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Crystallization and preliminary X-ray diffraction analysis of human transcobalamin, a vitamin B_{12}-transporting protein

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Transcobalamin is a cobalamin-binding protein in mammalian plasma that facilitates the cellular uptake of vitamin B_{12}. Human transcobalamin was crystallized using polyethylene glycol and ethanol as precipitants. Crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 49.04$, $b = 145.27$, $c = 164.96$ Å. A complete data set to 3.2 Å resolution was collected from a single crystal using synchrotron radiation. Estimation of the crystal packing ($V_M = 3.2$ Å^3 Da^{-1}) and self-rotation function analysis suggest the presence of two molecules in the asymmetric unit related by non-crystallographic twofold symmetry.

1. Introduction

Vitamin B_{12} derivatives (cobalamins, Cbl) are important as cofactors for methylmalonyl-CoA mutase and methionine synthetase in both humans and prokaryotes and for several other enzymes found exclusively in prokaryotes (Banerjee, 1999). Whereas the molecular structure of several B_{12} enzymes is becoming available (Drennan *et al.*, 1994; Dixon *et al.*, 1996; Mancia *et al.*, 1996; Shibata *et al.*, 1999), no structural information on B_{12}-transporting proteins has so far been reported.

Higher organisms have developed a transportation mechanism with three binding proteins, intrinsic factor (IF), transcobalamin (TC) and haptocorrin (HC), for the intestinal absorption, transport and cellular uptake of the vitamin (Glass *et al.*, 1954; DiGirolamo & Huennekens, 1975; Kräutler, 1998; Nexø, 1998; Rothenberg *et al.*, 1999). The lack of synthesis of these proteins leads to Cbl deficiency, resulting in megaloblastic anaemia and abnormalities in the peripheral and central nervous system (Stabler *et al.*, 1990; Li *et al.*, 1993). Released during digestion, Cbl molecules bind to gastric IF and the complex IF–Cbl enters the ileal mucosal cells by a receptor-mediated mechanism (Allen *et al.*, 1978). Inside the enterocytes, Cbl is transferred from IF to TC and transported into portal plasma, where Cbl complexed to TC is endocytosed by membrane receptors (Cooper & Paranchych, 1961; Schneider *et al.*, 1976; Bose *et al.*, 1995). Inside the target cells, the Cbl molecules are metabolized to the two cofactors, 5′-deoxyadenosyl-Cbl (coenzyme for methylmalonyl-CoA mutase) and methyl-Cbl (coenzyme for methionine synthetase) (Nexø, 1998). The third Cbl transporter, haptocorrin (HC), withdraws occasional Cbl analogues from plasma circulation by the liver, preventing incorporation of the analogues into tissues, where they may inhibit B_{12} enzymes (Kolhouse & Allen, 1977).

Cbl-binding proteins consist of a protein core with approximately 400 amino acids (Nexø, 1998). TC does not contain any carbohydrates, whereas IF and HC are highly glycosylated (15% of carbohydrates in IF and 33–40% in HC; Alpers & Russel-Jones, 1999). Alignment of the amino-acid sequence of human TC with other mammalian TCs (rat, mouse and bovine) shows about 70% identity (Fedosov *et al.*, 1999), whereas alignment with other Cbl-binding proteins (human, rat and mouse IF and human and porcine HC) reveals 26–32% identity, with four regions of greater than 80% homology (Li *et al.*, 1993). These regions encompass the majority of the hydrophobic areas of these proteins and are probably involved in Cbl binding (Li *et al.*, 1993; Nexø, 1998; Fedosov *et al.*, 1999). Determination of the ternary structure of a member of the B_{12} transporters is essential in order to obtain a better insight into the nature of the interaction between Cbl and its carriers.

The cloning of the cDNA encoding human TC, a 46 kDa globular protein of 409 amino acids, has provided sufficient quantities of this Cbl-binding protein in a pure form for X-ray structure determination (Quadros *et al.*, 1993; Fedosov *et al.*, 2000). In this report, we present results of its crystallization and data collection.

2. Experimental and results

2.1. Expression and purification of the recombinant human TC

Details concerning the construction of the expression plasmid, expression and purifica-

