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Crystallization and molecular replacement solution of human heparin binding protein

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Abstract

The highly glycosylated protein, human heparin binding protein, has been crystallized in the primitive orthorhombic space group $P2_12_12_1$ with cell dimensions $a = 39.0$, $b = 66.2$ and $c = 101.4$ Å. Ethanol was used as precipitant and glycerol as additive. A full data set has been collected to 3.1 Å and diffraction was observed to at least 2.3 Å. A molecular replacement solution using human neutrophile elastase as a search model was obtained, showing one molecule per asymmetric unit. The crystal packing showed no bad contacts and the R factor was 44.8% after ten cycles of rigid-body refinement.

1. Introduction

The heparin binding protein (HBP) also known as CAP37 or azurocidin is an inactive serine protease homologue (Flodgaard *et al.*, 1991). The inactivity is caused by selective mutations in the active site triad, serine for a histidine at position 41 and glycine for a serine at position 175. The human HBP exhibits 44% sequence identity with human neutrophile elastase (Flodgaard *et al.*, 1991). HBP consists of 225 amino acids, is highly glycosylated and possess three putative *N*-glycosylation sites. HBP purified from human neutrophile azurophile granules is mainly a 28 kDa protein (Flodgaard *et al.*, 1991). A 37 kDa human HBP (cationic antimicrobial protein of 37 kDa, CAP37) from human neutrophiles has been reported (Shafter, Martin & Spitznagel, 1986). Human HBP has been expressed in the baculovirus system as a 28 kDa protein, resulting in a glycosylation degree of 13% (Rasmussen *et al.*, 1996).

HBP is believed to be involved in host defence during infection and inflammation (Østergaard & Flodgaard, 1992). Specific monocyte chemotaxis is activated by HBP. Also, monocyte survival and differentiation are induced by HBP towards a macrophage phenotype (Flodgaard *et al.*, 1991; Østergaard, Nielsen & Flodgaard, 1992). In addition, it has been shown that HBP binds strongly to lipid A and endotoxin (lipopolysaccharide) with $K_{\rm ass} = 0.8 \times 10^9 M^{-1}$ (Flodgaard & Göricke, 1994) and possess antibacterial properties against a number of Gram-negative bacteria (Shafer *et al.*, 1986). The strong heparin-binding potential of HBP is believed to be related to the basic nature (calculated pI 9.21) of the protein (Flodgaard *et al.*, 1991).

The multifunctionality of HBP makes it highly relevant for structural investigation.

2. Experimental

2.1. *Expression and purification*

Human HBP was expressed in the baculovirus system. A four-step purification procedure was applied. After filtration, two consecutive ion-exchange chromatography steps were carried out where the latter only served to concentrate the protein solution before the final gel-filtration step. The purification procedure generates a pure preparation of HBP as judged by sodium dodecyl sulfate polyacrylamide gel electrophoresis and high pressure liquid chromatography analysis (Rasmussen *et al.*, 1996).

The protein was concentrated to a 5 mg ml^{-1} solution using a Millipore microconcentrator (Ultrafree-MC 20.000 NMWL filter unit) in 5 mM Hepes buffer pH 7.8.

2.2. *Crystallization*

The 5 mg ml^{-1} protein solution was screened for crystallization conditions using the Hampton Crystal Screen and the hanging-drop vapour-diffusion method. Under the best conditions large crystalline aggregates were obtained with 20% ethanol in 0.1 M Tris buffer pH 8.5 at 293 K. Optimization over the above standing conditions did not lead to any improvements in the crystal quality. The crystals used for data collection were grown by repeated seeding technique. First, microcrystals were made of crushed crystalline aggregates. 4 µl drops, consisting of 2 µl 5 mg ml^{-1} protein solution and 2 µl reservour solution containing 14–20% ethanol, 5–10% glycerol and 0.1 M Tris buffer pH 7.0 were allowed to equilibrate 30–60 min before seeding with the microcrystals. Seeded microcrystals grew overnight to the size of 0.1–0.2 × 0.005 × 0.03 mm. Secondly, single crystals were seeded under similar conditions as the microcrystals, and grew overnight to the size of 0.5–0.8 × 0.2 × 0.07 mm, see Fig. 1. The reservoir volume was 1 ml for all experiments.

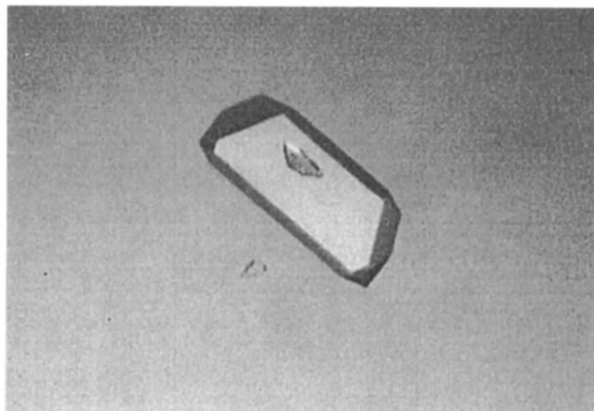

Fig. 1. HBP crystals obtained after repeated seeding. With microseeding as well as macroseeding crystal growth stopped within 24 h.

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2.3. *Data collection*

Diffraction data were collected on an R-AXIS II image-plate detector using a Rigaku RU200 rotating-anode generator ($\lambda = 1.542$ Å, 50 kV, 180 mA). The data collection was performed at 285 K. The crystal to image plate distance was 100 mm and the oscillation range was 2°. Auto-indexing and data processing were performed with *DENZO* (Otwinowski, 1986) and the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994).

