A note on graphing helical parameters of dynamics structure of DNA

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A graphical procedure for analysis of helical parameters in dynamic structure of DNA is described. The performance of the procedure is illustrated by analysis of a 20 ps dynamics simulation of the non–self-complementary ninemer, 5'CAAACAGGA:5'TCCTGTTTG, which is a part of DNA from lacI *gene. The dynamics trajectory of the duplex shows sequence-dependent fluctuations of helical parameters.*

Keywords: DNA dynamics, helical parameters, fluctuations of DNA

INTRODUCTION

It has been well established that the DNA double helical structure is altered upon the change in relative humidity and salt concentration of the sample[1,2] and during interactions with bound antibiotics[2,3] and proteins,[4] among others. Also DNA itself shows sequence-dependent microheterogeneity of the helix[5,6] that indirectly can be seen, e.g., on sequencing gels showing distributions of nuclease-generated fragments of DNA,[7] or by direct antibiotic-induced cleavage of DNA.[8] An alternative way to explore sequence-dependent microheterogeneity of DNA structure is to perform computer simulations of DNA models. For example, Monte Carlo and molecular mechanics simulations have been performed on short fragments of kinetoplast DNA from *Crithidia fascinata*, which show sequence-dependent curvature.[9,10] Also, molecular dynamics simulations were employed to study sequence-dependent variations of helical parameters[11,12] and interactions between antibiotics and DNA,[13] among others. This communication presents graphical analysis of dynamics trajectory of ninemer, that shows sequence-dependent cleavage by neocarzinostatin chromophore.[8,14] Also, advantages and limitations of the approach are described.

INITIAL DNA MODEL

The Cartesian coordinates of the ninemer, $5'C_1A_2A_3A_4C_5A_6G_7G_8A_9(+):5'T_{10}C_{11}C_{12}T_{13}G_{14}T_{15}T_{16}T_{17}G_{18}(-)$, were generated from the cylindrical polar coordinates of DNA components derived from the X-ray fiber diffraction[15] using the CORGEN program.[16] The generated model had global helical twist (Ω) predicted using the procedure described by Tung and Harvey with the VIEWDNA program.[10] The modification of (Ω) and global helical rise (D_z) were made through the rotation-translation matrix and general subgroup rotation matrix as recently described.[16] All energy minimizations were carried out with the CHARMM program[17] using the DNA topology and parameters described by Nilsson and Karplus.[18] All computations were performed with a VAXstation 3500, and the CPU requirements are as follows: 5 ps thermalization followed by 5 ps equilibration at 300 K took 32 CPU hours, whereas each 10 ps of the trajectory production step took up to 15 CPU hours.

GRAPHICAL DISPLAYING OF HELICAL PARAMETERS

The helical parameters of DNA were calculated with the CORDAN program.[20] The program can utilize either CHARMM dynamics file (binary file) or a set of consecutive ASCII/binary dynamics frames created from the parent dynamics file. If dynamics trajectory of DNA were generated with a program other than CHARMM, the original dynamics file would have to be rewritten into "CHARMM format." Alternatively, a small part of "READ DYN" subroutine should be modified accordingly. An average analysis of dynamics trajectory of DNA supplies from 200 to 1000 graphics files that correspond to variations of different helical parameters in the function of time of dynamics simulation. Various helical parameters can be monitored during analysis of dynamics trajectory, and graphics files are in the format, helical parameter vs. time. This simple format assures that the files can be displayed on virtually any graphical device.

RESULTS

The optimized initial structure and optimized 20 ps averaged dynamics structure of the ninemer are shown in Figure 1. A few of the graphical representations of fluctuations of helical parameters in the ninemer are shown in Figures 2–4