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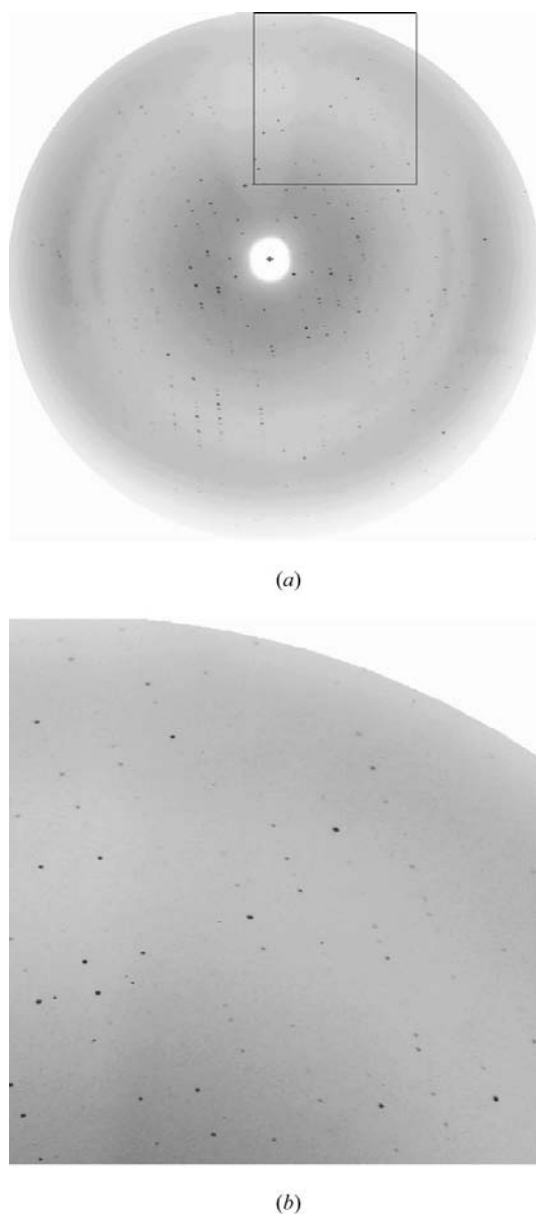
tion of human TC have previously been reported (Fedosov *et al.*, 2000).

2.2. Crystallization

The TC was crystallized using the hanging-drop vapour-diffusion method with 4 µl drops (2 µl protein solution plus 2 µl of reservoir solution) equilibrated against 1 ml reservoir solution at 289 K. Initial crystallization experiments were based on the sparse-matrix method (Jancarik & Kim, 1991) using commercially available reagents (Hampton Research). Crystalline precipitates were obtained using polyethylene glycol (PEG) 4000 as precipitant. The final optimized conditions for growing crystals suitable for X-ray analysis were 6 mg ml^{-1} protein, 20% PEG 4000, 20% ethanol and 100 m*M* tris(hydroxymethyl)aminomethane buffer pH 8.0. The red-coloured crystals of TC generally grew in about two weeks, emerging as clusters from which it was possible to separate isolated plate-like crystals with maximum dimensions of 0.30 × 0.30 × 0.02 mm.



(*a*)

(*b*)

Figure 1
(*a*) X-ray diffraction pattern from a crystal of human TC. The resolution of the outer edge of the image is at 3.1 Å; (*b*) an enlarged image of the indicated area in (*a*).

2.3. X-ray data collection and analysis

X-ray diffraction experiments were carried out at the Elettra Synchrotron, Trieste, Italy. Data were collected using a monochromatic radiation with wavelength of 1.200 Å and a MAR CCD detector. A crystal of TC was mounted directly on a 0.4 mm cryoloop (Hampton Research) and flash-frozen in a stream of N_2 at 100 K. The crystal diffracted to better than 3.2 Å resolution (Fig. 1).

The diffraction pattern was indexed using an orthorhombic unit cell, with parameters a = 49.04, b = 145.27, c = 164.96 Å. Axial reflections along h00, 0k0 and 00l were systematically absent when h, k or l = 2n + 1, identifying the space group as $P2_12_12_1$. Table 1 reports a summary of data-collection and crystallographic statistics. The determination of unit-cell parameters, integration of reflection intensities and data scaling were performed using *MOSFLM* and *SCALA* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994).

Assuming two crystallographically independent molecules of TC in the asymmetric unit, the Matthews coefficient is 3.2 Å^3 Da^{-1} (Matthews, 1968) and the solvent content is approximately 61%. Alternatively, the Matthews coefficient may be 2.1 Å^3 Da^{-1} and the solvent content 41% assuming three molecules in the asymmetric unit.

Self-rotation functions were calculated in the resolution range 20–3.2 Å with a 30 Å radius of integration using the program *POLARRFN* (Collaborative Computational Project, Number 4, 1994). Analysis of self-rotation peaks revealed the presence of a non-crystallographic twofold symmetry in the [011] direction. The stereographic projection (κ = 180° section) of the self rotation is shown in Fig. 2. The presence of a peak at ω = 45, φ = 90° of height 35% of the origin peak suggests it is most likely that two crystallographic independent molecules of TC are present in the asymmetric unit.

Attempts to improve the resolution of the crystal diffraction and to search for heavy-atom derivatives are under way.

Table 1
Data-collection statistics for human transcobalamin.

Values in parentheses are for the highest resolution shell (3.37–3.20 Å).

