Multiple Electrostatic Interaction of Polycationic Chitosan Oilgomers with Nucleic Acids: Polyamine Promoted Stabilization of the A-Form of RNA and DNA in Aqueous Solutions

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(Received January 10, 1992)

Polycationic chitosan oligomers $\mathbf{1}_n$ (n=2—6), i.e., β-1,4-linked glucosamine oligomers, were found to bind to nucleic acids in aqueous solutions at pH 7.2 via multiple electrostatic interaction. The binding of trimer and higher homologues ($\mathbf{1}_n$, $n\geq3$) is especially efficient owing to an (at least) three-point simultaneous ammonio phosphate formation. Upon binding of $\mathbf{1}_n$ ($n\geq3$), the double-helical structure in the A-form of poly(A)·poly(U), a synthetic RNA, is significantly stabilized as revealed by enhancement of its helix-to-coil transition (melting) temperatures. Upon binding of $\mathbf{1}_n$ ($n\geq3$), calf thymus DNA in the B-form undergoes a drastic conformational change to the A-form, as revealed by circular dichrosim spectroscopy. In marked contrast, chitosan dimer ($\mathbf{1}_2$) and diamine and monoamine references only induce the B-to-C transition within the B-family. The significance of the multiple electrostatic interaction and the cooperativity of the glucosamine residues in $\mathbf{1}_n$ are discussed.

A rapidly growing area in the molecular recognition of nucleic acids is concerned with the development of DNA- and RNA-binding molecules that are capable of multiple interaction.[1-5] The basic structural elements of double helical DNA are π–π stacked heteroaromatic bases, hydrogen-bonded base pairs having peripheral polar groups, and polyanionic phosphodiester backbones.[6] These moieties provide the sites of intercalation,[3,7] hydrogen bonding,[2] and electrostatic interaction, respectively, for DNA- and RNA-binding proteins,[8] antibiotics,[2,4,9] and synthetic molecules.[1-3]

Multiple electrostatic interaction seems to provide a potential DNA-binding strategy. It also has biological significances; DNA's are often associated with basic proteins such as histones and protamines having many arginine and lysine residues as well as with polycationic amines spermine and spermidine.[10] The DNA(RNA)-polyamine interactions,[11] especially those involving oligolysine derivatives,[12] have been reported; thermodynamic analyses thereof have been performed to reveal the significance of DNA–polycation interactions.[13] We noticed the usefulness of chitosan oligomers as another series of readily available and chain-length controllable polycationic derivatives. Chitosan oligomers are β-1,4-linked glucosamine oligomers. We report here on their multiple electrostatic binding to RNA and DNA that results in pronounced stabilization of the A-form of double-helical structure.

Experimental

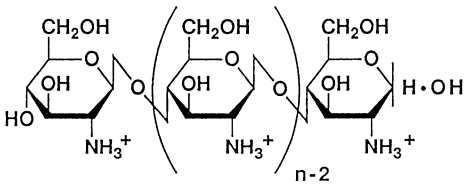
Sodium salt of polyadenylate–polyuridylate duplex (poly(A)·poly(U)) was a product of Yamasa Shoyu Co. Ltd. Highly polymeric calf thymus DNA (Pharmacia) was dissolved in aqueous NaCl (2 M, 1 M=1 mol dm^{-3}) and sonicated with a probe-type sonicator. The DNA solution was chilled in an ice bath during the sonication procedure.[14] The DNA was recovered by centrifugation. Chitosan oligomers $\mathbf{1}_n$, chitooligosaccharides $\mathbf{2}_n$, and cellooligosaccharides $\mathbf{3}_n$ were obtained from Seikagaku Kogyo Co., Ltd. Glucosamine ($\mathbf{1}_1$) and other chemicals were commercial products of Nacalai Tesque Inc.

A series of sample solutions in a cell of 1-cm path-length were prepared by dissolving poly(A)·poly(U) or calf thymus DNA in citrate buffer (poly(A)·poly(U); sodium citrate (10 mM) and sodium chloride (1 mM): Calf thymus DNA; sodium citrate (150 mM) and sodium chloride (1.5 M) in redistilled, ion-exchanged water, pH 7.2) in the absence or presence of varying amounts of $\mathbf{1}_n$ or a reference salt. The concentrations of a nucleic acid were maintained at 9.36×10^{-5} M with respect to P or nucleotide unit, as determined spectrophotometrically using the known molar extinction coefficient (per P or nucleotide) at 260 nm; 6.58×10^3 and 6.60×10^3 for poly(A)·poly(U) and calf thymus DNA, respectively. The ratio of absorbance at 500 nm and that at 260 nm for any sample solution was $A_{500}/A_{260}\leq0.06$, indicating that the solution was free from turbidity.

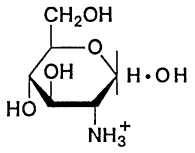
The electronic-absorption and circular dichroism spectra were recorded with a Hitachi 320 or JASCO Ubest-50 spectrophotometer and a JASCO J-600 spectropolarimeter, respectively. A computer-controlled variable-temperature unit was used for the measurement of melting temperatures. The rate of heating was 0.5 degree per min and the actual temperature of the sample solution was measured with a platinum thermocouple.

