Crystallization and Preliminary X-ray Analysis of the cAMP-Dependent Protein Kinase Catalytic Subunit from *Saccharomyces cerevisiae*†

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ABSTRACT: A truncated variant of TPK1, the yeast cAMP-dependent protein kinase catalytic subunit, was overexpressed in an engineered strain of *Saccharomyces cerevisiae*, purified by liquid chromatography, and crystallized from solutions of 2-propanol and magnesium at alkaline pH. The crystals are hexagonal dipyramids, space group $P6_122$ ($P6_522$), with unit-cell parameters $a = b = 61$ Å, $c = 320$ Å. Large single crystals suitable for diffraction analysis are obtainable by microseeding, and diffract beyond 2.8-Å resolution. Crystal density measurements reveal 12 kinase monomers per unit cell with a single kinase monomer per asymmetric unit.

Protein kinases are regulatory enzymes that catalyze the transfer of phosphate from ATP to protein substrates (Edelman et al., 1989). The large number of proteins phosphorylated in eukaryotic cells (Chelsky et al., 1985) points to the existence of a protein kinase superfamily, each member of which carries out an important regulatory role (Hunter, 1987). Yet despite clear amino acid sequence homology among eukaryotic protein kinases (Hanks et al., 1988), and the probable existence of a common enzymatic mechanism of phosphoryl transfer, each protein kinase retains a unique (though potentially overlapping) recognition selectivity for protein substrates. For the handful of kinases studied in detail, the ability of a protein to serve as a substrate is determined by the amino acid sequence surrounding its phosphorylatable hydroxyamino acid (i.e., serine, threonine, or tyrosine), with the location and spacing of charged amino acids being especially important (Kemp & Pearson, 1990).

Because we wish to understand the general folding pattern of eukaryotic protein kinases, and to identify the structural features of kinases involved in catalysis, regulation, and substrate selectivity, we initiated a crystallographic study of the well-characterized cAMP-dependent protein kinase catalytic subunit from yeast, TPK1. It, along with its isozymes (TPK2 and TPK3), regulates the ability of that organism to grow. We chose this enzyme for structure determination because (1) its gene has been cloned (Toda et al., 1987), (2) it can be overexpressed in a soluble form in yeast (Zoller et al., 1988), (3) mutant proteins with altered catalytic properties can be obtained through genetic screens in yeast and overexpressed in soluble form (Cameron et al., 1988; Levin et al., 1989; Levin & Zoller, 1990), and (4) it retains many of the well-characterized structural and kinetic features of the mammalian enzyme, including an ability to recognize the substrate consensus sequence exemplified by the peptide substrate Leu-Arg-Arg-Ala-Ser-Leu-Gly (Kemptide)[1] (Zoller et al., 1988; Taylor, 1989), and an ability to form a tetrameric holoenzyme with a regulatory subunit (the *BCY1* gene product). Thus, the yeast system allows us to study protein kinase enzymology with diverse techniques.

In this paper, we describe the design, synthesis, characterization, and crystallization of a truncated variant of TPK1 that we refer to as TPK1Δ. Determination of this protein's structure, along with that of its mammalian homologue (Knighton et al., 1991), will yield much needed structural information on this important family of regulatory proteins.

Experimental Procedures

Materials. All solvents (including 2-propanol) were HPLC grade and obtained from Burdick and Jackson. Hydroxylapatite was obtained from Bio-Rad (HTP grade); Sephacryl S-100HR and TSKGel phenyl-5PW media were from Pharmacia/LKB. The detergent Brij 35 and the polycation poly(ethylenimine) were from Sigma, as was the assay reagent Kemptide. [γ-^{32}P]ATP was obtained from Amersham.

Mutagenesis. The *TPK1* gene, isolated in phagemid vector pUC118 (Vieira & Messing, 1987), was prepared for mutagenesis by the method of Kunkel et al. (1988) as described previously for *BCY1* (Kuret et al., 1988). Deletion mutagenesis (Eghtedarzadeh & Henikoff, 1986) of the *TPK1* coding sequence was performed with the oligonucleotide 5′-TGTAAACTATACTTCGGCATTGTAAGCTTACA-3′ (noncoding sequence). Simultaneously, the mismatched guanosine residues (underlined) created a proline residue adjacent to the initiating methionine. The deletion was confirmed by DNA sequence analysis performed by the dideoxynucleotide chain termination method (Sanger et al., 1977; Biggin et al., 1984) on denatured double-stranded templates. These and other basic molecular cloning techniques were performed as described by Sambrook et al. (1990).