Space group	$P2_12_12_1$
Temperature (K)	100
Resolution range (Å)	32.0–3.2
Unit-cell parameters (Å)	a = 49.04, b = 145.27, c = 164.96
No. of observations	105390
Unique reflections	19677
R_{merge} (%)	14.9 (43.4)
Completeness (%) [$I > \sigma(I)$]	95.5 (93.8)
$I/\sigma(I)$	5.8 (2.2)

† $R_{merge} = \sum |I - \langle I \rangle| / \sum I$.

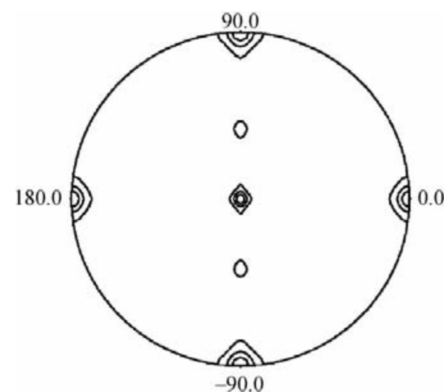


Figure 2
Plot of the self-rotation function at κ = 180°. The section has ω = 0 or 180° at the centre, ω = 90° around the edge and φ as marked around the periphery. The view is down the c axis; ω = 90, φ = 0° corresponds to the direction of the a axis and ω = 0° to the direction of the c axis.

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References

Allen, R. H., Seetharam, B., Allen, N. C., Podell, E. R. & Alpers, D. H. (1978). *J. Clin. Invest.* **61**, 1628–1634.

Alpers, D. H. & Russel-Jones, G. J. (1999). *Chemistry and Biochemistry of B₁₂*, edited by R. Banerjee, pp. 411–440. New York: Wiley.

Banerjee, R. (1999). *Chemistry and Biochemistry of B₁₂*. New York: John Wiley.

Bose, S., Seetharam, S. & Seetharm, B. (1995). *J. Biol. Chem.* **270**, 8152–8157.

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

Cooper, B. A. & Paranchych, W. (1961). *Nature (London)*, **191**, 393–395.

DiGirolamo, P. M. & Huennekens, F. M. (1975). *Arch. Biochem. Biophys.* **168**, 386–393.

Dixon, M., Huang, S. Matthews, R. G. & Ludwig, M. L. (1996). *Structure*, **4**, 1263–1275.

Drennan, C., Huang, S., Drummond, J. T., Matthwes, R. G. & Ludwig, M. L. (1994). *Science*, **266**, 1669–1674.

Fedosov, S. N., Berglund, L., Nexø, E. & Petersen, T. E. (1999). *J. Biol. Chem.* **274**, 26015–26020.

Fedosov, S. N., Fedosova, N. U., Nexø, E. & Petersen, T. E. (2000). *J. Biol. Chem.* **275**, 11791–11798.

Glass, G. B. J., Boyd, L. J. & Stephenson, L. (1954). *Science*, **120**, 74–75.

Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.

Kolhouse, J. F. & Allen, R. H. (1977). *J. Clin. Invest.* **60**, 1381–1392.

Kräutler, B. (1998). *Vitamin B_{12} and B_{12} Proteins*, edited by B. Kräutler, D. Arigoni & T. Golding, pp. 3–43. Weinheim: Wiley–VCH.

Li, N., Seetharam S., Lindemans, J., Alpers, D. H., Arwert, F. & Seetharam, B. (1993). *Biochim. Biophys. Acta*, **1172**, 21–30.

Mancia, F., Keep, N. J., Nakagawa, A., Leadly, P. F., McSweeney, S., Rasmussen, B., Bosecke, P., Diat, P. & Evans, P. R. (1996). *Structure*, **4**, 339–350.

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

Nexø, E. (1998). *Vitamin B_{12} and B_{12} Proteins*, edited by B. Kräutler, D. Arigoni & T. Golding, pp. 461–475. Weinheim: Wiley–VCH.

Quadros, E. V., Sai, P. & Rothenberg, S. P. (1993). *Blood*, **261**, 1239–1245.

Rothenberg, S. P., Quadros, E. V. & Regec, A. (1999). *Chemistry and Biochemistry of B_{12}*, edited by R. Banerjee, pp. 441–473. New York: John Wiley.

Schneider, R. J., Burger, R. L., Mehlmen, C. S. & Allen, R. H. (1976). *J. Clin. Invest.* **57**, 27–38.

Shibata, N., Masuda, J., Tobimatsu, T., Toraya, T., Suto, K., Morimoto, Y. & Yasuoka, N. (1999). *Structure*, **7**, 997–1008.

Stabler, S. P., Allen, R. H., Savage, D. G. & Lindenbaum, J. (1990). *Blood*, **76**, 871–881.