SHORT COMMUNICATIONS

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Crystallization and preliminary analysis of two crystal forms of human Clara cell 16 kDa protein (CC10).

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Abstract

The human Clara cell 16 kDa protein (CC10), isolated from lung lavage fluid, has been crystallized in two crystal forms. The first is in space group $P1$ and has cell parameters a = 43.04, b = 45.90, c = 51.29 Å and α = 62.46, β = 69.74, γ = 69.43°. Two molecules are present in the unit cell. The second form is in space group $P222$, with cell parameters a = 42.24, b = 84.06, c = 40.05 Å and $\alpha = \beta = \gamma = 90°$, and four molecules per unit cell. Its diffraction pattern displays pseudo-body-centered symmetry. Both crystal forms diffract X-rays beyond 2.0 Å.

Introduction

The human Clara cell 16 kDa protein (CC10) is secreted specifically from the Clara cells, which predominate in the bronchioles of the lung (Singh *et al.*, 1988). Clara cells are non-ciliated non-mucous secretory cells, which have been reported to play a role in the metabolism of xenobiotics and the renewal of damaged distal airway epithelium (Boyd, 1977; Evans, Cabral-Anderson & Freeman, 1978). Human CC10 is a homodimer with a molecular weight of 15 800 Da, composed of two identical 70 amino-acid residue chains. These chains are covalently bound together in an antiparallel manner by two disulfide bonds. The apparent molecular weight based on gel electrophoresis was originally reported to be 10 kDa (Singh, Katyal & Gottron, 1985), and hence the protein has been referred to as CC10 in the literature.

The protein binds polychlorinated biphenyls (PCB's) (Andersson *et al.*, 1991). It has also been reported to inhibit phospholipase A_2 (Singh *et al.*, 1990) and to be a substrate of transglutaminase (Mantile *et al.*, 1993). The physiological function of this protein is unknown, but it has been proposed to be an immunosuppressive agent and to prevent potentially harmful inflammatory responses of the lung. CC10 has a 55.7% sequence homology to rabbit uteroglobin and a 54.3% sequence homology to the corresponding rat Clara cell protein (Singh *et al.*, 1990).

Crystallization

Two different crystal forms of human CC10 have been grown, a triclinic form and an orthorhombic form. The triclinic CC10 crystals were grown using the vapor-diffusion method, and the crystallization conditions were based on the parameters under which rat CC10 crystals may be grown (Swaminathan *et al.*, 1990; Umland *et al.*, 1992). The crystallization droplet was composed of 100 µl of protein at a concentration of 100 µg ml^{-1} and 20 µl of reservoir solution. This was equilibrated against a reservoir solution of 60% of saturated ammonium sulfate in 10 m*M* Tris–HCl buffer (pH 7.0). After the appearance of a small amount of precipitate, the reservoir was changed to 70% saturated ammonium sulfate and 10 m*M* Tris–HCl buffer (pH 7.0). The crystals grew to a size of ~0.15 × 0.15 × 0.10 mm in about a week, and had a rectangular prism shape. However, if the crystals were undisturbed for several months, they occasionally grew to a larger size, ~0.35 × 0.25 × 0.2 mm. As well as diffracting X-rays to a much higher resolution, these larger crystals were much more stable in the X-ray beam. The crystals are in space group $P1$, with unit-cell parameters of a = 43.05, b = 45.78, c = 51.06 Å, and α = 62.83, β = 70.16, γ = 69.63°. The Matthews coefficient (Matthews, 1968) was determined to be 2.59 Å^3 Da^{-1}, assuming two dimers per unit cell.

Large diffraction-quality crystals of CC10 of the triclinic form could not be reproducibly obtained. Thus, variations in the crystallization conditions were attempted in order to bring about more controlled growth of crystals. Together with the normal fine screening of the variables for the conditions which produced the triclinic crystals (pH, precipitating agent concentration, buffer *etc.*) the use of glycerol was investigated (Sousa, Lafer & Wang, 1991; Pechik, Nachman, Ingham & Gilliland, 1993). A crystallization droplet of 20 µl of protein at 100 µg ml^{-1} and 20 µl of reservoir solution was equilibrated against a reservoir of 70% saturated ammonium sulfate, 20 m*M* Tris–HCl buffer (pH 7.5) and 16–18%(*v*/*v*) glycerol. A limited number of small crystals appeared within three or four days. These continued to grow slowly for approximately a week after the crystals first appeared. The crystals were diamond-shaped plates, with generally poorly formed faces. The maximum size that these crystals grew to was ~0.20 × 0.20 × 0.07 mm. These crystals were readily grown and crystallized in space group $P222$ with cell dimensions a = 42.24, b = 84.06, c = 40.05 Å, and $\alpha = \beta = \gamma = 90°$. The Matthews coefficient is 2.25 Å^3 Da^{-1}, assuming four dimers per unit cell.