Strains and Vectors. The strain built for overexpression of *TPK1*Δ, JK920, extends the expression system described previously for TPK1 (Zoller et al., 1988; Levin & Zoller, 1990), and derives from the haploid yeast strain T162-1a (*Matα ade8 his3 leu2 trp1 ura3 tpk2::HIS3 tpk3::TRP1*; Toda et al., 1987b). T162-1a was transformed sequentially with YEp(ADE8)BCY1-Asn^{144}-Ala^{145} (a shuttle plasmid that drives high-level expression of the Asn^{144}Ala^{145}-*BCY1* double mutant while conferring adenine prototrophy; Levin & Zoller, 1990) and then with pAD4/*TPK1*Δ. The latter vector was constructed by inserting a *Hin*dIII fragment containing the *TPK1*Δ coding sequence into *Hin*dIII-digested pAD4. pAD4

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[1] Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Kemptide, Leu-Arg-Arg-Ala-Ser-Leu-Gly; MOPS, 3-(*N*-morpholino)propanesulfonic acid; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate.

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is identical to pADNS [described by Colicelli et al. (1989)] with the exception that it does not contain the additional restriction sites (*Not*I, *Sac*II, and *Sfi*I) in its pUC18-based polylinker. The final vector (pAD4/*TPK1*Δ) places *TPK1*Δ behind the strong *ADH1* promoter (Ammerer, 1983) and confers leucine prototrophy. We used the lithium acetate method for all yeast transformations (Ito et al., 1983).

*Expression and Purification of TPK1*Δ. JK920 cells were grown overnight at 30 °C in 1 L of selective medium (SC minus uracil; Sherman et al., 1989) until an A_{660nm} of about 4 was obtained, at which point the culture was used to inoculate 10 L of rich medium (YPD: 1% yeast extract, 2% Bacto-peptone, and 2% glucose). After another night of growth to an A_{660nm} of approximately 12, the cells were harvested by centrifugation (15 min at 3000*g*; 4 °C), washed with water, and stored at −70 °C. A 10-L growth typically yields about 130 g (wet weight) of cells.

All subsequent steps were carried out at 4 °C. Frozen cells were thawed, placed in a 375-mL Biospec Bead Beater homogenizer half-filled with 500-μm glass beads (Biospec Products, Bartlesville, OK), and resuspended with enough homogenization buffer (200 mM potassium phosphate, pH 7.0, 5 mM EGTA, 0.1% 2-mercaptoethanol, 1.0 mM PMSF, and 0.01% Triton X-100) to fill the chamber. Cells were ruptured with 20 cycles of alternate homogenization and cooling, each lasting 30 s. The resulting homogenate was filtered through a coarse fritted-disk Buchner funnel, which trapped glass beads and other particulate matter. The retentate was washed with an additional 100 mL of homogenization buffer and refiltered. The wash and the lysate were combined and centrifuged 20 min at 20000*g*, yielding a cloudy supernatant taken as fraction 1 (crude extract).

The extract was brought to 0.8% poly(ethylenimine) by the addition of a 10% stock solution, stirred 10 min, and centrifuged 20 min at 20000*g*. The clear amber supernatant was brought to 45% saturation with solid ammonium sulfate, stirred 30 min, and then centrifuged 20 min at 20000*g*. The resulting pellet was resuspended in 50 mL of dialysis buffer (10 mM potassium phosphate, pH 7.0, 150 mM NaCl, 1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mM PMSF, and 0.01% Brij 35), dialyzed 4 h against 1 L of dialysis buffer with one change, and finally clarified by centrifugation (20 min at 20000*g*). The resulting supernatant was taken as fraction 2.

Fraction 2 was loaded onto a 100-mL (5 × 5 cm) hydroxylapatite column equilibrated in dialysis buffer. After the column was washed overnight with 10 bed volumes of wash buffer (85 mM potassium phosphate, pH 7.0, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 0.5 mM PMSF, and 0.01% Brij 35), TPK1Δ was eluted selectively with the same buffer containing 100 μM cAMP. The protein peak was pooled and taken as fraction 3 (hydroxylapatite pool).

