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Human plasminogen binding protein tetranectin: crystallization and preliminary X-ray analysis of the C-type lectin CRD and the full-length protein

JETTE SANDHOLM KASTRUP,[a]* HANNE RASMUSSEN,[a] BETTINA BRYDE NIELSEN,[a] INGRID KJØLLER LARSEN,[a] THOR LAS HOLTET,[b] JONAS HEILSKOV GRAVERSEN,[b] MICHAEL ETZERODT[b] AND HANS CHRISTIAN THØGERSEN[b] *at* [a]*Department of Medicinal Chemistry, Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark, and* [b]*Laboratory of Gene Expression, Department of Molecular and Structural Biology, University of Aarhus, DK-8000 Aarhus C, Denmark. E-mail: kastrup@medchem.dfh.dk*

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Abstract

The recombinant human plasminogen binding protein tetranectin (TN) and the C-type lectin CRD of this protein (TN3) have been crystallized. TN3 crystallizes in the tetragonal space group $P4_22_12$ with cell dimensions $a = b = 64.0$, $c = 75.7$ Å and with one molecule per asymmetric unit. The crystals diffract X-rays to at least 2.0 Å resolution. A complete diffraction data set has been collected to 2.7 Å resolution. The crystals of TN, obtained by the vapour-diffusion reverse salting-in method at 280 K, are rhombohedral, space group $R3$, with the hexagonal axes $a = b = 89.1$, $c = 75.8$ Å, and diffract to at least 2.5 Å. A full data set has been collected to 3.0 Å. The asymmetric unit contains one monomer of TN. Molecular replacement solutions for TN3 and TN have been obtained using the structure of the C-type lectin CRD of rat mannose-binding protein as search model. The rhombohedral space group indicates that trimers of TN are formed in accordance with the observation of trimerization in solution.

1. Introduction

The plasma protein tetranectin (TN) was found and characterized about ten years ago (Clemmensen, Petersen & Kluft, 1986). It has been isolated from plasma, but is also present in various tissues and cells (Christensen, Johansen, Jensen & Clemmensen, 1987; Christensen & Clemmensen, 1989; Borregaard, Christensen, Bjerrum, Birgens & Clemmensen, 1990). TN has been found in the extracellular matrix of certain human carcinomas, whereas no or very little TN is present in the corresponding normal tissues (Christensen & Clemmensen, 1989, 1991; Høgdall, Christensen & Clemmensen, 1993; Wewer & Albrechtsen, 1992).

TN binds specifically to the kringle 4 domain of plasminogen (Clemmensen *et al.*, 1986) and is consequently involved in fibrinolysis. The fibrinolytic proteins of the plasminogen/plasmin system are involved in extracellular proteolysis (Danø *et al.*, 1985; Carmellet *et al.*, 1994). The precise biological function of TN is not known, but the specific binding to kringle 4 of plasminogen combined with its abundant occurrence in plasma and various tissues suggest that the protein has a decisive function in fundamental biological processes, *e.g.* tissue degradation and cell migration (Christensen & Clemmensen, 1991; Nielsen, Clemmensen & Kharazmi, 1993).

The TN polypeptide chain consists of 181 amino-acid residues with three intrachain disulfide bridges (Fuhlendorff, Clemmensen & Magnusson, 1987; Berglund & Petersen, 1992). The protein was originally proposed to occur as a homo-tetramer in solution (Clemmensen *et al.*, 1986). However, it has been shown recently that recombinant TN, as well as human TN from plasma, is a trimeric protein (Holtet, Thøgersen, Clemmensen & Etzerodt, 1997). Each of the monomers consists of three exons or domains, TN1, TN2 and TN3. Sequence identity has been found among the C-terminal domain TN3 and other proteins containing a calcium-dependent C-type lectin carbohydrate recognition domain (CRD) (Fuhlendorff *et al.*, 1987; Day, 1994). Like other C-type lectins TN has a high affinity for calcium ions (Clemmensen *et al.*, 1986; Kluft, Los & Clemmensen, 1989) and binds sulfated polysaccharides (Clemmensen, 1989).

