5. Crystallographic Methods

5.1.   Crystallization

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) was used for initial setups. Pure protein and DNA solutions were dialyzed separately against 5 mM HEPES pH 7.5, 20 mM NaCl and concentrated to 1.2 mM. Mixing the concentrated stock solutions to yield a solution containing a final concentration of 600 µM of both protein and DNA formed the protein-DNA complex. The mixture was equilibrated for 30 min at room temperature. 1-2 µl of the preformed Zα-DNA complex solution was mixed with 1-2 µl of reservoir solution on a 22 mm silanized glass cover slip. The cover slip was placed over a well containing 500 µl of reservoir solution in a 24-well VDX crystallization dish (Hampton Research, Laguna Hills, CA). Setups were incubated at 24 °C and examined for several days/weeks. Promising conditions were varied by changing pH, temperature, precipitant concentration, ionic strength, ion composition and the use of various additives.

5.2.   X-Ray Data Collection

The crystal was mounted in a cryo loop and flash-frozen in a gaseous nitrogen stream prior to the diffraction experiment. Data collection under cryo conditions, rather than ambient temperature, largely improves the data quality. This is primarily due to less radiation damage to the crystal and less background noise. Rotation images were collected at –150 °C from single crystals. The obtained datasets were reduced with the program package DENZO/SCALEPACK (Otwinowski & Minor, 1997). The space group was determined from analysis of the Laue symmetry and the systematic absences.

5.3.   Structure Determination Using Isomorphous Replacement Techniques

The three iodine positions in the derivative Iodo-6 (see 7.2) were obtained using the automated solution procedure implemented in the program SOLVE (Terwilliger & Berendzen, 1996). The resulting single isomorphous replacement phases, including anomalous scattering (SIRAS), were used to calculate an initial electron-density map. This map was further improved by phase extension and solvent flattening with the program DM (Cowtan & Main, 1998) of the CCP4 package (CCP4, 1994).

5.4.   Structure Refinement

The initial solvent flattened SIRAS map to 2.8 Å allowed for identification of the DNA bases and the backbone of the three strands in the asymmetric unit. The peptide backbone and a large number of side chains of two of the three protein molecules in the asymmetric unit were also built unambiguously into the electron density map. One of the two protein backbones was transposed into the less well-defined density for the third protein molecule in the asymmetric unit. The resulting symmetry matrix, which transposes the three protein-DNA complexes into one another, was used together with solvent flattening and phase extension to 2.4 Å to obtain an improved SIRAS map. This map allowed for further improvement of the initial model. Prior to refinement with the program X-PLOR (Brünger, 1992a), 10% of the reflections were selected in thin shells evenly over the entire resolution range and set aside. These reflections were used for cross validation of the refinement process by using the R-factor R_{free} (Brünger, 1992b). Reflections that contribute to R_{free} were not used throughout the entire refinement process, however they were included for map caalculations. Positional refinement was carried out using the parameters deduced by Engh and Huber (1991). The model was carefully rebuilt and refined to improve the values for R_{cryst} and R_{free} at the same rate. Manual rebuilding was done with the program vuSette zc (M. A. Rould, personal communication). After several rounds of positional refinement the experimental map was improved by combining the experimental phases with calculated phases. The model was refined initially against data collected from a native crystal. Subsequent phase extension to 2.1 Å was carried out with data from the Iodo-1 derivative. In the final stage of the refinement, individual,

restrained B-factor refinement was used to improve the R-factors. Water molecules were positioned independently in the three complexes in the asymmetric unit. Non-crystallographic symmetry (NCS) restraints were not used in any step of refinement. The rationale for this was that model bias should be avoided at any step of the refinement. Including NCS restraints in the refinement process limits the independence of the complexes in the asymmetric unit.

Water molecules in the final model fulfill the following criteria:

- appearance in both a $2F_o$-F_c map and an F_o-F_c omit map contoured at 1σ and 2σ, respectively,

- stereochemical rationale,

- refined temperature factors are less than 65 Å^2.