Fraction 3 was brought to 0.8 M ammonium sulfate and loaded onto a 3.8-mL (8 × 75 mm) TSKGel phenyl-5PW HPLC column equilibrated in phenyl buffer (10 mM MOPS, pH 7.0, 1 mM EGTA, and 0.1% 2-mercaptoethanol) containing 0.8 M ammonium sulfate. The column was washed with 2 bed volumes of this buffer and developed with a 25-mL linear gradient of decreasing ammonium sulfate (from 0.8 to 0 M). TPK1Δ elutes as a sharp peak centered about 0.2 M ammonium sulfate. The protein peak was taken as fraction 4 (phenyl-TSK pool).

Fraction 4 was loaded directly onto a 190-mL (1.6 × 95 cm) column of Sephacryl S-100 HR gel filtration medium equilibrated and run in 10 mM HEPES, pH 7.4, 150 mM NaCl, and 0.1 mM EGTA at 30 mL/h. TPK1Δ eluted at V_e/V_o = 1.41 (K_d = 0.333) and was taken as fraction 5 (gel filtration pool).

The gel filtration pool was concentrated by pressure filtration (YM30 membrane; Amicon, MA) followed by centrifugal filtration (Centricon-10; Amicon, MA). The concentrate was made 1 mM in dithiothreitol, diluted to 10 mg/mL protein, and stored at 4 °C for up to 48 h before use.

Analytical Methods. Purified TPK1Δ was quantified spectrophotometrically, using an absorbance coefficient ($A^{1\%}_{280nm}$ = 14.3) calculated from the amino acid content (Perkins, 1986), whereas the protein content of extracts and impure column fractions was estimated by the method of Bradford (1976) using purified TPK1Δ as standard. We assayed kinase activity with Kemptide as described previously (Kuret et al., 1988).

SDS–polyacrylamide gel electrophoresis (10–15% gradient) and isoelectric focusing (pH 4–6) were performed with PhastSystem (LKB/Pharmacia). All phastgels were stained with silver (Heukeshoven & Dernick, 1988).

We obtained amino acid sequence data by subjecting intact TPK1Δ to automated Edman degradation. Approximately 5 μg of purified protein was dialyzed against 50 mM NH_4HCO_3 containing 0.01% SDS, lyophilized to dryness, resuspended in 100 μL of water, and applied to a gas-phase sequenator operated as described by Hunkapillar and Hood (1983).

Calculations of molecular weight, isoelectric point, and amino acid composition were performed with protein sequence analysis programs (Intelligenetics, CA) run on an SUN3 computer.

Crystallography. TPK1Δ crystals were grown at 16 °C by vapor diffusion in 30-μL sitting drops containing equal volumes of protein solution and crystal buffer (20 mM Tris-HCl, pH 8.5, 10% 2-propanol, and 10 mM $MgCl_2$). Nine such drops were equilibrated against a 20-mL reservoir of crystal buffer in a sealed container for 5 min and then microseeded (Fitzgerald & Madsen, 1986) as follows: a single, well-developed crystal (produced from a previous crystallization) was suspended in 10 μL of crystal buffer and crushed with a fine glass fiber. The resulting microcrystal suspension was diluted serially 3 times. Dilutions were performed by adsorbing a small volume of suspension onto a fresh glass fiber and transferring it to a 5-μL drop of fresh protein solution mixed with an equal volume of crystal buffer. The resulting dilute suspension then served as the source of microcrystals for the next dilution. Each dilution lowered the concentration of microcrystals 10–100-fold. The third and final dilution served as the source of seeds for sitting drops. As with the serial dilutions, seed microcrystals were transferred to sitting drops with a glass fiber. A successful seeding produced drops containing one to six crystals that were visible in 1 h and grew to full size (1–1.5 mm in length) in 3–4 days. Individual crystals were harvested into crystal buffer containing 1 mg/mL TPK1Δ. They were stable for 1–2 weeks when stored under these conditions at 16 °C.

Single TPK1Δ crystals were mounted in thin-walled glass capillaries (0.7–1.5-mm-inner diameter; Charles Supper Co., Natick, MA) between plugs of crystal buffer as described by McPherson (1982). X-ray diffraction data were collected on an Enraf–Nonius FAST area detector attached either to an Elliott GX-21 rotating-anode X-ray generator producing nickel-filtered Cu Kα radiation (operating at 45 kV, 95 mA) or to the National Synchrotron Light Source beamline X12C (tuned to deliver 0.95-Å radiation). The FAST area detector was controlled by the program MADNES (Messerschmidt &

Pflugrath, 1987). The resulting data were scaled and merged with the PROTEIN program package (Steigemann, 1974; Remington et al., 1982). Precession photographs were taken with a precession camera (Huber) mounted on our GX-21 generator. All data were collected at 16 °C.

