Thermodynamic Analysis of Duplex Formation of the Heterochiral DNA with L-Deoxyadenosine

Junji KAWAKAMI,^{*,**} Kazuhiko TSUJITA,^{**} and Naoki SUGIMOTO^{*,**†}

^{*}Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University,
8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan

^{**}Department of Chemistry, Faculty of Science and Engineering, Konan University, 8-9-1 Okamoto,
Higashinada-ku, Kobe 658-8501, Japan

An L-DNA, the mirror-image isomer of natural DNA, has extraordinary nuclease resistance, and thus the molecules should be promising reagents for many applications, such as antisense technology. However, little is known about the structural and thermodynamic properties of DNAs with this modified nucleotide. In this study, we prepared the L-nucleotide (L-dA) and introduced it into oligodeoxyribonucleotides to assess the ability of the L-nucleotide as a functional molecule for many applications based on the DNA hybridization. Two decamers with an L-dA at the center were synthesized and duplexes with the complementary DNA strand were applied to structural and thermodynamic analyses. The structural study by CD spectra showed that the structures of both modified "L/D-D" duplexes were the typical B-form. This result suggests that the global structure of DNA was not collapsed by the introduction of an L-DNA. Thermodynamic parameters (ΔH° , ΔS° , and ΔG°_{37}) of the duplex formation, determined by UV melting experiments, indicated that the both duplexes were destabilized at about 2.5 to 3.0 kcal mol⁻¹ by the introduced L-dA, mainly due to an unfavorable enthalpic effect. In conjunction with information by other researchers, these results suggest that the L-DNA affect on the duplex structure and the stability vary locally; thus, the thermodynamic stability of modified L/D-D duplexes should be predictable by the nearest-neighbor thermodynamic parameters.

(Received September 13, 2004; Accepted November 8, 2004)

Introduction

Gene expression can be regulated at the translational step by using synthetic oligodeoxyribonucleotides, namely antisense oligonucleotides (AS-ODNs).¹ The efficiency of a synthetic AS-ODN to regulate gene expression in living cells depends on the thermodynamic stability of the AS-ODN-mRNA duplex, the nuclease resistance of the AS-ODN and so on. Therefore, a modified oligonucleotide that has sufficient nuclease resistance and is able to hybridize to the complementary sequence of natural DNA or RNA would be a candidate with good prospects for an efficient AS-ODN. Mainly for the acquisition of nuclease resistance, many modifications were introduced into AS-ODNs at the phosphate backbone: alkylphosphonate,² phosphorothioate,^{3,4} phosphoroselenoate,⁵ phosphorodithioate,^{4,6} phosphate triester,⁷ phosphoramidate,⁸ phosphormorpholidate,⁹ deoxyribonucleic guanidine,¹⁰ peptide nucleic acid,^{11,12} and so on. Though the phosphorothioate oligonucleotide has been the most widely investigated among these modifications, its nonspecific binding to proteins was reported,¹³ and therefore the phosphorothioate oligonucleotide is still not the best modified one.

We have noticed an enantiomer of natural DNA, β -L-2'-oligodeoxyribonucleotide,¹⁴ as a good candidate of AS-ODN, because the enantiomer exhibits highly resistance against a nuclease treatment,^{15,16} and the metabolite products (bases, sugars, and phosphates) do not have any poisonous property.

Thus, the "mirror image" DNA, L-DNA, should be a good antisense reagent for pharmaceutical use. Nevertheless, little is known about the ability of duplex formation that directly relates to the antisense efficiency of L-DNA. A homo hexamer of L-deoxyadenosine, L-(dA)₆, bound to poly(U), but not to poly(A), poly(G), poly(C), or poly(dT).¹⁷ Similarly, L-(U)₁₂ and L-(dU)₂₀ bound to poly(A), but not to poly(dA).¹⁸ In both cases, L-oligomers distinguished RNA from DNA. In contrast, L-DNA oligomers containing all four bases did not hybridize to either complementary natural DNA or RNA.¹⁹ Only the chimeric oligomers with both D- and L-2'-deoxyribose sugars (L/D-DNA) achieved both nuclease resistance and duplex formation, though some decrease of duplex stability was found.²⁰⁻²² NMR studies for duplexes composed of L/D-DNA and complementary natural DNA (L/D-D duplexes) in which single nucleotide was substituted by L-deoxyguanosine (L-dG) or L-thymidine (L-T) revealed that the structures of the duplexes were normal B-form, and the introduced L-nucleotide formed Watson-Crick hydrogen bonds as normal nucleotides.^{23,24} However, these findings do not account for the destabilization of duplexes in the other experiments.

