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Expression, purification and crystallization of a human tau-tubulin kinase 2 that phosphorylates tau protein

Tau-tubulin kinase 2 (TTBK2) is a Ser/Thr kinase that putatively phosphorylates residues Ser208 and Ser210 (numbered according to a 441-residue human tau isoform) in tau protein. Functional analyses revealed that a recombinant kinase domain (residues 1–331) of human TTBK2 expressed in insect cells with a baculovirus overexpression system retains kinase activity for tau protein. The kinase domain of TTBK2 was crystallized using the hanging-drop vapour-diffusion method. The crystals belong to space group $P2_12_12_1$, with unit-cell parameters $a = 55.6$, $b = 113.7$, $c = 117.3$ Å, $\alpha = \beta = \gamma = 90.0^\circ$. Diffraction data were collected to 2.9 Å resolution using synchrotron radiation at BL24XU of SPring-8.

1. Introduction

Tau-tubulin kinases (TTBK) have been found in various species such as human, mouse, zebrafish, *Caenorhabditis elegans* and *Drosophila melanogaster* (Manning *et al.*, 2002; Tomizawa *et al.*, 2001; Sato *et al.*, 2006). They belong to the casein kinase 1 (CK1) group, which consists of the CK1, TTBK and vaccinia-related kinase families. Two isoforms, referred to as TTBK1 and TTBK2, have been identified in the TTBK family. In the CK1 group, the crystal structures of CK1-family enzymes such as CK1 from *Schizosaccharomyces probe* and CK1δ from rat testis have been solved (Xu *et al.*, 1995; Longenecker *et al.*, 1996). Human TTBK2 is the most probable candidate to phosphorylate the tau-protein residues Ser208 and Ser210 (numbering according to a 441-residue human tau isoform; Goedert *et al.*, 1989), which are found in the paired helical filament-tau (PHF-tau) in the brain tissues of Alzheimer's disease patients (Morishima-Kawashima *et al.*, 1995; Hanger *et al.*, 1998). Based on an automated computational analysis in the 'kinome' project (Manning *et al.*, 2002), human TTBK2 (KinBase ID 16057) consists of 1244 amino acids and contains a kinase domain flanked by a putative noncatalytic long peptide region at the C-terminus. The alignment of the kinase domain of human TTBK2 and rat CK1δ (Graves *et al.*, 1993) shows 38% identity (51% homology). We previously demonstrated that the bovine and mouse brain TTBK2s, which were observed as 36 kDa species in brain extracts, display kinase activities for tau protein and tubulin and that a recombinant kinase domain of mouse TTBK2 corresponding to residues 1–316 of human TTBK2 (98% identity) uniquely phosphorylates Ser208 and Ser210 in tau protein (Takahashi *et al.*, 1995; Tomizawa *et al.*, 2001). Rat TTBK2 has not only been identified in brain, but also various other tissues including heart, muscle, liver, thymus, spleen, lung, kidney, testis and ovary (Takahashi *et al.*, 1995; Tomizawa *et al.*, 2001). In contrast, a recent study of a neuron-specific human TTBK1 (KinBase ID; 16054) proposed that the enzyme phosphorylates Ser198, Ser199, Ser202 and Ser422 in tau protein, solely depending on its kinase domain (residues 38–319), which shares 84% identity with the corresponding region in human TTBK2 (Sato *et al.*, 2006). These findings suggest that human TTBK2 is an orthologue of the bovine and mouse TTBK2s and that the 36 kDa N-terminal kinase domain of TTBK2 is essential for tau phosphorylation in the brain, although the detailed molecular mechanisms of the enzyme remain unclear, including a post-

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Figure 2
Crystal of TTBK2 (1–331). The dimensions of the crystals were approximately $0.15 \times 0.08 \times 0.08$ mm.

5. Results and discussion

Human TTBK2 (1–331), which is identical to human TTBK2 (KinBase ID 16057), was heterologously expressed using a baculovirus overexpression system as a fusion protein with GST at the N-terminus and a hexahistidine tag at the C-terminus. After cleavage of the GST tag, the purified enzyme migrated as a single band with a molecular weight of 39 kDa on SDS–PAGE (Fig. 1), which is in good agreement with the calculated value of 38 621 Da. A gel-filtration experiment gave a molecular weight of 35 kDa, suggesting that TTBK2 (1–331) is a monomeric enzyme.

As mentioned above, the bovine and mouse TTBK2 proteins phosphorylate Ser208 and Ser210 in tau protein (Takahashi *et al.*, 1995; Tomizawa *et al.*, 2001). To confirm the kinase activities of the expressed and purified recombinant TTBK2 (1–331) for tau protein, a functional analysis was carried out using the same procedure as described previously (Takahashi *et al.*, 1995). The recombinant human TTBK2 (1–331) was functionally identical to the bovine and mouse TTBK2s (data not shown), suggesting that TTBK2 (1–331) contains the entire functional kinase domain that directly phosphorylates Ser208 and Ser210 in tau protein. The phosphorylation at Ser208 and Ser210 found in PHF-tau in the brains of Alzheimer's patients is indeed caused by human TTBK2.

The crystals appeared reproducibly within 2 d and grew to average dimensions of $0.15 \times 0.08 \times 0.08$ mm (Fig. 2). A complete data set was collected to 2.9 Å resolution. Data-processing statistics are shown in Table 1. The crystallographic asymmetric unit contains two molecules of TTBK2 (1–331), with a Matthews volume (V_M ; Matthews, 1968) of $2.5 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 48%, which is in the range normally observed for protein crystals. A self-rotation function analysis calculated on data in the resolution range 15.0–

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Space group	$P2_12_12_1$
Unit-cell parameters	
a (Å)	55.6
b (Å)	113.7
c (Å)	117.3
$\alpha = \beta = \gamma$ (°)	90.0
Resolution (Å)	30.0–2.9 (3.00–2.90)
Unique reflections	17074
Redundancy	5.3 (5.3)
Completeness (%)	100.0 (100)
$\langle I/\sigma(I) \rangle$	27.6 (6.5)
$R_{\text{sym}}^\dagger$ (%)	10.2 (39.3)

$^\dagger R_{\text{sym}} = \sum_h \sum_i |I(h)_i - \langle I(h) \rangle| / \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of reflection h , $\sum_h$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection h .

4.0 Å using *CNS* (Brünger *et al.*, 1998) indicated the presence of a twofold noncrystallographic symmetry axis. Further structural analysis is in progress.

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