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Crystallization and preliminary X-ray analysis of pig pancreatic α-amylase in complex with a bean lectin-like inhibitor†

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Abstract

Pig pancreatic α-amylase (PPA, E.C. 3.2.1.17, 496 amino-acid residues) has been crystallized as a complex with a lectin-like inhibitor from bean *Phaseolus vulgaris* (224 amino-acid residues for the inhibitor monomer). The hanging-drop vapour-diffusion method was used to grow crystals from solutions containing 2-methyl-2,4-pentanediol as precipitant. The crystals belong to monoclinic space group $C2$ with $a = 152.5$, $b = 80.3$, $c = 68.8$ Å, $\beta = 91.4$° and diffract to 2.9 Å resolution. A molecular-replacement solution of the structure has been obtained using the refined PPA and LoLI (*Lathyrus ochrus* isolectin I) atomic coordinates as starting models. Low-resolution refinement of the model is underway. The analysis reveals that the functional inhibitor molecule is dimeric and interacts with two molecules of enzyme.

1. Introduction

α-Amylases (α-1,4-glucan-4-glucanohydrolase) constitute a group of enzymes found in microorganisms, plants and animal secretions. They catalyze the hydrolysis of α-(1,4) glycosidic linkages of starch components, glycogen, and various oligosaccharides. The amino-acid sequence comparison of porcine, human, mouse and rat pancreatic α-amylases demonstrates a high degree of homology (Pasero, Mazzei-Pierron, Abadie, Chicheportiche & Marchis-Mouren, 1986).

The three-dimensional molecular model of porcine pancreatic α-amylase (PPA) has been described in detail (Qian, Haser & Payan, 1993). The interactions between the enzyme and a carbohydrate inhibitor are defined in the refined structure at 2.2 Å resolution of the PPA/acarbose complex (Qian, Haser, Buisson, Duée & Payan, 1994). At present a hen egg-white lysozyme-like mechanism is considered as the most probable for glycoside hydrolases including amylases. However, further structural information is required for a better knowledge of enzyme function (Mazur, Haser & Payan, 1994). Particularly, the mechanism of inhibition by the widely occuring natural inhibitors is mostly unclear.

A number of proteinaceous inhibitors of α-amylases have been characterized in higher plants and microorganisms (Whitaker, 1988). The seeds of common bean, *Phaseolus vulgaris*, were shown to contain a family of plant defence proteins comprising phytohemaglutinin (PHA), arcelin (ARL) and α-amylase inhibitor (α-AI) (Chrispeels & Raikhel, 1991). The three proteins have different modes of action in protecting seeds from being eaten by mammals or infested by bruchid larvae that burrow into the seeds; α-AI inhibits the α-amylase in the digestive tract of mammals and coleoptera (Powers & Whitaker, 1977*b*). A comparison of the amino-acid sequences of lectins (PHA and LoLI from the seeds of *Lathyrus ochrus*) and lectin-like proteins (arcelin and α-AI) from *P. vulgaris* shows a high degree of both identity and homology (Rougé, Barre, Causse, Chatelain, Porthe, 1993). α-AI strongly inhibits PPA (Powers & Whitaker, 1977*a,b*) ($K_d = 3.5 \times 10^{-11}$ *M* at a neutral pH). According to Rousseau *et al.* (unpublished data) a functional heterotetramer complex consisting of the inhibitor dimer (I_2) and of two molecules of enzymes (E_2) is expected to occur ($M_r = 150\,000$). Previous measurements of the stoichiometry of inhibition (Marshall & Lauda, 1975), suggested the formation of a 1:1 complex of α-amylase and α-AI with a molecular weight of about 100 000. That, would be consistent with an EI_2 complex.

Detailed analysis of the interactions between PPA and α-AI would be of interest to improve our understanding of both enzyme function and the role of α-AI proteins in the protection of plants against some predatory insects.

2. Materials and methods

2.1. *Purification and crystallization*

PPA was prepared as described by Marchis-Mouren & Pasero (1967). The concentration for crystallization attempts was about 15 mg ml^{-1}. The α-AI inhibitor was purified from seed flour of *P. vulgaris cv.* Tendergreen by precipitation at 40% ammonium sulfate saturation of heat-resistant (343 K) proteins, chromatofocusing on PBE94 (Pharmacia) in the pH range 6.2–4.0 and subsequent chromatography on Sephadex G200 in Tris-buffered saline (pH 7.4) (Rousseau *et al.*, unpublished results). The fractions corresponding to α-AI were pooled and lyophilized. For crystallization attempts, the lyophilized α-AI was rehydrated to a concentration of 10 mg ml^{-1} in 0.1 *M* sodium acetate (pH 5.7), 0.01 m*M* $CaCl_2$. Solutions of both the enzyme and inhibitor were mixed together stoichiometrically and incubated at room temperature for 1 h. Initial crystallization trials were carried out by the hanging-drop vapour-diffusion method (McPherson, 1982), using the Hampton Research Grid Screen MPD (Weber, 1991). Single crystals suitable for X-ray analysis ($0.2 \times 0.2 \times 0.2$ mm) were obtained after 2 d, at room temperature in hanging drops consisting of 2 µl of enzyme complex solution and 2 µl from the reservoir which contained 0.1 *M* sodium acetate (pH 5) and 20% MPD (Grid screen MPD reagent B2).

2.2. *X-ray diffraction pattern*

Crystals were mounted and sealed in capillaries with a drop of mother liquor. X-ray diffraction data for the PPA/α-AI

† This work is dedicated to the memory of Patrice Rousseau who died April 30, 1995.

