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Crystallization and preliminary X-ray analysis of W120K mutant of streptavidin

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Bacterial streptavidin and chicken avidin are homotetrameric proteins that share an exceptionally high affinity towards the vitamin biotin. The biotin-binding sites in both proteins contain a crucial tryptophan residue contributed from an adjacent subunit. This particular tryptophan (W110 in avidin and W120 in streptavidin) plays an important role in both biotin binding and in the quaternary stabilities of the proteins. An intriguing naturally occurring alteration of tryptophan to lysine was previously described in the C-terminal domain of sea-urchin fibropellins, which share a relatively high sequence similarity with avidin and streptavidin. Avidin (Avm-W110K) and streptavidin (Savm-W120K) mutations show substantially reduced affinities towards biotin as well as the dissociation of their tetrameric structure into stable avidin and streptavidin dimers. Savm-W120K was crystallized at 293 K using the hanging-drop vapour-diffusion method. The crystals diffract to 1.7 Å resolution using synchrotron radiation and belong to the monoclinic space group $P2_1$, with unit-cell parameters a = 50.43, b = 100.41, c = 52.51 Å, β = 112.12°. The asymmetric unit contains four molecules of Savm-W120K, with a corresponding V_M of 2.3 Å^3 Da^{-1} and a solvent content of 46%.

1. Introduction

Bacterial streptavidin and chicken avidin are homotetrameric proteins which share remarkably high affinities towards the vitamin biotin ($K_d \simeq 10^{-15}$ M). Avidin and streptavidin have a similar tertiary fold and quaternary assemblies and contain four identical biotin-binding sites. Comparison of the primary structures of the two proteins show only moderate sequence homology (30% identity, 41% similarity; Livnah *et al.*, 1993) yet near-identical arrangements of amino acids in the respective biotin-binding sites. The tetrameric arrangement of avidin and streptavidin can be divided into two different categories of dimer pairs. Monomers 1 and 2 and monomers 3 and 4 (numbered according to Livnah *et al.*, 1993) form 'functional' dimers, whereas 'structural' dimers are formed between monomers 1 and 4 and monomers 2 and 3. In the 'functional' dimers, the conserved Trp120 in streptavidin (Trp110 in avidin) of one subunit plays a substantial role in the biotin-binding site of the adjacent monomer by forming the 'lid' of the hydrophobic box that accommodates biotin (Livnah *et al.*, 1993; Weber *et al.*, 1989; Hendrickson *et al.*, 1989). Mutation studies indicate that this tryptophan residue also plays a significant role in the stability of the quaternary assembly of streptavidin and avidin (Laitinen *et al.*, 1999; Chilkoti *et al.*, 1995).

Fibropellins are epidermal growth-factor (EGF) homologues found in the hyaline layer of the extracellular matrix in sea-urchin embryos. The C-terminal domain of fibropellins exhibits a similar sequence to avidin and streptavidin (Hunt & Barker, 1989; Laitinen *et al.*, 1999). The function of the domain in the sea-urchin fibropellin homologous to avidin and streptavidin has yet to be discovered. It is not even known whether this domain binds biotin. Sequence comparison of the C-terminal domain of fibropellin with avidin and streptavidin reveal that most of the residues participating in the biotin-binding sites in avidin and streptavidin are indeed conserved. One of the intriguing differences between fibropellin and avidin or streptavidin, however, is the replacement of Trp120 in streptavidin (and the equivalent Trp110 in avidin) with a lysine residue in all four fibropellins (Bisgrove *et al.*, 1995). Using this 'natural' mutation as a model, we designed similar mutations of avidin and streptavidin in the analogous residue (denoted Avm-W110K and Savm-W120K, respectively). The mutated proteins were shown to display significantly reduced affinities towards biotin ($K_d \simeq 10^{-8}$ M). The binding to biotin was found to be reversible, unlike that of the wild-type proteins which is virtually irreversible in both cases. Using an HPLC assay, Avm-W120K and Savm-W120 were found to form stable dimers in

Figure 1
A monoclinic crystal of Savm-W120K.

solution in the apo and biotin complex forms. As a first step towards the structure determination of Savm-W120K and subsequent structural understanding of how the mutation alters the biochemical properties of streptavidin, we report here the crystallization and preliminary X-ray data analysis of the protein.

