Nucleus Accumbens-Associated Protein 1 Binds DNA Directly through the BEN Domain in a Sequence-Specific Manner

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Supplementary Materials

Table 1. Structural Statistics for NAC1 (322-485).

| NMR restraints                             |       |
|--------------------------------------------|-------|
| Distance restraints                        |       |
| Total NOE                                  | 2364  |
| Intra-residue                              | 648   |
| Inter-residue                              |       |
| Sequential (|i - j| = 1)                     | 640   |
| Medium-range (1 < |i - j| < 5)                        | 499   |
| Long-range (|i - j| ≥ 5)                       | 577   |
| Hydrogen bonds restraints a                | 248   |
| Dihedral angle restraints a                |       |
| φ and ψ                                    | 111/111|
| χ1 and χ2                                 | 22/14 |

| Structure statistics (20 conformers)       |       |
| CYANA target function (Å²)                 | 3.56  |
| Residual NOE violations                     |       |
| Number > 0.1 Å                             | 14    |
| Maximum (Å)                                | 0.59  |
| Residual dihedral angle violations          |       |
| Number > 5°                                 | 2     |
| Maximum (°)                                | 12.20 |
| AMBER energies (kcal/mol)                  |       |
| Mean AMBER energy                          | -7217 |
| Mean restraints violation energy            | 29.02 |

| Ramachandran plot statistics (%) b         |       |
| Residues in most favored regions           | 93.0  |
| Residues in additionally allowed regions   | 6.0   |
| Residues in generously allowed regions     | 0.9   |
| Residues in disallowed regions             | 0.1   |

| Average R.M.S.D. to mean structure (Å) c   | Region A | Region B |
| Protein backbone                           | 0.67      | 0.49     |
| Protein heavy atoms                         | 1.12      | 1.05     |

a Used only in CYANA calculations. b Calculated with RAMPAGE server [1]. c Region A: For residues A345-I351, K368-E370, Y378-T380, R381-S390, H395-A404, S434-F447, and E453-A461. Region B: For residues K368-E370, Y378-T380, R381-S390, H395-A404, S434-F447, and E453-A461.
Table S2. Thermodynamic parameters (average of three experiments) for the interactions between oligonucleotides and NAC1 (322-485).

| oligonucleotide | Binding event | $K_d$ (μM) | $\Delta H$ (kcal/mol) | $\Delta S$ (cal/mol/deg) | N  |
|-----------------|--------------|------------|-----------------------|--------------------------|----|
| GP1-dsDNA       | first        | 0.16       | 1.8                   | 37.1                     | 0.77 |
|                 | second       | 18.0       | -30.4                 | -80.2                    | 0.32 |
| GP1 mut-dsDNA   | first        | 135        | -1162                 | -3880                    | < 0.1 |
|                 | second       | 0.37       | -51570                | 29.2                     | 1.03 |
| GP1-ssDNA       | first        | 0.2        | -11000                | -36861                   | < 0.1 |
|                 | second       | 0.46       | -11.4                 | -9.1                     | 1.13 |
| de novo motif 1 | single       | 3.1        | 2.3                   | 33.0                     | 0.67 |
| de novo motif 2 | single       | 0.83       | 2.2                   | 35.2                     | 0.87 |

Figure 1. (a) BSA, bacterially expressed and purified GST-NAC1(2-527; full-length), GST alone, and NPM1 as a positive control were each incubated with linearized pBluescript SK II plasmid DNA, followed by gel mobility shift assays. The agarose gel was then stained with ethidium bromide. Lanes 1 and 6 contain free linearized plasmid DNA. (b) Bacterially expressed and purified GST-NAC1, GST-NAC1 (2-250), and GST-NAC1 (251-527) were each subjected to a bacterial genome carry-over assay. The agarose gel (upper panel) and SDS-PAGE gel (lower panel) were stained with ethidium bromide.
and Coomassie Blue, respectively. (c) Bacterially expressed and purified GST alone, GST-NAC1, and GST-NAC1 (L432N) were each subjected to a bacterial genome carry-over assay. (d) DNase or RNase treatment of carry-over materials. Bacterially expressed and purified GST-NAC1 and GST-NAC1 (251-527) were each treated with DNase or RNase respectively, and then subjected to a bacterial genome carry-over assay.
Figure 2. The promoter region of human GADD45GIP1, PSAT1 and AZU1 genes harboured the consensus DNA-binding sequence of NAC1. The promoter regions (GADD45GIP1, NSR000000343712; PSAT1, ENSR00001306399; AZU1, ENSR00000341277) were predicted by the Ensembl regulatory build database [2]. The consensus DNA-binding sequences of NAC1 are underlined in blue. Sequences corresponding to the first methionine codon ATG, first exon, and TATA boxes are highlighted with red, green and magenta characters, respectively.

Figure 3. Isothermal titration calorimetry. Upper panels are raw titration data plotted as heat (μcal/sec) versus time (min). Each experiment consisted of 28 injections of 10 μl of 50 μM de novo motif1 (left panel) or of de novo motif2 (right panel) into a solution of 500 μM NAC-1(322-485) at 25°C. The lower panels are integrated heat responses plotted as normalised heat per mole of injectant. Smooth curves represent best fits of the data to the equation as described under “Materials and methods” using software provided by the instrument manufacturer. Data shown is representative of three independent experiments.
Figure 4. The 20 conformers representing the solution conformation of the BEN domain of NAC1322-485.

Figure 5. A representative HeLa cell stably expressing GFP, GFP-NAC1, GFP-NAC1 (251-527) or GFP-NAC1 (251-527, L432N). Images were obtained under a 473 diode laser. Bars, 10 μm.

References

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2. Zerbino, D.R., Wilder, S.P., Johnson, N., Juettemann, T. and Flicek, P.R. The ensembl regulatory build. Genome Biol. 2015, 16, 56.