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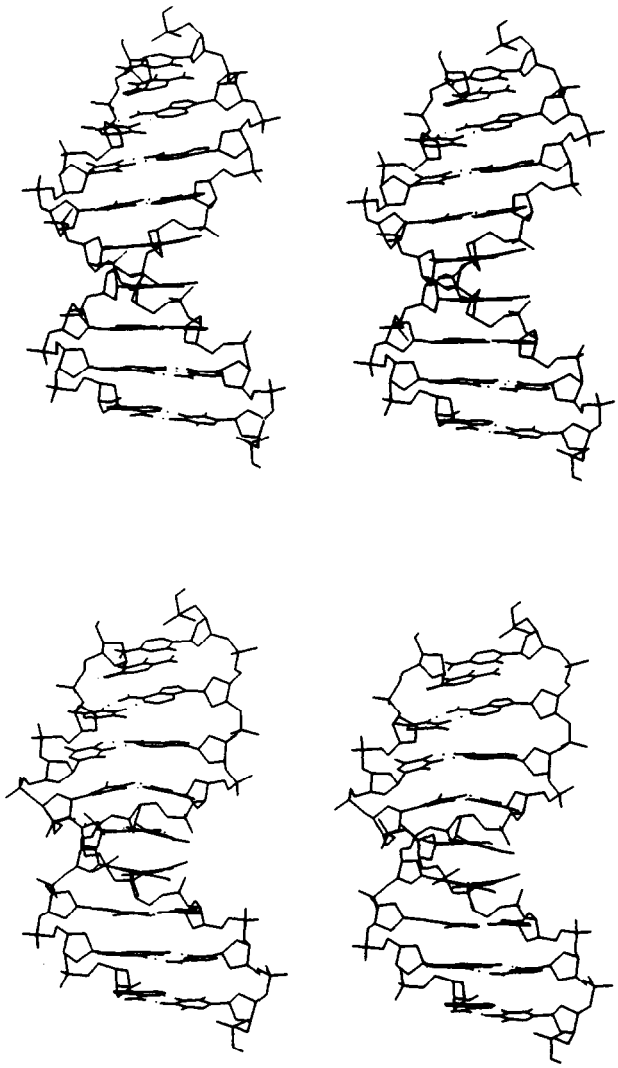

Figure 1. The optimized initial structure (upper panel) and optimized averaged dynamics structure (lower panel) of 5'CAAACAGGA:5'TCCTGTTTC duplex. The initial structure had the following Ω introduced from the Cyt*1*-Gua*18*/Ade2-Thy*17 base step: 27.2°, 39.8°, 37.7°, 35.6° 39.8°, 35.6°, 37.7° and 31.4°. The initial model structure was optimized with 200 of constrained minimization*[19] *followed by 1800 steps of unconstrained minimization using the adapted basis Newton-Raphson method, ABNR.*[17] *The mean Ω was 35.3°. The molecular dynamics simulation of the duplex was started from its optimized structure as described.*[11,13] *The terminal base-pairs were constrained with a harmonic force of 10 kcal/mol/Å on each atom. The structure was heated from 10 K to 300 K over 10 ps followed by 10 ps of equilibration. The production portion of the dynamics simulation was then carried out for 20 ps. Dynamics frames (coordinates) were stored every 0.025 ps.*

(summarized helical parameters can be supplied upon request). Changes of RMS difference between the optimized average dynamics structure and dynamics trajectory for the three central base-pairs during a 20 ps dynamics simulation are shown in Figure 2. The variations of RMS values are sequence- and position-dependent. For example, there are smaller RMS fluctuations for *Ade*6 and *Thy*15 than for the residues of *Ade*4 and *Gua*. Specifically, large fluctuations occurred at *Ade*3 and *Ade*4, and they were primarily dominated by movements of the phosphate atoms and sugar moieties. In the (-)strand, the fluctuations of RMS values were smaller. RMS larger than 1.0 occurred for *Thy*13, and they were due to considerable fluctuations of the phosphate backbone and even more by movements of the sugar moiety. These fluctuations reflect the microstability of the double helix, which is higher at *Gua-Cyt*–rich regions than at *Ade-Thy*–rich regions. Relatively large fluctuations for *Ade*4 and *Thy*15 can result from interstrand purine-purine clashes *Ade*4/*Gua*14/*Ade*6.[21,22]