Results and Discussion

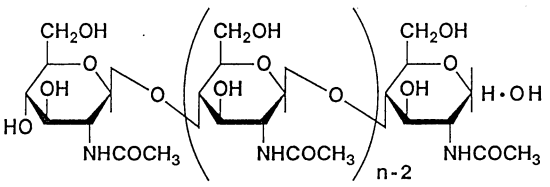
Polyamine Enhanced Stability of the Double-Helical Structure of Poly(A)·Poly(U). It is well-known that double helical DNA and RNA undergo thermal helix-to-coil transitions with loss of hypochromicity.[15] The transition or melting temperatures depend not only on the chain length[16] and GC/AT(AU) content[17] of nucleic acids or oligonucleotides but also on the concentration of additives such as salts[17a] and intercalators.[18] The melting temperatures of poly(A)·poly(U), a double-helical synthetic RNA composed of polyadenylate–polyuridylate duplex, were measured in the presence and absence of a series of chitosan oligomers $\mathbf{1}_n$ (n=2—6) and parent glucosamine ($\mathbf{1}_1$) at pH 7.2 (citrate); $\mathbf{1}_n$ were



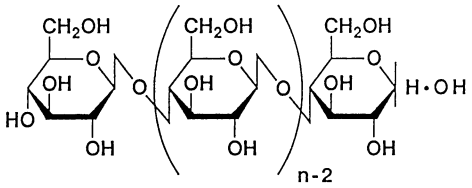
$\mathbf{1}_n$ (n = 2 - 6)



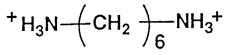
$\mathbf{1}_1$



$\mathbf{2}_n$ (n = 2 - 6)



$\mathbf{3}_n$ (n = 2 - 5)



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actually protonated polyammonium derivatives at this pH. As references were used chitooligosaccharides $\mathbf{2}_n$ (n=2—6; *N*-acetyl derivatives of chitosan oligomers $\mathbf{1}_n$), cellooligosaccharides $\mathbf{3}_n$ (n=2—5; β-1,4-linked glucose oligomers), and hexamethylenediamine (**4**). Poly(A)·poly(U) was chosen because it is a *homopolymer* containing only wealky hydrogen-bonded AU base pairs and thus gives rise to sharp melting in a relatively low and hence conventional temperature range.[16]

Figure 1 shows some typical melting curves for

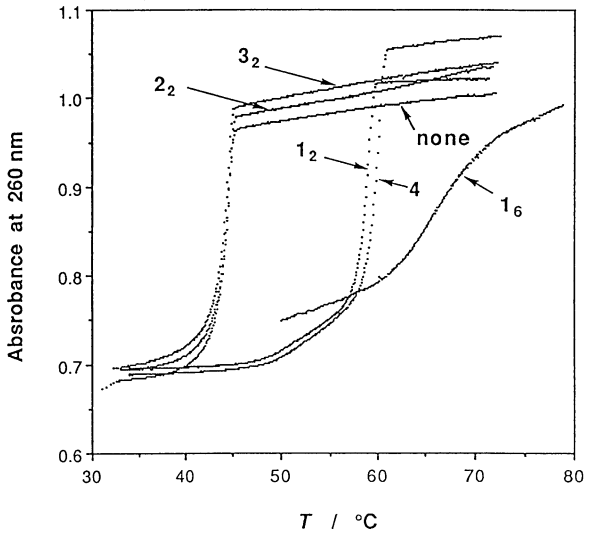

Fig. 1. Temperature dependence of the absorbance at 260 nm for a solution of poly(A)·poly(U) (9.36×10^{-5} M with respect to P or nucleotide unit) in citrate buffer at pH 7.2 in the absence (none) or presence of chitosan dimer ($\mathbf{1}_2$), chitosan hexamer ($\mathbf{1}_6$), chitobiose ($\mathbf{2}_2$), cellobiose ($\mathbf{3}_2$), or hexamethylenediamine (**4**): $[\mathbf{1}_2]=[\mathbf{2}_2]=[\mathbf{3}_2]=[\mathbf{4}]=2.90\times10^{-3}$ M and $[\mathbf{1}_6]=1.45\times10^{-3}$ M.