Results and discussion

X-ray diffraction data from single crystals of both crystal forms of CC10 were collected using a Siemens X100 multiwire area

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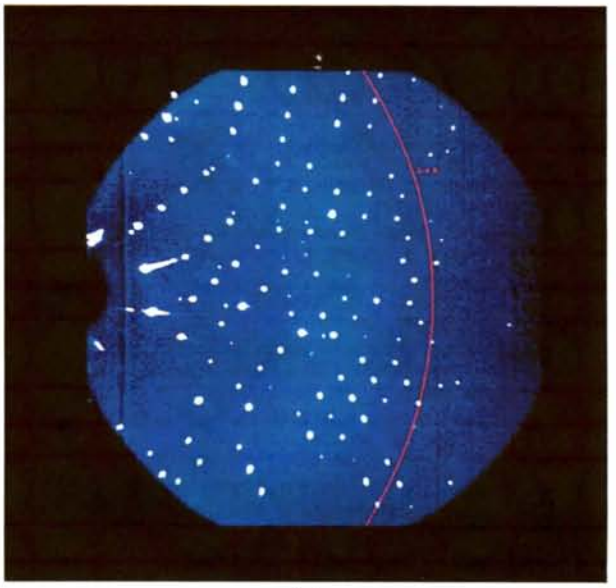
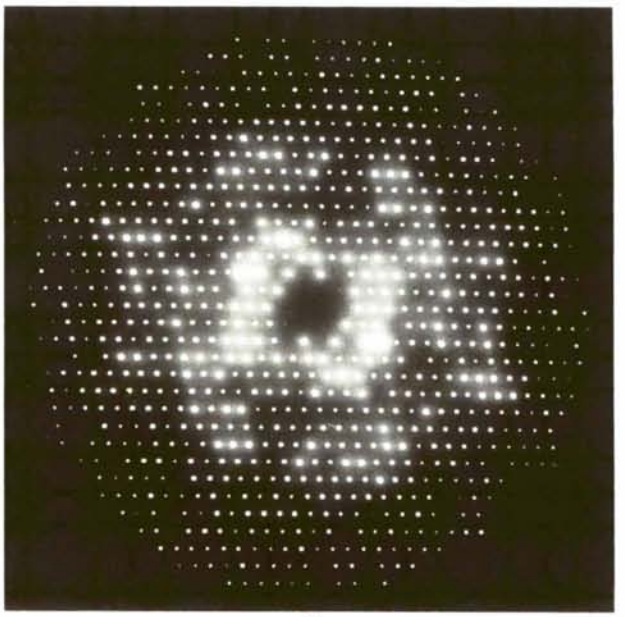
detector. The Cu $K\alpha$ X-rays were produced by a Rigaku RU200 rotating-anode X-ray generator operated at 42 kV and 65 mA, and were filtered using a Ni foil and focused using Franks mirrors.

The software by Blum, Metcalf, Harrison & Wiley (1987) was used for data collection. The data was then processed using *XENGEN* (Howard, Gilliland, Finzel & Poulos, 1987) through the integration of the reflections, and then the locally modified scaling programs of Weissman (1982) were used to merge the data.

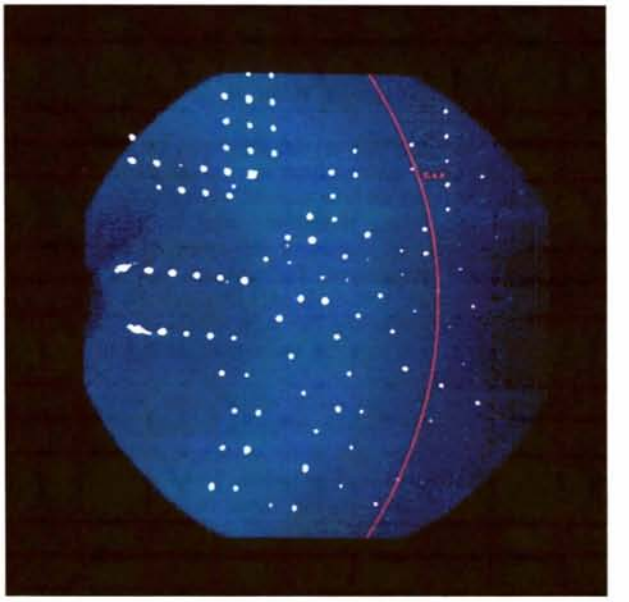


(*a*)