Variations of Ω and D_z are shown in Figure 3. The Ω and D_z values differ somewhat from those in the optimized structure. For example, the mean Ω value for the *Cyt*5-*Gua*14/*Ade*6-*Thy*13 base step increased to 34°, whereas the value for the *Ade*4-*Thy*15/*Cyt*5-*Gua*14 base step reached 45°, and the *Ade*6-*Thy*13/*Gua*7-*Cyt*12 base step reached 39°. The highest fluctuation of Ω was noted for the *Ade*4-*Thy*15/*Cyt*5-*Gua*14 base step. Also, there are some changes in the D_z values that are above the average value for the *Cyt*5-*Gua*14/*Ade*6-*Thy*13 base step and below the average value for the preceding step. This finally leads to unusual shifting of the sugar moiety of *Ade*6 over the mean plane of the base-pair. The variations of pseudorotation phase angle (*P*) during 20 ps dynamics simulation of the ninemer for the six central residues are shown in Figure 4. The sugar pucker of *Ade*4, *Cyt*5, *Gua*14 and *Thy*15 remained unchanged. However, there was a change of the pucker in *Ade*6 and *Thy*13, namely, *Ade*6 switched from the 04'-exo into the more stable C3'-endo pucker, whereas *Thy*13 stabilized with the C4'-exo pucker, which is also often found in the X-ray elucidated structures.[6] The latter could be imposed by interstrand clashes between purines in the *Ade*4-*Gua*14-*Ade*6 triangle. These clashes are relieved by smaller than average global helical twist and positive slide of the *Cyt*5-*Gua*14/*Ade*6-*Thy*13 base step. As a result of these interstrand interactions and DNA bending, there is a substantial increase of cleavage at *Ade*6 by neocarzinostatin antibiotic.[12] In fact, the sugar moiety of *Ade*6 is the strongest cleavage site in the 66 base-pair fragment (*Ade*6 corresponds to *Ade*15 in the 66 base-pair fragment) that was generated from the *lac*I gene[23] by cleavage with a number of restriction enzymes.

CONCLUSIONS

Analysis of dynamics trajectories of the ninemer shows apparent sequence-dependent fluctuations of helical parameters. However, these fluctuations are typical of the model under consideration, and they may change if the helix is solvated or if the explicit cations and ionic strength are introduced. Also, the helical parameters in the ninemer were influenced by the alterations of Ω in the generated initial structure (predicted Ω). Moreover, DNA is a flexible molecule whose structure is globally determined by the conditions of the phase, whereas local variations of helical parameters are sequence-dependent.[1–4,24,25] Thus, any con-