poly(A)·poly(U) (9.36×10^{-5} M with respect to P or nucleotide unit) in the absence (none) and presence (2.90×10^{-3} M unless otherwise indicated) of $\mathbf{1}_2$, $\mathbf{1}_6$, $\mathbf{2}_2$, $\mathbf{3}_2$, or **4**. A couple of important points are immediately noted. First, the melting temperature (T_m), defined as the temperature corresponding to a 50% transition, is raised by an ammonio-functionalized chitosan oligomer, more effectively by $\mathbf{1}_6$ than by $\mathbf{1}_2$, but not by neutral species such as chitobiose ($\mathbf{2}_2$) and cellobiose ($\mathbf{3}_2$). Higher homologues of these such as $\mathbf{2}_5$ and $\mathbf{3}_5$ could not raise T_m, either. Second, chitosan dimer $\mathbf{1}_2$ and simple diamine **4** show almost the same effects on T_m. These results indicate that the multiple ammonio phosphate formation between poly(A)·poly(U) and $\mathbf{1}_n$ is responsible for the enhancement of T_m. The essential role of polycations $\mathbf{1}_n$ is to partially neutralize the negative charge on the phosphate moieties of poly(A)·poly(U). This would weaken the phosphate–phosphate electrostatic repulsion and thus stabilize the double-helical structure as a consequence.[19]

In Fig. 2 are shown the correlations of T_m and $[\mathbf{1}_n]$ for all the members of $\mathbf{1}_n$ in the concentration ranges which cause no precipitation. Clearly, the higher homologues of $\mathbf{1}_n$ having a large number of cationic centers are the more effective in raising T_m as a result of *intramolecular* cooperation of the glucosamine residues in $\mathbf{1}_n$ in a similar manner as the higher homologues of oligolysines show the higher affinities to synthetic RNA's.[12] The intramolecular cooperativity becomes clearer when the T_m vs. $n\cdot[\mathbf{1}_n]$ correlations for different $\mathbf{1}_n$, as shown in Fig. 3, are compared. The quantity $n\cdot[\mathbf{1}_n]$ represents the total concentration of the glucosamine residue ([glu-N^+]) and hence the total concentration of the unit cationic center. The efficiency of *one* glucosamine unit on this criterion still increases markedly on going from

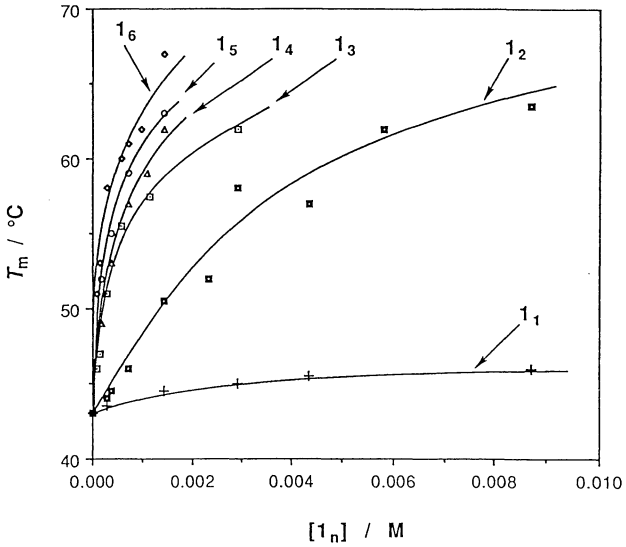

Fig. 2. Correlations of melting temperatures (T_m) with [$\mathbf{1}_n$] (n=1—6) for a series of solutions of poly(A)·poly(U) (9.36×10^{-5} M with respect to P or nucleotide unit) in citrate buffer pH 7.2 in the presence of a chitosan oligomer ($\mathbf{1}_n$, n=2—6) or glucosamine ($\mathbf{1}_1$).

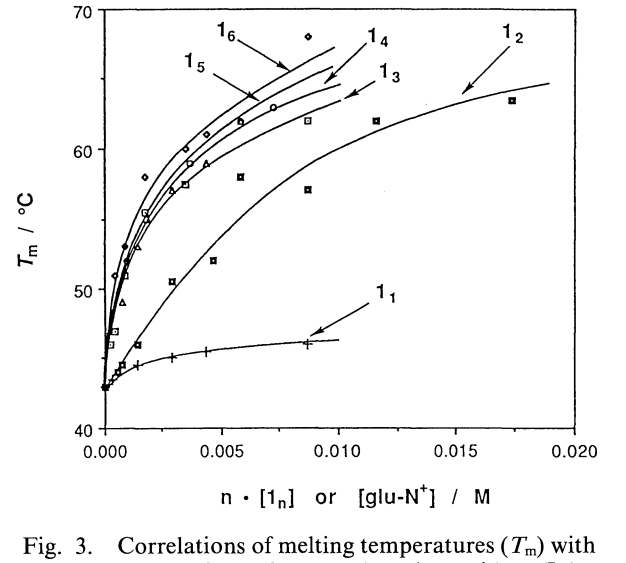

Fig. 3. Correlations of melting temperatures (T_m) with total concentrations of the glucosamine residues ([glu-N$^+$] or n·[$\mathbf{1}_n$], n=1—6) for a series of solutions of poly(A)·poly(U) (9.36×10^{-5} M with respect to P or nucleotide unit) in citrate buffer at pH 7.2 in the presence of a chitosan oligomer ($\mathbf{1}_n$, n=2—6) or glucosamine ($\mathbf{1}_1$).