The introduction of L-nucleotides should lead to a similar situation as mismatches introduced into a DNA duplex. In the case of single mismatches, the stability of a duplex should depend on both the mismatch pair and the closing base pairs.^{25,26} Indeed, thermodynamic studies for L/D-D DNA duplexes containing an L-nucleotide, L-T; L-deoxycytidine (L-dC); and L-dG, were reported and it was suggested that the influence of L-nucleotide substitution should depend on the duplex circumstances around the L-nucleotide, as expected.^{27,28} In these studies, the thermodynamic effect of an L-dG introduction

[†] To whom correspondence should be addressed.
E-mail: sugimoto@konan-u.ac.jp

seemed to be different from that for the L-pyrimidine nucleotides. In this study, we focused on the effect of L-purine nucleotide introduced in DNA duplexes. L-dA phosphoramidite was synthesized, and seven duplexes were prepared for the analysis. The property of the L/D-D duplexes for structure and thermodynamic stability have been investigated by means of CD (circular dichroism) spectroscopy, UV melting experiments, and MD (molecular dynamics) calculations. As a result, the effect of an L-dA modification on the duplex structure is very local, and the stability of a base pair containing L-dA is strongly affected by the surrounding sequence context, suggesting that the nearest-neighbor model would be applicable to the modified L/D-D duplexes. By using the nearest-neighbor parameters, one can easily predict the stability of the desired duplexes; thus, the hybridization ability or the binding constant of the L/D-D duplexes to a target should be predictable before use.

Materials and Methods

Synthesis of oligonucleotides

β -Cyanoethyl phosphoramidite of β -L-2'-deoxyadenosine was synthesized from L-arabinose, as reported previously, with a total yield of 1.1%.²⁹ β -Cyanoethyl phosphoramidites of β -D-2'-deoxyribonucleotides and abasic nucleotide were purchased commercially (GREN RESEARCH). All oligodeoxyribonucleotides were synthesized on a solid support by using phosphoramidite chemistry, and purified by reverse-phase column chromatography after deblocking operations.³⁰ Synthesized 7 decamers were desalted with a C-18 Sep-pak cartridge column (Waters). The final purity of the decamers was confirmed to be greater than at least 98% by HPLC.

CD measurements

All experiments of CD and UV spectroscopies were carried out in a buffer containing 1 M NaCl, 10 mM Na₂HPO₄, and 1 mM Na₂EDTA (pH 7.0). Single-strand concentrations of the decamers were determined by measuring the absorbance at 260 nm at a high temperature, as described previously.³¹ Single-strand extinction coefficients were calculated from mononucleotide and dinucleotide data by using a nearest-neighbor approximation.³² The L/D-DNA strand and its complementary D-DNA strand were mixed with a 1:1 concentration ratio and annealed to obtain the L/D-D duplex.

Circular dichroism (CD) spectra were obtained from a JASCO J-600 spectropolarimeter at 5.0°C. The cuvette-holding chamber was flushed with a constant stream of dry N₂ gas to avoid water condensation on the cuvette exterior. All of the CD spectra were measured from 350 to 200 nm in a 0.1 cm path length cuvette. The concentration of the samples was 70 μ M in a 10 mM phosphate buffer containing 1 M NaCl (pH 7.0).

UV measurements

Measurements of the UV absorption were carried out on Hitachi U-3200, U-3210, and Beckman DU640 spectrophotometers. Melting curves (absorbance vs. temperature curves) were measured at 260 nm with these spectrophotometers connected to Hitachi SPR-7 and SPR-10 thermoprogrammers. The heating rate was 0.5 or 1.0°C per min. The water condensation on the cuvette exterior at a low temperature range was avoided by flushing with a constant stream of dry N₂ gas. The melting temperatures (T_m) were obtained with a curve-fitting procedure, described previously.^{33,34} The thermodynamic parameters for each duplex formation were determined as average values obtained by the

following two methods:^{31,33} (I) curve fitting of individual melting curves with a two-state approximation, and (II) reciprocal melting temperature, T_m^{-1} vs. $\ln(C_t/4)$ plots to give enthalpy and entropy change. For method (II), the following equation was applied to the analysis:³⁵

$$T_m^{-1} = [R \ln(C_t/4) + \Delta S^\circ] / \Delta H^\circ, \quad (1)$$

where T_m is the melting temperature of a double helix, ΔH° and ΔS° are calculated enthalpy and entropy changes for duplex formation, respectively, R is the gas constant, and C_t is the total strand concentration of oligonucleotides. A free-energy change associated with the duplex formation at 37°C, ΔG°_{37} , was calculated from the obtained ΔH° and ΔS° values by

$$\Delta G^\circ_{37} = \Delta H^\circ - (310.15 \times \Delta S^\circ). \quad (2)$$

