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Crystallization and molecular-replacement solution of a truncated form of human recombinant tetranectin

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The two C-terminal domains, TN23 (residues 17–181), of human recombinant tetranectin, a plasminogen kringle 4 binding C-type lectin, have been crystallized in two different space groups. Using PEG 8000 as precipitant and at a pH of 8.5, crystals belonging to the monoclinic space group $C2$ are obtained, with unit-cell parameters $a = 160.4$, $b = 44.7$, $c = 107.5$ Å, $\beta = 127.6^\circ$. Using sodium formate as precipitant and at a pH of 5.0, TN23 crystallizes in a rhombohedral space group, with unit-cell parameters $a = b = c = 107.4$ Å, $\alpha = \beta = \gamma = 78.3^\circ$. A full data set to 4.5 Å has been collected from the monoclinic crystals. Using the structure of full-length tetranectin, a molecular-replacement solution has been obtained. The crystal packing shows that TN23 crystallizes as a trimer, with one trimer in the asymmetric unit.

1. Introduction

Human tetranectin (TN) is a plasminogen-binding C-type lectin. The protein was first isolated from plasma, where it is present at concentrations of 10 mg ml^{-1} (Clemmensen *et al.*, 1986). TN binds specifically to the kringle 4 domain of plasminogen (Clemmensen *et al.*, 1986) and binding is sensitive to both calcium and lysine (Graversen *et al.*, 1998). TN is expressed by a variety of cells and various tissues. In particular, TN is accumulated in the extracellular matrix of certain human carcinomas (for a review, see Høgdall, 1998) and has been found co-localized with plasmin/plasminogen in the invasive front of cutaneous melanoma lesions, indicating a coordinated role of these proteins in the invasive process (De Vries *et al.*, 1996). In addition, the protein interacts with complex sulfated polysaccharides (Clemmensen, 1989) and, in a calcium-dependent manner, with fibrin (Kluft, Los *et al.*, 1989) as well as with lipoprotein(a) (Kluft, Jie *et al.*, 1989). A role for tetranectin might be the targeting of plasminogen to carbohydrate ligands on cell surfaces or to carbohydrates in the extracellular matrix. Recent studies indicate that TN plays a role in osteogenesis and myogenesis (Wewer *et al.*, 1994; Iba *et al.*, 1995; Wewer *et al.*, 1998).

TN is a homotrimeric protein, each polypeptide chain consisting of 181 amino-acid residues (Holtet *et al.*, 1997). TN is encoded by three exons, of which exon 3 encodes TN3, the long-form carbohydrate-recognition domain (CRD) of TN. TN and TN3 have been crystallized (Kastrup *et al.*, 1997) and the structures have been determined to 2.8 Å resolution (Nielsen *et al.*, 1997) and 2.0 Å resolution (Kastrup *et al.*, 1998), respectively. TN is a trimer in the crystals as well as in solution, whereas TN3, which is a monomer in solution (Holtet *et al.*, 1997), forms dimers in the crystal. The same phenomenon is observed for a structurally similar C-type lectin, rat mannose-binding protein (Weis *et al.*, 1991, 1992; Weis & Drickamer, 1994).

The trimerization of TN is mainly a consequence of the formation of a triple α-helical coiled coil. The monomers of TN each consist of a long N-terminal helix with the CRD on top of the helix. The coiled coil holds the CRDs of the monomers apart and only a few contacts are observed between the CRDs. Two Ca^{2+}-binding sites in the CRD have been located and characterized (Nielsen *et al.*, 1997; Kastrup *et al.*, 1998). Recently, the plasminogen kringle 4 binding site has been located on the CRD of TN by site-directed mutagenesis and involves residues of the putative carbohydrate-binding site (Graversen *et al.*, 1998).

Cross-linking studies on different constructs of TN have shown that the polypeptide TN1–16 is trimeric at high concentration, whereas TN23 (residues 17–181) appeared to be monomeric. Gel-filtration analysis, however, indicated that TN23 is a trimer (Holtet *et al.*, 1997), as is TN. To address the ambiguity concerning the oligomerization state of TN23, crystallization of human recombinant TN23 was undertaken.

2. Experimental

2.1. Crystallization

Overexpression and purification of human recombinant TN23 have been described by Holtet *et al.* (1997). A solution of TN23 in 50 mM Tris–HCl pH 8.0, 100 mM NaCl was

Table 1
Data-collection statistics for crystal modification I of TN23.

Space group	*C*2
Unit-cell parameters (Å, °)	a = 160.4, b = 44.7, c = 107.5, β = 127.6
Resolution range (Å)	25.0–4.5
Completeness (%)	97.4
Multiplicity	2.8
$R_{\rm merge}$ (I) (%)	
25.0–4.50 Å	15.9
25.0–9.53 Å	6.6
9.53–7.64 Å	8.8
7.64–6.69 Å	17.6
4.66–4.50 Å	35.0
$\langle I/\sigma(I)\rangle$	
25.0–4.50 Å	6.5
25.0–9.53 Å	12.4
9.53–7.64 Å	11.2
7.64–6.69 Å	6.1
4.66–4.50 Å	3.2

concentrated to the appropriate concentration using Centricon 10 concentrators (Amicon).