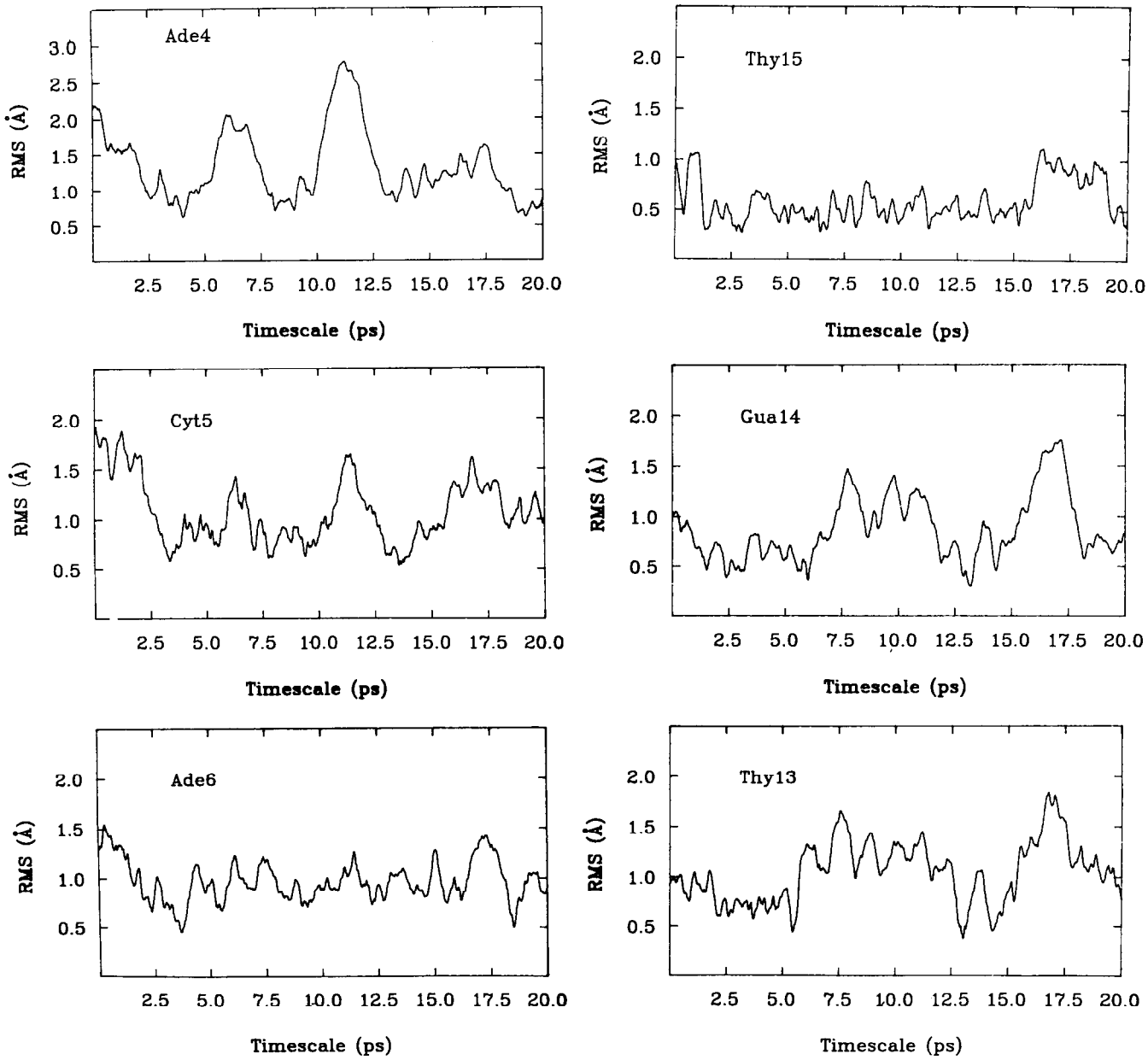

Figure 2. Changes of RMS values for six central residues of the ninemer. Average RMS for Ade*4, 1.337;* Cyt*5, 1.075; and* Ade*6, 0.966 whereas in the (−)strand,* Thy*13, 1.044;* Gua*14, 0.875; and* Thy*15, 0.589. Root-mean-squares (RMS) fluctuations of individual residues are calculated with respect to the averaged dynamics structure by:*

$$RMS = \left[\frac{1}{N}\sum_{i=1}^{N}(r_i - \bar{r}_i)^2\right]^{1/2}$$

where r_i *are the atomic coordinates of the molecular fragment in consecutive dynamics trajectories,* r_i *are corresponding atomic coordinates of the averaged dynamics structure, and* N *is the number of atoms.*

clusions on sequence-dependence of DNA structure can be exclusively related to the conditions of the phase.[1,2,24,25] To include the latter in computer simulations of model DNA would require parameters derived from experimental conditions under which the local variations of helical parameters were observed. Such a task certainly is challenging, and will parallel increase of computer speed and development of more accurate potential functions and parameters for solute-solvent interactions.