monomer $\mathbf{1}_1$ through dimer $\mathbf{1}_2$ to trimer $\mathbf{1}_3$. Those for higher homologues ($\mathbf{1}_n$, $n\geq3$), however, are similar to each other. These results indicate that the intramolecular cooperativity in $\mathbf{1}_n$, even when $n\geq3$, is such that allows at best a three-point simultaneous ammonium-phosphate electrostatic interaction between $\mathbf{1}_n$ and poly(A)·poly(U). Thus, trimer $\mathbf{1}_3$ gives a maximal economy of multiple interaction. Trimer and higher

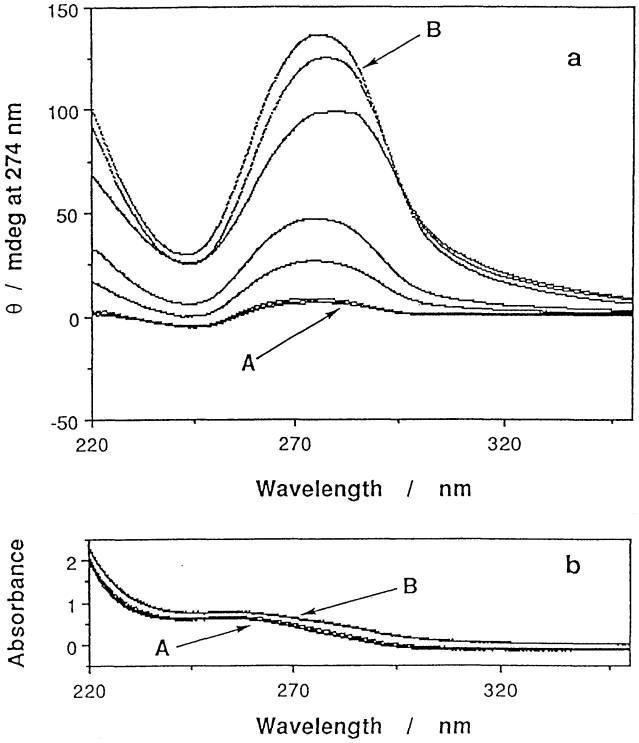

Fig. 4. Circular dichroism (a) and electronic absorption (b) spectra for a solution of calf thymus DNA (9.36×10^{-5} M with respect to P or nucleotide unit) in citrate buffer at pH 7.2 in the absence and presence of varying amount of chitosan trimer ($\mathbf{1}_3$) at 25°C; [$\mathbf{1}_3$]/10^{-2} M=0.00, 1.60, 1.67, 1.70, 1.75, 1.90, 2.00, 2.20, and 2.50, read from A to B.

homologues ($\mathbf{1}_n$, $n\geq3$) in a concentration range [$\mathbf{1}_n$]/[P]=9.30—31.0 give rise to an enhancement of $T_m\geq20$ (referring to Fig. 2). Similar enhancement of T_m can be brought about by KCl, but only when its concentration is two-orders of magnitude larger than those required for $\mathbf{1}_n$ ($n\geq3$) under otherwise identical conditions, i.e., [KCl]/[P]≈3100.

Polyamine Induced B-to-A Transition of the Structure of Calf Thymus DNA. The structures of right-handed double-helical DNA's are classified into A and B families.[20] The A- and B-DNA in solutions can be readily distinguished by the circular dichroism (CD) spectroscopy.[21] The CD spectrum of calf thymus DNA in water (I=1.5 with NaCl) at pH 7.2 (citrate) is composed of positive and negative Cotton effects of similar intensities (Fig. 4a) and is characteristic of B-DNA. Addition of chitosan trimer ($\mathbf{1}_3$) to the solution resulted in increase in the intensity of the longer-wavelength positive-component (Fig. 4a) with almost no change in the electronic absorption spectra (Fig. 4b). This change in CD spectra is charateristic of a transition of DNA from the B-form to the A-form.[21]

In Fig. 5 are shown the correlations of the CD intensities (ellipticities) at 274 nm with [$\mathbf{1}_n$] (Fig. 5a) or with n·[$\mathbf{1}_n$] (Fig. 5b). Thus, trimer and higher