Results and Discussion

TPK1Δ: Design and Rationale. The first step in our mutagenic approach to kinase structure was to simplify the primary structure of TPK1 as much as possible while retaining its enzymatic properties. We reasoned that by reducing main chain flexibility and microheterogeneity, we could maximize the resolution obtainable from X-ray crystallography (McPherson, 1982). Moreover, by minimizing the mass of TPK1, we could employ the widest possible range of heavy-atom compounds for isomorphous replacement. A similar strategy proved valuable in the successful crystallization of other proteins, including c-H-*ras* p21 (de Vos et al., 1988).

Examination of TPK1's primary structure reveals an amino terminus extending far beyond the beginning of the canonical protein kinase domain (Toda et al., 1987b) and having no homology with any known kinase structure, including *TPK2* or *TPK3* (Hanks et al., 1988). Moreover, this amino-terminal region contains the most hydrophilic region of the protein, as predicted by the method of Hopp and Woods (1981), which is consistent with it being highly solvated and flexible (Ragone et al., 1989).

Considering that the structural features potentially detrimental to crystallography are located in the amino-terminal 83 amino acid residues of TPK1, that the sequence similarity among TPK1, TPK2, and TPK3 begins 14 residues from the first glycine of the putative nucleotide binding loop (Wierenga & Hol, 1983), and that all protein kinases contain at least 10 residues amino terminal to this glycine, we decided to eliminate residues 1 through 80 of TPK1 and replace them with the synthetic amino terminus Met-Pro-. The truncated TPK1 (termed TPK1Δ) has the amino-terminal sequence Met-Pro-Lys-Tyr-Ser-Leu-Gln-Asp-Phe-Gln-Ile-Leu-Arg-Thr-Leu-**Gly**- where the glycine residue marks the beginning of the nucleotide binding loop. We chose Met-Pro- as the amino terminus because this sequence is a good substrate for the yeast methionyl aminopeptidase and is a poor substrate for known posttranslational modifications (Arfin & Bradshaw, 1988). Thus, the new sequence (TPK1Δ) eliminates nearly 20% of TPK1's mass, reduces main chain flexibility, retains homology with TPK2 and TPK3 at the amino terminus, and cannot be modified posttranslationally.

Overexpression. Constitutive overexpression of TPK1 in yeast is complicated by its role in yeast cell growth regulation; yeast strains that express *TPK1* in excess of *BCY1* (the yeast cAMP-dependent protein kinase regulatory subunit) grow poorly (Toda et al., 1987a), whereas strains completely devoid of TPK1 do not grow at all (Toda et al., 1987b). We have shown, however, that this problem of toxicity is overcome by expressing both subunits of the kinase simultaneously on two separate multicopy epichromosomal plasmids (Zoller et al., 1988). Although it is possible that TPK1 accumulates in these strains because BCY1 binds and protects TPK1 from proteolytic degradation, a more likely explanation is that BCY1 inhibits and controls the activity of TPK1, allowing normal yeast cell growth. Whatever the mechanism, constitutive TPK1 expression is increased still further by placing both *TPK1* and *BCY1* behind powerful yeast promoters and increasing the affinity between BCY1 and TPK1 (Levin & Zoller, 1990). The first of these strategies is accomplished by cloning *TPK1* and *BCY1* into the expression plasmids pAD4 and pADE8, respectively. These vectors contain the strong, constitutive *ADH1* promoter (Ammerer, 1983) and together drive TPK1 expression 7-fold higher than achieved previously (data not shown). The second strategy is accomplished by mutating Ser^{145} of BCY1 to Ala, which increases its affinity for TPK1 by an order of magnitude (Kuret et al., 1988). Although this modification further increases TPK1 expression only modestly, the resulting yeast strains are healthier, more stable, and faster growing than strains lacking the BCY1 mutation. Double mutation of BCY1 to $Asn^{144}Ala^{145}$ increases the intersubunit affinity still further (Yamano et al., 1987), but this has not been confirmed in vitro. Yeast strains incorporating these modifications to the original expression system accumulate TPK1 to about 1% of the total *soluble* protein in yeast, which corresponds to about 9 mg of catalytic subunit per liter of culture (data not shown). Overexpression of TPK1Δ is less efficient but still yields about 3 mg of catalytic subunit per liter of culture (prior to purification; Table I).

