Crystallization and Preliminary X-ray Investigation of Sarcoplasmic Calcium-binding Protein from Nereis diversicolor*

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Crystals of sarcoplasmic calcium-binding proteins from Nereis diversicolor have been grown from solutions of ammonium sulfate. The crystals are monoclinic, space group P21; the axes are a = 43.65 (1), b = 56.05 (1), c = 65.77 (1) Å, and β = 92.58 (2)°. The crystals are quite stable to X-rays and diffract beyond 2.5 Å resolution. The asymmetric unit contains two protein molecules.

Sarcoplasmic calcium-binding proteins (SCPs)1 are an important Ca2+-binding system in both vertebrate and invertebrate muscle (1). Those found in vertebrates are called parvalbumins, and in invertebrates those Ca2+-binding proteins that includes calmodulin and troponin C. The SCPs found in invertebrates display properties distinct from those of parvalbumins, although they share some sequence homology. Unlike parvalbumins, which are essentially confined to striated skeletal muscle, SCPs are present in a wide range of muscle types. The function of SCPs is not yet known.

SCPs from two species of sandworm have been isolated and sequenced: Nereis diversicolor (2, 3) and Perinereis vancaucurata tetradentata (4). Each is a single polypeptide chain that contains 174 amino acids and has a molecular weight of about 19,500. There are 23 amino acid differences between the two proteins. It has been shown that sandworm SCP has three Ca2+-Mg2+ mixed sites, although Ca2+ uptake causes a release of Mg2+ (2). Sequence homology of sandworm SCPs with Ca2+-binding proteins of known three-dimensional structure suggests that they originally contained four Ca2+-binding domains with the so-called EF-hand structure (5). Three EF-hand type Ca2+-binding domains (I, III, and IV) can be identified in each protein by sequence homology with related Ca2+-binding proteins. The portion of the sequence corresponding to domain II has had a number of amino acid replacements and deletions and probably does not bind Ca2+.

EXPERIMENTAL PROCEDURES

Nereis SCP was isolated and purified as described by Cox and Stein (2). For crystallization experiments, the lyophilized material was dissolved in distilled water containing 2.5 mM CaCl2. Crystals were obtained by vapor diffusion equilibration of 2-μl drops hanging from siliconized cover slips inverted on Linbro plates. The drops consisted of 1 μl of a solution containing 15 mg of SCP/ml of distilled water and 2.5 mM CaCl2, plus 1 μl of a solution containing 72% ammonium sulfate in 0.05 M HEPES buffer, pH 7.6. These drops were equilibrated against 1 ml of a solution containing 72% ammonium sulfate in 0.05 M HEPES buffer, pH 7.6. After 6–8 days at 22 °C, small monoclinic plates with dimensions up to 0.06 × 0.06 × 0.02 mm were obtained. However, these never grew larger than about 0.1 mm on edge, so seeding was used. A single, medium-size crystal was added to a 2-μl drop containing 15 mg of SCP/ml, 2.5 mM CaCl2, and 63% ammonium sulfate in 0.05 M HEPES buffer, pH 7.8. These drops were equilibrated against 1 ml of a solution containing 63% ammonium sulfate in 0.05 M HEPES buffer, pH 7.8. After 2–3 days at 22 °C, large, monoclinic prisms with dimensions up to 0.4 × 0.4 × 0.3 mm were obtained. For X-ray studies, crystals are transferred to a stabilizing solution of 80% ammonium sulfate in 0.05 M HEPES buffer, pH 7.8.

RESULTS AND DISCUSSION

X-ray precession photographs show that the crystals are monoclinic; space group P21 is indicated by the systematic absence of reflections 0k0 with k odd (Fig. 1). Unit cell parameters, which were obtained by least-squares refinement of the angular settings for 25 reflections measured on a diffractometer, are a = 43.65 (1), b = 56.05 (1), c = 65.77 (1) Å, and β = 92.58 (2)°. Assuming an occupancy of two 19,500-dalton chains per crystallographic asymmetric unit, and a

![Fig. 1. Precession photograph of the h00 zone of Nereis SCP, β = 15° (3.0 Å resolution).](image-url)
Crystal Data for Sarcoplasmic Ca\textsuperscript{2+}-binding Protein

partial specific volume of 0.74 ml/g, $V_m = 2.06 \, \text{Å}^3$/dalton (6), and the solvent volume fraction is 40%. For one molecule in the asymmetric unit, the corresponding values are 4.12 \, \text{Å}^3/dalton and 70%, respectively, so it appears likely that the asymmetric unit contains two protein molecules.

The crystals are stable indefinitely in the artificial mother liquor at room temperature. They diffract to at least 2.5 \, \text{Å} resolution on a rotating anode and show very little decomposition in the x-ray beam after 4 days of data collection on a diffractometer. Therefore, they are suitable for high resolution x-ray analysis.

REFERENCES