Adenovirus proteinase: crystallization and preliminary X-ray diffraction studies to atomic resolution

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Acta Crystallographica Section D
Biological
Crystallography
ISSN 0907-4449

Adenovirus proteinase (AVP) is required for the synthesis of infectious virus and is a target for antiviral therapy. The enzyme requires two viral cofactors for activation: pVIc, an 11-amino acid peptide, and the viral DNA. The structure of the enzyme in the absence of cofactors has not been observed. Single crystals of AVP were obtained via microseeding using the hanging-drop vapour-diffusion method with sodium acetate and sodium citrate as precipitants. At the National Synchrotron Light Source at Brookhaven National Laboratory, the native crystal diffracted to a resolution of 0.98 Å and an isomorphous heavy-atom derivative diffracted to 1.9 Å. Comparison of the structure of AVP with that of the AVP-pVIc complex should reveal the structural basis of activation of the enzyme by pVIc.

1. Introduction

Many medically important viruses contain a gene for a virus-coded proteinase whose activity is essential for the synthesis of infectious virus. Among these is human adenovirus (Weber & Tihanyi, 1994). Late in adenovirus infection, the adenovirus proteinase (AVP) becomes activated inside young virions and cleaves multiple copies of six virion precursor proteins. L3 23k is the gene for AVP (Yeh-Kai et al., 1983) and it has been cloned, expressed in Escherichia coli (Anderson, 1993; Mangel et al., 1993; Tihanyi et al., 1993; Tihanyi et al., 1993) or baculovirus-infected insect cells (Webster et al., 1993) and the resultant 204 amino-acid protein purified.

Recombinant AVP alone is relatively inactive. Two viral cofactors were discovered that stimulate proteinase activity. One cofactor is pVIc, the 11-amino-acid peptide from the C-terminus of adenovirus precursor protein pVI (Baniecki et al., 2001; Mangel et al., 1993; Webster et al., 1993). Its sequence is GVQSLKRRRCF. Cys104 of AVP can form a disulfide bond with Cys10 of pVIc in vitro (Ding et al., 1996; McGrath, Baniecki, Peters et al., 2001) and does so in vivo in the virus particle (McGrath, Aherne et al., 2002; McGrath, Ding et al., 2002). A second cofactor is the viral DNA (Mangel et al., 1993; McGrath, Baniecki, Li et al., 2001). The cofactors increase the specificity constant \( k_{cat}/K_m \) for substrate hydrolysis (McGrath, Baniecki, Li et al., 2001). In the presence of pVIc, \( k_{cat}/K_m \) for AVP increases 110-fold; in the presence of Ad2 DNA, \( k_{cat}/K_m \) increases 34 100-fold compared with that of AVP alone.

The disulfide-linked complex AVP-pVIc has been crystallized (Keefe et al., 1995; McGrath et al., 1996) and its structure determined at 2.6 Å resolution (Ding et al., 1996). More recently, a 1.6 Å resolution structure of AVP-pVIc has been determined (McGrath, Aherne et al., 2002; McGrath, Ding et al., 2002). AVP-pVIc is a cysteine proteinase. The order of the amino acids involved in catalysis along the polypeptide chain is unique, indicating AVP-pVIc to be the first member of a new class of cysteine proteinases. However, the amino acids involved in catalysis by AVP can be superimposed with similar residues involved in catalysis by papain. Upon superimposing AVP and papain, Cys122 in AVP is in an identical position to the nucleophile Cys25 of papain. Furthermore, two other residues of AVP, His54 and Glu71, are in identical positions to those of His159 and Asn175, the two other residues of papain that constitute the charge-relay system (Blow et al., 1969). Glu19 of papain (Drenth et al., 1976), presumed to participate in formation of an oxyanion hole (Robertus et al., 1972), and Glu115 of AVP also overlap. This remarkable juxtaposition of catalytic elements strongly suggests that AVP employs the same catalytic mechanism as papain (Polgar, 1974). Because the fold of AVP is different from the fold of papain, yet the positions of the residues involved in catalysis are the same, AVP is an example of convergent evolution. More recently, other proteinases have been added to the adenovirus proteinase family: Ulp1, a proteinase involved in sumoylation (Li &
alignment for efficient catalysis, because AVP is relatively inactive. What structural signals are transduced towards the active site upon the binding of pVIc?

2. Materials, methods, results and future refinement

2.1. Cloning and purification

The L3 23k gene was cloned and expressed in E. coli strain BL21 (DE3pLysS) and the resultant AVP purified as previously described (Mangel et al., 1996). The final step in the purification procedure involved dialysis against nitrogen-saturated 10 mM HEPES pH 8.0, 5 mM NaCl and 0.1 mM EDTA. AVP was stored at a concentration of 300 μM at 193 K.

