Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary studies of the DNA-binding domain Za from ADAR1 complexed to left-handed DNA

Thomas Schwartz,[a] Karen Shafer,[b] Ky Lowenhaupt,[a] Eugene Hanlon,[b] Alan Herbert[a] and Alexander Rich[a]*

[a]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA, and [b]George R. Harrison Spectroscopy Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Correspondence e-mail: cbeckman@mit.edu

Received 5 February 1999
Accepted 28 April 1999

The proteolytically defined Z-DNA binding domain Za of human adenosine deaminase type 1 (hADAR1) has been crystallized in complex with the DNA oligomer d(TCGCGCG). The crystals were obtained from a solution containing ammonium sulfate as precipitating agent and belong to the tetragonal space group $P42_12$. A complete diffraction data set has been collected to a resolution of 2.4 Å. The unit-cell dimensions are $a = b = 85.9$, $c = 71.3$ Å. A Raman spectrum of the complex indicates that the DNA in the complex adopts the left-handed Z conformation.

1. Introduction

Double-stranded RNA-dependent adenosine deaminase (ADAR1) is a candidate enzyme for nuclear pre-mRNA editing (Maas *et al.*, 1997). The 136 kDa protein has several functional domains. Intriguingly, one of these has been identified as a Z-DNA binding protein (Herbert *et al.*, 1993, 1995). The DNA-binding activity was mapped to the N-terminus of the protein (Herbert *et al.*, 1997). Although the connection between RNA editing and Z-DNA binding has yet to be elucidated, a plausible model has been proposed (Herbert & Rich, 1996): ADAR1 substrate sites in double-stranded RNA involve a pairing of exonic and intronic sequences; therefore, editing must take place before splicing occurs. Since splicing is an early event, often concurrent with transcription, ADAR1 must be directed to transcribing genes. Z-DNA is stabilized by the negative supercoiling formed by a moving RNA polymerase. The Z-DNA binding activity may thus be used to target ADAR1 to transcribing genes.

The Z-DNA binding domain in human ADAR1 is bipartite, with two similar motifs, Zα and Zβ, separated by a duplicated 49 amino-acid linker module (Schwartz *et al.*, 1999). The Zα moiety has been extensively characterized and is sufficient to bind left-handed DNA in a conformation-specific manner (Herbert *et al.*, 1998). However, proteolytic experiments show that the binding characteristics of Zα are significantly changed by Zβ (Schwartz *et al.*, 1999).

Here, we report the crystallization of the proteolytically defined Z-DNA binding domain Za containing the Zα motif, in complex with a short DNA substrate capable of adopting the Z conformation. Raman spectroscopic measurements have been carried out to study the DNA conformation in the crystal. The Raman studies show that the DNA has fully adopted the Z conformation. This confirms previous studies of a Zα peptide in solution, which have shown that the bound substrate DNA is left-handed (Berger *et al.*, 1998).

2. Materials and methods

2.1. Protein preparation

The proteolytically defined core DNA-binding domain Za (residues 133–209) from human ADAR1 was purified as described by Schwartz *et al.* (1999). Homogeneity of the protein was confirmed by sodium dodecyl sulfate gel electrophoresis and by matrix-assisted laser desorption ionization–time of flight (MALDI–TOF) mass spectrometry.

2.2. DNA preparation

The synthetic DNA oligomer $d(TCGCGCG)_2$ (DNAgency, Malvern, PA) was dissolved in 10 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl. The solution was incubated for 10 min at 353 K and then slowly cooled to less than 293 K within 1 h. The annealed double-stranded oligomer was then purified using FPLC on a Mono-Q HR5/5 (Pharmacia, Piscataway, NJ) anion-exchange column. A 30 ml salt gradient from 50 to 400 m*M* NaCl in 50 m*M* Tris–HCl pH 8.0, 2 m*M* EDTA at a flow rate of 1 ml min^{-1} was applied.

2.3. Crystallization of the Za–DNA complex

Pure protein and DNA were dialyzed separately against 5 m*M* HEPES pH 7.5, 20 m*M* NaCl and concentrated to 1.2 m*M*. Protein–DNA complex was formed by mixing the concentrated stock solutions to yield a solution

containing a final concentration of 600 µ*M* of both Za and d(TCGCGCG). The mixture was equilibrated for 30 min at room temperature. Crystallization was performed using the vapor-diffusion method with hanging drops in combination with an incomplete factorial approach (Carter & Carter, 1979). A subset of 24 conditions from the 96 conditions used in commercially available screening kits (Hampton Research, Laguna Hills, CA, USA) was used. 2 µl of the preformed Za–DNA complex solution was mixed with 2 µl reservoir solution containing 1.8 *M* ammonium sulfate and 10% glycerol. Single crystals formed reproducibly at room temperature within 4–7 d. The crystals grew to 1.0 mm in their largest dimension.

2.4. X-ray crystallographic studies

For diffraction studies, crystals were harvested and directly flash-frozen at 123 K.

