JOURNAL OF STRUCTURAL BIOLOGY **117,** 77–79 (1996)
ARTICLE NO. 0072

CRYSTALLIZATION NOTE

Preparation and Crystallization of a Complex between Human Adenovirus Serotype 2 Proteinase and Its 11-Amino-Acid Cofactor pVIc

WILLIAM J. MCGRATH, JIANZHONG DING, ROBERT M. SWEET, AND WALTER F. MANGEL[1]

Biology Department, Brookhaven National Laboratory, Upton, New York 11973

Received December 1, 1995, and in revised form March 22, 1996

Crystals have been obtained of the recombinant human adenovirus serotype 2 proteinase (AVP) in a complex with its 11-amino-acid cofactor pVIc. AVP–pVIc complexes were formed by the incubation of AVP with a 1.2-fold molar excess of pVIc prior to the crystallization trials. Diffraction-quality crystals were obtained at 18°C by the vapor-diffusion method with 5.6 mg/ml AVP–pVIc in 1.4 *M* sodium acetate and 0.1 *M* Hepes, pH 7.5. Diffraction data (99% complete to 2.6 Å resolution with R_{merge} of 0.077) were collected from native crystals at room temperature at beamline X12-C at the National Synchrotron Light Source. The crystals belong to space group $P6_1$ with unit cell dimensions $a = b = 114.2$ Å, $c = 50.1$ Å; $\alpha = \beta = 90°$, $\gamma = 120°$. The unit cell dimensions and likely mass of the molecular species in the crystals were consistent with there being one 25 000-Da complex (1:1) per asymmetric unit. Additionally, one heavy-atom derivative, obtained by the soaking of preformed crystals, was isomorphous to the native crystal. Diffraction data obtained on these crystals were 95% complete to 3.0 Å resolution with an R_{merge} of 0.076. Difference-Patterson analysis indicates three heavy atom sites in the derivative asymmetric unit.

INTRODUCTION

Many animal and plant viruses contain a gene for a proteinase whose correct expression is absolutely required for the synthesis of infectious virus (Krausslich and Wimmer, 1988). Because of this and because virus-coded proteinases are highly specific enzymes, they are appealing targets for antiviral therapy. The human adenovirus proteinase is required to process 6 of the 12 major polypeptides from which adenovirus virions are assembled. Weber (1976) isolated a temperature-sensitive mutant H2ts1 (ts1) of human adenovirus serotype 2 (Ad2) that lacks proteinase activity at the nonpermissive temperature. Virions of ts1 assemble at the nonpermissive temperature but contain precursor proteins in place of the mature components present in wild-type virus. Such immature virions attach to cells but fail to initiate a productive infection (Hannan *et al.*, 1983; Mirza and Weber, 1980). The mutation in ts1 was identified as a single base-pair change in a 204-codon open reading frame (L3 23 kDa) at the 3′ end of the L3 family of late messages (Yeh-Kai *et al.*, 1983). The L3 23K gene has been cloned and expressed in *Escherichia coli*, and the resultant protein has been purified (Anderson, 1990; Mangel *et al.*, 1995).

Recombinant human adenovirus serotype 2 proteinase (AVP) has little activity compared to that in disrupted virions. This prompted a search for cofactors; two were discovered. One was the 11-amino-acid peptide from the carboxy terminus of the virion precursor protein pVI, pVIc (Mangel *et al.*, 1993; Webster *et al.*, 1993). It stimulates AVP activity 350-fold (Mangel *et al.*, 1995). The other cofactor is the viral DNA, which in the presence of pVIc stimulates AVP activity 6000-fold (Mangel *et al.*, 1993). The requirement of two cofactors for proteinase activity is rare; for one to be DNA is unprecedented.

AVP is a difficult proteinase to classify. Analysis of the AVP sequence of 12 different adenovirus serotypes reveals no homology with any proteins (Rancourt *et al.*, 1994). Inhibitor profiles imply it may be a serine (Bhatti and Weber, 1979; Chatterjee and Flint, 1987; Tremblay *et al.*, 1983) or cysteine proteinase (Grierson *et al.*, 1994; Rancourt *et al.*, 1994; Tihanyi *et al.*, 1993; Weber and Tihanyi, 1994; Webster *et al.*, 1993). AVP exhibits a very selective

[1] To whom correspondence and reprint requests should be addressed at Biology Department, Brookhaven National Laboratory, Upton, NY 11973. Fax: (516) 344-3407.

