltd. Metylenediamine·2HCl, ethylenediamine·2HCl, 1,3-diaminopropane·2HCl and 1,6-diaminohexane·2HCl were obtained from Tokyo Kasei. 1,4-Diaminobutane·2HCl (putrescine) and 1,5-dianinopentane·2HCl (cadaverine) were obtained from Wako Pure Chemical Industries.

DNA was dissolved in 45 mM Tris-HCl buffer solution of pH 5.3–7.2 with 40 mM NaCl, 0.6 μM DAPI and 4% (v/v) 2-ME. Diaminoalkane $NH_2(CH_2)_nNH_2$ (n = 1–6), DA_n, was added to the DNA solution at the desired concentration. It has been confirmed that the persistent length and the contour length in DNA remain essentially constant at such a low concentration of DAPI [18].

Fluorescence images of T4DNA molecules were observed using a Carl Zeiss microscope, Axiovert 135 TV, equipped with a 100 × oil-immersed objective lens and with a high sensitive Hamamatsu SIT TV camera, and were recorded on video tapes. The video image was analyzed with an image processor, Arugus 50 (Hamamatsu Photonics). Observation was carried out at room temperature, 20°C. The long-axis length l, which was defined as the longest distance in the outline of the DNA image, was obtained as the ensemble average of at least 40 DNA molecules. Due to the blurring effect in the observation with the highly sensitive TV system, the size of observed DNA image was assumed to be slightly larger than the actual size of the DNA by the order of 0.3 μm [16].

3. Results and discussion

Fig. 1 exemplifies the fluorescence images of T4DNA molecules together with the corresponding quasi three-dimensional

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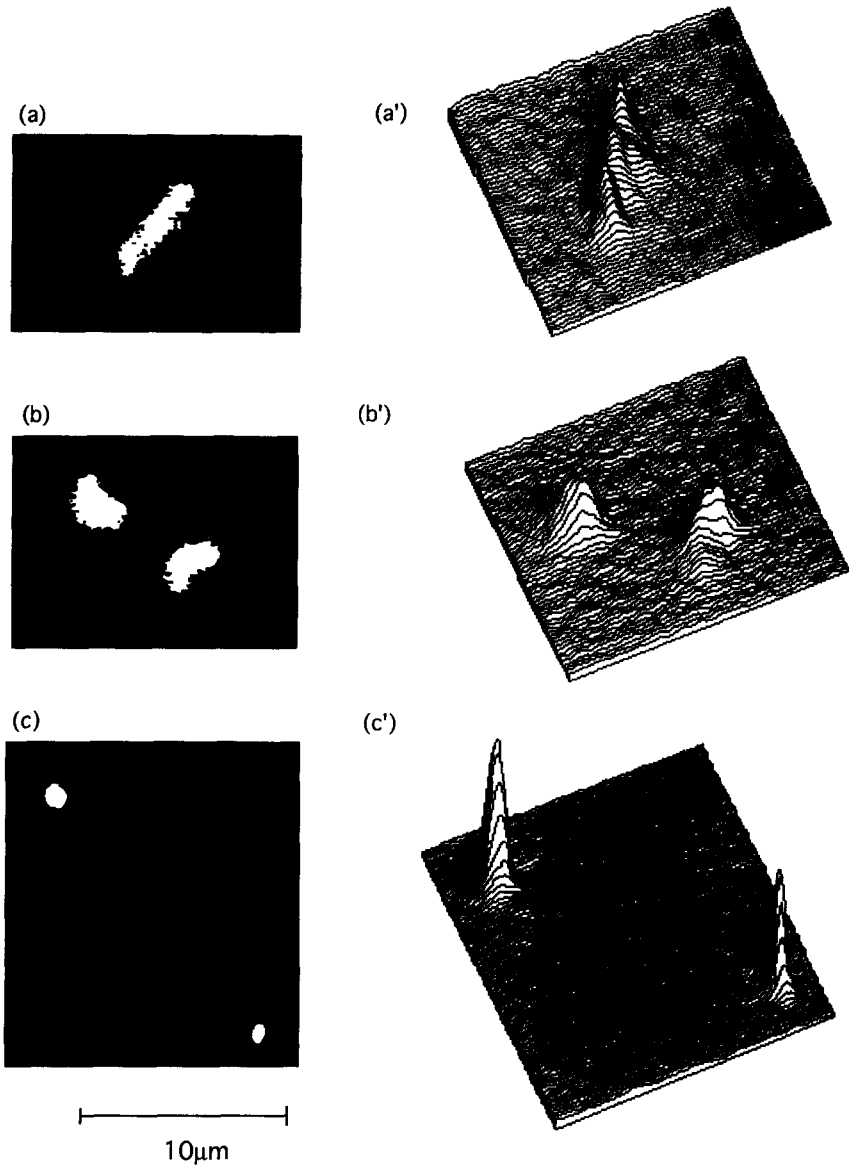