Table I: Purification of TPK1Δ from 10 L (130 g) of Yeast Strain JK920

fraction	volume (mL)	protein[a] total (mg)	protein[a] TPK1Δ (mg)	purification (x-fold)	yield (%)
(1) extract	300	11020	30	1	100
(2) precipitations[b]	130	2350	29	5	97
(3) hydroxylapatite	100	39	21	210	70
(4) phenyl-TSK HPLC	6	15	15	230	53
(5) gel filtration[c]	15	13	13	230	50

[a] Total protein was measured by Coomassie Blue binding (steps 1 through 4; Bradford, 1976) or by A_{280} measurement (step 5; Perkins, 1986). TPK1Δ protein is estimated from specific activity measurements, calibrated with material from step 5. [b] 0.8% poly(ethylenimine) and 45% $(NH_4)_2SO_4$. [c] Before concentration.

Purification. Although our original immunoaffinity-based method of TPK1 purification (Zoller et al., 1988) is rapid and convenient, it also is expensive, nonrenewable, and difficult to scale up to the quantities of protein needed for structural studies. Fortunately, the chromatographic behavior of TPK1Δ is sufficiently different from its parent that we were able to develop a procedure that separates it completely from TPK1 while retaining the high efficiency of affinity purification (Table I).

A typical purification from 10 L of yeast is summarized in Table I. The most important step in the process is affinity chromatography over hydroxylapatite. When loaded onto hydroxylapatite and washed with moderate concentrations of phosphate salt, the holoenzyme and individual TPK1 catalytic subunits bind tightly to the resin, but TPK1Δ does not. Apparently, TPK1Δ holoenzyme binds to hydroxylapatite solely through BCY1. Thus, the addition of 100 μM cAMP to the wash buffer dissociates the subunits and elutes TPK1Δ selectively in high yield and purity. Successive hydrophobic interaction and gel filtration chromatography steps remove detectable contaminants and yield a preparation that is ≥99% pure as judged by silver-stained SDS gels (Figure 1). Our standard 10-μL preparation yields 12–14 mg of purified TPK1Δ.

Characterization. We characterized TPK1Δ kinetically using assays described previously (Kuret & Schulman, 1984; Kuret et al., 1988; Zoller et al., 1988). As summarized in Table II, TPK1Δ is very similar to its parent in substrate affinity, maximum velocity, and sensitivity to the protein kinase inhibitor analogue PKI_{5-24}. We conclude that TPK1Δ retains all the structural features necessary for protein kinase activity

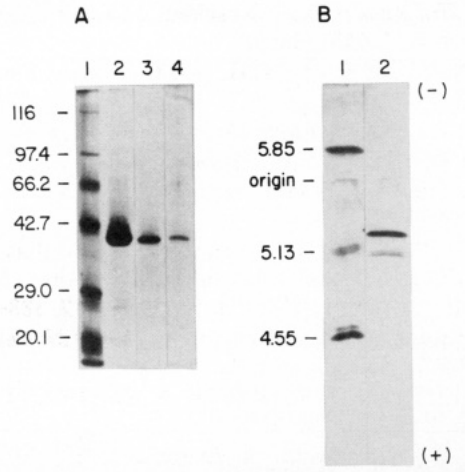

FIGURE 1: Electrophoretic analysis of purified TPK1Δ. (A) A silver-stained, 10–15% gradient SDS–polyacrylamide gel containing (lane 1) molecular mass standards and (lanes 2–4) 900, 30, and 3 ng of purified TPK1Δ, respectively. (B) A silver-stained, native isoelectric focusing gel (pH 4–6) containing (lane 1) p*I* standards and (lane 2) 200 ng of purified TPK1Δ. The origin marks the site of sample application.

Table II: Properties of TPK and TPK1Δ

	kinase	
	TPK1[a]	TPK1Δ
physical properties		
quaternary structure	monomer	monomer
amino acid residues	396	318
molecular mass	45950	37290
p*I* (calculated/measured)	5.8/ND[b]	5.8/5.3
N-terminal blocking group	+	–
kinetic properties		
V_{max} (μmol mg^{-1} min^{-1})	13.2	11.1
K_m(Kemptide) (μM)	101	83
K_m(MgATP) (μM)	33	45
K_i(PKI$_{5-24}$) (nM)[c]	280	125

[a] Zoller et al. (1988). [b] ND, not determined. [c] Calculated from IC_{50} data assuming simple competitive inhibition (Cheng & Prusoff, 1973).

and that determination of its three-dimensional structure will help us localize the amino acid residues responsible for catalysis and substrate recognition.