1047-8477/96 $18.00

substrate specificity, (I,L,M)XGG↓X or (I,L,M)XGX↓G, where X is any residue (Webster *et al.*, 1989). In developing an assay for AVP, only synthetic, fluorogenic substrates with the consensus P_4, P_2, and P_1 amino acids were cleaved by AVP (McGrath *et al.*, 1996). To exploit this selective substrate specificity in the design of proteinase inhibitors as antiviral agents, we need to know not only the type of proteinase but also its three-dimensional structure.

Here we report the crystallization and preliminary X-ray diffraction studies of preformed complexes of Ad2 AVP–pVIc as a prelude to the determination of the three-dimensional structure. The Ad2 AVP–pVIc crystal structure should reveal the type of proteinase and how pVIc interacts with AVP to stimulate the rate of catalysis. There is a report on the crystallization of the Ad2 proteinase with a heterologous pVIc, the adenovirus serotype 12 pVIc (Keefe *et al.*, 1995). Their crystallization conditions are totally different from ours; their crystals were grown in 20–40% 2-methyl-2,4,pentanediol, 0.1–0.2 *M* sodium citrate, and 0.1 *M* sodium Hepes, pH 5.0–7.0. The space group was $P3_121$ or $P3_221$ ($a = b = 41.3$ Å, $c = 197.0$ Å, one molecule per asymmetric unit), and apparently their crystals have not yielded a structure.

MATERIALS AND METHODS

Cloning and Purification of AVP

Recombinant AVP was expressed in *E. coli* strain BL21(DE3) and purified as described (Anderson, 1990; Mangel *et al.*, 1996). Purified AVP, at a concentration of 315 µ*M*, was dialyzed against nitrogen-saturated 0.01 *M* Hepes, 5 m*M* NaCl, 0.1 m*M* EDTA, pH 8.0, prior to use in crystallization trials.

Formation of AVP–pVIc Complexes

Ad2 pVIc (GVQSLKRRRCF) was purchased from Multiple Peptide Systems (San Diego, CA) and dissolved in 0.1% acetic acid prior to use. The concentration of pVIc was confirmed by amino acid analysis. The oxidation state of its cysteine residue was determined by titration with Ellman's reagent (Riddles *et al.*, 1979). All complex formations were initiated with fully reduced pVIc. Complexes of recombinant AVP with pVIc were formed in 10 m*M* Hepes, 5 m*M* NaCl, 0.1 m*M* EDTA, pH 8.0, by incubation of 210 µ*M* AVP with 252 µ*M* pVIc at room temperature for 20 min.

Crystallization of the AVP–pVIc Complex

The hanging drop, vapor-diffusion technique (McPherson, 1990) was used to crystallize the AVP–pVIc complex in 24-well Linbro culture plates. An incomplete factorial screen (Hampton Research Crystal Screen 1) was employed in which 3 µl of AVP–pVIc complex was mixed with 1 µl of the precipitant. From the initial screen, crystals were obtained reproducibly at room temperature after 3 to 5 days under two conditions. Under the first condition the precipitant solution consisted of 0.1 *M* sodium cacodylate and 1.4 *M* sodium acetate, pH 6.5; under the second it was 0.1 *M* Hepes, 0.8 *M* sodium, potassium tartrate, pH 7.5. The crystals were poor, so both sets of conditions were refined by grid screening.

X-ray Diffraction Data Collection and Analysis

For X-ray diffraction studies, a crystal was mounted in a sealed quartz capillary in the presence of a small drop of the natural mother liquor. The X-ray diffraction was performed at beamline X12-C at the National Synchrotron Light Source at Brookhaven National Laboratory. Complete data were collected at room temperature on a MAR Research 300-mm-diameter imaging plate scanner, mounted on the θ arm of a FAST (Enraf-Nonius) diffractometer. Diffraction data for the native crystal was carried out using a crystal-to-detector distance of 300 mm. The X-ray wavelength was 1.15 Å.

Heavy-Atom Derivatization of AVP–pVIc Crystals and Preliminary Phasing Analysis

Crystals of AVP–pVIc were prepared as described above and washed in 0.11 *M* Hepes, 1.54 *M* sodium acetate, pH 7.5. K_2PtCl_4 was added to a final concentration of 10 m*M*, and the crystals were soaked for 24 hr at 18°C. The crystals were then mounted and a data set was collected as described above, except the X-ray wavelength was 1.07 Å, selected spectroscopically to match the intense "white line" of absorption by the Pt atoms in the crystals. These crystals were isomorphous to the native crystals. A data set was obtained to 95% completeness at 3.0 Å resolution.