A sparse-matrix screen (Crystal Screens I and II, Hampton Research) and the hanging-drop vapour-diffusion method at 293 K were used to find initial crystallization conditions. A drop containing 3 µl of a 5 mg ml^{-1} solution of TN23 in 25 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl, 20 m*M* $CaCl_2$ was mixed with 3 µl of reservoir solution and equilibrated against a 0.5 ml reservoir. Crystals were obtained with the following reservoir solutions. Crystal modification I: 0.1 *M* Tris–HCl pH 8.5, 0.2 *M* $MgCl_2$, 30% PEG 4000, where tiny needles appeared. Crystal modification II: 0.1 *M* sodium acetate pH 4.6, 2.0 *M* sodium formate, which resulted in a rod-shaped crystal.

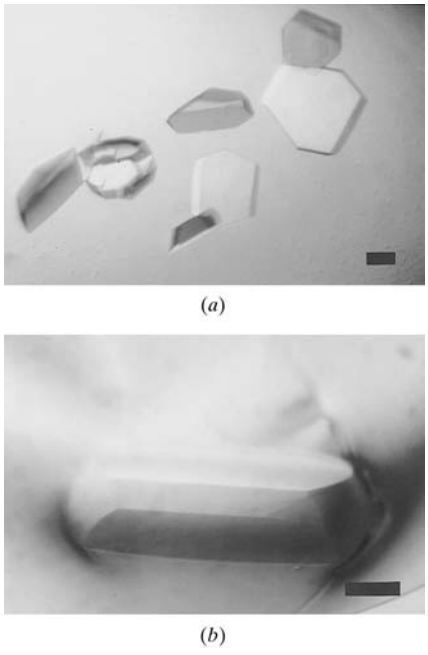

Figure 1
Crystals of TN23. (*a*) Thin crystals belonging to space group *C*2. (*b*) Rectangular crystals belonging to a rhombohedral space group. The bar corresponds to 100 µm.

2.1.1. Crystal modification I. The crystallization conditions were optimized to a reservoir solution consisting of 0.1 *M* Tris–HCl pH 8.5, 0.05 *M* $MgCl_2$, 20% PEG 8000 and a protein solution containing 5 mg ml^{-1} TN23 in 25 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl, 2 m*M* $CaCl_2$. The drop size was 4 µl, containing equal amounts of protein solution and reservoir solution. Crystallization at 279 K gave slightly larger crystals than at 293 K. The crystals are thin plates, with maximum dimensions of 0.3 × 0.3 × 0.08 mm.

2.1.2. Crystal modification II. A high concentration of sodium formate was used to grow these crystals. The crystallization conditions were optimized to a drop size of 4 µl. Each drop contained 2 µl of a reservoir solution consisting of 0.1 *M* Tris–maleate pH 5.0, 2.5 *M* sodium formate and 2 µl of a protein solution consisting of 10 mg ml^{-1} TN23 in 25 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl, 20 m*M* $CaCl_2$. The crystals had dimensions of 0.3 × 0.3 × 0.5 mm after 12 months.

2.2. Data collection

A complete diffraction data set to 4.5 Å resolution was collected from crystal modification I on an R-AXIS II imaging-plate detector, using a rotating-anode generator (Rigaku RU-200) operated at 50 kV and 180 mA, λ = 1.542 Å. Data collection was carried out at 293 K with a crystal-to-detector distance of 110 mm and an oscillation range of 2° per image. Autoindexing and processing were performed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997) and the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994). For crystal modification II, diffraction data were collected at 278 K to about 5 Å resolution using an oscillation range of 2° per image at a crystal-to-detector distance of 200 mm using synchrotron radiation of wavelength λ = 0.9076 Å (EMBL beamline X11 at the DORIS storage ring, DESY, Hamburg) and a MAR345 detector. Autoindexing was performed using *DENZO* (Otwinowski & Minor, 1997).

2.3. Molecular replacement

The program *AMoRe* implemented in the *CCP*4 suite of programs (Navaza, 1994) was used to find a molecular-replacement solution. The coordinates of full-length TN (PDB code 1htn; residues 26–181) in the trimeric form were used as a search model in the resolution range 25.0–4.5 Å for the rotation function and in the resolution range 10.0–4.5 Å for the translation function. The 50 highest peaks of the rotation solutions were used in the translation search. The three best solutions were subjected to 25 cycles of rigid-body refinement (25.0–4.5 Å resolution) also using the program *AMoRe*, allowing each of the three monomers to refine independently.

3. Results and discussion

TN23 has been crystallized in two different crystal modifications (Fig. 1). Crystal modification I crystallizes in space group *C*2, with unit-cell parameters a = 160.4, b = 44.7, c = 107.5 Å, β = 127.6°. A diffraction data set to 4.5 Å was collected from a single crystal. Data-collection statistics are shown in Table 1. V_m was calculated to be 2.77 Å^3 Da^{-1} (Matthews, 1968), assuming one trimer in the asymmetric unit. This corresponds to a solvent content of 56%.