Fig. 1. Fluorescence images (left side) and the corresponding light intensity distributions (right side) on the video flames. The obstacles are T4 DNA molecules; (a) and (a'), in 45 mM Tris-HCl solution with the 40 mM NaCl at pH7.2; (b) and (b'), with the addition of 20 mM DA_2 to the buffer solution; and (c) and (c'), with the addition of 20 mM DA_3 to the buffer solution.

representation of the fluorescence intensity distribution. The DNA molecules exhibit extended conformation, i.e. a coil state, in buffer solution (Fig. 1a) and in a solution of 20 mM DA_2 (Fig. 1b). While, the DNA molecules take on shrunken, globular conformation with 20 mM DA_3 (Fig. 1c). As in Fig. 1c, we have succeeded in the observation of the individual compact globular obstacles made from single double-stranded DNA's under the condition that the DNA concentration is quite low, 0.3 μM in the base-pair unit. Taking into consideration that the full-stretched length or the contour length of T4 DNA is ca. 55 μm [16] and that the blurring effect is ca. 0.3 μm, structures of the elongated coil and the shrunken globule may be depicted as in Fig. 2.

The effect of diamines on the long axis length of DNA is summarized in Fig. 3. Without the diamines, the average long-axis length l for DNA molecules is 3.5 μm at pH 7.2, which is almost the same as that at pH 5.3 (3.3 μm). This figure indicates that, more or less, diamines decrease the length of the DNA molecules. The effect on the decrease of l is significant for DA_3 (pH 5.3 and 7.2), and DA_5 (pH 5.3). Although it has been put forward that diamines cannot cause compaction of DNA in aqueous solution at room temperature [22], in the present study it has become clear that diamine can cause compaction of DNA. On the other hand, DA_2, DA_4 and DA_6 do not show a marked effect on the compaction of DNA at either pH 5.3 or 7.2. We have noticed that DA_4 tends to induce the aggregation between different DNA molecules, instead of the compaction of individual molecules. Especially at pH 5.3, we observed multi-molecular aggregation of DNA at DA_4 concentrations higher than 5 mM. Suwalskey et al. found that spermine induces aggregation between DNA's [23]. They assumed that the length of the central aliphatic chain of spermine, which is four

