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Expression, purification and crystallization of human tau-protein kinase I/glycogen synthase kinase-3β

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Human tau-protein kinase I (TPK-I; also known as glycogen synthase kinase-3β, GSK-3β) is a serine/threonine protein kinase. Full-length TPK-I/GSK-3β was expressed in *Escherichia coli* as a fusion protein with a 6×His tag at the C-terminus and was crystallized using the hanging-drop vapour-diffusion method. Prismatic crystals of dimensions 0.4 × 0.2 × 0.1 mm were obtained using 12–15%(*w*/*v*) polyethylene glycol 6000 as a precipitant at 278 K. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 82.9, b = 86.1, c = 178.1 Å measured at 100 K, diffract to 2.3 Å resolution and seem to contain two enzyme molecules per asymmetric unit.

1. Introduction

Histopathological features of Alzheimer's disease (AD) include extracellular deposits of amyloid β protein in the core of senile plaques (Yankner *et al.*, 1990), intracellular neurofibrillary tangles (Kidd, 1963) and extensive neuronal death. The neurofibrillary tangles are formed with paired helical filaments (PHF) which consist of a microtubule-associated protein tau in a highly phosphorylated form (Grundke-Iqbal, Iqbal, Quinlan *et al.*, 1986; Grundke-Iqbal, Iqbal, Tung *et al.*, 1986; Morishima-Kawashima *et al.*, 1995). It is suspected that the phosphorylation of tau protein in AD brain results in the depolymerization of microtubules, impaired axonal transport and neuronal degeneration (Alonso *et al.*, 1997).

Identifications of kinases responsible for the phosphorylation have been performed by several groups in order to understand the pathological change in AD brain (Baudier & Cole, 1987; Ishiguro *et al.*, 1988; Correas *et al.*, 1992; Drewes *et al.*, 1992). One of the kinases purified from the microtubule fraction of brain tissue was named tau-protein kinase I (TPK-I; E.C. 2.7.1.135); the amino-acid sequence of TPK-I was identical to that of glycogen synthase kinase 3β (GSK-3β; Ishiguro *et al.*, 1992, 1993). TPK-I/GSK-3β phosphorylates tau protein more extensively than any other serine/threonine kinases so far examined (Ishiguro *et al.*, 1992) and the phosphorylation sites on tau by TPK-I/GSK-3β account for those commonly found in PHF-tau (Imahori & Uchida, 1997). Structural knowledge of TPK-I/GSK-3β will provide us with a phosphorylation mechanism of tau protein by TPK-I/GSK-3β and will shed light on the pathology of AD.

TPK-I/GSK-3β consists of not only the kinase domain, but also extended N- and C-terminal domains which are not found in other kinases (Hanks & Quinn, 1991; Ishiguro *et al.*, 1993). The phosphorylation of Ser9 located at the N-terminal domain inactivates TPK-I/GSK-3β, suggesting Ser9 as a key regulatory site of TPK-I/GSK-3β function (Stambolic & Woodgett, 1994; Eldar-Finkelman *et al.*, 1996). Thus, TPK-I/GSK-3β was not synthesized as a form consisting of the kinase domain (~285 residues), but as the full-length form with a His-tag at the C-terminus (428 residues). In this study, we present the expression, purification, crystallization and preliminary X-ray analysis of full-length TPK-I/GSK-3β.

2. Expression and purification

A cDNA of human TPK-I/GSK-3β was inserted into a vector pET24a (Novagen, Inc.) and expressed in *E. coli* strain BL21(DE3) as a fusion protein with the sequence Leu-Glu-6×His at the C-terminus under transcriptional control of a T7 promoter. An amino-acid residue at position 350 of human TPK-I/GSK-3β used in this study is leucine instead of histidine as described in P49841, SWISS-PROT. The substitution is not a cloning artifact but arises from polymorphism in the human TPK-I/GSK-3β gene (Sato, 2000). Two $tRNA^{Arg}$ that recognize arginine rare codons in *E. coli* (AGA and AGG) were coexpressed to achieve efficient high-level expression of the protein. Cells in 2×YTA medium were grown to the late-log phase (OD_{600} = 0.8) at 298 K and then induced by IPTG and incubated for another 5 h.

Table 1
Crystal data and intensity statistics.

Values given in brackets are for the highest resolution shell (2.85–2.70 Å).

Space group	$P2_12_12_1$
Unit-cell parameters† (Å)	a = 82.9 (1), b = 86.1 (1), c = 178.1 (1)
V_M (Å^3 Da^{-1})	3.3
Number of molecule in unit cell	8
Solvent content (%)	58.5
Number of crystals	2
Resolution (Å)	44.5–2.7
Number of observations	393547
Number of unique reflections	35746
Measurement temperature† (K)	100 (1)
Completeness‡ (%)	100.0 [100.0]
Redundancy§	11.0 [11.2]
R_{merge}¶ (%)	8.8 [37.1]
$I/\sigma(I)$	7.8 [2.0]

† Standard deviations in parentheses. ‡ The completeness is the percentage of obtained reflections compared with the theoretically obtainable measurements. § Redundancy is the number of multiple measurements. ¶ R_{merge} is the internal R factor, $R_{merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$.