Further analysis of purified TPK1Δ by automated Edman degradation reveals our strategy for producing a homogeneous amino terminus was only partially successful; although approximately two-thirds of the protein has the predicted Pro-Lys- amino terminus, about one-third of the protein retains the initiating methionine residue (Met-Pro-Lys-; data not shown). This may be the source of heterogeneity observed when the preparation is subjected to native isoelectric focusing; 70% of TPK1Δ focuses as a single isoelectric species of p*I* = 5.2 with the remainder being more acidic (Figure 1).

Crystallization. We searched for suitable TPK1Δ crystallization conditions using the hanging-drop vapor diffusion technique combined with a sparse matrix sampling procedure (Jancarik & Kim, 1991) analogous to the incomplete factorial search procedures advocated by others (Carter & Carter, 1979; Carter et al., 1988), and found hexagonal crystals growing over a period of 1–4 days in solutions containing 2-propanol and $MgCl_2$ at 16 °C, pH 6 through 8. These conditions have consistently yielded crystals from several separate preparations of the protein. Our best crystals are obtained by seeding, are hexagonal dipyramids (Figure 2), and have the dimensions 0.6 × 0.6 × 1.5 mm. Apparently, microheterogeneity at the TPK1Δ amino terminus does not interfere with crystallization under these conditions.

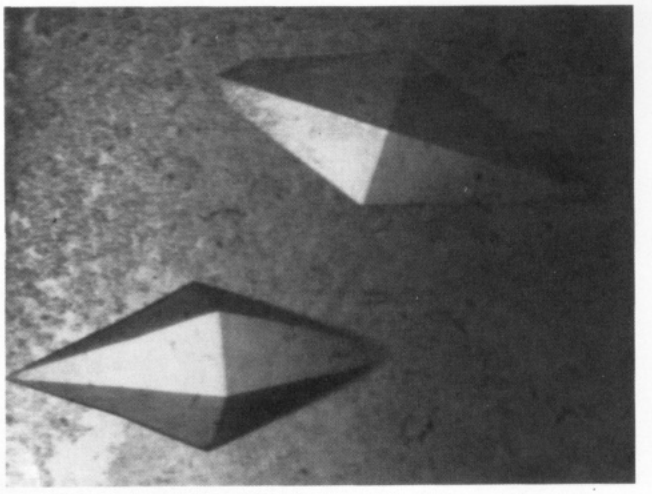

FIGURE 2: TPK1Δ crystals. These crystals were 0.3-mm wide when photographed.

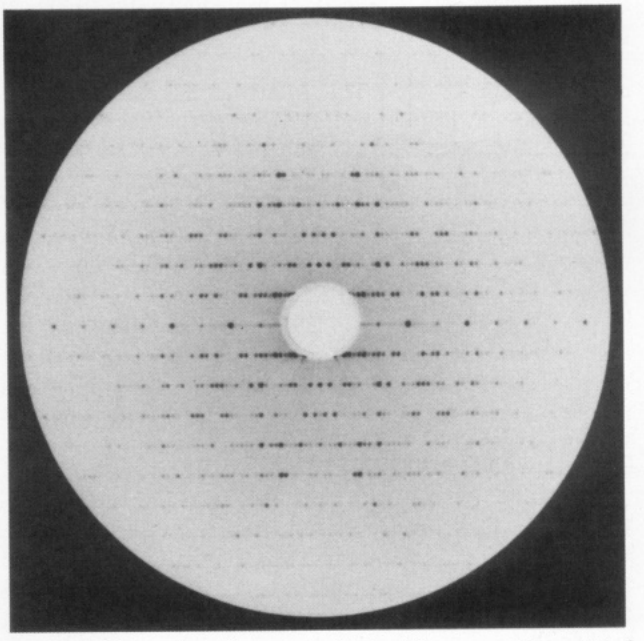

FIGURE 3: Precession photograph obtained from a small crystal of TPK1Δ. Reflections in this 00*l* zone appear only when $l = 6n$. The photograph has been enlarged to resolve the closely spaced reflections along c^* (oriented horizontally); the circumference of the diffraction pattern corresponds to approximately 4-Å resolution.