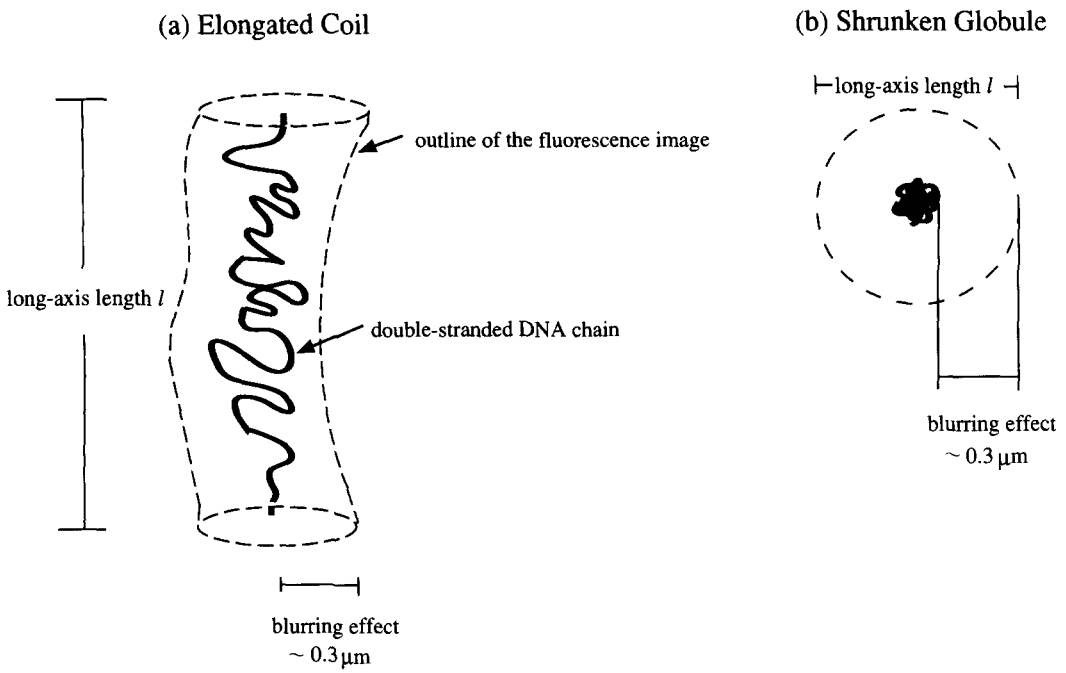

Fig. 2. Schematic illustration of the fluorescence image of double-stranded T4 DNA molecules, (a) in an elongated coil and (b) in a shrunken globule.

carbon atoms long, was suitable to bridge the segments of different DNA molecules.

Fig. 4 shows the distributions of l values for T4DNA molecules at different concentrations of DA_3. At 1 mM, DNA molecules show the distribution of a single maximum with an average length of 3.0 μm. At 8 mM of DA_3, the distribution is bimodal, indicating that elongated coil and compact globular conformations coexist. At the concentrations higher than 16 mM, almost all DNAs take the globular form.

Due to the blurring effect with the high-sensitive SIT camera, the real size should be somewhat smaller than the apparent size for the fluorescent obstacles (see Fig. 2). Such an effect may be significant especially for the compact, globular DNA molecules. In order to evaluate the real size of DNA, we have performed the measurement of the translational diffusion constant D for the individual DNA obstacles observed with the fluorescence microscopy. The value D can be obtained from the mean square displacement (MSD) of the center of mass for DNA. Although we have tried to minimize the convective flow in the aqueous sample, there remained non-negligible convective flow during the measurement, possibly due to the effect of the illumination. As the convective motion was almost constant both in the flow rate and flow direction during the period of observation, we could eliminate the effect of convective flow using the relationship of Eq. 1 [24].

$$<(\boldsymbol{R}(t) - \boldsymbol{R}(0))^2> = 4Dt + At^2, \quad (1)$$

where $\boldsymbol{R}(t) = (R_x, R_y)$ is the position of the center of mass for a DNA, $<(\boldsymbol{R}(t) - \boldsymbol{R}(0))^2>$ is the mean square displacement, and A is a numerical constant related to the convective flow. The effective hydrodynamic radius ξ of a single DNA molecule was calculated from the D value based on the Stokes–Einstein equation given in Eq. 2 [25,26],

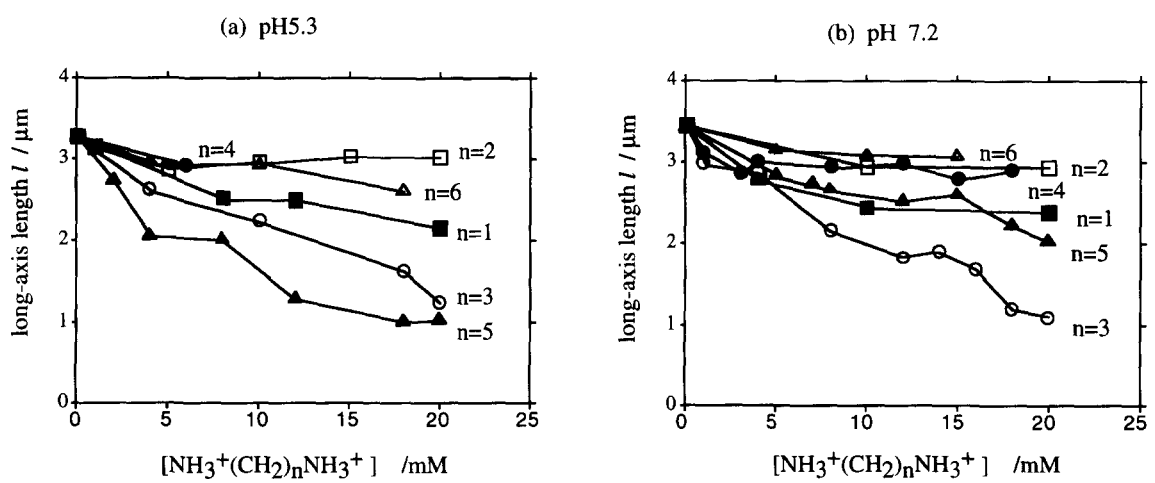

Fig. 3. Change of long-axis length l of T4 DNA with the concentration of diamines. pH = (a) 5.3, (b) 7.2.