Structure determination of TN was initiated in order to obtain an understanding of the molecular interactions between TN and plasminogen and components from the extracellular matrix. The three-dimensional structure of tetranectin and of a TN–kringle 4 complex constitute an essential background for the design and development of drugs and/or model compounds interfering with the function of TN by activation or blockade. In this report crystallization and preliminary crystallographic studies of the C-type lectin CRD TN3 and of the full-length TN are described.

2. Experimental

2.1. *Protein purification*

Recombinant human TN3 and TN, expressed in *E. coli*, were isolated and purified as described earlier (Thøgersen, Holtet & Etzerodt, 1994; Holtet *et al.*, 1997). The TN3 solution was concentrated to 5 mg ml^{-1} in 25 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl and 20 m*M* $CaCl_2$. TN was concentrated to a 5 mg ml^{-1} solution in 25 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl and 2 m*M* $CaCl_2$. Millipore microconcentrators (Ultrafree-MC 10.000 NMWL filter unit) were used to concentrate the protein solutions.

2.2. *Crystallization*

The 5 mg ml^{-1} TN3 solution was screened for crystallization conditions using the sparse-matrix sampling screen of Jancarik & Kim (1991) from Hampton Research and the hanging-drop vapour-diffusion method at 293 K. Small crystals were observed with 5% isopropanol and 2.0 *M* ammonium sulfate. Optimization of the crystallization conditions gave crystals which appeared within one week and with a size up to 1.0 × 0.3 × 0.3 mm (Fig. 1*a*). 0.5 ml of 5% ethanol in 2.1 *M* ammonium sulfate was used as well solution. The drops contained 4 µl protein solution and 3 µl well solution.

Screening of the 5 mg ml^{-1} TN solution at 293 and 280 K gave no useful leads, but when a solution of TN (5 mg ml^{-1} in 25 m*M* Tris–HCl pH 8.0, 12.5 m*M* NaCl and 2 m*M* $CaCl_2$), was stored at 277 K, spontaneous formation of microcrystal-

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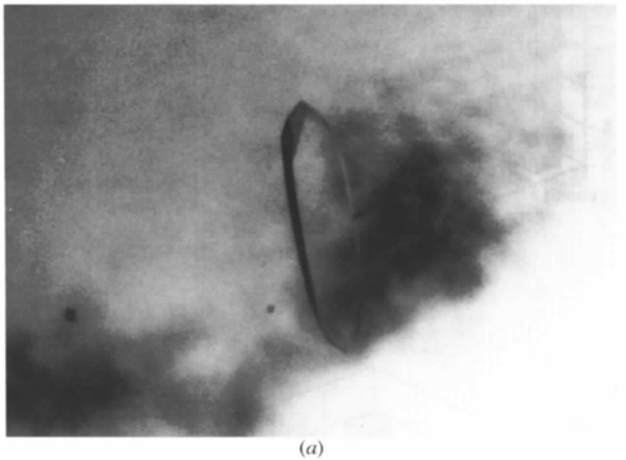
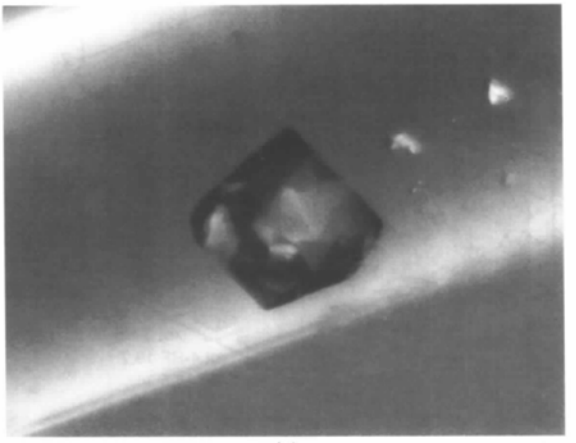
line precipitate was observed. Subsequently, crystals suitable for X-ray work were obtained by the hanging-drop vapour-diffusion reverse salting-in method at 280 K with drops of 2–8 µl containing 5 mg ml^{-1} protein, 12.5–25 m*M* Tris–HCl pH 8.0, 25–50 m*M* NaCl and 2 m*M* $CaCl_2$. Linbro plates (Flow Laboratories Inc., USA) were used with 0.5–1 ml water as reservoir solution. Crystals appeared after 3 weeks and grew to a size of about 300 µm within 2 months (Fig. 1*b*).