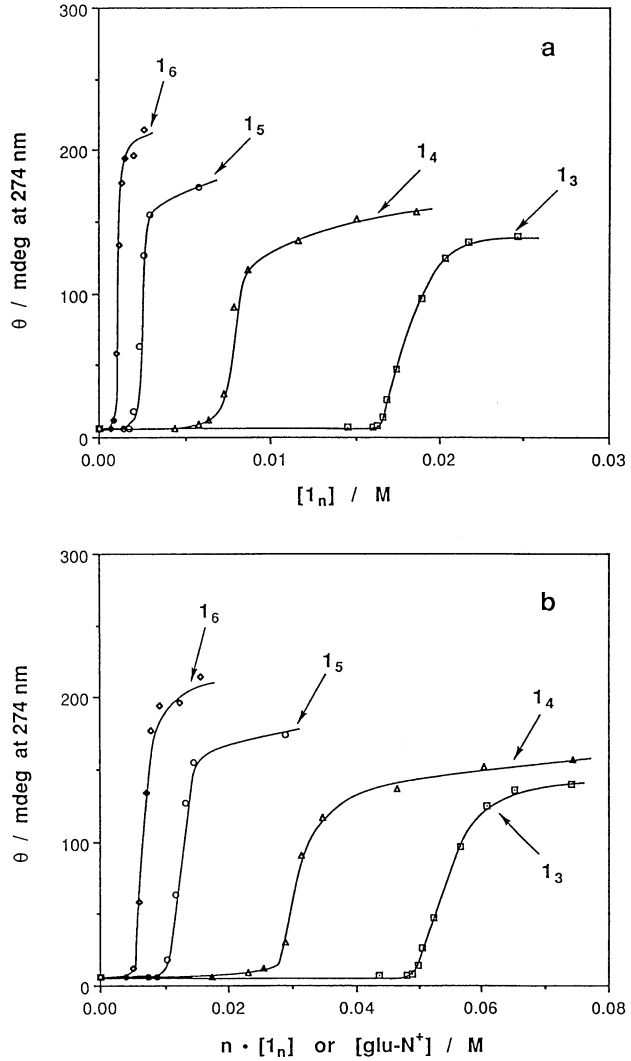

Fig. 5. Correlations of observed ellipticities (θ) at 274 nm with $[\mathbf{1}_n]$ (n=3—6) (a) and with total concentrations of the glucosamine residues ($n\cdot[\mathbf{1}_n]$ or [glu-N$^+$], n=3—6) (b) for a series of solutions of calf thymus DNA 9.36×10^{-5} M with respect to P or nucleotide unit) in citrate buffer at pH 7.2 in the presence of varying amounts of chitosan trimer ($\mathbf{1}_3$), tetramer ($\mathbf{1}_4$), pentamer ($\mathbf{1}_5$), or hexamer ($\mathbf{1}_6$) at 25°C.

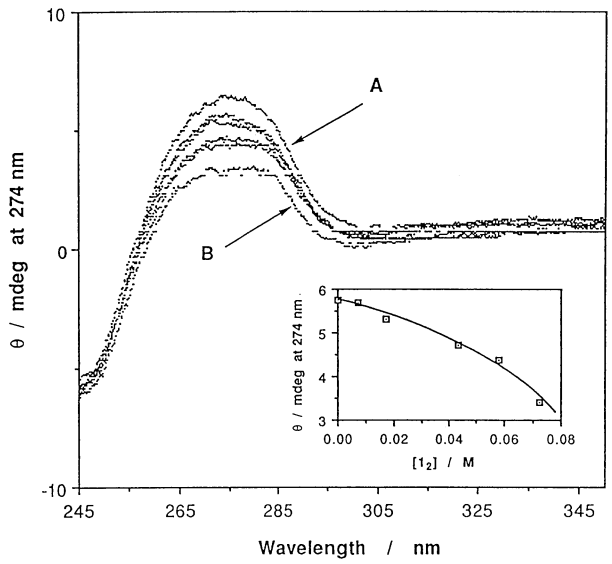

Fig. 6. Circular dichroism spectra for a solution of calf thymus DNA (9.36×10^{-5} M with respect to P or nucleotide unit) in citrate buffer at pH 7.2 in the presence of varying amounts of chitosan dimer ($\mathbf{1}_2$) at 25°C; $[\mathbf{1}_2]/10^{-2}$ M=0.00, 0.70, 1.70, 4.30, 5.80, and 7.20, read from A to B). Inset: correlation of observed ellipticities (θ) at 274 nm with $[\mathbf{1}_2]$.

homologues ($\mathbf{1}$, $n\geq3$) are very effective in inducing the B-to-A transition. In fact, it occurs with such a low concentration range of $\mathbf{1}_n$, especially pentamer $\mathbf{1}_5$ and hexamer $\mathbf{1}_6$, as $[\mathbf{1}_n]/[P]<31$ (n=5 or 6) (referring to Fig. 5). In addition, several important points are noted. First of all, the $\mathbf{1}_n$-induced B-to-A transition takes place sharply at narrow concentration ranges of $\mathbf{1}_n$ and is thus a highly cooperative process. The critical concentrations of $\mathbf{1}_n$ required for this transition show little dependence on the ionic strength (e.g., 2×10^{-3} M for $\mathbf{1}_5$ at I=1.5 and at I=0.15) and decrease in the order $\mathbf{1}_3$ (18×10^{-3})>$\mathbf{1}_4$ (8×10^{-3})>$\mathbf{1}_5$ (2×10^{-3})>$\mathbf{1}_6$ (1×10^{-3} M). The critical concentrations of the total glucosamine unit ($n\cdot[\mathbf{1}_n]$ or [glu-N$^+$]) are still significantly chain-length dependent (Fig. 5b). This is apparently in contrast to the similar effects of $\mathbf{1}_n$ ($n\geq3$), in terms of *one* glucosamine unit, on the melting temperatures of poly(A)·poly(U) (Fig. 3). The intramolecular cooperativity of the glucosamine residues in higher homologues, e.g., hexamer $\mathbf{1}_6$, must be more effective in the case of the B-to-A transition of calf thymus DNA than in the case of the thermal helix-to-coil transition of poly(A)·poly(U). It is also interesting to note that the B-to-A transition results in an enormous enhancement of the CD intensity, which is somewhat dependent on the chain length of $\mathbf{1}_n$ (Fig. 5) and is 40-fold in the case of $\mathbf{1}_6$.

