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Crystallization and initial X-ray analysis of β-crustacyanin, the dimer of apoproteins A_2 and C_1, each with a bound astaxanthin molecule

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Abstract

Crystals of β-crustacyanin, a carotenoid-binding protein from lobster carapace, have been grown under oil from solutions containing sodium potassium phosphate as precipitant. They grow slowly over a period of months to reach maximal dimensions of 0.5 × 0.1 × 0.1 mm, and belong to space group *P*622 with cell dimensions: $a = b = 124.39$, $c = 188.86$ Å and $\gamma = 120°$. The crystals diffract to beyond 3 Å but are very radiation sensitive, limiting the resolution of usable data. The unit-cell volume suggests that there are two β-crustacyanin molecules per asymmetric unit.

1. Introduction

The blue astaxanthin-binding carotenoprotein, crustacyanin, in the calcified outer layer of the lobster carapace helps to camouflage the crustacean in its natural environment. The native protein, α-crustacyanin, has a molecular weight of about 350 kDa and dissociates at low ionic strength to form, irreversibly, the purple derivative β-crustacyanin. α-Crustacyanin consists of eight β-crustacyanin units, each of which binds two astaxanthin molecules. Removal of astaxanthin results in reversible dissociation into apoprotein subunits which are half the size of β-crustacyanin (Quarmby, Norden, Zagalsky, Ceccaldi & Daumas, 1977). The apoprotein consists of five electrophoretically distinct forms, which fall into two types: C_1, C_2 and A_1 (type I), and A_2 and A_3 (type II) (Keen, Caceras, Eliopoulos, Zagalsky & Findlay, 1991). β-Crustacyanin is a heterodimer of a type I subunit and a type II subunit, each with a bound astaxanthin molecule. Sequence analyses have revealed that the apoproteins belong to the lipocalin family, whose members are small extracellular proteins with high affinity for small hydrophobic molecules, *e.g.* retinol-binding protein, bilin-binding protein and mouse major urinary protein. They share a common tertiary structure, which contains an eight-stranded β-barrel (North, 1991). Astaxanthin undergoes a large bathochromic spectral shift in the crustacyanins, similar to that of retinal in the visual pigment, rhodopsin (Wald, Nathanson, Jencks & Tarr, 1948).

Extensive crystallization experiments have so far failed to yield well ordered crystals of α-crustacyanin. Crystals of type I apoprotein have been obtained diffracting to high resolution both without (Wright *et al.*, 1992) and with bound ligand (E. J. Gordon & C. E. Wright, unpublished results); recently, crystals of type II apoprotein, diffracting to lower resolution (5–4.5 Å) have been prepared (EJ. Gordon, unpublished results). In this communication we report the crystallization of β-crustacyanin, the dimer formed from the main apoproteins, A_2 and C_1, with two molecules of astaxanthin. The structure of β-crustacyanin will provide information about the carotenoid binding and provide a structural basis for understanding the observed bathochromic shift for astaxanthin upon oligomerization. β-Crustacyanin is of great structural interest as it bridges two important structural families: the lipocalins, small extracellular proteins which bind small hydrophobic molecules; and a large diverse family of biologically important proteins which form polyene–protein complexes, for example, visual pigments and bacteriorhodopsin.

This work is part of a larger project encompassing the structure solution of the individual subunits, and structural analysis of the intact α-crustacyanin and other carotenoid-binding proteins.

2. Experimental procedures

2.1. *Protein purification*

α-Crustacyanin was extracted from lobster carapace and purified by ion-exchange chromatography (Keen *et al.*, 1991). The protein was dissociated into β-crustacyanins by extensive dialysis against 0.3 m*M* Tris–HCl, pH 8.8. The relevant β-crustacyanin (β_{1a}) was resolved from the mixture of dimers by fast protein liquid chromatography on a MonoQ HR5/5 column with a linear gradient (0–60% *B*) made from buffer *A* (5 m*M* Tris–HCl, pH 7) and buffer *B* (5 m*M* Tris–HCl–0.05 *M* KCl, pH 7) and run at 0.5 ml min^{-1}.

2.2. *Crystallization*

Extensive crystallization experiments using vapour-diffusion techniques failed to yield well ordered crystals of β-crustacyanin. Initial conditions for successful crystallization were obtained in 2 µl drops by the microbatch under-oil method (Chayen, Shaw Stewart, Maeder & Blow, 1990; Chayen, Shaw Stewart & Blow, 1992), using a sparse-matrix screening protocol (Jancarik & Kim, 1991).

Crystallization conditions were optimized in further microbatch trials. The best crystals were grown at 277 K in 4 µl drops under oil, by mixing 2 µl of a solution containing 15 mg ml^{-1} protein in 10 m*M* Tris–HCl buffer (pH 8.0) with 2 µl of a solution containing 2.5 *M* $(NaH_2/K_2H)PO_4$ at pH 7 and 0.1 *M* HEPES buffer at pH 7.5.

3. Results and discussion

Single purple-coloured crystals appeared as hexagonal rods within 1–4 weeks, and required 3–4 months to reach maximum size (0.5 × 0.1 × 0.1 mm).

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X-ray diffraction data were collected at 278 K as 0.25° rotation images (240 s exposure per image) using a Xentronics area detector on a Rigaku rotating-anode X-ray generator (45kV, 600 mA with a 200 μm focus). 90° of data were collected about the apparent sixfold axis of the crystal, then a further 30° were collected about a second axis perpendicular to the first.

Data were processed with *XDS* (Kabsch, 1988). The autoindexing routine indicated a hexagonal space group with cell dimensions $a = b = 124.39$, $c = 188.86$ Å and $\gamma = 120°$, and the data were integrated using a triclinic cell with these cell dimensions. The data were scaled and reduced using both *P*1 and *P*3 symmetry. Pseudo-precession pictures were produced using *HKLPLOT* (Collaborative Computational Project, Number 4, 1994), where only reflections with $F > 6$ were included. Sixfold symmetry was present on both the *hk*0 and *hkl* zones. The *hk*0 zone clearly showed *mm* symmetry in both pseudo-precession pictures and a small-angle precession photograph. 00*l* reflections did not show systematic absences, suggesting that the unique axis is a pure rotation rather than a screw axis.

The data were merged in space groups *P*3, *P*6, *P*622 with no significant differences in the R_{merge}. The relatively high value of R_{merge}, however, is probably due to radiation damage which can be seen as an increase in the scale factor over the course of the data collection.

The data were finally merged in *P*622 using *ROTAVATA* and *AGROVATA* (Collaborative Computational Project, Number 4, 1994). The native data set contains 8506 unique reflections, and is 94.2% complete to 3.8 Å, with redundancy 4.2 and R_{merge} 12.2%. In the highest resolution shell 60% of the reflections have an intensity greater than 3. The crystals diffract weakly, and the average $I/(I)$ is only 6.1, even with a relatively long exposure time per image.

Self-rotation functions have been calculated using *POLARRFN* (Collaborative Computational Project, Number 4, 1994) over several resolution ranges and with different Patterson radii, with inconclusive results.

Preliminary studies have shown that the crystals diffract to better than 3 Å at station PX9.6 of the Synchrotron Radiation Source (CLRC Daresbury), but the diffraction pattern decayed rapidly in the intense beam. Flash-freezing methods will need to be optimized before higher resolution data can be obtained.

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