Molecular modeling

Molecular models of the L/D-D duplex was built by a molecular-mechanics calculation performed on a QUANTA 97.1/CHARMm 23.2 (Molecular Simulations Inc.) by using the parameters of a modified residue topology file (RTF), which made calculations of duplex stacking possible. MD simulations of energy-minimized structures were performed to obtain various conformations for the calculation. In an MD simulation, the VERLET algorithm with a time step of 1 fs was used, and covalent bonds involving hydrogens were constrained to their equilibrium values with the SHAKE algorithm for a shorter computation time. In this case, because we used the MD simulation for the purpose of a conformational search, the system was heated from 0 to 1000 K. The system was then equilibrated at 1000 K for 3 ps and simulated for 6 ps. Thirty conformations were obtained for each duplex during the simulated annealing from 1000 to 0 K, and the optimal structure was obtained by energy-minimization at 300 K. All of the calculations were performed on a Silicon Graphics Indigo2 workstation running IRIX 5.3.

Results

Design of the DNA sequences

Two normal DNAs, two DNAs with an abasic nucleotide, and two heterochiral DNAs were synthesized to prepare the following duplexes [1] to [7]:

dCGGCAAGCGC / dGCGCTTGCCG	[1]
dCGGCAAGCGC / dGCGCTTGCCG	[2]
dCGGCAAGCGC / dGCGCTTGCCG	[3]
dCGGCAAGCGC / dGCGCTXGCCG	[4]
dCGGCAAGCGC / dGCGCTTGCCG	[5]
dCGGCAAGCGC / dGCGCTXGCCG	[6]
dCGGCAAGCGC / dGCGCTXGCCG	[7]

where **A** and **X** indicate L-dA and abasic D-deoxyribonucleotide, respectively. The effect of introducing an L-dA can be extracted and evaluated by comparing duplexes [1] and [2] or [3]. At the same time, by comparing duplexes [2] and [3], the effect of the sequence context, especially the contribution of the 5' adjacent nucleotide, is accountable.²⁷ To investigate the base pairing in the duplexes, the differences in the thermodynamic effect between [4] or [5] from [1] and [6] from [2] or [7] from [3] can be used.

Fig. 1 CD spectra of dCGGCAAGCGC / dGCGCTTGCCG (bold line), dCGGCAAGCGC / dGCGCTTGCCG (thin line) and dCGGCAAGCGC / dGCGCTTGCCG (broken line). Each oligomer concentration was 70 μ M, and measurements were performed in a 10 mM phosphate buffer containing 1 M NaCl (pH 7.0) at 5.0°C.

Structural analysis of the L/D-D duplexes by CD spectra

Overall structural information of duplexes [1], [2], and [3] (L/D-D duplexes and the corresponding natural DNA duplex) was obtained with CD. Figure 1 shows the CD spectra of the duplexes at 5.0°C, the temperature at which all of the duplexes were stably formed, as described in the following UV analysis section. All of these duplexes show a similar CD pattern in their spectra. Characteristic peaks, a positive peak around 285 nm and a negative peak around 252 nm were observed for all duplexes, and a peak shift was not observed by introducing L-dA. This spectral feature indicates that all of those duplexes are typical B-form DNA.³⁶ However, for duplexes [2] and [3], the intensity of the peaks decreased to some degree, indicating that base stacking in the heterochiral duplexes is rather distorted, as compared with duplex [1]. The decrement of the peak intensity at 252 nm of duplex [2] was greater than that of duplex [3] in spite of the fact that the intensity at 285 nm of duplex [3] was weaker than that of duplex [2], indicating that the two L/D-D duplexes folded into a very similar structure, but were not the same.

Thermodynamic analysis of the L/D-D duplexes by UV spectra

All of the UV melting curves were measured in the range from 0°C to 90°C. The two-state transition of the melting behavior was confirmed by a comparison of the thermodynamic parameters (ΔH° , ΔS° , and ΔG°_{37}) derived from the two methods: T_m^{-1} vs. $\ln(C_t/4)$ plots and the curve-fitting procedure. When the difference between these thermodynamic values was within 10%, we decided that the melting behavior of the duplex was the two-state transition. All of the melting curves of the L/D-D duplexes showed normal melting behaviors, being regarded as the same two-state transition as DNA double helices. Thus, the melting temperature and the thermodynamic parameters for each duplex formation were able to be determined, as described in the materials and methods section.^{31,33,34} Figure 2 shows T_m^{-1} vs. $\ln(C_t/4)$ plots for duplexes [1], [2], and [3]. As shown in Fig. 2, the melting temperatures decrease with decreasing total strand concentration, and the T_m^{-1} vs. $\ln(C_t/4)$ plots are linear. The averaged thermodynamic parameters for the duplex formation of duplexes [1] to [7] are

Fig. 2 T_m^{-1} vs. $\ln(C_t/4)$ plots of dCGGCAAGCGC / dGCGCTTGCCG (●; bold line), dCGGCAAGCGC / dGCGCTTGCCG (▲; thin line), and dCGGCAAGCGC / dGCGCTTGCCG (□; broken line). All measurements were carried out in a 10 mM phosphate buffer containing 1 M NaCl (pH 7.0).

listed in Table 1.

