

FOR THE RECORD

X-ray grade crystals of a designed α -helical coiled coil

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Parallel α -helical coiled coils are found in fibrous proteins such as tropomyosin, myosin, paramyosin, and intermediate filament proteins (Cohen & Parry, 1986). This coiled coil motif has been recognized by its distinctive signature, a periodic repeat of apolar residues at positions *a* and *d* in a heptad repeat of the form *abcdefg*. Recently, short stretches of this heptad repeat have been discovered in the eukaryotic transcription activators such as Fos, Jun, and GCN4 (Johnson & McKnight, 1989; Mitchell & Tjian, 1989). The significantly higher occurrence of leucine at the heptad repeat position *d* of the transcription activators led to an alternative model for the structure of this class of proteins. In the alternative model, the leucine side chains in the *d* position of two straight parallel helices interdigitate with one another at the dimer interface to form a leucine zipper (Landschulz et al., 1988). However, both two-dimensional nuclear magnetic resonance (Oas et al., 1990) and X-ray diffraction studies (O'Shea et al., 1991) confirm that this peptide is of the family of the coiled coil motifs. In the manner proposed by Crick (1953), the side chains in the *a* and *d* positions of the heptad repeat are packed "knobs-into-holes" (O'Shea et al., 1991).

To gain an atomic model of another coiled coil, O'Neil and DeGrado (1990) synthesized a peptide that is designed to dimerize into a parallel α -helical two-stranded coiled coil. This design originates with the work of Hodges and coworkers, who have synthesized a series of repeating heptapeptides to mimic the structural properties of tropomyosin (Hodges et al., 1981). Their most successful heptapeptide repeat was modified and extended to the sequence Ac-EWEALEKKLAALX-KLQALEKKLEALEHG-CONH₂, where Ac- is CH₃CO- and X is the host to any

one of the 20 natural amino acids. These peptides are named coil-X, where X is the name of the guest amino acid. Like the GCN4 peptide, all four *d* positions are occupied by Leu. Analytical ultracentrifugation and circular dichroism indicate that coil-X dimerizes in aqueous solution to form stable α -helical dimers as designed (O'Neil & DeGrado, 1990).

Crystals of coil-Ser were grown by the hanging drop method. Drops were prepared by mixing 5 μ L of a 2-mg/

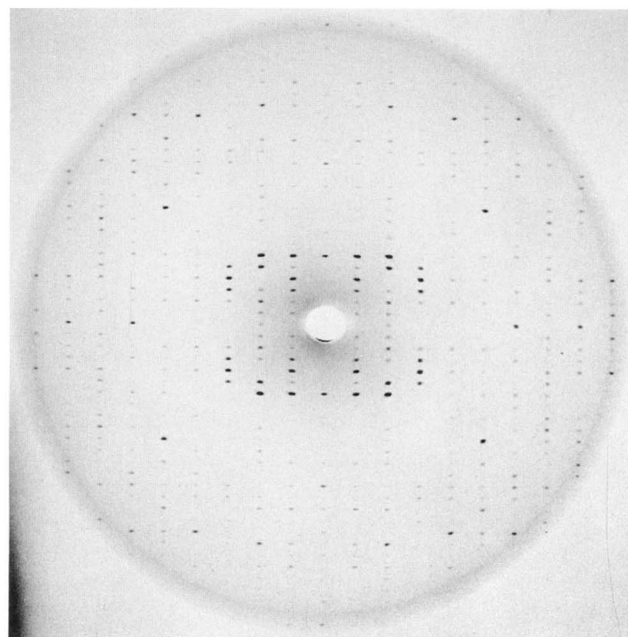


Fig. 1. A screened 15° precession photograph of the *h0l* zone. This photograph reveals Laue symmetry *mm* and the absence of reflections with odd indices of the classes *h00* and *00l*. An additional precession photograph of the *hk0* zone reveals Laue symmetry *mm* and the additional absence of reflections with odd indices of the class *0k0*, indicating the space group *P2₁2₁2₁*.

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mL peptide solution, and 5 μ L of reservoir solution, which consisted of a 0.05 M potassium phosphate-sodium hydroxide buffer, pH 5.05, and 2.95 M ammonium sulfate. All drops were prepared using an Accuflex pipetting station (ICN Micromedic, Huntsville, Alabama). Crystalline rods measuring $0.5 \times 0.1 \times 0.1$ mm grew within approximately 3 weeks at 22 °C.

The crystals are suitable for structure determination by X-ray diffraction. Screened 15° precession photographs were taken of the $hk0$ and $h0l$ zones (Fig. 1). These photographs reveal Laue symmetry mm and the absence of reflections with odd indices of the classes $h00$, $0k0$, or $00l$, indicating the space group $P2_12_12_1$. The unit cell dimensions are $a = 27.81$ Å, $b = 38.81$ Å, and $c = 77.34$ Å. There is a peptide trimer or dimer in the asymmetric unit (corresponding, respectively, to $V_m = 2.07$ or 3.11 Å³ Da⁻¹) (Mathews, 1968). Although a trimer seems inconsistent with the analytical centrifugation data (O'Neil & DeGrado, 1990), three-stranded coiled-coil proteins are found in nature and we cannot rule out this possibility (for a review see Cohen & Parry, 1990). Native data to 2.15 Å resolution have been collected, and heavy atom adducts are being sought to solve the structure of coil-Ser by multiple isomorphous replacement.

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