Population Analysis of the Intermediate Complex States During B-Z Transition of Non-CG-repeat DNA Duplexes Induced by the Za Domain of Human ADAR1

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Z-DNA contains nucleic acid bases in alternating anti- and syn-conformations along the nucleotide chain and has only one groove that is similar to the minor groove of B-DNA.1-3 Z-DNA is in a higher energy conformation than B-DNA and is stabilized by negative supercoiling generated in vivo.2,3 Human ADAR1 has two left-handed Z-DNA binding domains at its NH2-terminus, Za and ZB, preferentially binds Z-DNA, rather than B-DNA, with high binding affinity.4-6 The co-crystal structure of the Za domain of human ADAR1 (ZαADAR1) bound to Z-DNA revealed that one monomeric ZαADAR1 domain binds to one strand of double-stranded DNA and a second ZαADAR1 monomer binds to the opposite strand with two-fold symmetry with respect to the DNA helical axis.7 A structural study showed that ZαADAR1 binds to the Z-conformation of non-CG-repeat DNA duplexes through a common structural feature rather than by a specific sequence or structural alternations.8 A previous NMR study on a d(CGCGCG)2-ZαADAR1 complex9 suggests an active-mono B-Z transition mechanism (see Fig. 1) in which the ZαADAR1 protein first binds to B-DNA and then converts it to left-handed Z-DNA, a conformation that is then stabilized by the additional binding of a second ZαADAR1 molecule.

Recently, we have reported NMR hydrogen exchange data of complexes between ZαADAR1 and the non-CG-repeat DNA duplexes, d(CACGTG)2 [referred to as CA6] or d(CGTACG)2 [referred to as TA6], with a variety of protein-to-DNA (P/N) molar ratios.10 The kex data for the G4b of the CA6-ZαADAR1 complex and for the G2b of the TA6-ZαADAR1 complex showed significant changes as the Z-DNA fraction (fz) was increased (meaning that the P/N ratio increased) (see Fig. 2). These changes of the kex data can be explained by the presence of mixtures of two imino protons from B-form DNA (referred to as B) and B-DNA-ZαADAR1 complex (referred to as BP) in the imino peaks as given by Eq. 1:10

\[
\begin{align*}
    k_{ex} &= \frac{[B]k_{ex}^B + [BP]k_{ex}^{BP}}{[B] + [BP]} = k_{ex}^B + \frac{[BP]}{1 + fZ} (k_{ex}^{BP} - k_{ex}^B) \\
    k_{ex}^B \text{ and } k_{ex}^{BP} & \text{ are the } k_{ex} \text{ of the imino protons for the B and BP.}
\end{align*}
\]

Figure 1. Active-mono B-Z transition mechanism of a 6-bp DNA duplex by two Z-DNA binding proteins. B and Z indicate the B-form and Z-form of the DNA duplex and P indicates the Z-DNA binding proteins.

Figure 2. (A) The kex values of the G4b imino proton for the CA6-ZαADAR1 complex determined at 25 °C and (B) kex values of the G2b imino proton for the TA6-ZαADAR1 complex determined at 15 °C as a function of the fz. Black solid lines are the best fit to Eq. 1, where the kex data were weighted by the inverse of their variance. The grey lines indicate their upper and lower confidence limits (95% confidence level).
BP states, respectively, and \([B] \) and \([BP] \) are the concentrations of the B and BP states, \(Z_t\) is the total concentration of Z-conformation. Thus, the correlation between the \(k_e\) and \(f_z\) data can be expressed by Eq. 2 as described in previous report.\(^{10}\)

\[
k_{ex} = k_{ex}^B + \frac{k_{ex}^B - k_{ex}^\alpha}{2(1 - \alpha)(1 - f_z)} \left[1 + (K_{BZ}^1 - 1)f_z\right] - \sqrt{(1 + (K_{BZ}^1 - 1)f_z^2 - 4k_{ex}^\alpha(1 - \alpha)f_z(1 - f_z)}
\]  

(2)

where \(K_{BZ}^1\) is the ratio of the association constants \((K_a)\) of the ZP and BP complex states. In the previous report,\(^{10}\) the \(\alpha\) (CA6: 1.42; TA6: 13.9), \(K_{BZ}^1\) (CA6: 0.4 ± 0.1; TA6: 6.3 ± 3.1), \(k_{ex}^\alpha\) (CA6: 39.2 ± 0.6 s\(^{-1}\); TA6: 11.5 ± 0.5 s\(^{-1}\)), and \(k_{ex}^B\) (CA6: 10.2 ± 3.1 s\(^{-1}\); TA6: 22.2 ± 5.3 s\(^{-1}\)) values of CA6 and TA6 complexed with Z\(_{\alpha ADAR1}\) were determined by curve fitting \(k_e\) of the imino protons as a function of \(f_z\) with Eq. 2 (Fig. 2).\(^{10}\)

In order to estimate the reliability of the proposed model in the previous study, we performed the iterative non-linear curve fitting \(k_e\) of the imino protons in the CA6-Z\(_{\alpha ADAR1}\) and TA6-Z\(_{\alpha ADAR1}\) complexes as a function of \(f_z\) with Eq. 2 using program Origin 7. The upper and lower confidence limits on the \(k_e\) data of CA6 and TA6 complexed with Z\(_{\alpha ADAR1}\) were evaluated by iterative non-linear curve fitting and the 95% confidence bands of the \(k_e\) data are shown in Fig. 2. This result shows that the active-mono B-Z transition mechanism, which was proposed in the previous study,\(^{10}\) is a suitable approach to understand the DNA sequence discrimination step of the Z\(_{\alpha ADAR1}\) protein during B-Z transition.