The B-to-A transition could be induced neither with neutral references such as chitooligosaccharides ($\mathbf{2}_n$) and cellooligosaccharides ($\mathbf{3}_n$) nor with chitosan dimer ($\mathbf{1}_2$). The former, i.e., $\mathbf{2}_n$ (n=2 or 5) or $\mathbf{3}_n$ (n=2 or 5), gave no change in the CD spectrum of calf thymus DNA in water under otherwise identical conditions as above. Addition of chitosan dimer, on the other hand, resulted in a spectral change but in the opposite direction. The CD intensity in concern *decreases* upon addition of dimer $\mathbf{1}_2$, as shown in Fig. 6. In the inset of this figure is shown the intensity–$[\mathbf{1}_2]$ correlation. A similar spectral change was observed also with hexamethylenediamine, glucosamine ($\mathbf{1}_1$), methylamine, or even KCl. This type of spectral change is characteristic of a transition of B-DNA to C-DNA;[21] C-DNA is a member of the B-family.

Polyamine Promoted Stabilization of the A-Form of Double Helix. X-Ray crystallography indicates that well-hydrated DNA's in crystals, fibers, and films usually take the B-form, while at lower relative humidities they

prefer the A-form.[20] The essential difference between A-DNA and B-DNA, or more strictly DNA in the A-family and that in the B-family, lies in the puckering modes of the sugar moieties (C_3'-*endo* for A and C_2'-*endo* for B). The intrastrand P–P distance for two adjacent phosphate moieties is ca. 1 Å shorter in A-DNA (5.9 Å) than in B-DNA is (7.0 Å), and the rise per nucleotide along the helical axis again shorter in A-DNA (2.56—3.29 Å) than in B-DNA (3.30—3.37 Å). The displacement of the gravity center of base-pairs from the helical axis is slight for B-DNA but significantly large for A-DNA (4.4—4.9 Å into the major groove). These structural charateristics indicate that A-DNA's are more tightly packed than B-DNA's.

As referred to above, the relative humidity is the most important factor governing the structure (A or B) of DNA. When it is high, the B-form is preferred, where the base and sugar moieties in addition to the phosphate moieties are hydrated to give the *hydration spine* in the minor groove.[13e] If it is low, the A-form is preferred, where only phosphate moieties are hydrated to give the interstrand phosphate–H_2O–phosphate bridges in the major groove.[22]

Hydration must be extensive in an aqueous *solution*, where DNA naturally takes the B-form. However, transition to the A-form can take place upon addition of a large amount (e.g., 70—80%) of an organic cosolvent such as ethanol.[21,23] Some of naturally-occurring polyamines such as spermine and spermidine but not diamine putrescein are known to further promote the B-to-A transition of DNA in aqueous ethanol.[23b] In the absence of organic cosolvent, however, these polyamines as well as simple inorganic salts[21,23b,24] only induce a winding of the DNA helix within the same B-family in the direction of B to C. This transition is continuous (or non-cooperative) as indicated by a gradual spectral change shown in the inset of Fig. 6, in marked contrast to the sharp or cooperative B-to-A transition (Fig. 5). In this respect, chitosan dimer ($\mathbf{1}_2$) and reference salts in the present study behave as expected. To the best of our knowledge, there has been no example of chemically induced B-to-A transitions of DNA in *water*.

It is interesting to note that RNA's[25] including poly(A)·poly(U)[26] take only the A-family form, which binds to the higher homologues of chitosan oligomers ($\mathbf{1}_n$, $n\geq3$) with a remarkable efficiency and is thereby further stabilized. This fact provides the simplest explanation of the present B-to-A transition of calf thymus DNA in water. The binding of $\mathbf{1}_n$ ($n\geq3$) to the present DNA would involve an at least three-point ammonio phosphate electrostatic interaction, in a similar manner as suggested above for the $\mathbf{1}_n$–poly(A)·poly(U) system. Such a multiple interaction may be sterically more favorable when the DNA is in the A-form than it is in the B-form.[27] Chitosan dimer ($\mathbf{1}_2$) can only induce an intra-family B-to-C transition, in marked contrast to $\mathbf{1}_n$ ($n\geq3$). This strongly suggests that a simultaneous three-point ammonio phosphate formation between $\mathbf{1}_n$ and DNA is very important for the B-to-A transition or, equivalently, for the stabilization of the A-form.