2. Baculovirus overexpression and purification

Construction and expression of Savm-W120K in the baculovirus expression system (Bac-To-Bac) was performed by a modification of the previously described procedure (Laitinen *et al.*, 1999). Instead of purifying the protein from the cells, viral infection was allowed to continue for 5 d, causing lysis of the majority of the cells. Remaining cells and cell debris were removed from the culture medium by centrifugation at 1500*g* for 15 min at room temperature. The supernatant was then clarified by recentrifugation in the subsequent step (15 000*g*, 277 K, 20 min). NaCl was then added to a final concentration of 1 *M* and Savm-W120K was purified by affinity chromatography on a biotin-agarose column. Elution of the bound and washed protein was performed using biotin-saturated PBS.

3. Crystallization and data collection

Initial scanning for crystallization conditions for Savm-W120K (10 mg ml^{-1}) were examined by hanging-drop vapour diffusion at 293 K using Hampton Research Crystal Screen I (Jancarik & Kim, 1991). Crystallization experiments were prepared by mixing 1.5 µl protein and 1.5 µl reservoir solutions. Large (0.6 mm in length) crystals were obtained after 24 h in a single crystallization experiment (condition No. 28) where the reservoir solution contained 15% polyethene glycol (PEG) 8000, 0.2 *M* sodium acetate and 0.1 *M* sodium cacodylate pH 6.45. Crystallization conditions were further refined by varying the crystallization parameters, *e.g.* buffer content, pH, sample size, concentrations of precipitating agents and various additives. Improved crystals were obtained at 293 K with reservoir solutions consisting of either 20% PEG 8000, 0.2 *M* sodium acetate and 0.1 *M* cacodylate buffer pH 7.05 (Fig. 1) or 25% PEG 6000, 0.3 *M* sodium acetate and 0.1 *M* cacodylate buffer pH 6.45.

Initial diffraction data to 2.2 Å resolution were collected at room temperature on an RAXIS-IV image plate mounted on a direct-drive Rigaku 18S rotating-anode generator with Max-Flux optics. Using synchrotron radiation at the European Synchrotron Radiation Facility (ESRF), Grenoble, France at beamline ID14-1, diffraction extended to 1.7 Å. Crystals were initially introduced into a cryoprotectant stabilizing solution, wherein the crystallization reservoir solution was supplemented with either 25% ethylene glycol or 25% xylitol. Data were collected at 100 K using an Oxford Cryosystems Cryostream device on a MAR 165 mm CCD detector with an oscillation range of 0.5° and a radiation wavelength of 0.934 Å. Data were processed and scaled using the *HKL* package (Otwinowski & Minor, 1997). The asymmetric unit contained four molecules of Savm-W120K, resulting in a crystal volume per protein mass (V_M) of 2.3 Å^3 Da^{-1} (Matthews, 1968) and a solvent content of 46%. Crystal data-collection statistics are summarized in Table 1.

Table 1
Synchrotron data-collection statistics.

Values in parentheses refer to the highest resolution shell (1.76–1.7 Å).

Space group	$P2_1$
Unit-cell parameters	
a (Å)	50.43
b (Å)	100.41
c (Å)	52.51
β (°)	112.12
Resolution range (Å)	40–1.7
No. of reflections	51529
Redundancy	2
Data completeness (%)	97.3 (98.6)
R_{sym}† (%)	4.1 (29)
$\langle I/\sigma I\rangle$	21.6 (3.9)

† $R_{sym} = \sum |I - \langle I\rangle| / \sum I$.

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