Communications to the Editor

Conversion of λ Phage Cro into an Operator-Specific Nuclease

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Received January 23, 1991

The nucleolytic activity of chemical nucleases can be targeted by tethering them to a carrier ligand which recognizes a unique structural feature of the target nucleic acid. In this communication, we report the conversion of the λ phage Cro protein[1] into a selective nucleolytic agent by linking it to the oxidative chemical nuclease 1,10-phenanthroline–copper.[2,3]

The design of the Cro-based nucleolytic agent, shown schematically in Figure 1, has been guided by the structural model of Cro binding to DNA based on crystallographic studies and the chemical mechanism of scission of DNA by the nuclease activity of 1,10-phenanthroline–copper. The X-ray studies have suggested that Cro makes sequence-specific contacts with the recognition sequence in the major groove via a helix–turn–helix motif and directs its five C-terminal residues toward the minor groove.[4-7] Since mechanistic studies have shown that the scission chemistry of 1,10-phenanthroline–copper is initiated by oxidative attack on the C-1 hydrogen of the deoxyribose within the minor groove, an efficient, preorganized nucleolytic activity should result if a 1,10-phenanthroline moiety is linked to the C-terminus of Cro. *A novel feature of this semisynthetic nuclease is that Cro retains its high affinity to the major groove while directing the nucleolytic activity of 1,10-phenanthroline–copper toward its chemically reactive site in the minor groove.*

The nucleolytic agent has been generated by replacing the C-terminal alanine of the wild-type protein with cysteine (A66C Cro-Cys) followed by alkylation of this unique sulfhydryl group with 5-(iodoacetamido)-1,10-phenanthroline. Gel retardation assays[10,11] demonstrated that wild-type Cro, A66C Cro-Cys, and A66C Cro-Cys alkylated by 5-(iodoacetamido)-1,10-phenanthroline (A66C Cro-Cys-OP) bind with equivalent affinity to OR-3, a high-affinity binding site for the repressor.[4-7,12] The

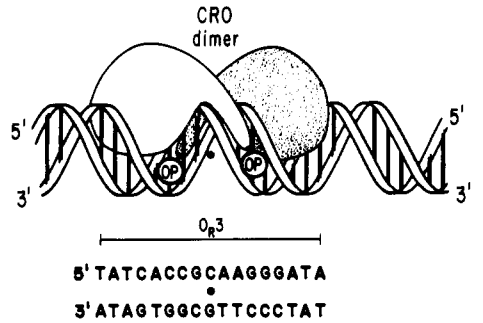

Figure 1. Schematic representation of postulated reactive complex of A66C Cro-Cys-OP to OR-3.

sulfhydryl oxidant sodium tetrathionate[13] inhibits the formation of the complex with A66C Cro-Cys but not wild-type Cro or A66C Cro-Cys-OP (Figure 2A).

The 1,10-phenanthroline–copper footprinting reaction carried out within the gel matrix of OR-3 complexes with wild-type Cro and A66C Cro are comparable[14] and confirm that the C-terminal alanine is not essential for sequence-specific interaction of the repressor protein (Figure 2B, lanes c and d).[15] To demonstrate protein-directed scission of OR-3 by the 1,10-phenanthroline–copper modified A66C Cro-Cys-OR-3 complex, the gel slice containing the retarded band (Figure 2A, lane designated (Cro-Cys-OP)$_2$ + $Na_2S_4O_6$) was immersed in a solution containing 23 μM cupric ion, 3 mM 3-mercaptopropionic acid, and 3 mM hydrogen peroxide. After 10 min, the reaction was quenched by the addition of 2,9-dimethyl-1,10-phenanthroline and the deoxyoligonucleotide products were isolated and analyzed on a sequencing gel. With OR-3 DNA labeled on either the top or botton strand, scission was observed *within* the recognition sequence. This is precisely the sequence that is *protected* from scission by the diffusible OP–Cu in the footprinting experiment. (Compare lane d and lane e of Figure 2B.) Prominent cleavages on one strand were associated with correspondingly intense sites of scission in the 3′ direction on the opposing strand, as would be expected from minor-groove attack.[16,17]

One advantage of juxtaposing the binding and reactive centers as in A66C Cro-Cys-OP is that high-affinity, specific binding to the intact substrate is unperturbed but the chemically reactive group is accessible and directed to its known primary target. The multiple sites of scission observed within the minor groove are consistent with the flexibility of the C-terminus proposed from

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(12) Bacterial cell extracts were prepared from cells, induced by IPTG and sonicated in ice-cold lysis buffer (ca. 10 mL/g cells) containing 0.1 M Tris·HCl, pH 7.5 (5 °C), 1 mM Na_2EDTA, 5% glycerol, 1.0 mM DTT, 100 μM PMSF, and 1 mg/L pepstatin. Following centrifugation to remove cell debris, the supernatant of a 5% streptomycin sulfate precipitation was precipitated with 80% ammonium sulfate. Protein pellets were resuspended in storage buffer (minus glycerol) containing 20 mM Tris·HCl, pH 7.9, 100 mM KCl, 0.2 mM Na_2EDTA, 0.5 mM DTT, 0.5 mM PMSF, ±1 mg/L pepstatin A. To alkylate with 5-(iodoacetamido)-1,10-phenanthroline, 4.0 μL of freshly prepared 100 mM MPA, pH 7-8, was added to desalted protein extracts (200 μL) containing wild-type Cro or A66C Cro-Cys, followed by incubation on ice for 60 min. Then 20 μL of freshly prepared 50 mM (solubilized by brief heating to <85°C) 5-(iodoacetamido)-OP in DMF (to 5.0 mM and 10% DMF) was added and incubated on ice for >60 min, followed by addition of 4.0 μL of 500 mM MPA, pH 7-8 (to 10 mM).