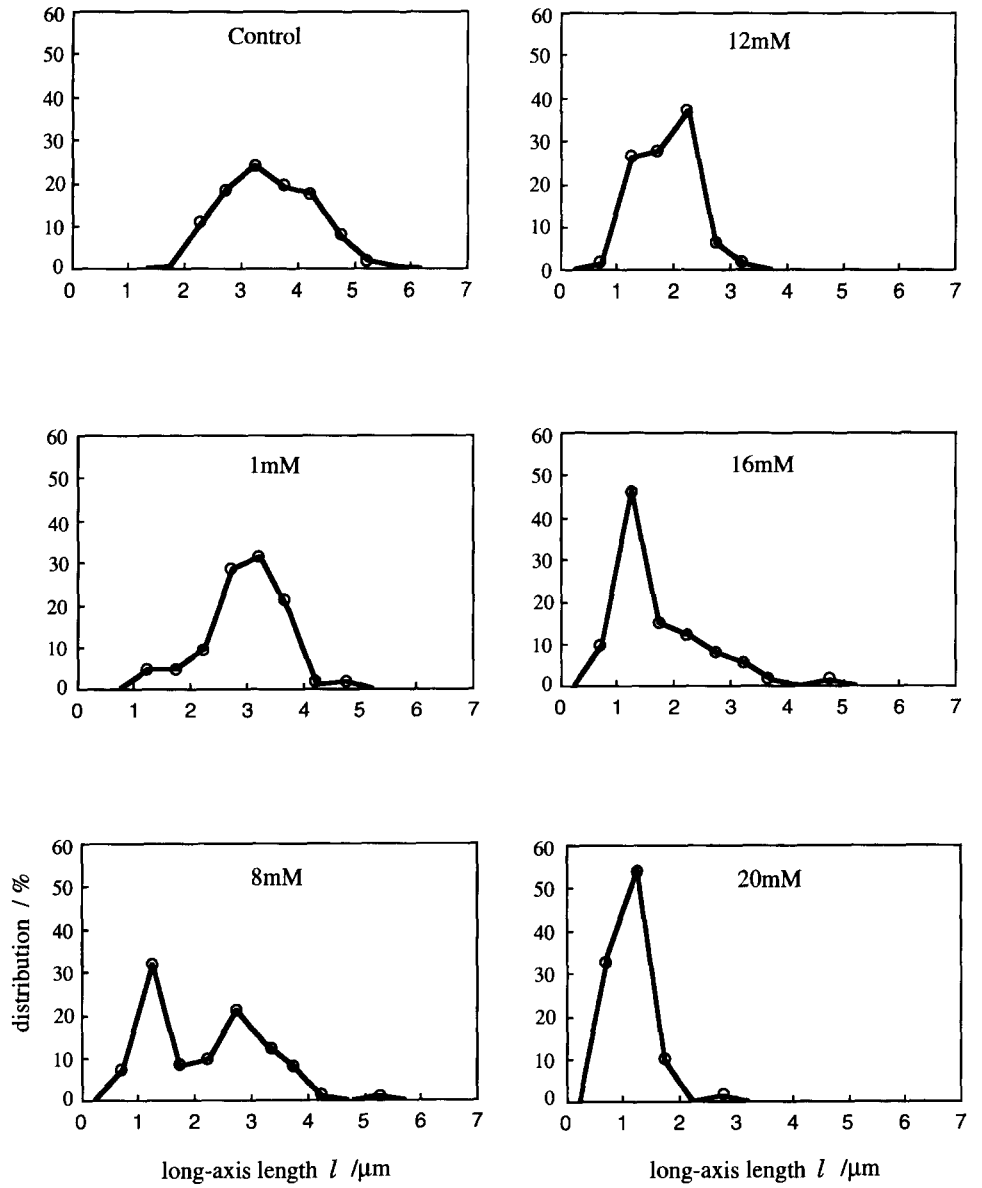

Fig. 4. Distribution of the long-axis length l of T4DNA at various DA_3 concentrations.