Another important factor is dehydration, which has been an essential aspect of the hitherto-known A-DNA's. In this viewpoint, the present B-to-A transition may be promoted by a relatively apolar microenvironment provided by the sugar backbone of $\mathbf{1}_n$. Furthermore, *each* sugar residue in $\mathbf{1}_n$ could contribute to the dehydration of DNA by undergoing hydrogen-bonding interaction with nucleoside moieties and/or nearby water molecules. This may explain why simple polyamine such as spermine and spermidine fail to induce the B-to-A transition in water and also why there is no *saturation* with respect to the increase in the chain length (n) of $\mathbf{1}_n$ for the critical values of $n\cdot[\mathbf{1}_n]$ or [glu-N^+] to induce the B-to-A transition (Fig. 5b). Such a critical concentration reflects the extent of intramolecular cooperativity of the glucosamine residues in $\mathbf{1}_n$.

Concluding Remarks

The affinities of chitosan oligomers $\mathbf{1}_n$ to both RNA and DNA increase dramatically but reasonably with increasing numbers (n) of the cationic centers in $\mathbf{1}_n$. Higher homologues ($\mathbf{1}_n$, $n\geq3$) undergo an at least three-point simultaneous electrostatic interaction with the phosphate moieties of nucleic acids. This interaction seems to be of a profound significance. It remarkably stabilizes the double-helical structure in an A-form of poly(A)·poly(U) and enhances its melting temperature as a consequence. It also induces a drastic and highly cooperative conformational change of calf thymus DNA from the B-form to the A-form. Such a B-to-A transition can never be observed when chitosan dimer ($\mathbf{1}_2$) or a simple reference cation is used. The intramolecular cooperativity of the glucosamine residues in $\mathbf{1}_n$ is so pronounced that hexamer $\mathbf{1}_6$, for example, in such a low concentration range as $N^+/P^-\approx62$ can induce the B-to-A transition.

The significance of this work may be dual. First, it provides a clue for the rational design of polycationic *high-affinity* RNA- and DNA-binding molecules. Second, it suggests that the conformation of DNA in *solution* is controllable by molecular-recognition directed choice of DNA-binding agents.[28] An interesting suggestion in this respect is that the B-to-A transition takes place in important biological processes such as transcription.[29] What remains to be further characterized is the interactions, both polar (hydrogen bonding) and apolar (hydrophobic), of the sugar backbones of chitosan oligomers $\mathbf{1}_n$.[30] Such a study may also shed light on the essential roles of the sugar moieties often found in DNA-binding antibiotics.[9,31,32]

We are grateful to Dr. Akira Suyama and Dr. Kunitsugu Soda (Nagaoka University of Technology) for

giving us facilities for the measurements of melting temperatures and CD spectra, respectively. We also thank Professor M. Takagi and Dr. S. Takenaka (Kyushu University) for their technical suggestions for handling DNA at the early stage of this work. Generous gifts of chitosan oligomers, chitooligosaccharides, and cellooligosaccharides by Sanwa Cornsrarch Co., Ltd. are greatly appreciated. This work was supported by a Grant-Aid for Scientific Research on Priority Areas No. 03241209 from the Ministry of Education, Science and Culture.

References

1) a) A. Pullman and B. Pullman, *Q. Rev. Biophys.*, **14**, 289 (1981). b) C. Zimmer and U. Wahnert, *Prog. Biophys. Mol. Biol.*, **47**, 31 (1986). c) D. S. Sigman, *Acc. Chem. Res.*, **19**, 180 (1986).

2) a) G. B. Preyer and P. B. Dervan, *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 968 (1985). b) P. B. Dervan, *Science*, **232**, 464 (1986). c) H. E. Moser and P. B. Dervan, *ibid.*, **238**, 745 (1987). d) L. C. Griffin and P. B. Dervan, *ibid.*, **245**, 967 (1989). e) D. A. Horne and P. B. Dervan, *J. Am. Chem. Soc.*, **112**, 2435 (1990). f) T. J. Povsic and P. B. Dervan, *ibid.*, **112**, 9428 (1990), and references cited therein.

3) a) J. K. Barton, *Science*, **233**, 727 (1986). b) A. M. Pyle, J. P. Rehmann, R. Meshoyrer, C. V. Kumar, N. J. Turro, and J. K. Barton, *J. Am. Chem. Soc.*, **111**, 3051 (1989), and references cited therein.

4) S. M. Hecht, *Acc. Chem. Res.*, **19**, 383 (1986).

5) D. C. Corog, D. Pei, and P. G. Schultz, *J. Am. Chem. Soc.*, **111**, 8523 (1989).

6) W. Saenger, "Principles of Nucleic Acid Structures," Springer-Verlag, New York (1984).

7) M. A. Rould, J. J. Perona, D. Soll, and T. A. Steitz, *Science*, **246**, 1135 (1989).

8) W. D. Wilson, F. A. Tanious, J. K. Barton, L. Strekowski, and D. W. Boykin, *J. Am. Chem. Soc.*, **111**, 1989 (1989), and references cited therein.