The relative population of each complex state (such as B, BP, ZP, and ZP2) as a function of the P/N ratio was determined from the \(f_z\) and \(k_e\) data, which were reported in previous study,\(^{10}\) as the following procedure. First, the \([BP]\) values are calculated from the exchange data, \(k_e\), \(k_{ex}^B\), and \(k_{ex}^\alpha\), by using Eq. 3:

\[
[BP] = \frac{k_{ex} - k_{ex}^B}{k_{ex}^B - k_{ex}^\alpha}(1 - Z_t)
\]

(3)

where \(Z_t\) are determined from relative peak intensities of the imino proton resonances of the Z-form DNA. Second, the \([B] \) values can be calculated by using the equation, \([B] = 1 - [ZP] - [BP]\). Third, the concentration of the ZP state ([ZP]) is calculated from the flowing relation, \([ZP] = [BP]/K_{BZ}^1\). Forth, the concentration of the ZP2 state ([ZP2]) can be calculated by using the equation, \([ZP2] = Z_t - [ZP]\). The relative populations (including estimated errors) of the B, BP, ZP, and ZP2 states in the CA6-Z\(_{\alpha ADAR1}\) and TA6-Z\(_{\alpha ADAR1}\) complexes as a function of the P/N ratio are shown in Fig. 3 and 4, respectively. Finally, the concentration of free Z\(_{\alpha ADAR1}\) ([P]) could be calculated by the Eq. 4:

\[
\]

(4)

where \(P_t\) is the total concentration of Z\(_{\alpha ADAR1}\). From these concentrations, the association constants, \(K_{\alpha BP} = [BP]/[\alpha P]\) and \(K_{\alpha ZP} = [ZP]/[\alpha P]\) for the CA6-Z\(_{\alpha ADAR1}\) and TA6-Z\(_{\alpha ADAR1}\) complexes were calculated. The \(K_{\alpha BP}\) and \(K_{\alpha ZP}\) values of CA6-Z\(_{\alpha ADAR1}\) complex are 3.9 ± 1.3 × 10\(^3\) and 5.5 ± 1.9 × 10\(^3\), respectively.\(^{10}\) This means that, unlike the d(CCGCGG)\(_2\)-Z\(_{\alpha ADAR1}\) complex, the Z\(_{\alpha ADAR1}\) protein can bind to the B and ZP complex states with similar binding affinity. The relative population of each complex state for the CA6-Z\(_{\alpha ADAR1}\) complex as a function of the P/N ratio could be calculated from these association constants and equilibrium constants for B-Z transition and the results are shown in Fig. 3 (solid lines). It was observed that \([B]\) was gradually decreased, but \([BP]\) and \([ZP]\) were increased as the P/N ratio increased up to 2 (Fig. 3). In addition, the observation that \([BP]\) is always smaller than \([ZP]\) could be explained by the fact that \(K_{BZ} < 1\) (Fig. 3). When the P/N ratio rose to 2, the ZP2 complex was dominantly produced but \([BP]\) and \([ZP]\) were decreased as the P/N ratio increased because the added P preferentially bound to the ZP complex rather than the B and BP (Fig. 3).

Similarly, the \(K_{\alpha BP}\) and \(K_{\alpha ZP}\) values of the TA6-Z\(_{\alpha ADAR1}\) complex as function of the P/N ratio are shown in Fig. 4 (solid lines). Similar to the CA6-Z\(_{\alpha ADAR1}\) complex, in both complexes, it was observed that \([B]\) was gradually decreased, but \([BP]\) and \([ZP]\) were increased as the P/N ratio increased up to 2 (Fig. 4). However, in contrast to the CA6-Z\(_{\alpha ADAR1}\) complex, it was observed that \([BP]\) is always larger than \([ZP]\), indicating that \(K_{BZ} > 1\) (Fig. 4). When the P/N ratio rose to 2, the ZP2 complex was dominantly produced but \([BP]\) and \([ZP]\) were decreased as the P/N ratio increased like CA6 (Fig. 4).

Interestingly, the simulated population (solid line in Fig. 3 and
Fig. 4 can explain how the Z_{ADAR1} protein recognizes the d(CGCGCG) sequence from d(CACGTG) and d(CGTACG) sequences in a long genomic DNA.

In summary, we derived the relative population of each complex state, which is thought to be produced during B-Z transition induced by Z_{ADAR1}, as a function of the P/N ratio. This approach provides the insight into the active B-Z transition mechanism and DNA sequence discrimination step of human Z-DNA binding protein, ADAR1.

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