$$\xi = \frac{k_B T}{6\pi\eta_S D} \quad (2)$$

where k_B is the Boltzmann constant and η_s is the viscosity of the solvent (1.002 mPa·s for pure water at $T = 293K$). The results showed that the values ξ's of DNA molecules in 20 mM DA_3 and DA_5 solutions at pH 5.3 are 0.18 mm and 0.19 mm, respectively, and 0.16 mm in 20 mM DA_3 solution at pH 7.2. As for these compact obstacles, the apparent long-axis length is 0.8–1.0 μm as is shown in Fig. 3. Taking into account of the blurring effect with the order of 0.3 μm, the difference between the hydrodynamic radius ξ and long-axis length for the globular forms is explained well as is schematically shown in Fig. 2b.

Next, let us discuss on the conformational change due to the binding of DA_3 and DA_5 to DNA molecules. According to the standard statistical theory of polymers [27], the contour length L, which is the length of a single DNA molecule measured along its backbone, is given by Eq. 3.

$$L = N\lambda, \quad (3)$$

where N and λ are the number of segments and persistence length, respectively. Here, it is noted that the curvature of the DNA chain is roughly equal to $1/\lambda$. Then, let us assume that the curvature is proportional to the binding degree of DA_n that binds to DNA molecule as in the following relationship.

$$(1/\lambda - 1/\lambda_0) \propto C_b, \quad (4)$$

where λ_0 is the natural persistence length and C_b is the number of the bound DA_n molecules per base-pair of DNA. This assumption seems appropriate, because the increase of the curvature may be proportional to the bending elastic energy of an elastic rod. When the conformation of the DNA molecule is described with the random flight model [27], the root mean square separation R_{rms} is given by Eq. 5.

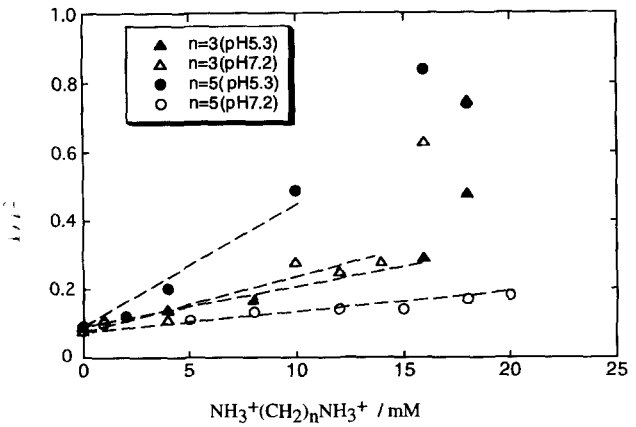

Fig. 5. $1/l^2$, inverse of the square of the long-axis length, plotted against the concentration of diamines.

$$R_{rms} = N^{1/2}\lambda. \quad (5)$$

As R_{rms} is expected to be proportional to the radius gyration, R_g, and also to the long-axis length, l, Eq. 6 is derived.

$$1/l^2 = kC_b + A, \quad (6)$$

where k and A are numerical constants. Fig. 5 shows the plots of $1/l^2$ vs. the concentration of DA_n. Here, we adapted the bulk concentrations of DA_n instead of the binding degree, C_b, because of the difficulty to evaluate C_b from the experiment. As shown in Fig. 5, the nearly linear relationship was noticed at least for the region of low concentration of the ligand, This may be due to the effect that, when the ligand concentration is low, the binding degree of DAn is almost proportional to the bulk concentration. On the other hand, at higher concentration of diamine, the relationship does not hold. The difference in the effect of DA_5 between pH 5.3 and 7.2 may be attributed to the difference in the degree of the dissociation of the amino groups.

It is expected that diamines form the complex with DNA, through the interaction between the amino groups and the negative charged phosphate groups along the double-stranded DNA chains. The different effect of diamines with odd and even carbon numbers on the compaction of DNA molecules may be related to the difference in the steric interaction between diamines and DNA. Difference in the effect between odd and even carbon atoms has been encountered in the study of the micellar rate constant of a series of alkanediamine bis(dodecanoate) salts [28].

The bimodal distribution at the intermediate ligand concentration (see e.g. Fig. 4 at 8 mM) is to be noted. We have found similar effect for the process of the compaction of DNA with polyethylene glycol, with polyarginine and also with cationic surfactant [19,29,30]. Considering that the persistence length of DNA is 500–1000 Å, T4 DNA is regarded as the linear polymer composed with ca. 1000 interconnecting segments [16]. In this condition, coexistence of the coil and globular structures seems to be a rather general phenomenon. Details of the theoretical considerations on the bimodality will be discussed in a forthcoming paper.

