Solution structure of the Z-DNA binding domain of PKR-like protein kinase (PKZ) from Carassius auratus and quantitative analyses of the intermediate complex during B–Z transition

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Supplementary Information

Details of Derivation of Equations 2
Supplementary Figure S1 4
Supplementary Figure S2 5
Supplementary Figure S3 6
Supplementary Figure S4 7
Supplementary Figure S5 8
Supplementary Figure S6 9
Details of Derivation of Equations.

The possible pathways of B–Z transition of the 6-bp DNA duplex induced by Z-DNA binding proteins are given by:

\[
\begin{align*}
B & \leftrightarrow P \rightarrow BP \leftrightarrow P \rightarrow BP_2 \\
\uparrow & \quad \uparrow \\
Z & \leftrightarrow P \rightarrow ZP \leftrightarrow P \rightarrow ZP_2
\end{align*}
\]  
(Scheme S1)

where \( P \) indicates the Z-DNA binding proteins and \( B \) and \( Z \) indicate the B-form and Z-form of 6-bp DNA duplexes, respectively. Based on the active B–Z transition mechanism reported previously, the B–Z transition pathway could be simply given by:

\[
B \leftrightarrow P \rightarrow BP \leftrightarrow P \rightarrow ZP_2
\]  
(Scheme S2)

The total amount of added proteins ([\( P \] \text{tot}) is the summation of the concentrations of the free protein (\( P \)) and protein complexes (\( BP \), \( ZP \), and \( ZP_2 \)):

\[
[P]_{\text{tot}} = [P] + [BP] + [ZP] + 2[ZP_2]
\]  
(S1)

The dissociation constants for the \( BP \) and \( ZP_2 \) complexes are given by:

\[
K_{d,BP} = \frac{[B][P]}{[BP]} \quad (S2)
\]

\[
K_{d,ZP_2} = \frac{[ZP][P]}{[ZP_2]} \quad (S3)
\]

The equilibrium constant between the \( BP \) and \( ZP \) complexes is given by:

\[
K_{BZ,1} = \frac{[ZP]}{[BP]} \quad (S4)
\]

The total concentration of DNA duplexes is given by:

\[
[N]_{\text{tot}} = [B] + [BP] + [ZP] + [ZP_2] \quad (S5)
\]

Eq. S2 – S4 and S5 give rise to:

\[
[N]_{\text{tot}} = [B] + \frac{[B][P]}{K_{d,BP}} + \frac{K_{BZ,1}[B][P]^2}{K_{d,BP}K_{d,ZP_2}} + \frac{K_{BZ,1}[B][P]^2}{K_{d,BP}K_{d,ZP_2}^2}
\]  
(S6)

The relative populations of each complex state compared to \([N]_{\text{tot}} \) are given by:

\[
\frac{[B]}{[N]_{\text{tot}}} = \frac{K_{d,BP}K_{d,ZP_2}}{K_{d,BP}K_{d,ZP_2} + K_{d,ZP_2}[P] + K_{BZ,1}K_{d,ZP_2}[P] + K_{BZ,1}[P]^2}
\]  
(S7)

\[
\frac{[BP]}{[N]_{\text{tot}}} = \frac{K_{d,ZP_2}[P]}{K_{d,BP}K_{d,ZP_2} + K_{d,ZP_2}[P] + K_{BZ,1}K_{d,ZP_2}[P] + K_{BZ,1}[P]^2}
\]  
(S8)

\[
\frac{[ZP]}{[N]_{\text{tot}}} = \frac{K_{BZ,1}K_{d,ZP_2}[P]}{K_{d,BP}K_{d,ZP_2} + K_{d,ZP_2}[P] + K_{BZ,1}K_{d,ZP_2}[P] + K_{BZ,1}[P]^2}
\]  
(S9)
\[
\frac{\{ZP_3\}}{\{N\}_{tot}} = \frac{K_{BZ,1}\{P\}^2}{K_{d,BP}K_{d,ZP2} + K_{d,ZP2}\{P\} + K_{BZ,1}K_{d,ZP2}\{P\} + K_{BZ,1}\{P\}^2}
\] (S10)

Eq. S7 – S10 and S1 give rise to:

\[
\{P\}_{tot} = \{P\} + \{N\}_{tot} \frac{K_{d,ZP2}\{P\} + K_{BZ,1}K_{d,ZP2}\{P\} + 2K_{BZ,1}\{P\}^2}{K_{d,BP}K_{d,ZP2} + K_{d,ZP2}\{P\} + K_{BZ,1}K_{d,ZP2}\{P\} + K_{BZ,1}\{P\}^2}
\] (S11)

Eq. S11 becomes Eq. S12:

\[
K_{BZ,1}\{P\}^3 + \{P\}^2 \{K_{BZ,1}(2\{N\}_{tot} - \{P\}_{tot}) + (1 + K_{BZ,1})K_{d,ZP2}\} + \{P\}(1 + K_{BZ,1})K_{d,ZP2}\{N\}_{tot} - \{P\}_{tot} + K_{d,BP}K_{d,ZP2} - K_{d,BP}K_{d,ZP2}\{P\}_{tot} = 0
\] (S12)