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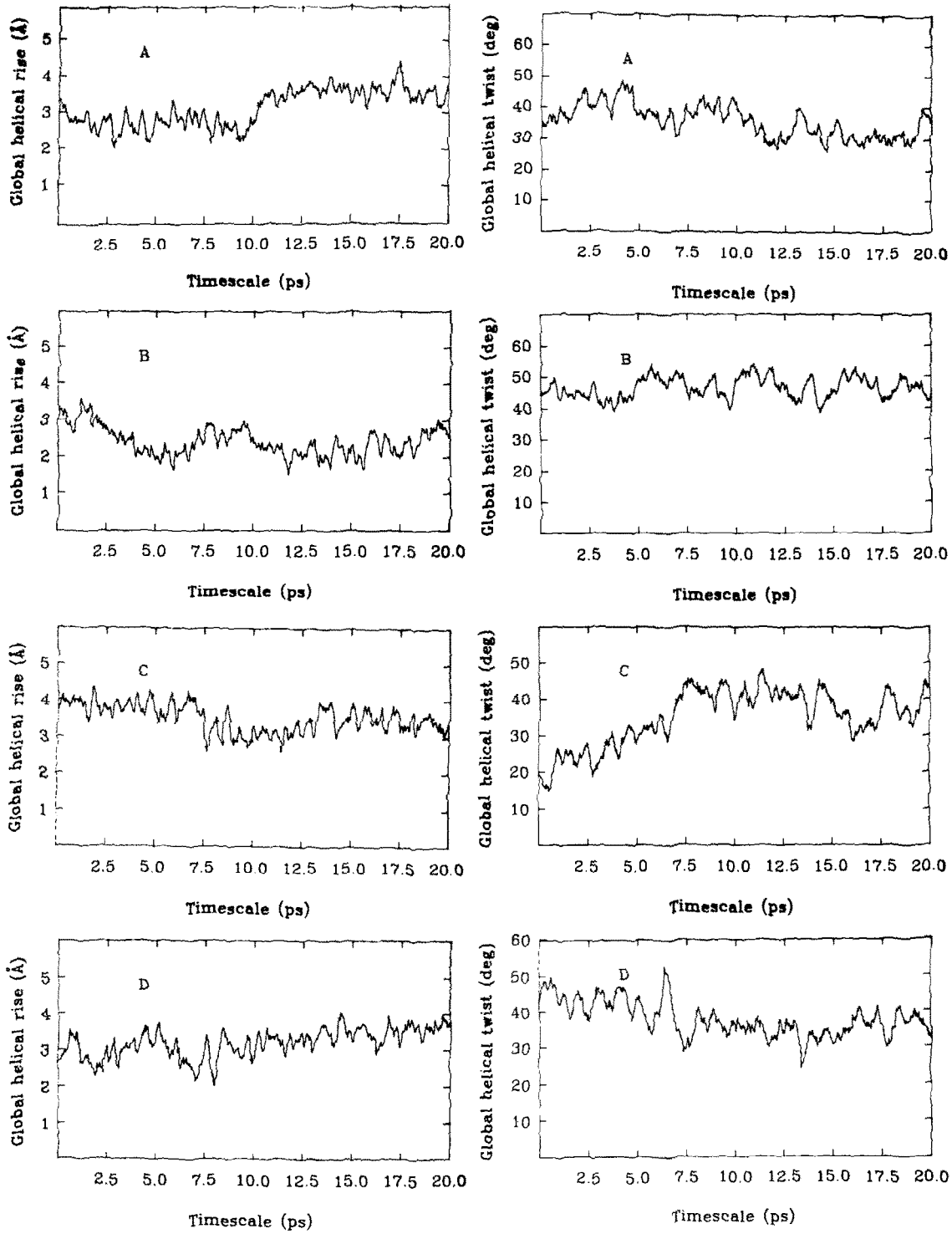

Figure 3. Fluctuations of Ω *and* D_z *for three central base steps of the ninemer; (A) the* Ade*3*-Thy*16*/Ade*4*-Thy*15 base step*, $\Omega = 35.3°$ (f = *5.4*), $D_z = 3.05$ Å (f = *0.61* Å); *(B) the* Ade*4*-Thy*15*/Cyt*5*-Gua*14 base step*, $\Omega = 45.3°$ (f = *3.2*°), $D_z = 2.27$ Å (f = *0.41* Å); *(C) the* Cyt*5*-Gua*14*/Ade*6*-Thy*13 base step*, $\Omega = 34.3°$ (f = *8.2*°), $D_z = 3.50$ Å (f = *0.33* Å); *and (D) the* Ade*6*-Thy*13*/Gua*7*-Cyt*12 base step*, $\Omega = 39.2°$ (f = *5.3*°), $D_z = 3.35$ Å (f = *0.37* Å). *Best overall helical axis was determined based on the C1′ and N1/N9 atoms, respectively. Averaged rms fluctuations of helical parameters* (f) *were calculated;*

$$f = \left[\frac{1}{N}\sum_{i=1}^{N}(p_i - p^a)^2\right]^{1/2}$$

where p^a *is the value of parameter in the averaged dynamics structure, and* N *is the number of dynamics trajectories.*