The ΔH° values of duplexes [1], [2], and [3] were -85.6 , -65.2 , and -56.0 kcal mol $^{-1}$, respectively. That means L-dA introduction is unfavorable for duplex formation in the enthalpic effect. Contrary to ΔH° , the entropic energy loss of the L/D-D duplex formation was less than that of the corresponding natural DNA duplex. The ΔS° value of duplexes [1], [2], and [3] were -233 , -176 , and -147 cal mol $^{-1}$ K $^{-1}$, respectively. As a result, the ΔG° of duplexes [1], [2], and [3] were -13.3 , -10.7 , and -10.3 kcal mol $^{-1}$, respectively. These data indicate that the introduction of the mirror-image DNA destabilizes the duplex, and that the instabilization is due to an unfavorable enthalpic effect.

Further information was obtained by the same UV analysis for duplexes [4] to [7]. These duplexes include an abasic deoxyribonucleotide, **X**, instead of a T of the **A**·T or A·T pair. Hydrogen bonds and stacking interaction at the mutated site could be eliminated by introducing the abasic nucleotide. However, a similar tendency appeared for duplexes [4] to [7] as duplexes [2] and [3]; enthalpy-dominated destabilization of the duplex formation was observed. Consequently, the difference in the thermodynamic parameters associated with the replacement of an **X** for a T of the **A**·T pair (1.5 kcal mol $^{-1}$ for [6] minus [2] and 2.8 kcal mol $^{-1}$ for [7] minus [3]) is smaller than that of corresponding unmodified base pair (4.8 kcal mol $^{-1}$ for [4] minus [1] and 5.9 kcal mol $^{-1}$ for [5] minus [1]).

Discussion

Structure of L/D-D duplexes

As shown in Fig. 1, the CD spectra of L/D-D duplexes are very similar to that of the corresponding normal DNA duplex. This finding suggests our L/D-D duplexes folded into a similar structure as the normal B-form DNA; the sugar pucker, glycosidic torsion angle χ , rise, shift, slide and tilt of base pairs should have almost the same values as a whole between the L/D-D and the DNA duplexes. This is consistent with previous structural studies of L/D-D duplexes with one nucleotide substitution.^{23,24} Urata *et al.* reported an NMR spectrum of an L/D-D dodecamer with an L-dG.²⁴ They showed that the exchangeable imino signals and NOE signals of the L/D-D duplex were quite similar to that of the B-form DNA duplex in

Table 1 Thermodynamic parameters of duplex formation in 1 M NaCl-phosphate buffer (pH 7.0)^a

Duplex	Sequence ^b	$-\Delta H^\circ/\text{kcal mol}^{-1}$ (kJ mol^{-1})	$-\Delta S^\circ/\text{cal mol}^{-1}\text{K}^{-1}$ ($\text{J mol}^{-1}\text{K}^{-1}$)	$\Delta G^\circ_{37}/\text{kcal mol}^{-1}$ (kJ mol^{-1})	T_m°/C
[1]	5'dCGGCAAGCGC3' 3'dGCCGTTCGCG5'	85.6 ± 1.3 (358 ± 5)	233 ± 2 (975 ± 8)	13.3 ± 1.5 (55.6 ± 6.3)	62.7 ± 1.4
[2]	5'dCGGCA $\underline{\text{A}}$ AGCGC3' 3'dGCCGTTCGCG5'	65.2 ± 7.2 (273 ± 30)	176 ± 9 (736 ± 38)	10.7 ± 0.7 (44.7 ± 2.9)	58.4 ± 3.4
[3]	5'dCGGCA $\underline{\text{A}}$ GCGC3' 3'dGCCGTTCGCG5'	56.0 ± 4.4 (234 ± 18)	147 ± 5 (615 ± 21)	10.3 ± 1.9 (43.3 ± 7.9)	59.3 ± 0.4
[4]	5'dCGGCAAGCGC3' 3'dGCCG $\underline{\text{X}}$ TCGCG5'	45.3 ± 7.0 (190 ± 29)	119 ± 9 (498 ± 38)	8.5 ± 0.2 (35.6 ± 0.8)	51.1 ± 1.8
[5]	5'dCGGCAAGCGC3' 3'dGCCGT $\underline{\text{X}}$ CGCG5'	36.0 ± 3.4 (151 ± 14)	92.3 ± 4.0 (386 ± 17)	7.4 ± 1.1 (31.0 ± 4.6)	44.3 ± 1.0
[6]	5'dCGGC $\underline{\text{A}}$ AGCGC3' 3'dGCCG $\underline{\text{X}}$ TCGCG5'	60.4 ± 5.1 (253 ± 21)	165 ± 6 (690 ± 25)	9.2 ± 1.1 (38.5 ± 4.6)	51.2 ± 0.4
[7]	5'dCGGCA $\underline{\text{A}}$ GCGC3' 3'dGCCGT $\underline{\text{X}}$ CGCG5'	45.4 ± 5.6 (190 ± 23)	122 ± 7 (510 ± 29)	7.5 ± 1.2 (31.4 ± 5.0)	43.9 ± 2.4

