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Preliminary X-ray crystallographic analysis of human salivary cystatin

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Abstract

Human salivary cystatin, a thiol proteinase inhibitor, has been implicated in potential antimicrobial and antiviral functions of saliva. A variant of human salivary cystatin SN expressed and purified in an *Escherichia coli* expression system lacking residues 12–16 near the N-terminus (Δ12–16) has been crystallized by the vapor-diffusion technique. The crystals are of the hexagonal space group *P*622 and have cell constants of $a = 85.41$, $b = 85.41$, $c = 131.6$ Å, $\alpha = \beta = 90$, $\gamma = 120°$, and contain two molecules of molecular weight 13 500 per asymmetric unit. The crystals diffract up to a resolution of 2.2 Å and are suitable for X-ray diffraction analysis.

1. Introduction

Human saliva contains multiple forms of cysteine proteinase inhibitors known as cystatins (Shomers, Tabak, Levine, Mandel & Hay, 1982; Ramasubbu *et al.*, 1991). The isoforms of salivary cystatins have been designated as neutral (SN), acidic (S and SA) and cystatin D (Bobek & Levine, 1992). Human salivary cystatins belong to family 2 of the cystatin superfamily, and are encoded by a multigene family comprising seven members (Saitoh, Kim, Smithies & Maeda, 1987; Bobek & Levine, 1992; Freije *et al.*, 1993). Two major functions of salivary cystatins are their thiol-proteinase inhibitory activity and their ability to bind to enamel (hydroxyapatite) surfaces (Johnsson, Richardson, Bergey, Levine & Nancollas, 1991). They are also involved in the formation of *in vivo* early enamel pellicle (Al-Hashimi & Levine, 1989) and have been implicated in the inhibition of the replication of herpes simplex virus (Bergey, Gu, Collins, Bradway & Levine, 1993; Gu, Haraszthy, Collins & Bergey, 1995). These properties of salivary cystatins suggest a role in the oral cavity.

The complete amino-acid sequence for the salivary cystatin SN has been deduced from the cDNA sequence (Al-Hashimi, Dickinson & Levine, 1988). The cystatin SN is a single polypeptide chain consisting of 121 amino-acid residues with a molecular weight of $\simeq 13\,500$ Da. Salivary cystatins show significant differences in their ability to inhibit thiol-proteinase activities against papain and cathepsins (Ramasubbu *et al.*, 1991; Bobek, Ramasubbu, Wang, Weaver & Levine, 1994). In our effort to study the structure and function of the salivary cystatins and their interaction with various thiol proteinases, we have recently expressed and purified several recombinant salivary cystatin variants using an *Escherichia coli* expression system, pGEX-2T (Bobek *et al.*, 1994). One of the variants (Δ12–16) lacks five amino-acid residues (G-G-I-Y-N) near the N-terminal end. Interestingly, this variant possesses higher inhibitory activity towards papain compared to the full-length recombinant cystatin (Bobek *et al.*, 1994).

In the proposed mechanism of action of chicken egg-white cystatin (CEW) based on a docking model between CEW and papain (Bode *et al.*, 1988), it has been suggested that the N-terminus may be involved in the formation of a tight turn (Leu8-Gly9). Because of this, Leu8 may interact with the S2 subsite on the papain surface with Gly9 close to the active-site thiol group. Cystatins, in general, possess an exposed central hairpin loop flanked by the amino-terminal segment and a second hairpin loop all of which have been shown to be important in the thiol proteinase inhibitory activity (Bode *et al.*, 1988; Machleidt *et al.*, 1989). Earlier work has shown that isoforms of cystatin members which lack the N-terminal residues possess significantly lower inhibitory activities against some thiol proteinases (Machleidt, *et al.*, 1989). Gly at position 9 of CEW (Gly12 in SN) is conserved among all members of the cystatin family 2 (Barrett, 1987). Because of the deletion, the Δ12–16 cystatin mutant may possess a Pro10–Ala16 segment instead of Pro11-Gly12 turn. Interestingly, Δ12–16 inhibits papain to a greater extent than the recombinant full-length SN or CEW and this ability may be attributed to this segment (Bobek *et al.*, 1994). Thus, Δ12–16 provides an opportunity to define precisely the role of the N-terminal segment in the inhibitory mechanism of cystatins.