2.3. *Data collection*

A complete diffraction data set of TN3 to 2.7 Å resolution was collected using one crystal on a Rigaku RU-200 rotating-anode generator with an R-AXIS II imaging plate detector ($\lambda = 1.542$ Å, 50 kV, 180 mA, normal focus). The data collection was performed at 277 K. The crystal-to-detector distance was 110.0 mm and 180° rotation in steps of 2° oscillation were measured. Auto-indexing and data processing were performed with *DENZO* (Otwinowski, 1986) and the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994).



(*a*)



(*b*)

Fig. 1. (*a*) Tetragonal crystals of TN3, the C-type lectin CRD of tetranectin. The largest dimension is about 500 µm. (*b*) Rhombohedral crystal of tetranectin obtained by the vapour-diffusion reverse salting-in method. The largest dimension is about 300 µm.

Table 1. *Statistics of the data sets for TN3, the C-type lectin CRD of tetranectin, and for the full-length tetranectin, TN*

	TN3	TN
Resolution range (Å)	25.0–2.7	25.0–3.0
Completeness (%)	99.8	99.7
Multiplicity	5.4	5.5
R_{merge} (I) (%)	9.9	9.0
	30.7 (2.8–2.7 Å)	33.4 (3.1–3.0 Å)
$I/\sigma(I)$	6.9	8.0
	2.5 (2.8–2.7 Å)	2.3 (3.1–3.0 Å)

The crystal of TN used for data collection was diamond shaped with a diameter of 300 µm. Diffraction data were collected as for TN3 but with a crystal-to-image-plate distance of 100 mm. Auto-indexing and data processing were performed with *DENZO* and *CCP*4.

2.4. *Molecular replacement*

The program *AMoRe* (Navaza, 1994) from *CCP*4 was used for the molecular replacement solutions of TN3 and TN, respectively. The structure of the C-type lectin CRD of the rat mannose-binding protein (MBP) (Weis, Drickamer & Hendrickson, 1992) was used as a search model with all amino-acid side chains included in both cases.

The 20 best rotation solutions were used to solve the translation function. Subsequently, the best solutions were refined with ten cycles of rigid-body refinement. A sphere radius of 15 Å was chosen. The resolution range for both the rotation and translation search was 25.0–3.1 Å for TN3 and 25.0–4.1 Å for TN.

3. Results and discussion

3.1. *The C-type lectin CRD TN3*

TN3 was crystallized in the tetragonal space group $P4_22_12$ with cell dimensions $a = b = 64.0$, $c = 75.7$ Å. The crystals diffract to at least 2.0 Å resolution (Fig. 2*a*). The solvent content was calculated to 53% assuming one molecule in the asymmetric unit and $V_m = 2.6$ Å^3 Da^{-1} (Matthews, 1968). A complete data set was obtained to 2.7 Å resolution. The statistics of the data set are shown in Table 1.

The molecular replacement method was used to obtain initial phases. A monomer of the C-type lectin CRD of rat MBP, which shows 30% sequence identity to TN3, was used as the search model. Using *AMoRe* one clear solution to the translation function appeared with the best solution from the rotation search (see Fig. 3*a*). After rigid-body refinement the correlation coefficient improved to 49.9 and the R value dropped to 50.0% (25.0–3.1 Å). Although having only 30% identity, all amino-acid side chains were included in the search model as a polyalanine model turned out to be less sensitive in the rotation search.