2.4. *Molecular replacement*

The *AMoRe* program (Navaza, 1994) from *CCP*4 was used for the molecular replacement solution. A human neutrophile elastase structure (1.8 Å) (Bode, Meyer & Powers, 1989) was used to conduct the rotation and translation search, including all residues with side chains. All rotation solutions with correlation coefficients between ten and 20 were used in the translation function. After the translation function all top solutions were refined with ten cycles of rigid-body refinement.

3. Results and discussion

A full data set was collected to 3.1 Å on one single crystal. Statistics of the data set are shown in Table 1. However, diffraction was observed to at least 2.3 Å, see Fig. 2. During data collection the high-resolution reflections tended to lose intensity or disappear, as evidence of slow crystal decay. The relatively low resolution data set compared to the maximum diffraction, might be a result of rapid crystal growth in combination with glycosylation of the protein. The very flexible glycosylation chains might add to the disorder in the crystal. Also, the crystal used was less than 0.1 mm in the smallest dimension, thus, increasing the volume of the crystal might improve the diffraction. Investigations are conducted to solve the kinetics of the crystal growth. The use of synchrotron radiation and/or flash-freezing of the crystal might also solve the resolution problem.

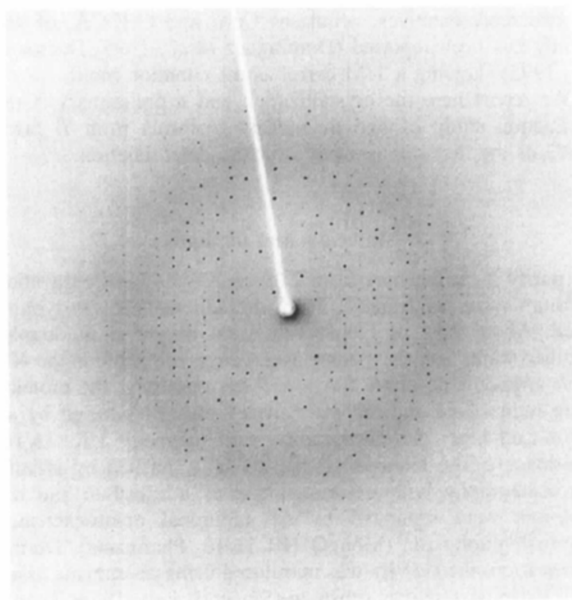

Fig. 2. Diffraction pattern obtained from an HBP crystal on an R-AXIS II image-plate system. The oscillation range for this frame was 2° and the crystal-to-image-plate distance was 100 mm. Diffraction is observed to 2.3 Å.

Table 1. *Crystal data and statistics of the data set*

Space group	$P2_12_12_1$
Cell dimensions (Å)	$a = 39.0$, $b = 66.2$ and $c = 101.4$
Z	4
Resolution range (Å)	25–3.1
Completeness (%)	98.3
Multiplicity	5.2
$R_{\rm merge}(I)$ (25–3.1 Å) (%)	9.3
$R_{\rm merge}(I)$ (3.26–3.1 Å) (%)	24.9
$\langle I/\sigma(I)\rangle$ (25–3.1 Å)	7.5
$\langle I/\sigma(I)\rangle$ (3.26–3.1 Å)	2.9

HBP crystallized in space group $P2_12_12_1$ and with cell dimensions $a = 39.0$, $b = 66.2$ and $c = 101.4$ Å. V_m was calculated to be 2.3 Å^3 Da^{-1} with $Z = 4$, corresponding to one molecule per asymmetric unit. The solvent content was determined to be 46% using the equation of Matthews (1968).

Human neutrophile elastase served as a good template for the molecular replacement solution. After the translation function only one significant solution was found, with correlation coefficient and R factor of 39.2 and 47.9%, respectively. The second best solution had a correlation score of 20.5 and an R factor of 53.9%. After ten cycles of rigid-body refinement the correlation coefficient increased to 50.4 and the R factor dropped to 44.8%.

The crystal packing was inspected using the program *O* (Jones, Bergdoll & Kjeldgaard, 1990). No bad contacts were observed between the molecules in the unit cell.

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References

Bode, W., Meyer Jr, E. & Powers, J. C. (1989). *Biochemistry*, **28**, 1951–1963.

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

Flodgaard, H. & Göricke, C. (1994). *J. Cell. Biochem. Suppl.* 18A, Abstract E505.

Flodgaard, H., Østergaard, E., Bayne, S., Svendsen, A., Thomsen, J., Engels, M. & Wollmer, A. (1991). *Eur. J. Biochem.* **197**, 535–547.

Jones, T. A., Bergdoll, M. & Kjeldgaard, M. (1990). *Crystallographic & Modelling Methods in Molecular Design*, edited by C. Bugg & S. Ealick, pp. 189–199. New York: Springer-Verlag.

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

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

Østergaard, E. & Flodgaard, H. (1992). *J. Leuko. Biol.* **51**, 316–323.

Østergaard, E., Nielsen, O. F. & Flodgaard, H. (1992). *APMIS*, **100**, 1073–1080.

Otwinowski, Z. (1986). *DENZO. An Oscillation Data Processing Program for Macromolecular Crystallography*. Yale University, CT, USA.

Rasmussen, P. B., Bjørn, S., Hastrup, S., Nielsen, P. F., Norris, K., Thim, L., Wiberg, F. C. & Flodgaard, H. (1996). *FEBS Lett.* In the press.

Shafter, W. M., Martin, L. E. & Spitznagel, J. K. (1986). *Infect. Immun.* **53**, 651–655.