a. Thermodynamic parameters were averaged with the data derived from curve fitting and $\log(C/4)$ plot. b. $\underline{\text{A}}$ and $\underline{\text{X}}$ indicate L-dA and abasic nucleotide, respectively. c. Melting temperatures were calculated for 10^{-4} M total strand concentration.

the same sequence. In another report, an NMR and thermodynamic study of an L/D-D heptamer with an L-dT was carried out by Blommers *et al.*²³ A distance geometry calculation based on the NOE intensity revealed the typical B-form structure of the L/D-D heptamer. In both previous studies, the L-sugar puckering was an S conformation, and the χ angle was close to 180°. Furthermore, NOE between the C2' or C2'' proton of the L-nucleotide and the C1' proton of the 5' adjacent nucleotide was observed. All of these findings suggest that an internal L-nucleotide was introduced into a normal DNA duplex form Watson-Crick hydrogen bonds to the corresponding base. Moreover, molecular modeling studies suggest that L-nucleotides can locate their bases at an appropriate position for Watson-Crick or Hoogsteen base pairing with natural nucleic acids.^{14,17,18,37-39}

However, in our present study, though the structure of duplexes [2] and [3] clearly folded into identical B-forms, as previously reported, an apparent $\underline{\text{A}}\cdot\text{T}$ pair seems not to form, as described in the following section. The CD peak intensities of the two L/D-D duplexes around 285 nm were slightly different; that is, the intensity at 285 nm of duplex [3] was weaker than that of duplex [2], though they have only one and the same $\underline{\text{A}}\cdot\text{T}$ pair. This is not due to the concentration of the duplex, because an opposite phenomenon was observed at 252 nm. Only the position (and of course the adjacent nucleotides also) of the introduced L-dA is the difference of the two L/D-D duplexes. Thus, these results strongly suggest that the stacking interaction of the 5' and 3' adjacent "nearest-neighbor" base pairs affects the local structure of an L-D base pair. This is consistent with other reports²⁷ and our results by an energetic consideration in the following.

Thermodynamics of L/D-D duplexes

As listed in Table 1, the L-dA leads to destabilization of the duplexes. Differences in the ΔH° value ($\Delta\Delta H^\circ = \Delta H^\circ(\underline{\text{A}}\cdot\text{T}) - \Delta H^\circ(\text{A}\cdot\text{T})$) associated with the enthalpic effect by the introduction of $\underline{\text{A}}$ instead of A were 20.4 and 29.6 kcal mol⁻¹ for duplexes [2] and [3], respectively. The corresponding differences of the ΔS° value, $\Delta\Delta S^\circ$, of duplexes [2] and [3] were 57 and 86 cal mol⁻¹ K⁻¹, and thus the differences of the entropic energy loss for duplex formation at 37°C ($-310.15 \times \Delta\Delta S^\circ$) were

Fig. 3 Thermodynamic effect of the modification for D-D and L/D-D duplex formation. The difference in the free-energy changes ($\Delta\Delta G^\circ_{37}$), energy changes by entropy changes ($-T\Delta\Delta S^\circ$) at 37°C, and enthalpy changes ($\Delta\Delta H^\circ$) are denoted by black, white, and gray bars, respectively.

