Inhibition of tomato yellow leaf curl virus replication by artificial zinc-finger proteins

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ABSTRACT

Previously, we designed an artificial zinc-finger protein (AZP) for blocking a replication protein (Rep) of beet severe curly top virus (BSCTV) from binding to its replication origin and demonstrated that transgenic Arabidopsis plants expressing the AZP are completely resistant to the virus infection. Here we applied the AZP technology to tomato yellow leaf curl virus (TYLCV) infective to an important agricultural crop, tomato. We designed an AZP binding to the direct repeat to block the TYLCV Rep binding and confirmed in gel shift assays that the designed AZP has a higher affinity to the replication origin than that of Rep. Furthermore, we demonstrated in competitive binding assays that the AZP effectively inhibited the Rep binding in vitro. We discuss properties of the AZP for inhibition of TYLCV replication in detail.

RESULTS AND DISCUSSION

The TYLCV Rep protein is known to bind to double strand DNA of the direct repeats (i.e., 5'-ATCGGTGT-ATCGGTGT-3') in the viral replication origin (Fig. 1a). To block the Rep binding to the direct repeats, a six-finger AZP was designed by using our nondegenerate recognition code table6 to bind to a 19-bp DNA (5'-GAAATCGGTGTTATCGGTGT-3') containing the direct repeats (underlined). The amino acid sequence was shown in Fig. 1b. The DNA encoding the AZP was cloned into a pET-21a plasmid (Novagen), and the corresponding protein was expressed in E. coli and purified by ion-exchange chromatography. The Rep protein was prepared in a same manner.

The dissociation constant of the AZP was determined by gel shift assays. Under our experimental condition, half-maximal binding of Rep was observed at a protein concentration of 30 nM. In contrast, half-maximal binding of the AZP was observed at <30 pM, indicating that the AZP has >1000-fold higher affinity for the direct repeats than that of Rep.

Next, to examine whether or not the 6-finger AZP can block the Rep protein of TYLCV from binding to the direct repeats, competitive binding assays were performed. In the experiment, the AZP was first mixed with the DNA probe containing the direct repeats. And then, the Rep protein was
a

--- ATGGCAATCGGTGATATCGGTGCTT ---

AZP

b

1 2 3 6
1 PYKCPKCSGKSFSTDLHLHRTHTGEK
29 PYKCPKCSGKSFSTDLHLHRTHTGEK
57 PYKCPKCSGKSFSTDLHLHRTHTGEK
85 PYKCPKCSGKSFSTDLHLHRTHTGEK
113 PYKCPKCSGKSFSTDLHLHRTHTGEK
141 PYKCPKCSGKSFSTDLHLHRTHTGEK

Fig. 1 AZP designed for TYLCV. (a) DNA sequence of the direct repeats of TYLCV, and the DNA target site of the six-finger AZP. The two open rectangles indicate the direct repeats. (b) Amino acid sequence of the AZP. The underlined amino acids in each finger domain show recognition amino acids at positions 1-2, 3 and 6 in the α-helix of the finger domain. These amino acids were chosen from our recognition code table.9

added to the binding mixture. This experimental condition seems to simulate BSCTV infection in the transgenic Arabidopsis plants constitutively expressing the AZP. In the actual virus infection in the transgenic plants, it is likely that the direct repeats are preoccupied by the AZP before Rep binding because there is a time-lag between generation of the dsDNA form of the viral genome by endogenous enzymes and appearance of the Rep protein (expressed from the dsDNA) in nuclei. In this experiment, the AZP was first mixed with the DNA probe to final concentrations of 1, 10, and 100 nM, respectively, where all of the probe molecules were bound to the AZP. After incubation for 30 min on ice, Rep protein was added to the binding reaction mixture to the final concentration of 1000 nM and the reaction mixture was incubated for 30 min on ice. By electrophoresis of the final reaction mixture under a native condition, we found that even 1 nM AZP inhibited binding of 1000 nM Rep to the direct repeats. The property of the AZP was same to that of an AZP previously designed for the Rep of BSCTV, which demonstrated efficient inhibition of the BSCTV infection to Arabidopsis thaliana.

CONCLUSION

A six-finger AZP was generated to block the Rep protein of TYLCV from binding to its replication origin. In competitive binding assays, the AZP inhibited Rep binding efficiently. Since we had previously demonstrated that an AZP that inhibited binding of Rep of BSCTV could inhibit the virus replication in Arabidopsis thaliana, our results strongly support that the AZP designed in the present study will inhibit TYLCV replication in tomato.

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