RESULTS AND DISCUSSION

Crystallization of the AVP–pVIc Complex

Initially we obtained crystals of the AVP–pVIc complex under two conditions. The crystal morphology was better with the sodium acetate precipitant.

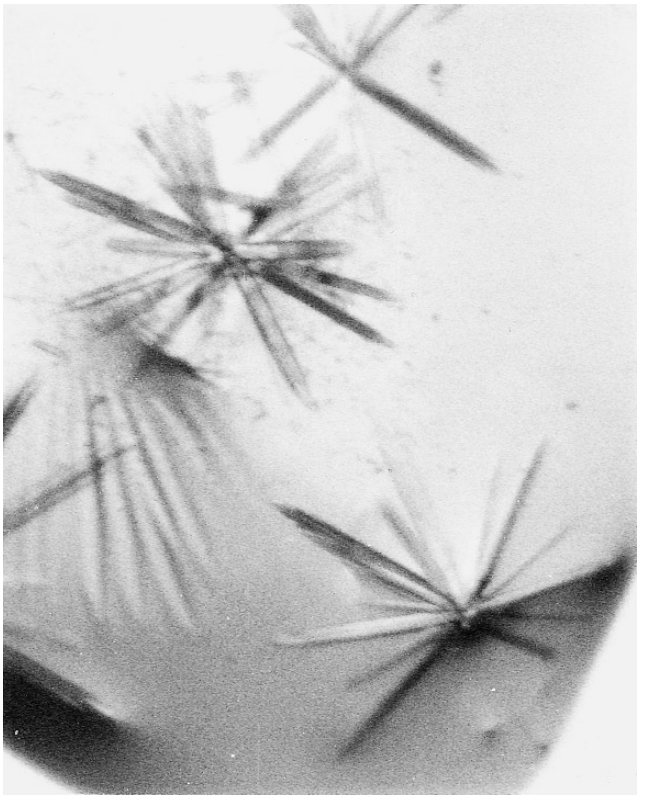

FIG. 1. Photomicrograph of AVP–pVIc crystals. The crystal dimensions are 0.6 × 0.1 × 0.1 mm.

TABLE I

Data Collection Statistics for Crystals of the AVP–pVIc Complex and the Platinyl Derivative

	AVP–pVIc	AVP–pVIc + K_2PtCl_4
Space group	$P6_1$	$P6_1$
Unique reflections	11559	7528
Resolution (Å)	2.6	3.0
Completeness	98.8%	95.4%
Average $I/\sigma(I)$	19.8	17.3
R_{merge}[a]	0.077	0.076
R_{iso} (%)[b]		10.5

[a] R_{merge} is $= \Sigma(|(I - \bar{I})/|)/\Sigma(I)$, where I is intensity.

[b] R_{iso} is the $\Sigma\|F_{PH}| - |F_P\|/\Sigma |F_P|$, where $|F_P|$ is the protein structure factor amplitude and $|F_{PH}|$ is the platinum derivative structure factor amplitude.

Optimization around this condition resulted in reproducible, diffraction quality crystals using 100 m*M* Hepes, pH 7.5, with 1.4–1.6 *M* sodium acetate as a precipitant. These crystals were hexagonal-rod shaped with dimensions approximately 0.6 × 0.1 × 0.1 mm (Fig 1). Analysis of the diffraction data indicate that the AVP–pVIc complex crystals belong to the hexagonal space group $P6_1$ with unit cell parameters $a = b = 114.2$ Å, $c = 50.1$ Å; $\alpha = \beta = 90°$, $\gamma = 120°$. The unit cell dimensions and likely mass of the molecular species in the crystals were consistent with there being one 25 000-Da complex (1:1) per asymmetric unit (The solvent content was 68% and $V_m = 3.9$ Å$^3/d$). A data set has been collected to 2.6 Å resolution from a single AVP–pVIc crystal, and the statistics are given in Table I.

Heavy-Atom Derivatization of AVP–pVIc Complexes

The chloroplatinite-containing crystals were more sensitive to X-ray exposure than were the native crystals. However, a data set, 95.4% complete, was obtained to 3.0 Å resolution using three crystals (Table I). Difference-Patterson analysis, using the PHASES package (Furey and Swaminathan, 1990), shows peaks about seven times above the rms on Harker sections. Calculations indicate the presence of three heavy atom sites.

The work was supported by National Institute of Allergy and Infectious Diseases Grant AI26049 and in part by the Office of Health and Environmental Research of the United States Department of Energy.