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complex crystals were collected using a MAR Research imaging-plate scanner (Hendrix and Lentfer, Hamburg) and Cu $K\alpha$ radiation from a Rigaku rotating-anode generator operating at 80 mA, 40 kV and graphite monochromator. Frames of data were recorded while the crystal was oscillated through 1° steps and were measured for 15 min. The diffraction data were auto-indexed and processed using the program *MARXDS* 2.0 (Kabsch, 1988*a*). The crystals belong to the space group $C2$ and have unit-cell dimensions $a = 152.5$, $b = 80.3$, $c = 68.8$ Å, $\beta = 91.4°$. Assuming half an E_2I_2 complex molecule of 75 kDa in the asymmetric unit, the V_m value is 2.8 Å^3 Da^{-1} corresponding to a solvent content of 56%. These values are within the normal range for proteins (Matthews, 1968).

A total of 56 888 diffraction intensities was collected to 2.9 Å; they were merged into 17 155 unique reflections using the program *MARSCALE* (Kabsch, 1988*b*). The data set is 94.5% complete up to 2.9 Å resolution (79.0% complete in the final shell), with $I/\sigma(I)$ above 2.7, and a merging R factor for symmetry-related reflections of 10%.

3. Results and discussion

The molecular-replacement calculations were performed at 4 Å resolution using the program suite *AMoRe* (Navaza, 1992). The search models were the refined 2.1 Å resolution structure of PPA (Qian, Haser & Payan, 1993) and one monomer of the 1.9 Å resolution structure of LoLI (Bourne *et al.*, 1990).

Two rotation functions were calculated (Navaza, 1992), one for PPA and one for the monomer of LoLI. They both unambiguously showed only one solution corresponding to the orientation of PPA and LoLI, respectively. After each translation function, the previously determined positions and orientations were refined as rigid bodies (Castellano, Oliva, Navaza, 1992). The final values of correlation coefficient and R factor were 42.1 and 42.8%, respectively. The molecular-replacement process yielded an E_2I_2 complex model consistent with good packing and two EI entities related by the twofold crystallographic axis. The structure was then refined using the simulated-annealing program *X-PLOR* (Brünger, Kuriyan & Karplus, 1987) and after the first cycle the R value was 27.7%. At the 1σ level of the final $(2F_{obs} - F_{calc})\exp(i\alpha_{calc})$ map, all enzyme atoms had very well defined density; concerning the inhibitor model, all residues that directly contact the enzyme molecule were clearly observed in the electron density, as were all the secondary structural elements. Some external turns in the inhibitor model had poor or broken density, and the last 19 residues at the C terminus were not seen in the electron density. The PPA/α-AI complex model presents large deviations from search models occurring in the long loops of the enzyme strongly interacting with the inhibitor and shows a loop segment from the inhibitor model bound into the catalytic cleft of the enzyme molecule. With data at higher resolution, the phasing will be extended, the refinement completed and the details of the structure reported elsewhere.

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References

Bourne, Y., Abergel, C., Cambillau, C., Frey, M., Rouge, P. & Fontecilla-Camps, J. C. (1990). *J. Mol. Biol.* **214**, 571–584.
Brünger, A. T., Kuriyan, J. & Karplus, M. (1987). *Science*, **35**, 458–460.
Castellano, E. E., Oliva, O. & Navaza, J. (1992). *J. Appl. Cryst.* **25**, 281–284.
Chrispeels, M. J. & Raikhel, N. V. (1991). *Plant Cell*, **3**, 1–9.
Kabsch, W. (1988*a*). *J. Appl. Cryst.* **21**, 67–71.
Kabsch, W. (1988*b*). *J. Appl. Cryst.* **21**, 916–924.
McPherson, A. (1982). *Preparation and Analysis of Protein Crystals*, pp. 82–127. New York: John Wiley.
Marchis-Mouren, G. & Pasero, L. (1967). *Biochim. Biophys. Acta*, **140**, 366–368.
Marshall, J. J. & Lauda, C. M. (1975). *J. Biol. Chem.* **250**, 8030–8037.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mazur, A. K., Haser, R. & Payan, F. (1994). *Biochem. Biophys. Res. Commun.* **204**, 297–302.
Moreno, J. & Chrispeels, M. J. (1989). *Proc. Natl Acad. Sci. USA*, **86**, 7885–7889.
Navaza, J. (1992). *AMoRe: A New Package for Molecular Replacement*, pp. 87–91. In *Proceedings of the CCP4 Study Weekend*, edited by E. J. Dodson, S. Grower, W. Wolf. Warrington: Daresbury Laboratory.
Pasero, L., Mazzei-Pierron, Y., Abadie, B., Chicheportiche, Y. & Marchis-Mouren, G. (1986). *Biochim. Biophys. Acta*, **869**, 147–157.
Powers, J. R. & Whitaker, J. R. (1977*a*). *J. Food Biochem.* **1**, 217–238.
Powers, J. R. & Whitaker, J. R. (1977*b*). *J. Food Biochem.* **1**, 239–260.
Qian, M., Haser, R., Buisson, G., Duée, E. & Payan, F. (1994). *Biochemistry*, **33**, 6284–6294.
Qian, M., Haser, R. & Payan, F. (1993). *J. Mol. Biol.* **231**, 785–799.
Rougé, P., Barre, A., Causse, H., Chatelain, C. & Porthe, G. (1993). *Biochem. Syst. Ecol.* **21**, 695–703.
Weber, P. C. (1991). *Adv. Protein Chem.* **41**, 1–36.
Whitaker, J. R. (1988). *α-Amylases Inhibitors of Higher Plants and Microorganisms*, pp. 354–380. In *Food Proteins*, edited by J. E. Kinsella & W. G. Soucle, Proc. of Prot. Co-prod. Symp., AM. Oil Chem. Soc., Champaign, Il, USA.