Crystal Properties. TPK1Δ crystals typically diffract beyond 2.8-Å resolution. The unit-cell dimensions determined by the AUTOINDEX command of MADNES were refined to 61 × 61 × 320 Å in a hexagonal system (cell angles 90°, 90°, 120°), and from the intensities of reflections with Miller indices *hk*1 and *hk*2, we find these crystals belong to point group 622. Precession photographs record reflections along 00*l* only when $l = 6n$ (where n is an integer), restricting the crystals to space group $P6_122$ or its enantiomorph $P6_522$ (Figure 3). From the molecular mass of TPK1Δ and the volume of the unit cell, we calculate a packing density of 2.66 Å^3/Da. This value is typical of protein crystals (Matthews, 1968). These and other properties of the crystals are summarized in Table III.

The relatively long c axis made data collection difficult. Reflections are spaced closely together along c^* and cannot be resolved on our detector except at a very large crystal to detector distance of over 300 nm. At that distance, however, air absorbs a significant fraction of the diffracted photons, and the detector samples a relatively small portion of the Ewald sphere. To overcome these complications, we positioned the

Table III: Properties of Yeast TPK1Δ Crystals

crystal morphology	hexagonal dipyramid
space group	$P6_122$ or $P6_522$
unit-cell dimensions (Å)	61 × 61 × 320
packing density (Å³/Da)	2.66
crystal density[a] (g/mL)	1.165
solvent content[b] (%)	42
molecules/unit cell	12
molecules/asymmetric unit	1
stability in X-ray beam	≥2 days at 16 °C

[a] Measured in Ficoll gradients as described by Westbrook (1985). [b] Calculated as described by Matthews (1985) assuming a partial specific volume of 0.779 mL/g. This value was calculated from the amino acid composition (Perkins, 1986), using the anhydrous, crystallographic residue volumes derived from intact proteins (Chothia, 1975).

Table IV: Native Data Collection Statistics

no. of crystals used	16
measurements	59181
unique reflections	9220
R_{merge}[a] (%)	4.7
completeness of data	
20–4.7 Å	89.0%
4.7–3.0 Å	97.0%
3.0–2.8 Å	97.3%

[a] R_{merge} was calculated as described previously (Pflugrath & Quiocho, 1988).

crystal so c^* coincided with the incident beam and a^* was parallel to the goniostat ω axis with the SETDATUM command of MADNES. By rotating the crystal around the ω axis during data collection, most reflections were easily resolved on the face of the detector for the first 70° of rotation at a crystal to detector distance of 70 mm. As much as 65–70% of the data can be collected in this way with approximately 3-fold redundancy. To collect the remaining 30% of the data lying along c^*, we employed the intense radiation available at the nearby National Synchrotron Light Source (NSLS). Using monochromatic, 0.95-Å radiation at a crystal to detector distance of 360 mm, we were able to complete our native data set to 2.8 Å with acceptable merging statistics as summarized in Table IV. In its outermost shell (2.6–2.5 Å), the dataset was 51.1% complete. A search for heavy-atom derivatives is underway.

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Registry No. PK, 9026-43-1; MgATP, 1476-84-2; kemptide, 65189-71-1.

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CORRECTION

Secondary Structure Analysis of the Scrapie-Associated Protein PrP 27–30 in Water by Infrared Spectroscopy, by Byron W. Caughey,* Aichun Dong, Kolari S. Bhat, Darwin Ernst, Stanely F. Hayes, and Winslow S. Caughey, Volume 30, Number 31, August 6, 1991, pages 7672–7680.

Page 7674. Due to a printing error, the detail in Figure 2 was lost. An appropriate representation is shown below.

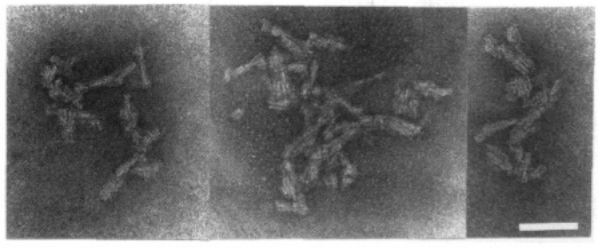

FIGURE 2: Electron microscopic analysis of PrP-res 27–30. Appearance of PrP-res 27–30 aggregates negatively stained with 0.5% ammonium molybdate. Magnification = 78750×. Bar = 100 nm.