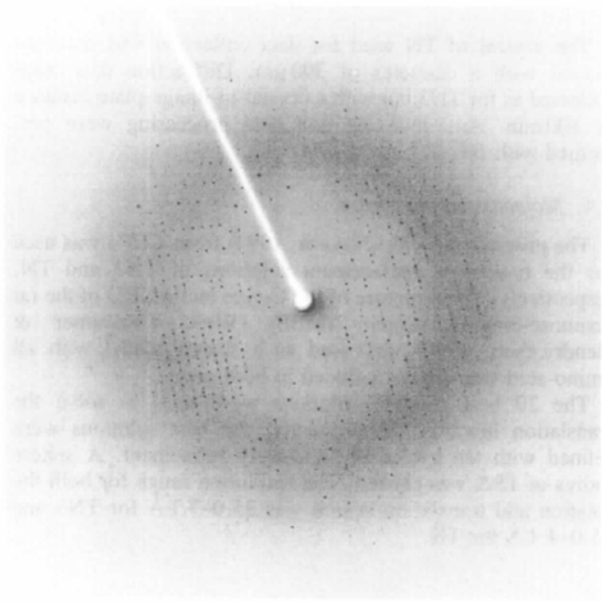
The packing of the molecules in the unit cell was inspected using the program *O* (Jones, Bergdoll & Kjeldgaard, 1990). No bad contacts between the molecules were observed.

3.2. *The full-length protein TN*

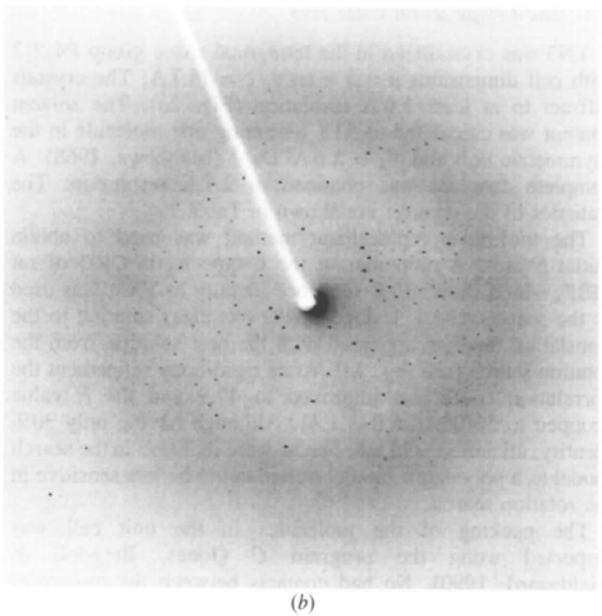
The crystals of TN obtained by the reverse salting-in method are rhombohedral. The space group is *R*3, and the cell

dimensions (hexagonal axes) are $a = b = 89.1$, $c = 75.8$ Å. The dimensions of the corresponding rhombohedral cell are $a = b = c = 57.3$ Å, $\alpha = \beta = \gamma = 102.1°$. V_m was calculated to be 3.1 Å^3 Da^{-1} with one monomer in the asymmetric unit corresponding to a solvent content of 60%. A full data set has been obtained to 3.0 Å. However, diffraction is observed at 2.5 Å (see Table 1 and Fig. 2*b*).

The molecular replacement method gave a convincing solution, though the search model, the structure of the C-type lectin CRD of MBP, corresponds to only about 3/4 of the TN molecule. As in the case of TN3, one clear solution to the translation function, combined with the best solution for the rotation search, appeared (see Fig. 3*b*). The correlation coefficient and *R* factor were 30.8 and 52.2%, respectively, and, after ten cycles of rigid-body refinement, 42.7 and 49.9%, respectively (25.0–4.1 Å). The rhombohedral space group indicates that TN crystallizes as trimers. No bad contacts were observed between the monomers in the unit cell.