Eq. S12 is simply expressed by:

\[
\{P\}^3 + a\{P\}^2 + b\{P\} + c = 0
\] (S13)

where

\[
a = 2\{N\}_{tot} - \{P\}_{tot} + \left(1 + \frac{1}{K_{BZ,1}}\right)K_{d,ZP2},
\]

\[
b = \left(1 + \frac{1}{K_{BZ,1}}\right)K_{d,ZP2}(\{N\}_{tot} - \{P\}_{tot}) + \frac{K_{d,BP}K_{d,ZP2}}{K_{BZ,1}},
\]

\[
c = -\frac{K_{d,BP}K_{d,ZP2}\{P\}_{tot}}{K_{BZ,1}}
\]

Thus the closed-form solution of Eq. S13 has been reported as:\footnote{31}

\[
\{P\} = \frac{a}{3} + \frac{2}{3} \sqrt{a^2 - 3b \cos \theta}
\] (S14)

where

\[
\theta = \arccos \frac{-2a^3 + 9ab - 27c}{2\sqrt{(a^2 - 3b)^3}}
\]
**Supplementary Fig. S1.** (A) Superimposition of $^1$H/$^{15}$N-HSQC spectra of free caZ$_{\alpha PKZ}$ (blue) and caZ$_{\alpha PKZ}$–dT(CG)$_3$ complex ($[N]_{tot}/[P]_{tot} = 0.6$, red) in NMR buffer (pH = 6.0) containing 10 mM NaCl at 35 °C. (B) The weighted average of $^1$H and $^{15}$N chemical shift changes ($\Delta\delta_{avg}$) of caZ$_{\alpha PKZ}$ upon binding to dT(CG)$_3$ at 10 (upper), 100 (middle), or 250 mM NaCl (lower). Residues whose cross-peaks disappear during titration are represented with green square symbols. The color used to illustrate the $\Delta\delta_{avg}$ is: red or blue, > 0.18 ppm; orange or cyan, 0.12 – 0.18 ppm; and yellow or pale green, 0.08 – 0.12 ppm.
Supplementary Fig. S2. The change in $^1$H/$^{15}$N-HSQC spectra of caZ$_{α}$PKZ by addition of dT(CG)$_3$ in NMR buffer (pH = 6.0) containing (A) 10 or (B) 100 mM NaCl at 35 °C. The cross-peak color changes gradually from blue (free) to red (bound) according to the [N]$_{tot}$/[P]$_{tot}$ ratio.
Supplementary Fig. S3. (A,B) The $^{15}$N (left) and $^1$H (right) chemical shift differences between the free and bound form for B-DNA (red) and Z-DNA binding (blue) of caZαPKZ to dT(CG)$_3$ at (A) 10 and (B) 100 mM NaCl at 35 °C. (C) The $^{15}$N (left) and $^1$H (right) chemical shift differences between [NaCl] of 10 and 100 mM for free caZαPKZ (yellow circle) and caZαPKZ bound to B-DNA (magenta circle) and Z-DNA (cyan circle) at 35 °C.
Supplementary Fig. S4. Superimposition of $^1$H/$^{15}$N-HSQC spectra of free caZ$_{\alpha PKZ}$ in NMR buffer (pH 6.0) containing 10 (red), 100 (blue), or 250 mM NaCl (green) at 25 °C.
Supplementary Fig. S5. (A,B) The concentrations of the total caZαPKZ ([P]_{tot}), total dT(CG)₃ ([N]_{tot}), free caZαPKZ ([P]), free B-DNA dT(CG)₃ ([B]), and each states of the caZαPKZ–dT(CG)₃ complex ([BP], [ZP], and [ZP₂]) as a function of the [N]_{tot}/[P]_{tot} ratio at (A) 10 or (B) 100 mM NaCl.
Supplementary Fig. S6. (A) Global fitting of the $^1$H/$^{15}$N-HSQC titration curves for caZ$_{\alpha}$PKZ with dT(CG)$_3$ as a function of $[N]_{tot}/[P]_{tot}$ ratio at pH 8.0. Data for the global fitting derived from $^1$H (left) and $^{15}$N (right) chemical shift changes of HSQC cross peaks of caZ$_{\alpha}$PKZ at 10 (upper) or 100 mM NaCl (lower). (B) Global fitting of the $^1$H/$^{15}$N-HSQC titration curves for caZ$_{\alpha}$PKZ with d(CG)$_3$ as a function of $[N]_{tot}/[P]_{tot}$ ratio at pH 8.0. Data for the global fitting derived from $^1$H (left) and $^{15}$N (right) chemical shift changes of HSQC cross peaks of caZ$_{\alpha}$PKZ at 10 mM NaCl.