# Crystallization and preliminary X-ray crystallographic analysis of yeast prion protein Ure2p with shortened N-terminal

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Abstract **An orthorhombic crystal form of a recombinant yeast prion protein with shortened N-terminal, 90Ure2p, has been obtained. Crystals were grown by the vapordiffusion technique against a mother liquor containing imidazole. Crystals belong to the primitive orthorhombic lattice with the cell parameters *a* = 54.5 Å, *b* = 74.7 Å, *c* = 131.0 Å. The crystals diffract to beyond 3.0 Å resolution at a synchrotron beamline.**

**Keywords: yeast prion, Ure2p, crystallization.**

Prions have been defined as the small proteinaceous particles which cause neurodegenerative disease in animals and humans. Such diseases in which the prion has been identified as the pathogen include Creutzfeld-Jakob and Kuru in humans, scrapie in sheep and bovine spongiform encephalopathy. It has been hypothesized that the infectious prion ($PrP^{Sc}$) is converted from its normal cellular form ($PrP^{C}$) by means of an α-helix to β-sheet conformational change[1—3]. This infectious form of the prion is believed to induce, through seeding, a similar conformational change in other $PrP^{c}$ proteins.

A yeast non-Mendelian element [URE3] in *Saccharomyces cerevisiae* which showed irregular segregation in meiosis was discovered in 1971 by Lacroute[4] and found to be propogated by cytoplasmic mixing[5]. It was proposed by Wickner in 1994 that [URE3] resulted from altered Ure2 protein and that the mechanism was similar to that found in prion disease[6—9]; indeed, this prion-like model explains the genetic properties of [URE3] extremely well. Similarly, [$PSI^{+}$] in *Saccharomyces cerevisiae* and [Het-s] in *Podospora anseria* also satisfy the genetic criteria as prion-like forms of normal proteins, Sup35p and Het-s respectively[9]. In contrast to mammalian prion proteins, in which their aggregated form can cause significant cell damage and is believed to induce cell apoptosis, these yeast prion proteins do not damage yeast cells but change their phenotypes. Yeast prions are not known to be harmful to humans and are easier to manipulate than their mammalian counterparts, thus they can be made ideal systems to study in order to understand further the infectious mechanism of mammalian prions.

The biological function of normal Ure2 protein is to repress the uptake of poor nitrogen source when a good nitrogen source becomes available[10,11]. The use of sequence alignment shows that Ure2p can be majorly divided into two domains: an N-terminal domain and a C-terminal domain. The C-terminal or N-repression domain has homology with glutathione S-transferase and is responsible for the nitrogen metabolism function. The N-terminal domain comprises approximately one quarter of the full length protein and is fully dispensable for the cellular function in nitrogen metabolism. The N-terminal domain is usually recognized as the prion-forming domain of Ure2p, in which residues 1—64 are sufficient to propagate the prion determinant[12,13], though the boundary between the prion-forming domain and the N-repression domain is not clear.

The N-terminal domain features an unusual amino acid composition rich in Gln and Asn residues, while the corresponding domain of mammalian prions has no such composition[14]. Recent experimental data have shown that several regions of Ure2p, excluding residues 1—64 but including some found in the N-repression domain, play an important role in prion induction[15,16]. Here we report the crystallization of Ure2p with a shortened N-terminal domain, comprising residues 90—345, which we named 90Ure2p.

## Methods and results

90Ure2p was highly expressed in *E. coli* C41(DE3) with 6 histine in soluble form. The LB medium with 100 μg/mL ampicillin was inoculated overnight with a culture of transformed cells in the same medium and grown at 37℃ with shaking. IPTG was not necessary in inducing the target protein since high concentration of IPTG would result in inclusion bodies. The purification of 90Ure2p was performed using a Ni-column, as described previously by Perrett and co-workers[17]. Crystals were obtained using the hanging drop/vapor diffusion method. The crystallization protocol involved mixing 2 μL of reservoir solution (0.6—1.0 mol/L imidazole, pH 7.0) with 1 μL 20 mmol/L glutathione in reduced form and 2 μL 8 mg/mL protein solution (20 mmol/L Tris-HCl, pH 8.4). Crystals were grown at 18℃ and the first crystals appeared after 6 d, growing to a final size of 0.2 mm × 0.02 mm × 0.03 mm (fig. 1).

Several crystals were picked from the drop and transferred into the reservoir solution in an Eppendorf tube. Each crystal was washed with reservoir solution three times to remove trace amounts of protein from the drop, and the reservoir solution was spun down at

4000 r/min for 15 min to collect the crystals. The crystals were treated as a common protein sample to run SDS-PAGE (fig. 2), which shows a clear band at 32 ku, strictly corresponding to the calculated molecular mass of 90Ure2p.

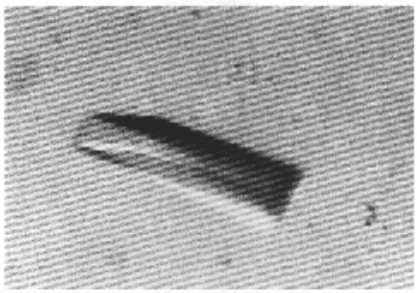

Fig. 1. Crystal of 90Ure2p.

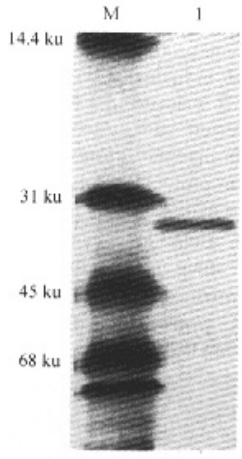


Fig. 2. Dissolved crystal. Protein was visualized by Coomassie-blue staining. M, protein marker; 1, 90Ure2p.

The crystals were tested and showed a bad diffraction ability in house, using a Rigaku2000 18 kW Cu Kα rotating–anode X-ray generator and a MAR Research MAR345 imaging plate. But at synchrotron beamline, the crystal can diffract to beyond 3.0 Å (fig. 3). A few images were collected on radiation station 9.5, at the synchrotron radiation source in Daresbury, UK. The radiation wavelength was set to 0.98 Å. When collecting, the crystal was frozen at 110 K, using mineral oil as a cryoprotectant. Data were auto-indexed, and integrated with the program DENZO on a Silicon Graphics $O_2$ workstation. The crystals belong to the primitive orthorhombic lattice with the cell parameters $a$ = 54.5 Å, $b$ = 74.7 Å, $c$ = 131.0 Å. The results for the data are given in table 1.

Further research is currently underway to solve the 3-dimentional structure of 90Ure2p which will create a structural basis to study the relationship between structure and pathogenic mechanism of prion protein.

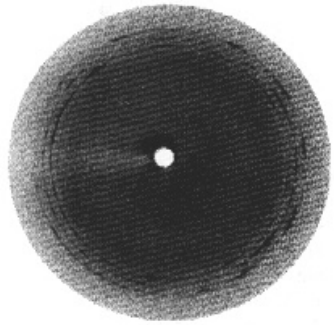

Fig. 3. A typical diffraction pattern of a crystal of 90Ure2p.

Table 1 Preliminary X-ray crystallographic analysis

| Lattice | Primitive orthorhombic |
|---|---|
| Cell dimensions / Å | |
| *a* | 54.5 |
| *b* | 74.7 |
| *c* | 131.0 |
| Wavelength/Å | 0.98 |
| Crystal-to-detector-distance /mm | 230 |
| Resolution range/Å | 30—3.0 |

## Note added in proof

After this note was submitted, two papers describing more shortened fragment[18,19] had just appeared.

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