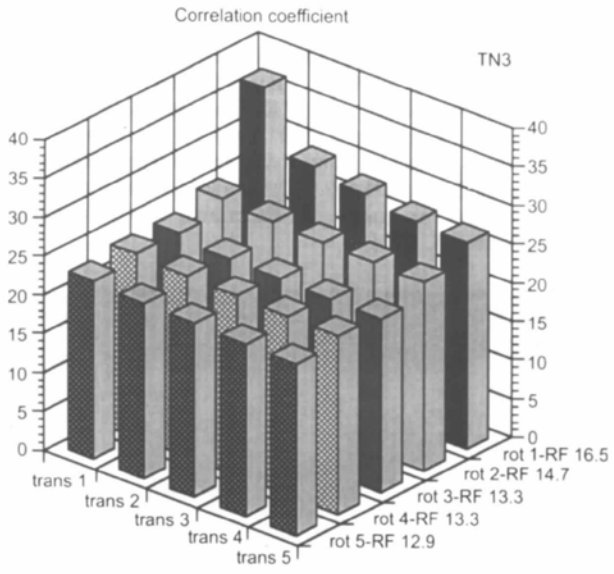


(*a*)

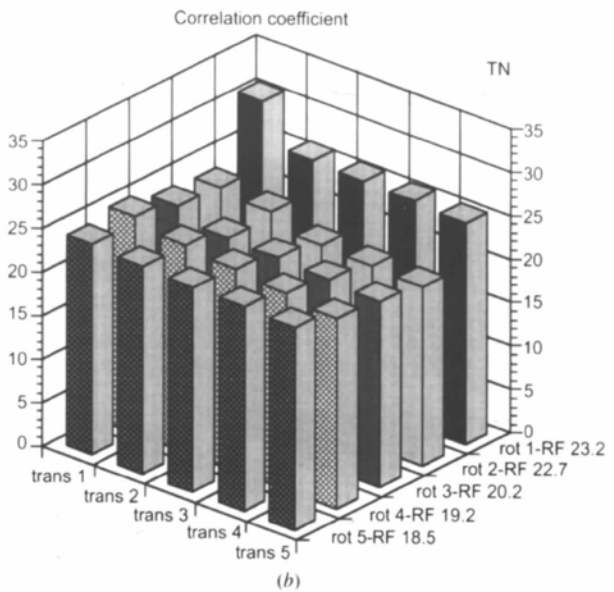


(*b*)

Fig. 2. Two degrees oscillation photographs obtained on an R-AXIS II imaging plate detector system with a Rigaku RU-200 rotating anode generator ($\lambda = 1.542$ Å, 50 kV, 180 mA) from (*a*) a crystal of TN3 and (*b*) a crystal of TN. The crystal-to-film distances were 110.0 and 100.0 mm, respectively.



(*a*)



(*b*)

Fig. 3. Molecular replacement of (*a*) TN3 (25.0–3.1 Å) and (*b*) TN (25.0–4.1 Å) using *AMoRe* from the *CCP*4 suite of programs. The RF values for the five best solutions in the rotation search (rot1–rot5) are indicated. The vertical axis specify the score in the translation search for the five best solutions (trans1–trans5).

3.3. *Comparison with the crystal packing of MBP's*

The three-dimensional structure of three C-type lectins have been determined, *i.e.* MBP from two different species: the human MBP (Sheriff, Chang & Ezekowitz, 1994) and MBP from rat (Weis, Kahn, Fourme, Drickamer & Hendrickson, 1991; Weis *et al.*, 1992; Weis & Drickamer, 1994) as well as the ligand binding region of human E-selectin (Graves *et al.*, 1994). As with MBP, the full-length TN seems to crystallize as a trimer. In MBP the neck region has been shown to be responsible for stabilization of the conformation of the C-terminal part of the trimer. The C-type lectin CRD of MBP without the neck region forms dimers in crystals. Accordingly, the C-type lectin CRD TN3 does not form trimers in the tetragonal crystals. This indicates that the domains TN1 and/or TN2 stabilize the trimerization of TN in agreement with the observations in solution (Holtet *et al.*, 1997).

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