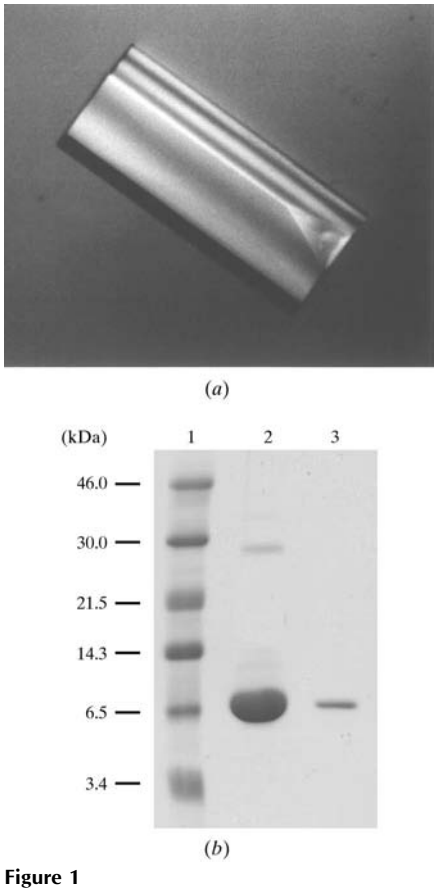

Figure 1
(*a*) Crystal of the Z-DNA binding domain Za of hADAR1 complexed to the DNA oligomer $d(TCGCGCG)_2$ viewed in polarized light. The tetragonal crystal (space group $P4_12$) grew in 2.5 m*M* HEPES pH 7.5, 10 m*M* sodium chloride and 1.8 *M* ammonium sulfate. The crystal size is 1.0 × 0.4 × 0.4 mm. (*b*) The presence of protein in the crystals was confirmed by SDS–PAGE (18%). Lane, 1, molecular weight markers; lane 2, purified Za protein; lane 3, dissolved crystal. Protein was visualized by Coomassie-blue staining.

X-ray diffraction data was collected on an R-AXIS IIc area detector using Cu $K\alpha$ radiation. A complete data set was collected from a single crystal using 0.5° oscillations. The data were indexed and integrated using the program *DENZO* (Otwinowski, 1993). The integrated reflections were scaled and merged using the program *SCALEPACK* (Otwinowski, 1993).

2.5. Spectroscopic studies

A Raman microscope was used to collect spectra of the protein–DNA complex crystal. The crystal was transferred from its mother liquor onto an MgF_2 support after briefly rinsing it with water. Near-infrared (830 nm) laser excitation was produced by an argon ion laser-pumped Ti–sapphire laser system (Coherent Innova 90 C/Spectra Physics 3900S) operating at 120 mW measured at the laser. The laser output was band-pass filtered (Kaiser Optical Systems, MI) before being focused onto the sample using an adjustable mirror, dichroic beam-splitter and a Zeiss Axioskop 50 fitted with a 10× objective (Zeiss Achroplan, NA 0.25). The Raman-scattered light was collected using the same microscope objective, collimated with a lens, filtered to reject the Rayleigh line (Kaiser Optical Systems, MI), and focused at the entrance slit of an imaging spectrograph (Chromex 2511S/SM) using an *f*-matching lens. Inside the spectrograph, a grating (600 lines mm^{-1}) dispersed the light onto a deep-depletion CCD detector (Princeton Instruments, NJ) cooled to 163 K. Spectra were collected for 50 s. Six spectra were averaged, corrected for cosmic rays and spectrograph response, and wavelength-calibrated using the spectral features of toluene. The background was subtracted using the spectrum of water only on the MgF_2 substrate. The spectral range of the data was 400–1900 cm^{-1}, with a resolution of 8 cm^{-1}.

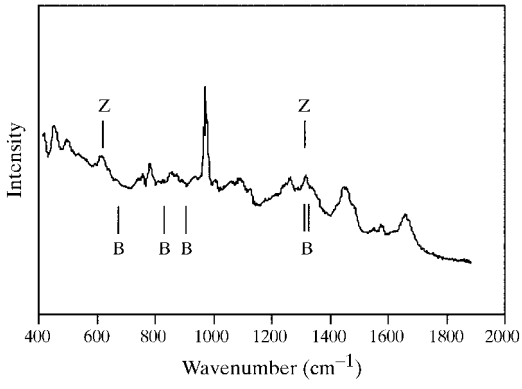

Figure 2
Raman spectrum of a Za–$d(TCGCGCG)_2$ complex crystal. Positions of marker bands for both B-DNA and Z-DNA are indicated. Only bands characteristic for Z-DNA are observed. Most obvious is the presence of the band for guanine in the *syn* conformation (Z-DNA, 625 cm^{-1}) and the absence of the band for the *anti* conformation (B-DNA, 675 cm^{-1}). The high peak at 978 cm^{-1} results from sulfate ions in the precipitant.

Table 1
Data collection and processing statistics.

Numbers in parentheses correspond to the 10% of all reflections in the highest resolution shell.