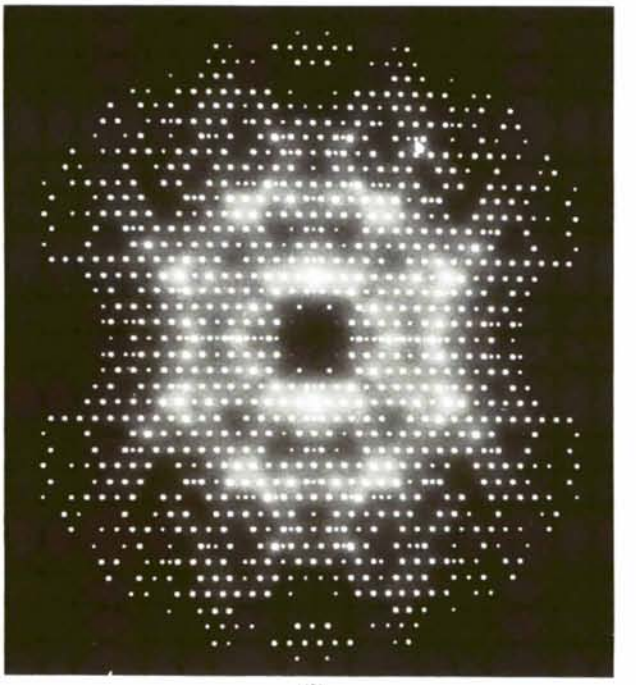


(*b*)

Fig. 1. (*a*) The superposition of eight consecutive area-detector data frames (0.25° oscillation per frame) collected from the *P*1 crystal form of human CC10. The red arc indicates 2.4 Å resolution. (*b*) A pseudo-precession photograph of the *h*0*l* zone of the *P*1 crystal form of human CC10. This was generated by *PRECESS* (W. Furey, unpublished work) from data collected on the Siemens area detector. Data up to 2.4 Å resolution are displayed.



(*a*)



(*b*)

Fig. 2. (*a*) The superposition of eight consecutive data frames (0.25° oscillation per frame) collected from the *P*222 crystal form of human CC10. The red arc indicates 2.4 Å resolution. (*b*) A pseudo-precession photograph of the 0*kl* zone of the *P*222 crystal form of CC10 generated by *PRECESS* (W. Furey, unpublished work). This contains data up to 2.0 Å resolution with $I \geq 2\sigma(I)$. The pseudo-body-centered nature of this crystal form is apparent, where many of the $h + k + l = 2n + 1$ reflections are either weak or absent. The arrow points at the 0,14,13 reflection.

Data were first collected on the triclinic crystal form. These crystals diffracted strongly to 2.4 Å, and reflections could be observed to beyond 2.0 Å. (See Fig. 1.) However, the size of the crystal was crucial. The smaller routinely obtained crystals tended to decay rapidly within the X-ray beam. However, it was possible to collect a data set from two crystals which was 97.2% complete to 2.5 Å, containing 16 342 unique reflections with $I \geq 1\sigma(I)$. The merging R factor, $R_{\rm merge} = \Sigma|I - \langle I\rangle|/\Sigma\langle I\rangle$, was 5.81%.

The possibility that this crystal form may be assigned to a crystal system other than triclinic was investigated. However, only the choice of the triclinic unit cell provided an acceptable indexing of the strong reflections used by *XENGEN* for auto-indexing and the subsequent refinement of unit-cell and camera-geometry parameters. This triclinic lattice was then analyzed to determine if it could also be described by a lattice containing higher symmetry. No such lattice was found, and thus it was concluded that the space-group assignment of $P1$ was correct.

Data were then collected on the orthorhombic crystal form of CC10. There was some initial difficulty in assigning the space group. The preliminary diffraction studies involved relatively short X-ray exposure times per frame. When this preliminary data was processed, very few reflections of the type $h + k + l = 2n + 1$ were observed. This raised the possibility that the unit cell was body centered, having the space group $I222$. However, the collection of data from higher quality crystals and with longer exposure times proved that the crystal lattice was not body centered. While in general the $h + k + l = 2n + 1$ reflections were weak, a small but significant number were considered observed, with $I \geq 2\sigma(I)$. The Patterson function indicated a pseudo-origin peak at $(u,v,w) = (\frac{1}{2},\frac{1}{2},\frac{1}{2})$ with a height 95.4% that of the origin peak. This was a further indication of the pseudo-body-centered nature of the unit cell. The self-rotation function indicated that the CC10 dimer twofold axes lie parallel to crystallographic twofold axes. The breaking of the centering symmetry may be due to the pseudo-body-centered dimers having slightly different rotational orientations about their dimer axes caused by crystal-packing forces. The crystals diffracted beyond 2.0 Å. Currently, the data set contains 4903 unique reflections with $I \geq 2\sigma(I)$, of which 4499 are of the type $h + k + l = 2n$ (84.3% of this type are complete at 2.0 Å), and 404 are of the type $h + k + l = 2n + 1$ (only 7.7% of this type are complete at 2.0 Å), and with $R_{\rm merge} = 7.26\%$. It is interesting to note that in any resolution shell up to 2.0 Å, the percentage of the $h + k + l = 2n + 1$ reflections which were observed remained relatively constant at ~10% of the total possible. (See Fig. 2.)

Currently, the structure determination of both crystal forms of human CC10 is underway. The molecular-replacement method is being employed, with the search model being the rat CC10 dimer (Umland *et al.*, 1992).

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