PROTEINS: Structure, Function, and Genetics 28:137–139 (1997)

Crystallization and Preliminary X-ray Studies of I-*Cre*I: A Group I Intron-Encoded Endonuclease From *C. reinhardtii*

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ABSTRACT **Group I intron endonuclease I-*Cre*I is encoded by an open reading frame contained within a self-splicing intron in the *Chlamydomonas reinhardtii* chloroplast 23S rRNA gene. I-*Cre*I initiates the lateral transfer or *homing* of this intron by specifically recognizing and cleaving a pseudopalindromic 19–24 bp homing site in chloroplast 23S rRNA genes that lack the intron. The gene encoding this enzyme has been subcloned, and the protein product has been purified and crystallized. The crystals belong to space group P321, with unit cell dimensions $a = b = 78.2$ Å, $c = 67.4$ Å. The crystal unit cell is consistent with an asymmetric unit consisting of the enzyme monomer. The specific volume of this unit cell is 3.3 Å^3/Da. The crystals diffract to at least 3.0 Å resolution after flash-cooling, when using a rotating anode x-ray source and an RAXIS image plate detector. Proteins 28:137–139, 1997.**

Key words: intron mobility; endonucleases; intervening sequences; DNA binding

INTRODUCTION

Mobile intron endonucleases are a diverse family of proteins encoded by open reading frames contained within Group I self-splicing introns, or in self-splicing protein introns termed "inteins." The catalytic activity of these endonucleases leads to their self-propagation by a targeted transposition mechanism termed intron mobility or intron homing. Mobility or homing is initiated and targeted by the endonuclease. The enzyme makes a highly site-specific DNA double-strand break in a 15–40 bp homing site on an allele that lacks the self-splicing intron (an "intron-less" allele). The intron containing the endonuclease gene is subsequently transferred to the cleaved recipient allele by a double-strand break repair/gene conversion event. Mobile intron endonucleases have been identified in a diverse collection of lower eukaryotes, Archea, and eubacteria, with the largest number encoded in organellar genomes.[1–4]

We have purified and crystallized a member of the Group I intron-encoded endonuclease family, the *Chlamydomonas reinhardtii* I-*Cre*I intron-encoded endonuclease. In the presence of Mg^{2+}, I-*Cre*I recognizes and asymmetrically cleaves both strands of the 19–24 bp I-*Cre*I homing site, to leave 4 base 3′ extended tails of sequence 5′-GTGA-3′ and 5′-TCAC-3′. The degree of sequence degeneracy that can be tolerated within this site has not yet been determined.[5,6] The I-*Cre*I endonuclease was originally identified as an open reading frame contained within a self-splicing Group I intron in the *C. reinhardtii* chloroplast 23S rRNA gene.[7] This open reading frame encodes a 163 amino acid protein of molecular weight ~18 kDa.[8,9] The I-*Cre*I endonuclease contains a single copy of the "dodecamer" or "LAGLIDADG" motif at residues 13–21[3–5]. This motif is believed to play a role in strand scission in other intron- or intein-encoded endonucleases.[3,4,10–12] We have begun structural studies of this protein to understand the processes of homing site recognition and cleavage by I-*Cre*I and by other intron-encoded endonucleases.

METHODS AND RESULTS

The I-*Cre*I endonuclease has been overexpressed and purified from an *Escherichia coli* host system.[13] The preparation of the enzyme consists of an ammonium sulfate fractionation followed by three chromatographic steps to yield a 60-fold purified protein. The final yield using this procedure was 13 mg protein from 4 l of bacterial cell culture. The protein used for crystallization was at 2 mg/ml in 20 mM Hepes, pH 7.5. Conditions for crystallization were initially determined using a commercially available sparse matrix screening kit.[14] Small, reproducible crystals were obtained from hanging drop experiments designed on the basis of results from the initial screening. The crystals used for data collection were grown by vapor diffusion in this geometry against reservoirs containing 20% PEG 6K, 100 mM acetate,pH 5.0. At 22°C, these grew within 1 month to average dimensions of 0.2 × 0.1 × 0.1 mm. The

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Received 3 December 1996; Accepted 3 December 1996

