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SHORT COMMUNICATIONS

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Crystallization and preliminary X-ray analysis of human endothelin.

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Abstract

Endothelin, a potent regulator of vasoconstriction and hypertension, is a naturally produced peptide of 21 amino acids containing two disulfide bonds. We have crystallized endothelin from humans using the vapor-diffusion technique, characterized the crystals by X-ray diffraction analysis, and have collected the X-ray intensities to a resolution of 1.8 Å. The crystals, which demonstrate physical properties similar to most protein crystals and have a comparable solvent content, are hexagonal prisms that frequently grow to lengths of 400 μm and widths of 150 μm. The space group of the crystals is $P6_122$ (or $P6_522$), with $a = 27.4$, $c = 79.6$ Å. There is one molecule of endothelin in the asymmetric unit of the crystals.

Introduction

Endothelin is a contractile peptide of 21 amino-acid residues isolated from aortic endothelial cells. It was discovered in 1988 and subsequent investigation has shown it to play an important role in the maintenance of blood pressure and in some pathophysiological phenomena such as hypertension and vasospasm (Vane, 1990). The exact nature and basis of these effects are, however, still not known.

The sequence of the peptide, though rather short, contains four cysteine residues which form two disulfide bridges. Endothelin is produced *in vivo* as a large peptide of 203 residues which is subsequently cleaved by processing enzymes in a series of steps to its active form. In isolated vascular strips endothelin is a potent and slow-acting contractile agent and *in vivo* a single dose elevates blood pressure in 20 to 30 min. Other properties of endothelin include inhibition of renin release, stimulation of ANF release, and a positive inotropic action in guinea pig atria (Anggard, Botting & Vane, 1990; Masaki, 1989; Vane, 1990).

Materials and methods

Synthetic human endothelin was purchased from Clinalfa Co. (Laufelfingen, Switzerland) as a 50 mg lot. Vapor-diffusion methods based on the Cryschem crystallization plate (Charles Supper Co., Natick, MA) were used according to McPherson (1990) to identify conditions that would yield crystals. Most of these initial crystals were morphologically disordered or of too small a size. X-ray examination showed them to be internally disordered and unsuitable for analysis. Further refinement of conditions, however, ultimately yielded crystals that were reproducible and entirely acceptable for X-ray data collection.

Prior to generating the crystallization trials, the lyophilized peptide was dissolved in distilled water. The crystallization samples yielding the best crystals were then formed by mixing 4 μl of a 15 mg ml^{-1} peptide solution with 2 μl of 0.2 *M* MES buffer, pH 6.0–7.0, and adding to this an equal volume of the reservoir which was 20 to 25% 2-methyl-2,4-pentanediol (MPD). Equilibration was carried out at 298 to 310 K for a period of three to seven days before the crystals were mounted for analysis in 0.5 mm quartz capillaries.

Generally many crystals grow in each trial, but a few are sufficiently large for diffraction purposes and the remainder can be recovered for subsequent experiments. A number of crystal habits have been obtained in addition to that employed for data collection, but we cannot be certain if they represent alternate unit cells.

The crystals were mounted by conventional methods in quartz capillaries and photographed with Ni-filtered Cu $K\alpha$ X-rays on a Buerger precession camera with a crystal-to-film distance of 90 mm. The X-ray source was an Enraf–Nonius generator fitted with a fine focus sealed X-ray tube and operated at 45 kV and 32 mA. Exposure times were generally no more than a few hours given that the crystals diffracted, in spite of their rather small size, very strongly.

The X-ray diffraction data to 1.8 Å resolution were collected using an area-detector system manufactured by San Diego Area Detector Systems with a crystal-to-detector distance of 420 mm. The X-ray source was a Rigaku RU-200 generator operated at 200 kV and 50 mA. A graphite monochromator was employed to obtain Cu $K\alpha$ radiation. Because of the relatively small unit cell, the strength of the intensities, and the slow decay of the crystals, the data could be collected very quickly. To insure accurate data all reflections or their symmetry equivalents were recorded a minimum of 12 times.

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Results

The best endothelin crystals were well formed hexagonal prisms of maximum length 400 μm and widths of 150 μm. Larger crystals were observed but these were generally twinned about the sixfold axis. Precession photography showed the reciprocal lattice to have *6mm* symmetry and all 00*l* reflections to be systematically absent except those for which $l = 6n$. The space group is therefore $P6_122$, or its enantiomorph $P6_522$, and the unit-cell dimensions are $a = 27.4$, $c = 79.8$ Å.

The volume of the unit cell is 5.06×10^4 Å^3 and if one assumes one molecule of endothelin, $M_r = 2460$, as the asymmetric unit, the volume-to-mass ratio for these crystals is 1.71 Å^3 per dalton. This value is near the low end, but well within the range observed for most protein crystals (Matthews, 1968). It is particularly reasonable for small proteins and peptides that might be expected to contain less solvent in proportion to protein mass and it represents a solvent volume of about 30%.

As already noted, the crystals diffract to beyond 1.8 Å resolution in their present state and very likely would produce data to higher resolution were they larger. The crystals do not decay at an unreasonable rate and we can normally collect several complete data sets from a single crystal.

Using our current data we are attempting to determine the three-dimensional structure of endothelin by a variety of techniques including molecular searches with model structures, direct methods and conventional MIR techniques.

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Structure refinement of commensurately modulated bismuth tungstate, Bi_2WO_6. Erratum.

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Abstract

Owing to a printer's error, Fig. 7 of the paper by Rae, Thompson & Withers [*Acta Cryst.* (1991), B**47**, 870–881] was published in the wrong orientation. The correct Fig. 7 is given.

All relevant information is given in the *Abstract*.

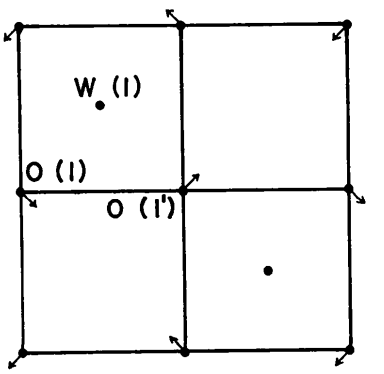

Fig. 7. The *Abam* displacive mode showing the 9·0° rotation of WO_6 octahedra about axes parallel to **c**. This mode can be either *A*-centred (*Abam* symmetry) or *B*-centred (*Bbam* symmetry). The majority component has *Abam* symmetry.

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