9) a) M. Otsuka, T. Masuda, A. Haupt, M. Ohno, T. Shiraki, Y. Sugiura, and K. Maeda, *J. Am. Chem. Soc.*, **112**, 838 (1990). b) A. Natrajan, S. M. Hecht, G. A. van der Marel, and J. H. van Boom, *ibid.*, **112**, 3997 (1990).

10) a) A. L. Lehninger "Biochemistry," Worth Publishers, New York (1970), Chap. 31. b) B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson, "Molecular Biology of the Cell," Garland Publishing, New York (1983), Chap. 8.

11) a) A. M. Liquori, L. Costantino, V. Crescenzi, V. Elia, E. Giglio, R. Puliti, M. de S. Savino, and V. Vitagliano, *J. Mol. Biol.*, **24**, 113 (1967). b) J. Liquier, M. P.-Lafaix, E. Taillandier, and J. Brahms, *Biochemistry*, **14**, 4191 (1975). c) E. C. Ong, C. Snell, and G. D. Fasman, *ibid.*, **15**, 468 (1976). e) P. Suau and J. A. Suburana, *J. Mol. Biol.*, **117**, 909 (1977). f) H. R. Drew and R. E. Dickerson, *ibid.*, **151**, 535 (1981).

12) S. A. Latt and H. A. Sober, *Biochemistry*, **6**, 3293 (1967).

13) M. T. Record, Jr., T. M. Lohman, and P. Haseth, *J. Mol. Biol.*, **107**, 145 (1976).

14) M. W. Davidson, B. G. Griggs, D. W. Boykin, and W. D. Wilson, *J. Med. Chem.*, **20**, 1117 (1977).

15) Ref. 6, Chap. 6.

16) D. Porschke, *Biopolymers*, **10**, 1989 (1971).

17) a) J. Marmur and P. Doty, *J. Mol. Biol.*, **5**, 109 (1962). b) O. Gotoh and Y. Takashira, *Biopolymers*, **20**, (1981).

18) Ref. 6, Chap. 16.

19) M. Haynes, R. A. Garrett, and W. B. Gratzer, *Biochemistry*, **22**, 4410 (1970).

20) Ref. 6, Chaps. 9, 11, and 17.

21) V. I. Ivanov, L. E. Minchenkova, A. K. Schyolkina, and A. I. Poletayev, *Biopolymers*, **12**, 89 (1973).

22) B. N. Conner, T. Takano, S. Tanaka, K. Itakura, and E. Dicjerson, *Nature*, **295**, 294 (1982).

23) a) V. I. Ivanov, L. E. Minchenkova, E. E. Minyat, M. D. Frank-Kemenetskii, and A. K. Schyolkina, *J. Mol. Biol.*, **87**, 817 (1974). b) E. E. Minyat, V. I. Ivanov, A. M. Kritzyn, L. E. Minchenkova, and A. K. Schyolkina, *ibid.*, **128**, 397 (1978).

24) V. I. Permogorov, V. G. Degabov, I. A. Sladkova, and B. A. Rebentish, *Biochin. Biophys. Acta*, **199**, 556 (1970).

25) Ref. 6, Chap. 10.

26) S. Arnott, D. W. L. Hukins, S. D. Dover, W. Fuller, and A. R. Hodgson, *J. Mol. Biol.*, **81**, 107 (1973).

27) DNA-poly(L-lysine) complexes in fibers can take the A-form (Ref. 11b).

28) For an approach to molecular-recognition directed probing of the microstructures of double-helical DNA, see: a) Ref. 3. b) A. M. Pyle, E. C. Long, and J. K. Barton, *J. Am. Chem. Soc.*, **111**, 4520 (1989). c) A. M. Pyle, T. Morii, and J. K. Barton, *ibid.*, **112**, 9432 (1990).

29) a) L. D. Hamilton, *Nature*, **218**, 633 (1968). b) S. Arnott, W. Fuller, A. Hodgson, and I. Prutton, *ibid.*, **220**, 561 (1968). c) V. L. Florentiev and V. I. Ivanov, *ibid.*, **228**, 519 (1970).

30) Cooperation of polar and apolar interactions is a general feature of the sugar–protein complexes. See: a) F. A. Quicho and N. K. Vyas, *Nature*, **242**, 1290 (1988). b) N. K. Vyas, M. N. Vyas, and F. A. Quicho, *ibid*, **327**, 635 (1987). c) F. A. Quicho, D. K. Wilson, and N. K. Vyas, *ibid.*, **340**, 404 (1989). d) D. R. Bundle, *Pure Appl. Chem.*, **61**, 1171 (1989). e) R. U. Lemieux, *Chem. Soc. Rev.*, **18**, 347 (1989). f) N. Sharon and L. Halina, *Chem. Br.*, **1990**, 679.

31) See, for example: M. D. Lee, G. A. Ellestad, and D. B. Borders, *Acc. Chem. Res.*, **24**, 235 (1991).

32) S. Walker, K. G. Valentine, and D. Kahne, *J. Am. Chem. Soc.*, **112**, 6428 (1990), and references cited therein.