Crystal modification II crystallizes in a primitive rhombohedral space group, with unit-cell parameters $a = b = c$ = 107.4 Å, $\alpha = \beta = \gamma$ = 78.3° ($a = b$ = 135.7, c = 220.6 Å, $\alpha = \beta$ = 90.0, γ = 120.0° in hexagonal axes). The crystals diffract to 7.5 Å using a rotating-anode generator and to about 5 Å using synchrotron radiation. Assuming space group *R*3 and trimeric TN23, a V_m of 3.55 Å^3 Da^{-1} corresponds to 65% solvent and two trimers in the asymmetric unit, whereas a V_m of 2.36 Å^3 Da^{-1} corresponds to 48% solvent and three trimers in the asymmetric unit. This crystal modification has not been further characterized.

The monoclinic crystals have a high mosaicity and the low resolution of the data might reflect disorder in the crystals. The resolution was only slightly improved by using synchrotron radiation (EMBL beamline X11 at the DORIS storage ring, DESY, Hamburg) or cryogenic temperatures. The maximum resolution observed was about 3.5 Å. Macroseeding has been applied to the monoclinic crystals; however, the resolution was not improved.

The 4.5 Å diffraction data were used to find a molecular-replacement solution in order to reveal the oligomeric state of TN23 in the crystals. The three highest peaks in the rotation function clearly correspond to the orientation of each of the monomers comprising the trimer. The correlation coefficients after the translation search are 31.2, 31.2 and 31.4, and the *R* values are 52.2,

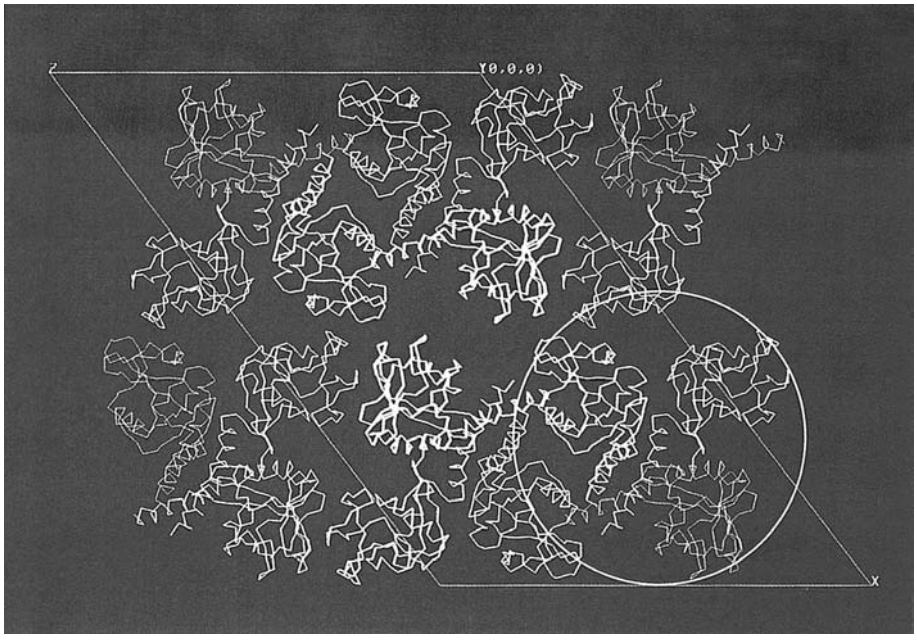

Figure 2
The crystal packing of TN23 shown in the *xz* plane, demonstrating that TN23 is a trimer, as is full-length TN. One TN23 trimer is marked with a circle.

52.1 and 52.1%, respectively. The fourth highest peak of the translation function has a correlation coefficient and an *R* value of 23.0 and 54.4%, respectively. Rigid-body refinement of the individual monomers of the top solution resulted in a correlation coefficient of 61.3 and an *R* value of 43.4%. The packing of the molecules in the unit cell was inspected using the program *O* (Jones *et al.*, 1991) and clearly shows the trimeric TN23 (Fig. 2). The crystal packing leaves space for the additional nine N-terminal residues of TN23 which were not included in the search model.

The oligomerization state of TN23 can unambiguously be assigned as trimeric, although the resolution of the structure of TN23 does not allow a detailed discussion of the structure. However, as no large movements of the individual monomers relative to one another were observed during the rigid-body refinement, the arrangement of the molecules in the trimer of TN23 seems to be very much the same as in full-length TN. TN23 crystallizes in a space group different to that of TN and with one trimer in the asymmetric unit, contrary to TN which crystallizes in space group *R*3 with the molecular threefold axis coinciding with the crystallographic threefold axis. As the first 25 residues of TN could not be located in the electron-density maps, it is difficult to ascribe a structural role to these residues. The first 16 residues are not necessary for the trimerization, as both TN23 and full-length TN form trimers. However, the residues may have a stabilizing effect on the trimer.

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