Space group	$P4_12$
Unit-cell parameters (Å)	$a = b = 85.9$, $c = 71.3$
Resolution range (Å)	40.0–2.4
Number of measured reflections	72043
Number of unique reflections	10896
$I/\sigma(I)$	33.2 (2.4)
R_{sym}† (%)	5.2 (56.7)
Completeness (%)	99.2 (97.2)

† $R_{sym} = \sum\sum|I(h)_i - \langle I(h)\rangle| / \sum\langle I(h)\rangle$, where $I(h)$ is the observed intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean intensity of reflection h.

3. Results and discussion

We have used the proteolytically defined core DNA-binding domain Za (residues 133–209) from human ADAR1 in an attempt to crystallize it with its DNA substrate. Initial microcrystals were obtained after 1 d at room temperature, using 2.0 *M* ammonium sulfate as the precipitant and the DNA oligomer $d(TCGCGCG)_2$ as the substrate. Large single crystals were obtained after reducing the precipitant concentration to 1.8 *M*. Adding 10%(*v*/*v*) glycerol resulted in minimization of the growth of twinned crystals. Under these conditions, well formed crystals with sharp edges and dimensions of up to 1.0 mm grew in 4–7 d (Fig. 1*a*). The presence of both protein and DNA within the crystals was confirmed. A crystal was dissolved in a small volume of 5 m*M* HEPES pH 7.5, 20 m*M* NaCl and electrophoresed on a denaturing polyacrylamide gel, resulting in a Coomassie-stained band at the predicted size for Za (Fig. 1*b*). The presence of DNA was confirmed by measuring the UV spectrum of a dissolved crystal, yielding an absorbance maximum at 260 nm. Diffraction data to a resolution of 2.4 Å were obtained under cryo-conditions at 122 K. It is of note that the crystals could be harvested and flash-frozen directly from their mother liquor containing 10%(*v*/*v*) glycerol without further addition of cryo-protectant. Excellent diffraction data were collected and are summarized in Table 1.

Raman measurements were used to analyze the DNA conformation

within the crystal. Earlier Raman spectroscopic studies of crystallized DNA oligomers have shown the power of this technique in discriminating between different DNA conformations. There are several spectral changes which can be used to distinguish between the Raman spectra of B-DNA and Z-DNA (Thamann *et al.*, 1981). The most obvious change is the shift of the guanine mode from 675 cm^{-1} in B-DNA to 625 cm^{-1} in Z-DNA, owing to the change in the conformation of guanine from *anti* to *syn*. Another important indicator is the absence of bands at 832 and 910 cm^{-1} in Z-DNA which are typically present in the Raman spectra of B-DNA. Finally, spectra of B-DNA include a guanine doublet at 1315/1331 cm^{-1}, whereas there is only a single peak at 1318 cm^{-1} in Z-DNA. A Raman spectrum of the Za–DNA complex is shown in Fig. 2. The band at 625 cm^{-1} is clearly observed, whereas no band is detectable at 675 cm^{-1}. Also, there are no spectral features present at 832 or 910 cm^{-1}. Finally, there is only a single peak at 1318 cm^{-1}. The combination of these spectral features indicates that the DNA has adopted the left-handed conformation.

Crystallization of heavy-atom derivatized complexes is under way in order to solve the structure.

This work was supported by grants from the National Institutes of Health and the American Foundation for Cancer Research to AR and by NIH grant P41-RR02594 to the Laser Biomedical Research Center at MIT. TS is the recipient of a predoctoral fellowship from the German Academic Exchange Service (DAAD).

References

Berger, I., Winston, W., Manoharan, R., Schwartz, T., Alfken, J., Kim, Y. G., Lowenhaupt, K., Herbert, A. & Rich, A. (1998). *Biochemistry*, **37**, 13313–13321.

Carter, C. W. Jr & Carter, C. W. (1979). *J. Biol. Chem.* **254**, 12219–12223.

Herbert, A., Alfken, J., Kim, Y. G., Mian, I. S., Nishikura, K. & Rich, A. (1997). *Proc. Natl Acad. Sci. USA*, **94**, 8421–6426.

Herbert, A., Lowenhaupt, K., Spitzner, J. & Rich, A. (1995). *Proc. Natl Acad. Sci. USA*, **92**, 7550–7554.

Herbert, A. & Rich, A. (1996). *J. Biol. Chem.* **271**, 11595–11598.

Herbert, A., Schade, M., Lowenhaupt, K., Alfken, J., Schwartz, T., Shlyakhtenko, L. S., Lyubchenko, Y. L. & Rich, A. (1998). *Nucleic Acids Res.* **26**, 3486–3493.

Herbert, A. G., Spitzner, J. R., Lowenhaupt, K. & Rich, A. (1993). *Proc. Natl Acad. Sci. USA*, **90**, 3339–3342.

Maas, S., Melcher, T. & Seeburg, P. H. (1997). *Curr Opin. Cell. Biol.* **9**, 343–349.

Otwinowski, Z. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 56–62. Warrington: Daresbury Laboratory.

Schwartz, T., Lowenhaupt, K., Kim, Y.-G., Li, L., Brown, B. A., Herbert, A. & Rich, A. (1999). *J. Biol. Chem.* **274**, 2899–2906.

Thamann, T. J., Lord, R. C., Wang, A. H. & Rich, A. (1981). *Nucleic Acids Res.* **9**, 5443–5457.