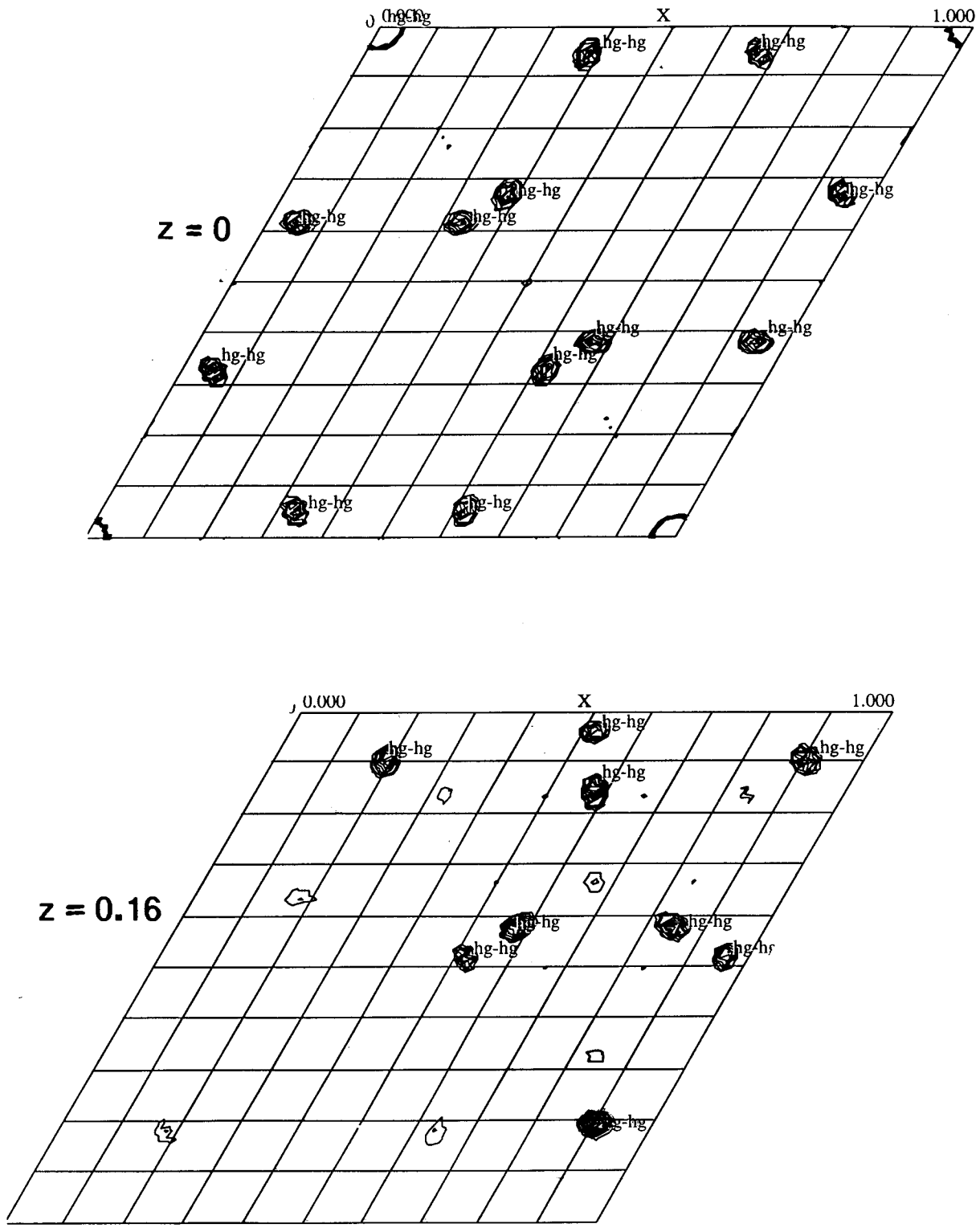

Fig. 1. Harker sections (z = 0 and 2z) showing strong self-symmetry peaks for single bound mercury atom in an isomorphous difference patterson map for I-*Cre*I. Phasing power of derivative to 3 Å resolution is 1.83.

largest specimens grew to approximately 0.3 mm in length. The crystals diffracted to 3.0 Å resolution. All data were collected using flash-cooled specimens: The crystals were stabilized for cooling by stepwise transfer to a final solution containing 20% PEG 6K, 20% glycerol, 20% glucose in 100 mM acetate, pH 5.0.

TABLE I. Native Data Statistics

Resolution shell				
Lower	Upper	R_{symm}	Reflections	Completeness
50.00	8.13	0.016	248	84.9
8.13	6.46	0.026	232	88.5
6.46	5.64	0.047	234	92.1
5.64	5.13	0.040	238	90.2
5.13	4.76	0.037	229	92.0
4.76	4.48	0.038	227	93.0
4.48	4.26	0.046	231	92.8
4.26	4.07	0.054	238	93.3
4.07	3.91	0.064	235	94.0
3.91	3.78	0.084	238	94.4
3.78	3.66	0.103	239	93.7
3.66	3.56	0.119	213	95.9
3.56	3.46	0.131	244	93.5
3.46	3.38	0.165	231	96.3
3.38	3.30	0.185	233	93.6
3.30	3.23	0.251	237	96.3
3.23	3.17	0.297	236	94.4
3.17	3.11	0.374	232	95.9
3.11	3.05	0.456	227	95.0
3.05	3.00	0.494	226	91.5
All hkl		0.059	4668	93.0

The crystal class was determined to be trigonal, space group P321, with cell dimensions of $a = b =$ 78.2 Å, $c = 67.4$ Å. The presence of the crystallographic twofold axis and the absence of a screw-axis along the principle threefold were verified by analysis of multiple datasets that were collected and initially indexed in the P3 space group by using the DENZO/SCALEPACK crystallographic data reduction package[15] and the CCP4 program HKLVIEW.[16] This space group assignment was confirmed by subsequent Patterson analysis of a single-site mercurial derivative (Fig. 1). The unit cell dimensions and diffraction are consistent with an asymmetric unit consisting of the enzyme monomer with a specific volume of 3.3 Å^3/Da.[17] A native dataset was collected from a single crystal by using Cu-Ka radiation from a Rigaku RU-200 rotating anode operating at 50 kV and 100 mA and an RAXIS-II imaging plate area detector (Molecular Structure Corporation). The crystal was looped directly from the stabilization solution described above after a 60-second soak, immersed in liquid nitrogen, and then immediately transferred for data collection to a stream of N_2 gas at −170°C. Reflections to 3.0 Å resolution were recorded at a crystal to detector distance of 160 mm. Data were reduced using the DENZO/SCALEPACK crystallographic data reduction package. The overall R factor of symmetry-related reflections for the 93% complete dataset was 0.056% on I (Table I).

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