able to be calculated as -17.8 and -26.6 kcal mol⁻¹, respectively. In other words, though L-dA substitution is entropically advantageous, a large enthalpic loss that overcomes the entropic effect arises. This findings is similar to the destabilization mechanism in L/D-D RNA, rather than L/D-D DNA,²⁸ suggesting the destabilization effect should arise in a sequence-dependent manner as well as from the difference between DNA and RNA. In our case, the $\Delta\Delta G^\circ_{37}$, 2.6 and 3.0 kcal mol⁻¹ for duplexes [2] and [3], are dominated by $\Delta\Delta H^\circ$. This tendency is in good agreement with previously reported thermodynamics of L/D-D duplexes.^{23,27} However, the absolute values of $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ for introducing an L-dA into the heptamer duplex dCGC $\underline{\text{A}}$ CGC / dGCGTGCG are surprisingly smaller ($\Delta\Delta H^\circ$, $\Delta\Delta S^\circ$, and $\Delta\Delta G^\circ_{37}$ were 3.0 kcal mol⁻¹, 5 cal mol⁻¹ K⁻¹, and 1.4 kcal mol⁻¹, respectively) than that of duplexes [2] and [3]. This might be due to the difference in the closing base pairs; the L-dA residue of the heptamer is between two G-C

Fig. 4 Energy-minimized structures of dCGGCAAGCGC / dGCGCTTGCCG (duplex [1], left) and dCGGC Δ AGCGC / dGCGCTTGCCG (duplex [2], right). a) Whole images of the duplexes viewed from the minor groove. b) Local views of the A·T and Δ ·T pairs with both adjacent base pairs.

base pairs, while the L-dA in duplex [2] or [3] is between G·C and A·T base pairs.

Nearest-neighbor parameters to predict the thermodynamic stability of a duplex for DNA/DNA,^{40,41} RNA/RNA,⁴² RNA/DNA,⁴³ and some mismatch base pairs^{26,44} have been reported. From the parameters, the thermodynamic properties of triplet nucleotides containing an A·T pair at the center was estimated. Of all the 16 triplets, CAC/GTG is the most stable triplet [ΔG°_{37} was calculated to be -3.2 kcal mol⁻¹ by our group⁴⁰ or -2.9 kcal mol⁻¹ by Allawi and Santalucia⁴⁴], while CAA/GTT and AAG/TTC, resembling the L-dA site of duplex [2] and [3], show moderate stability. Similarly, in the case of a triplet containing an A·G mismatch at the center, CAC/GGG is one of the most stable triplets, whereas CAA/GGT and AAG/TGC destabilize the duplex.²⁵ If the central Δ ·T pair is unfavorable for duplex formation, Watson-Crick-type hydrogen bonds at the base pair may not be formed.

Thermodynamic analyses for duplexes [4] to [7], with an abasic nucleotide instead of a T facing to L-dA or dA in the complementary strand, were carried out to investigate the detailed effect of L-dA introduction on the core helix. Because the abasic nucleotide does not have a nucleobase, hydrogen bonds and a stacking interaction at the abasic site would be lost in the duplexes. As listed in Table 1, enthalpy-dominated destabilization of the duplex formation was observed for all duplexes [4] to [7], as was seen for the cases of duplexes [2] and [3]. The thermodynamic effect by the modification is summarized in Fig. 3. In accordance with the same tendency, the difference in the thermodynamic parameters associated with the replacement of an $\underline{X}$ for a T of the Δ ·T pair is smaller than in the case of the corresponding unmodified base pair, as shown in Fig. 3. For example, from a comparison of duplexes [1], [3], [5], and [7], the introduction of $\underline{X}$ to A·T and Δ ·T leads 5.9 and 2.8 kcal mol⁻¹ destabilization, respectively. On the other hand, the introduction of Δ to A·T and A· $\underline{X}$ leads 3.0 kcal mol⁻¹ destabilization and 0.1 kcal mol⁻¹ stabilization (almost no effect), respectively. These values indicate that $\underline{X}$ destroys the base pair completely and the duplexes in which $\underline{X}$ introduced have similar thermodynamic stability regardless of the facing base, A or Δ . The Δ ·T pair has moderate stability between the A·T and Δ · $\underline{X}$ pair. This result of abasic nucleotide substitution also gives an interpretation that the Δ and T may not form a strong base pair with appropriate base stacking and hydrogen

bonding.

As a result, it was suggested that Δ can weakly interact with T in our L/D-D duplex, while some duplexes in other reports formed apparent base pairs.^{20,23,24} To consider the reason of this discrepancy from the viewpoints of the structural features, molecular modeling was carried out for duplexes [1] and [2]. Energy-minimized structures of the duplexes are shown in Fig. 4. As can be seen in the figure, duplex [2] folds into a clear B-form structure with stacked L-dA. In comparison with the bases in the normal B-form DNA, the Δ ·T pair had a high propeller angle (and abnormal buckle angle), and suitable hydrogen bonding could not be formed. Thus, there are some degrees of strain around the introduced L-dA residue in our duplexes. Though the strain of the backbone was infinitesimal, the strain affects the structures of adjacent base pairs. The effect of introducing L-dA seems to localize in three base pairs, of which the Δ ·T pair is in the center. These results suggest that the instabilization of the L/D-D duplex might be predicted by the expanded nearest-neighbor model with some new parameters, just like the thermodynamic parameters for mismatch triplets.^{25,26,44,45}