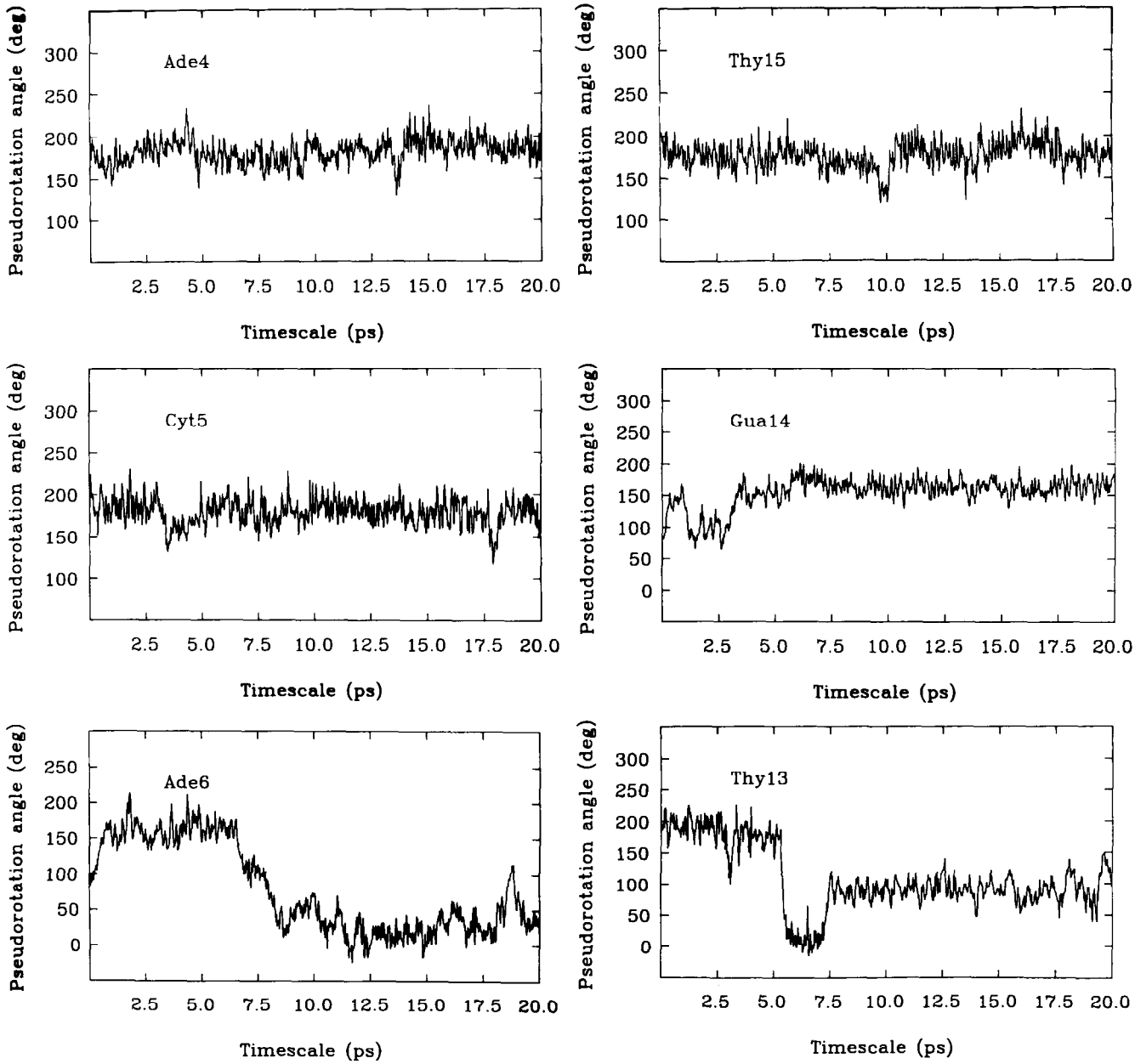

Figure 4. Fluctuations of pseudorotation phase angle (P) *for three central base-pairs in the ninemer; the (+)strand, (A)* Ade*4*, P = *182.3°* (f = *15.3°*)*; (B)* Cyt*5*, P = *179.8°* (f = *16.0°*)*; and (C)* Adc*6*, P = *78.7°* (f = *80.4°*)*. The (−)strand, (C)* Thy*13*, P = 109.3° (f = *58.1°*)*, (B)* Gua*14*, P = *154.7°* (f = *26.9°*) *and (A)* Thy*15*, P = *177.1°* (f = *16.0°*)*. The parameters were calculated as recently described.*[20]

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