The cells were suspended in 50 m*M* Tris–HCl pH 7.5 containing 200 m*M* NaCl, 5%(*v*/*v*) glycerol, 5 m*M* $MgCl_2$, 5 m*M* 2-mercaptoethanol and 0.5 mg ml^{-1} pefabloc SC (Pentapharm Ltd) and disrupted by 20 min of sonication in the presence of 0.3 mg ml^{-1} hen egg-white lysozyme. The supernatant was loaded on a cobalt-based immobilized metal-affinity column previously equilibrated with the buffer for cell suspension. The column was washed with the same buffer and eluted with a linear gradient of 0–200 m*M* imidazole. The effluents were dialyzed against 50 m*M* MES–NaOH pH 6.0 containing 10 m*M* DTT, 5%(*v*/*v*) glycerol, 5 m*M* $MgCl_2$ and 100 m*M* NaCl and applied to a methyl sulfonate-based cation-exchange column. The column was eluted with a linear gradient of 100–460 m*M* NaCl in the dialyzing buffer.

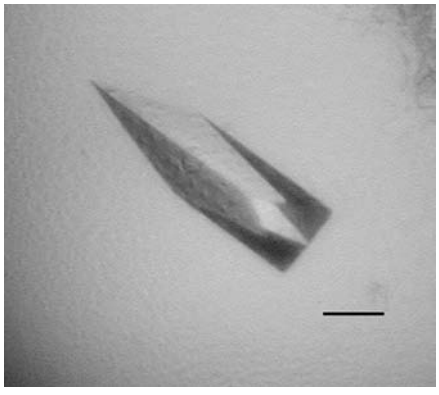

Figure 1
A crystal of TPK-I/GSK-3β from polyethylene glycol 6000 solution was grown to dimensions of 0.4 × 0.2 × 0.1 mm. Bar = 0.1 mm.

The active fractions were concentrated by ultrafiltration and applied to a gel-filtration column equilibrated with 50 m*M* MES–NaOH pH 6.5 containing 10 m*M* DTT, 5%(*v*/*v*) glycerol, 7 m*M* $MgCl_2$ and 175 m*M* NaCl. The fractions were collected and concentrated by ultrafiltration to 15–20 mg ml^{-1} protein. Protein concentration was determined with a Non-Interfering Protein Assay kit (Geno Technology, Inc.) using bovine serum albumin as a standard. The purified protein displayed a single major band on SDS–PAGE stained with Coomassie brilliant blue. Purified TPK-I/GSK-3β exhibited a specific activity as high as that of the authentic kinase from bovine brain as measured by ^{32}P incorporation into a phosphoglycogen synthase peptide (Ishiguro *et al.*, 1993).

3. Crystallization and X-ray diffraction data collection

Crystals of TPK-I/GSK-3β were obtained using the hanging-drop vapour-diffusion method. Prismatic crystals were grown within several days at 278 K from 12–15%(*w*/*v*) polyethyleneglycol 6000 containing 100 m*M* MES–NaOH pH 6.5, 10%(*v*/*v*) glycerol, 5 m*M* $MgCl_2$, 100 m*M* NaCl and 2 m*M* ATP (Fig. 1).

X-ray diffraction experiments were performed both at beamline BL-6A of Photon Factory with a Quantum 4CCD detector (Area Detector Systems Corp.) and beamline BL24XU of SPring-8 with an R-AXIS IV imaging-plate detector (Rigaku Corp.). The crystals were transferred to a buffer containing 30% D-sorbitol as a cryoprotectant and were flash-cooled in a nitrogen-gas stream at 100 K. A data set from a native crystal revealed significant diffraction to 2.3 Å resolution. Intensity statistics of data to 2.7 Å resolution [$I/\sigma(I) > 2.0$] are summarized in Table 1.

The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 82.9, b = 86.1, c = 178.1 Å. The asymmetric unit contains two molecules of TPK-I/GSK-3β (molecular weight 48 kDa), giving a Matthews constant (V_M) of 3.3 Å^3 Da^{-1}, which is within the acceptable range (Matthews, 1968).

Attempts to solve the phase problem by molecular replacement using known structures of kinase domains were unsuccessful, probably as a consequence of there being two independent molecules in the asymmetric unit, the insufficient size of the search model (kinase domain; *e.g.* 298 residues of cyclin-dependent kinase 2) against the His-tagged full-length protein (428 residues) and low amino-acid sequence homology between TPK-I/GSK-3β and other kinases (*e.g.* 38% with cyclin-dependent kinase 2). A search for heavy-atom derivatives is in progress.

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