L/D-D duplexes that have the ability of base-pair formation are in some trends:^{20,23,24} i) An introduced L-nucleotide is mainly either L-dG or L-dC, and ii) the number of incorporation of the L-nucleotide is restricted, and iii) the nearest-neighbor base pairs of the L-nucleotide are G·C pairs. These trends could be related to the nearest-neighbor parameters for DNA fullmatch and mismatch base pairs. Damha *et al.* reported on the property of L/D-D duplexes with terminally introduced L-dC nucleotides.²⁰ The modified duplexes not only exhibited ordinary stability against cellular nucleases, but also have sufficient stability that is the same as that of the normal DNA duplex.²¹ This phenomenon is also able to relate the stabilization of a duplex by dangling ends.⁴⁶

In summary, incorporated L-nucleotides into natural DNA could form hydrogen bonds when the proper base pairs are neighbored. And as indicated in this study, relatively stable stacked B-form structures are able to form, even if the base contact at the L-nucleotide is not sufficient. A thermodynamic prediction based on the nearest-neighbor model might be applicable for L/D-D duplexes, though an enormous number of parameters should be determined for exact prediction. The thermodynamic stability of these modified oligonucleotides was

assessed mainly by the melting temperature (T_m) of duplexes with the complementary oligonucleotides. Although the T_m measurement of a duplex is a convenient and useful method to judge the usefulness of a new modification, the stability of the duplex under the other conditions cannot be predicted only based on the T_m value. By using the nearest-neighbor parameters, researchers have been able to easily predict the stability of the desired DNA/DNA, RNA/RNA and DNA/RNA duplexes. Thus, the present study shows the ability of the thermodynamic prediction of a modified AS-ODN.