2.2. Crystallization

The hanging-drop vapor-diffusion technique (McPherson, 1990) was used to crystallize AVP in 24-well VDX plates (Hampton Research). Crystals grew after 2–3 months at 298 K in drops that contained 6.5 μl of enzyme and 3.5 μl of reservoir solution (1.0 M sodium citrate pH 5.0 and 1.8 M sodium acetate). These crystals were small and spherulitic in appearance and were not suitable for X-ray analysis. Crystal quality could not be improved by varying the precipitant, protein concentration, pH, buffer, additives or drop volume. However, crystal quality improved dramatically on using a microseeding procedure (Ducruix & Giege, 1992) in which small crystals were crushed and inoculated using a cat whisker into drops consisting of 6.5 μl of enzyme and 3.5 μl reservoir solution. Crystals suitable for X-ray analysis grew within 3–5 d. For AVP, their dimensions were 0.05 × 0.15 × 0.50 mm and their appearance was prismatic (Fig. 1).

2.3. X-ray diffraction data collection and analysis

Crystals of AVP were prepared for freezing in cryobuffer by transfer into a drop containing reservoir solution (1.0 M sodium citrate pH 5.0 and 1.8 M sodium acetate). Then, five 1 μl aliquots of synthetic mother liquor (1.0 M sodium citrate pH 5.0, 1.8 M sodium acetate and 30% glycerol) were added stepwise; the time interval between each addition was 4 min. The crystals were flash-frozen in the liquid-nitrogen stream of an Oxford Cryostream Cooler. X-ray intensities were recorded on a Brandeis 4k CCD Detector (Strauss et al., 1990; Phillips et al., 2000) at beamline X25 at the National Synchrotron Light Source, Brookhaven National Laboratory. Several passes, using a different exposure time, distance and beam attenuation for each pass, were undertaken and the data merged together, resulting in a very complete data set. The X-ray wavelength was 0.986 Å (Table 1).

2.4. Heavy-atom derivatization

A crystal of AVP was soaked for 24 h at room temperature in mother liquor containing 6 mM K2PtCl4. It was then put into cryobuffer, flash-frozen and a data set collected. The distance between the crystal and detector was 99.5 mm and the X-ray wavelength was 1.064 Å. A data set was obtained to 99.7% completeness to 1.9 Å. The crystal was isomorphous to the native crystal and Rint = 13.1% (Table 1). A single prominent Pt site could be identified from

Table 1

| Data-collection statistics for crystals of AVP and its platinyl derivative. |
|----------------|----------------|
|                | AVP            | AVP + K2PtCl4 |
| Space group    | P2₁            | P2₁           |
| Unique reflections | 92247 (6483)   | 12808 (1269)  |
| Resolution (Å) | 30.0–0.98 (1.0–0.98) | 30.1–1.90 (1.97–1.90) |
| Completeness (%) | 99.1 (97.1)    | 99.7 (98.8)   |
| Average | I/(σ²I) | I/(σ²I) | I/(σ²I) |
| Rmerge (%)     | 25.4 (1.6)     | 31.4 (20.6)   |
| Rfree (%)      | 5.7 (59.4)     | 3.8 (5.7)     |
| Rmax (%)       | 13.1           |              |

† Rmerge = Σ|I – 〈I〉|/Σ|I|, where 〈I〉 is the average intensity. † Rfree = Σ|Fobs| – |Fcalc|/Σ|Fobs|, where Fobs is the native protein structure amplitude and |Fcalc| is the platinum-derivative structure amplitude.

Figure 1

Photomicrograph of crystals of AVP. Prism-shaped crystals of AVP obtained by microseeding were used for X-ray diffraction. The typical crystal dimensions were 0.05 × 0.15 × 0.50 mm.
the isomorphous difference Patterson map (Fig. 2).

2.5. Results

The X-ray diffraction data were processed using DENZO and SCALEPACK from the HKL software suite (Otwinowski & Minor, 1997). Analysis of the X-ray diffraction data indicated that the AVP crystal belonged to space group $P2_1$, with unit-cell parameters $a = 36.2$, $b = 54.1$, $c = 42.1$ Å, $\beta = 100.5^\circ$. The unit-cell parameters and mass of the molecular species in the crystal were consistent with there being one 23 087 Da monomer of AVP per asymmetric unit, which gives a Matthews coefficient (Matthews, 1968) of 1.75 Å$^3$Da$^{-1}$, corresponding to a solvent content of 29.2%. The low solvent content may be the reason why this crystal diffracted to such high resolution (Dauter et al., 1997; Teeter et al., 1993). A data set was collected to 0.98 Å resolution from a single AVP crystal; the statistics are listed in Table 1.

2.6. Approach to solving the structure

Initially, molecular replacement will be attempted using as a search model the 1.6 Å structure of AVP-pVic or a portion of it (McGrath, Aherne et al., 2002; McGrath, Ding et al., 2002). A successful rotation and translation search would mean that only rigid-body refinement should be necessary for an adequate starting model. However, if molecular replacement fails, the data set with the Pt derivative of AVP, collected with the objective of obtaining heavy-atom phasing, will be used as previously (Ding et al., 1996). Since the resolution of the data extended beyond 1.0 Å, the structure will eventually be refined anisotropically with the program SHELXL (Sheldrick & Schneider, 1997).

We thank Dr Subramanyam Swaminathan for many insightful discussions and Dr Michael Becker for invaluable assistance at beamline X25 at the National Synchrotron Light Source. This research was supported by the Office of Biological and Environmental Research of the US Department of Energy under Prime Contract No. DE-AC0298CH10886 with Brookhaven National Laboratory and by National Institutes of Health Grant AI41599.

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