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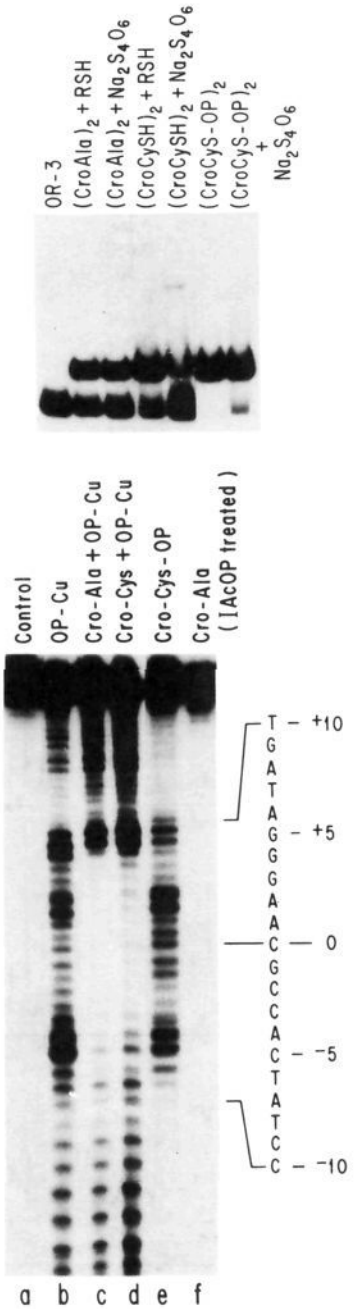

Figure 2. Top (A): Binding of Cro variants to OR-3 analyzed by gel retardation. Cro derivatives and OR-3 double-stranded DNA were incubated in a buffer composed of 10 mM Tris·HCl, pH 7.9, 1.0 mM Na_2EDTA, 50 mM KCl, 0.1 mg of BSA/mL, 10% glycerol in 15–30 μL. Cro derivatives were pretreated with either 2-mercaptoethanol (1–3 μL of 1/150 2-mercaptoethanol) or sodium tetrathionate (1–3 μL of 40 mM) for 30 min on ice (as indicated) prior to addition of nucleic acids (2 μL of 2 mg pdIdC-pdIdC/mL; 0.1 mL) (5′-^{32}P-labeled -OR-3-, ca. 3.0–12.0 × 10^5 cpm) and incubated at ambient temperature for 20 min. Except for lane designated OR-3, all other lanes also contain specific complexes formed with Cro variants and prebinding treatments as indicated above each gel lane. Bottom (B): Scission of nontemplate (upper) strand of OR-3. Lane a: probe alone. All other lanes are scission patterns of reactions carried out within the acrylamide matrix following separation of Cro-OR-3 complex by gel retardation (see Figure 2A). Footprinting reactions proceeded for 5 min; A66C Cro-Cys-OP mediated reactions, *for 10 min. All reactions were quenched by addition of 2,9-dimethyl*-1,10-phenanthroline to ca. 1.4 mM.[14] DNA fragments were recovered and analyzed as previously described.

crystallographic[6] and NMR[7] studies. Because of its ability to direct functional groups to the minor groove without sacrificing binding affinity, A66C Cro-Cys may be useful for comparing the efficiency of different DNA scission reagents. Isolation of A66C Cro-Cys variants with high affinity to different recognition sequences may provide a family of nucleolytic agents valuable in the analysis of chromosomal DNAs.

Acknowledgment. We thank Dr. C.-H. B. Chen for helpful discussions. These studies were supported by *USPHS 21199* and ONR N00014-89-J-3045.

Registry No. DNA nuclease, 9003-98-9; copper, 7440-50-8; 3-mercaptopropionic acid, 107-96-0.

High-Resolution Electrospray Mass Spectra of Large Molecules

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Impressive ionization methods for molecules of greater than 10 000 molecular weight (MW) include plasma desorption,[1] fast atom bombardment,[1] matrix-assisted laser desorption,[1] and electrospray ionization (ESI).[2,3] ESI, effective even with bovine albumin dimer (MW 133 000), has the unique advantage of producing multicharged molecular ions whose peaks are thus in the much more convenient mass/charge region of m/z 500–2000. For an ESI spectrum containing relatively few masses, m values can be derived from the larger number of m/z values because each z must be an integer value. As an alternative applicable to even complex spectra containing many masses, such as those of dissociated primary ions from tandem mass spectrometry (MS/MS, MS^n), resolution sufficient to separate the $^{13}C/^{12}C$ isotopic peaks will also define their charge state because the peak mass separation must be 1.0034 Da (daltons).[3] For instrumentation employed to date for ESI[2,3] (as well as other methods),[1] unit resolution has not been achieved for MW > 10 000, although mass-measuring accuracy of ~0.01% has been reported for samples up to MW 40 000 despite resolving powers (RP) of ~1000.[2,3] Using Fourier transform mass spectrometry (FTMS)[4] with ESI[3] of femtomole samples, we now report greatly improved RP and mass accuracy with fast broadband recording of all ions of m/z >400.

For each spectrum,[3] ions are introduced (through five pumping stages) during 7 ms (~3×10^{-15} mol of sample expended; front trapping plate 0 V, back 8 V) and allowed to cool for 1000 s (both trapping plates 8 V), the ions are excited (sweep −100 Hz μs^{-1}; both trapping plates 1 V), and the broadband signal is received (bandwidth 75 kHz, 1.7-s scan). Figure 1A clearly shows eight

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