Acknowledgements

We thank Dr. H. Urata for helpful suggestions on the chemical synthesis of L-nucleotides. This work was supported in part by Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1. C. Hélène and J. J. Toulme, *Biochim. Biophys. Acta*, **1990**, 1049, 99.
2. C. M. Chen, Q. Z. Ye, Z. M. Zhu, B. L. Wanner, and C. T. Walsh, *J. Biol. Chem.*, **1990**, 265, 4461.
3. F. Eckstein, *Annu. Rev. Biochem.*, **1985**, 54, 367.
4. P. Furrer, T. M. Billeci, A. Donati, C. Kojima, B. Karwowski, A. Sierzchala, W. Stec, and T. L. James, *J. Mol. Biol.*, **1999**, 285, 1609.
5. K. Mori, C. Boiziau, C. Cazenave, M. Matsukura, C. Subasinghe, J. S. Cohen, S. Broder, J. J. Toulme, and C. A. Stein, *Nucleic Acids Res.*, **1989**, 17, 8207.
6. W. S. Marshall and M. H. Caruthers, *Science*, **1993**, 259, 1564.
7. C. McGuigan, S. M. Tollerfield, and P. A. Riley, *Nucleic Acids Res.*, **1989**, 17, 6065.
8. B. Froehler, P. Ng, and M. Matteucci, *Nucleic Acids Res.*, **1988**, 16, 4831.
9. H. Ozaki, M. Kitamura, K. Yamana, and T. Shimidzu, *Nucleic Acids Symp. Ser.*, **1989**, 21, 35.
10. R. O. Dempcy, K. A. Browne, and T. C. Bruice, *Proc. Natl. Acad. Sci. USA*, **1995**, 92, 6097.
11. P. E. Nielsen, M. Egholm, R. H. Berg, and O. Buchardt, *Science*, **1991**, 254, 1497.
12. N. Sugimoto, N. Satoh, K. Yasuda, and S. Nakano, *Biochemistry*, **2001**, 40, 8444.
13. D. A. Weidner, B. C. Valdez, D. Henning, S. Greenberg, and H. Busch, *FEBS Lett.*, **1995**, 366, 146.
14. I. Tazawa, S. Tazawa, L. M. Stempel, and P. O. Ts'o, *Biochemistry*, **1970**, 9, 3499.
15. U. Asseline, J. F. Hau, S. Czernecki, T. Le Diguarher, M. C. Perlat, J. M. Valery, and N. T. Thuong, *Nucleic Acids Res.*, **1991**, 19, 4067.
16. K. P. Williams, X. H. Liu, T. N. Schumacher, H. Y. Lin, D. A. Ausiello, P. S. Kim, and D. P. Bartel, *Proc. Natl. Acad. Sci. USA*, **1997**, 94, 11285.
17. S. Fujimori and K. Shudo, *J. Am. Chem. Soc.*, **1990**, 112, 7436.
18. G. W. Ashley, *J. Am. Chem. Soc.*, **1992**, 114, 9731.
19. A. Garbesi, M. L. Capobianco, F. P. Colonna, L. Tondelli, F. Arcamone, G. Manzini, C. W. Hilbers, J. M. Aelen, and M. J. Blommers, *Nucleic Acids Res.*, **1993**, 21, 4159.
20. M. J. Damha, P. A. Giannaris, P. Marfey, and L. S. Reid, *Tetrahedron Lett.*, **1991**, 32, 2573.
21. M. J. Damha, P. A. Giannaris, and P. Marfey, *Biochemistry*, **1994**, 33, 7877.
22. J. Kawakami, K. Tsujita, and N. Sugimoto, *Nucleic Acids Symp. Ser.*, **1998**, 39, 55.
23. M. J. Blommers, L. Tondelli, and A. Garbesi, *Biochemistry*, **1994**, 33, 7886.
24. H. Urata, Y. Ueda, H. Suhara, E. Nishioka, and M. Akagi, *J. Am. Chem. Soc.*, **1993**, 115, 9852.
25. H. T. Allawi and J. SantaLucia Jr., *Biochemistry*, **1998**, 37, 9435.
26. N. Peyret, P. A. Seneviratne, H. T. Allawi, and J. SantaLucia Jr., *Biochemistry*, **1999**, 38, 3468.
27. H. Urata and M. Akagi, *Tetrahedron Lett.*, **1996**, 37, 5551.
28. H. Urata, H. Shimizu, H. Hiroaki, D. Kohda, and M. Akagi, *Biochem. Biophys. Res. Commun.*, **2003**, 309, 79.
29. Detailed procedures for synthesis of β -L-2'-deoxyadenosine phosphoramidite is available as supplemental information at http://fiber.konan-u.ac.jp/support/Anal_Sci/supplement.pdf.
30. R. Kierzek, M. H. Caruthers, C. E. Longfellow, D. Swinton, D. H. Turner, and S. M. Freier, *Biochemistry*, **1986**, 25, 7840.
31. N. Sugimoto, R. Kierzek, S. M. Freier, and D. H. Turner, *Biochemistry*, **1986**, 25, 5755.
32. E. G. Richard, in "Handbook of Biochemistry and Molecular Biology: Nucleic Acids", ed. G. D. Fasman, 3rd ed., **1975**, Vol. 1, CRC Press, Cleveland, OH, 596 - 603.
33. M. Petersheim and D. H. Turner, *Biochemistry*, **1983**, 22, 256.
34. Y. Mizukami, H. Nakatsuji, M. Hata, M. Sasaki, and N. Sugimoto, *Chem. Lett.*, **1991**, 2119.
35. C. E. Longfellow, R. Kierzek, and D. H. Turner, *Biochemistry*, **1990**, 29, 278.
36. V. I. Ivanov, L. E. Minchenkova, A. K. Schyolkina, and A. I. Poletayev, *Biopolymers*, **1973**, 12, 89.
37. C. A. van Boeckel, G. M. Visser, R. A. Hegstrom, and J. H. van Boom, *J. Mol. Evol.*, **1987**, 25, 100.
38. N. Tomioka and A. Itai, *Biopolymers*, **1992**, 32, 1593.
39. M. Kieninger and S. Suhai, *Anticancer Drug Des.*, **1995**, 10, 189.
40. N. Sugimoto, S. Nakano, M. Yoneyama, and K. Honda, *Nucleic Acids Res.*, **1996**, 24, 4501.
41. J. SantaLucia Jr., H. T. Allawi, and P. A. Seneviratne, *Biochemistry*, **1996**, 35, 3555.
42. S. M. Freier, N. Sugimoto, A. Sinclair, D. Alkema, T. Neilson, R. Kierzek, M. H. Caruthers, and D. H. Turner, *Biochemistry*, **1986**, 25, 3214.
43. N. Sugimoto, S. Nakano, M. Katoh, A. Matsumura, H. Nakamuta, T. Ohmichi, M. Yoneyama, and M. Sasaki, *Biochemistry*, **1995**, 34, 11211.
44. H. T. Allawi and J. SantaLucia Jr., *Biochemistry*, **1997**, 36, 10581.
45. M. Wu, J. A. McDowell, and D. H. Turner, *Biochemistry*, **1995**, 34, 3204.
46. M. J. Doktycz, T. M. Paner, M. Amaratunga, and A. S. Benight, *Biopolymers*, **1990**, 30, 829.