References

[1] Ames, B.N. and Dubin, D.T. (1960) J. Biol. Chem. 235, 769–775.
[2] Tabor, C.W. and Tabor, H. (1984) Annu. Rev. Biochem. 53, 749–790.
[3] Hafner, E.W., Tabor, C.W. and Tabor, H. (1979) J. Biol. Chem. 254, 12419–12426.
[4] Alhonen-Hongisto, L. and Jänne, J. (1980) Biochem. Biophys. Res. Commun. 93, 1005–1013.
[5] Oshima, T. (1982) J. Biol. Chem. 257, 9913–9914.
[6] Laemmli, U.K. (1975) Proc. Natl. Acad. Sci. USA 72, 4288–4292.
[7] Gosule, L.C. and Schellman, J.A. (1976) Nature 259, 333–335.
[8] Chattoraj, D.K., Gosule, L.C. and Schellman, J.A. (1978) J. Mol. Biol. 121, 327–337.
[9] Widom, J. and Baldwin, R.L. (1980) J. Mol. Biol. 144, 431–453.
[10] Marx, K.A. and Ruben, G.C. (1984) J. Biol. Struct. Dynam. 1, 1109–1132.
[11] Baeza, I., Gariglio, P., Rangel, L.M., Chavez, P., Cervantes, L., Arguello, C., Wong, C. and Montanez, C. (1987) Biochemistry 26, 6387–6392.
[12] Plum, G.E., Arscott, P.G. and Bloomfield, V.A. (1990) Biopolymers 30, 631–643.
[13] Arscott, P.G., Li, A.-Z. and Bloomfield, V.A. (1990) Biopolymers 30, 619–630.
[14] Yanagida, M., Hiraoka, Y., Katsura, I. (1983) Cold Spring Harbor Symp. Quant. Biol. 47, 177–187.
[15] Bustamante, C. (1991) Annu. Rev. Biophys. Biophys. Chem. 20, 415–416.
[16] Yoshikawa, K., Matsuzawa, Y., Minagawa, K., Doi, M. and Matsumoto, M. (1992) Biochem. Biophys. Res. Commun. 188, 1274–1279.
[17] Minagawa, K., Matsuzawa, Y., Yoshikawa, K., Masubuchi, Y., Matsumoto, M., Doi, M., Nishimura, C. and Maeda, M. (1993) Nucleic Acids Res. 21, 37–40.
[18] Matsuzawa, Y. and Yoshikawa, K. (1994) Nucleosides and Nucleotides 13, 1415–1423.
[19] Minagawa, K., Matsuzawa, Y., Yoshikawa, K., Khokhlov, A.R. and Doi, M. (1994) Biopolymers 34, 555–558.
[20] Perkins, T.T., Smith, D.E. and Chu, S. (1994) Science 264, 819–822.
[21] Perkins, T.T., Quake, S.R., Smith, D.E. and Chu, S. (1994) Science 264, 822–826.
[22] Gosule, L.C. and Schellman, J.A. (1978) J. Mol. Biol. 121, 311–326.
[23] Suwalskey, M., Traub, W., Shmueli, U. and Subirana, J.A. (1969) J. Mol. Biol. 42, 363–373.
[24] Matsumoto, M., Sakaguchi, T., Kimura, H., Doi, M., Minagawa, K., Matsuzawa, Y. and Yoshikawa, K. (1992) J. Polym. Sci.: Part B: Polym. Phys. 30, 779–783.
[25] Doi, M. and Edwards, S.F. (1986) The Theory of Polymer Dynamics, Clarendon, Oxford.
[26] Oono, Y. and Kohmoto, M.J. (1983) J. Chem. Phys. 78,, 520–528.
[27] Flory, P.J. (1969) Statistical Mechanics of Chain Molecules, Wiley, New York.
[28] O'Connor, C.J. and Lomax, T.D. (1983) Aust. J. Chem. 36, 907–916.
[29] Minagawa, K., Matsuzawa, Y., Yoshikawa, K., Matsumoto, M. and Doi, M. (1991) FEBS Lett. 295, 65–67.
[30] Melnikov, S.M., Sergeyev, V.G. and Yoshikawa, K. (1995) J. Am. Chem. Soc., in press.