REFERENCES

Anderson, C. W. (1990) The proteinase polypeptide of adenovirus serotype-2 virions, *Virology* **177,** 259–272.

Bhatti, A. R., and Weber, J. (1979) Protease of adenovirus type 2: Partial characterization, *Virology* **96,** 478–485.

Chatterjee, P. K., and Flint, S. J. (1987) Adenovirus type 2 endoproteinase: An unusual phosphoprotein enzyme matured by autocatalysis, *Proc. Natl. Acad. Sci. USA* **84,** 714–718.

Furey, W., and Swaminathan, S. (1990) A Program Package for the Processing and Analysis of Diffraction Data for Macromolecules, Poster, ACA meeting.

Grierson, A. W., Nicholson, R., Talbot, P., Webster, A., and Kemp, G. D. (1994) The protease of adenovirus serotype 2 requires cysteine residues for both activation and catalysis, *J. Gen. Virol.* **75,** 2761–2764.

Hannan, C., Raptis, L. H., Dery, C. V., and Weber, J. (1983) Biological and structural studies with adenovirus type 2 temperature-sensitive mutant defective for uncoating, *Intervirology* **19,** 213–223.

Keefe, L. J., Ginell, S. L., Westbrook, E. M., and Anderson, C. W. (1995) Crystallization and preliminary X-ray diffraction studies of human adenovirus serotype 2 proteinase with peptide cofactor, *Protein Sci.* **4,** 1658–1660.

Krausslich, H.-G., and Wimmer, E. (1988) Viral proteinases. *Annu. Rev. Biochem.* **57,** 701–754.

Mangel, W. F., McGrath, W. J., Toledo, D. L., and Anderson, C. W. (1993) Viral DNA and a viral peptide can act as cofactors of adenovirus virion proteinase activity, *Nature* **361,** 274–275.

Mangel, W. F., Toledo, D. L., Brown, M. T., Martin, J. H., and McGrath, W. J. (1996) Characterization of human adenovirus proteinase activity *in vitro*, purification of components, *J. Biol. Chem.* **271,** 536–543.

McGrath, W. J., Abola, A. P., Toledo, D. L., Brown, M. T., and Mangel, W. F. (1996) Characterization of human adenovirus proteinase activity in disrupted virus particles, *Virology* **217,** 131–138.

McPherson, A. (1990) Current approaches to macromolecular crystallization, *Eur. J. Biochem.* **189,** 1–23.

Mirza, A., and Weber, J. (1980) Infectivity and uncoating of adenovirus cores, *Intervirology* **13,** 307–311.

Rancourt, C., Tihanyi, K., Bourbonniere, M., and Weber, J. M. (1994) Identification of active-site residues of the adenovirus endopeptidases, *Proc. Natl. Acad. Sci. USA* **91,** 844–847.

Riddles, P. S., Blakeley, R. L., and Zerner, B. (1979) Ellman's reagent: 5,5′-Dithiobis(2-nitrobenzoic acid)—A reexamination, *Anal. Biochem.* **94,** 75–81.

Tihanyi, K., Bourbonniere, M., Houde, A., Rancourt, C., and Weber, J. M. (1993) Isolation and properties of adenovirus type 2 proteinase, *J. Biol. Chem.* **268,** 1780–1785.

Tremblay, M. L., Dery, C. V., Talbot, B. G., and Weber, J. (1983) *In vitro* cleavage specificity of the adenovirus type 2 proteinase, *Biochim. Biophys. Acta* **743,** 239–245.

Weber, J. (1976) Genetic analysis of adenovirus type 2, III. Temperature sensitivity of processing of viral proteins, *J. Virol.* **17,** 462–471.

Weber, J. M., and Tihanyi, K. (1994) Adenovirus Endopeptidases, *Methods Enzymol.* **244,** 595–604.

Webster, A., Hay, R. T., and Kemp, G. (1993) The adenovirus protease is activated by a virus-coded disulphide-linked peptide, *Cell* **72,** 97–104.

Webster, A., Russell, S., Talbot, P., Russell, W. C., and Kemp, G. D. (1989) Characterization of the adenovirus proteinase: substrate specificity, *J. Gen. Virol.* **70,** 3225–3234.

Yeh-Kai, L., Akusjärvi, G., Aleström, P., Petterson, U., Tremblay, M., and Weber, J. (1983) Genetic identification of an endopeptidase encoded by the adenovirus genome, *J. Mol. Biol.* **167,** 217–222.