Cystatin Δ12–16 was successfully crystallized using the following conditions. The protein used was at a concentration of 15 mg ml^{-2} in water. Crystallization attempts were carried out initially by the hanging-drop vapor-diffusion technique (McPherson, 1982) in 24-well cell-culture plates (Corning). Using Crystal Screen I (Hampton Research), conditions No. 9 and 15 yielded crystals. Crystals were obtained from No. 15 within a week and grew to a size of 0.2 × 0.2 × 0.1 mm. Larger crystals with well defined morphology were obtained using a silica-gel-mediated sitting-drop method (Cudney, Patel & McPherson, 1994). The sitting drop consisted of 5 µl of a 30 mg ml^{-1} solution of protein in water, 5 µl of the silica gel and 10 µl of the Hampton drop No. 15. For this purpose, cylindrical glass rods that fit within the wells of the tissue-culture plate were used (Gribskov, 1989). A crystal of size 0.5 × 0.3 × 0.2 mm was mounted directly from the silica-gel sitting drop into a 0.7 mm Lindemann glass capillary along with a small amount of the well solution. The ends of the capillary were sealed using dental wax. The crystals diffracted to a Bragg spacing of about 2.2 Å measured using a MAR Research imaging-plate detector (300 mm). Initial characterization of the crystals using a set of three 1° oscillation frames exposed for 10 min each were processed and indexed using the autoindexing routines of the MAR software. This resulted in the identification of the hexagonal symmetry with the point group *P*622 and the unit-cell constants of $a = 85.41$, $b = 85.41$, $c = 131.6$ Å, $\alpha = \beta = 90$, $\gamma = 120°$ with two molecules per asymmetric unit. The value of V_m is 2.6 Å^3 Da^{-1} which is within the normal range of 1.6–3.6 Å^3 Da^{-1} for water-soluble protein crystals (Matthews, 1968).

Native data were collected at room temperature to a resolution of 2.5 Å using the imaging plate mounted on a

Rigaku RU200 rotating anode with Cu $K\alpha$ radiation. A total of 60 frames at 1° intervals were measured using a crystal-to-detector distance of 150 mm. The rotating anode was operated at 50 mA and 200 kV. The data were processed using *MOSFLM* of the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). A total of 210 673 reflections were collected of which 19 445 were unique. In the native data set compete to 2.5 Å, there are 9740 reflections with $I > 1\sigma(I)$. The $\langle I \rangle / \sigma(I)$ for the outer shell (2.8–2.5 Å) is 5.9. The data set represents about 94% of the theoretically observable reflections in the resolution range from 2.5 to 60 Å. The $R_{\rm merge}$ for the reduced data set was 5.1% in this range. The considerable sequence similarity (46% identity) existing between SN and chicken egg-white cystatin (CEW) have prompted us to use molecular-replacement techniques for the structure determination of cystatin SN (Δ12–16) as implemented in the program *AMoRe* (Navaza, 1995).*

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* Supplementary material has been deposited with the IUCr. Free copies may be obtained through the Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England (Reference: GR0602).

References

Al-Hashimi, I., Dickinson, D. P. & Levine, M. J. (1988). *J. Biol. Chem.* **263**, 9381–9387.

Al-Hashimi, I. & Levine, M. J. (1989). *Arch. Oral Biol.* **34**, 289–295.

Barrett, A. J. (1987). *Trends Biochem. Sci.* **12**, 193–196.

Bergey, E. J., Gu, M., Collins, A. R., Bradway, S. D. & Levine, M. J. (1993). *Oral Microbiol. Immunol.* **8**, 89–93.

Bobek, L. A. & Levine, M. J. (1992). *Crit. Rev. Oral Biol. Med.* **3**, 307–332.

Bobek, L. A., Ramasubbu, N., Wang, X., Weaver, T. R. & Levine, M. J. (1994). *Gene*, **151**, 303–308.

Bode, W., Engh, R., Musil, D., Thiele, U., Huber, R., Karshikov, A., Brzin, J., Kros, J. & Turk, V. (1988). *EMBO J.* **7**, 2593–2599.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Cudney, B., Patel, S. & McPherson, A. (1994). *Acta Cryst.* D**50**, 479–483.

Freije, J. P., Balbin, M., Abrahamson, M., Velasco, G., Dalboge, H., Grubb, A. & Lopez-Otin, C. (1993). *J. Biol. Chem.* **268**, 15737–15744.

Gribskov, C. L. (1989). *Am. Cryst. Assn Abstracts Ser* 2, Abstracts 49, 17, 90.

Gu, M., Haraszthy, G. G., Collins, A. R. & Bergey, E. J. (1995). *Oral Microbiol. Immunol.* **10**, 54–59.

Johnsson, M., Richardson, C. F., Bergey, E. J., Levine, M. J. & Nancollas, G. H. (1991). *Arch. Oral Biol.* **36**, 631–636.

Machleidt, W., Thiele, U., Laber, B., Assfalg-Machleidt, I., Esterl, A., Wiegand, G., Kos, J., Turk, V. & Bode, W. (1989). *FEBS Lett.* **243**, 234–238.

McPherson, A. (1982). *Preparation and Analysis of Protein Crystals*, pp. 82–159. New York: John Wiley.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.

Ramasubbu, N., Reddy, M. S., Bergey, E. J., Haraszthy, G., Soni, S.-D. & Levine, M. J. (1991). *Biochem. J.* **280**, 341–352.

Saitoh, E., Kim, H.-S., Smithies, O. & Maeda, N. (1987). *Gene*, **61**, 329–338.

Shomers, J. P., Tabak, L. A., Levine, M. J., Mandel, I. D. & Hay, D. I. (1982). *J. Dent. Res.* **61**, 397–399.