# SHORT COMMUNICATIONS

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## Preliminary X-ray studies of a new crystal form of PHO4–DNA complex

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### Abstract

A new crystal form of PHO4 DNA-binding domain–DNA complex was obtained by the truncation of the N-terminal segment. This diffracts to a higher resolution than any forms reported previously. The present crystal belongs to orthorhombic space group $P2_12_12_1$, with unit-cell dimensions $a = 53.51$, $b = 68.30$, $c = 108.77$ Å. The crystal contains one protein dimer–DNA complex in the asymmetric unit.

### 1. Introduction

The phosphate regulon in *Saccharomyces cerevisiae* responds to intracellular levels of an essential nutrient, phosphate. The PHO4 protein is a positive regulatory factor in this regulon and is proposed to be indispensable for transcription of several genes including *PHO*5 (encoding p60, a major fraction of repressible acid phosphatase), *PHO*8 (repressible alkaline phosphatase) *etc*. PHO4 consists of 312 amino-acid residues with four functional domains (Ogawa & Oshima, 1990). The C-terminal region is identified as a DNA-binding domain composed of a basic region connected to the N terminus of an amphipathic helix which is separated by a loop from half of the C-terminal helix. The substructure is referred to as a basic helix–loop–helix (bHLH) motif. PHO4 can bind to the six base pair CACGT(G/T) motif of upstream activation sites (UAS's) in the promoter regions of *PHO*5 (Ogawa *et al.*, 1994; Vogel, Hörz & Hinnen, 1989) and *PHO*8 (Barbaric, Fascher & Hörz, 1992; Hayashi & Oshima, 1991) genes *via* the bHLH motif (Ogawa & Oshima, 1990). Although some three-dimensional structures of bHLH domain–DNA complexes (Ferré-D'Amaré, Prendergast, Ziff & Burley, 1993; Ferré-D'Amaré, Pognonec, Roeder & Burley, 1994; Ma, Rould, Weintraub & Pabo, 1994; Ellenberger, Fass, Arnaud & Harrison, 1994) have provided information, there are still some important questions to be asked about the bHLH proteins and about subtle differences in binding-site preferences. Therefore, we have attempted to crystallize the complex in order to obtain a more refined insight into specific protein–DNA interactions. In this study, crystallization was carried out with a 17 base pair oligomer, designated UASp2(17) derived from the UASp2 site of the *PHO*5 gene, since the binding of PHO4 to the UASp2 site is stronger than the other UAS sites (Ogawa *et al.*, 1994).

In this paper, we report crystallization of the complex between UASp2(17) and a DNA-binding domain of PHO4, consisting of 63 residues of the C-terminal [PHO4(63)]. This truncated protein forms new crystals that are more suitable for structural analysis than the crystals of UASp2(17)–PHO4(85) complex previously reported (Hakoshima *et al.*, 1993).

### 2. Materials and methods

PHO4(63) was overexpressed in *Escherichia coli* BL21(DE3) (Studier & Moffatt, 1986) using the T7 RNA polymerase system. The protein was purified by three column-chromatographic steps, using SP-sepharose, heparin–sepharose CL-6B and Mono-S (Pharmacia Biotech). The DNA-binding experiment using surface plasmon resonance measurement with BIAcore (Pharmacia Biosensor, Uppsala, Sweden) and the Beacon Fluorescence Polarization System (PanVera Corp., Madison, WI, USA) shows that the binding affinity of PHO4(63) is almost the same as that of the intact PHO4. The DNA used in the crystallization attempts was synthesized by standard phosphoramidite chemistry and was purified by reverse-phase high-pressure liquid chromatography and ion-exchange chromatography. Crystallization conditions were screened again using the hanging-drop vapor-diffusion method with various precipitants in a wide pH range. The final protein concentration was determined by UV absorption at 280 nm (Gill & von Hippel, 1989). The best crystals of PHO4(63)–UASp2(17)

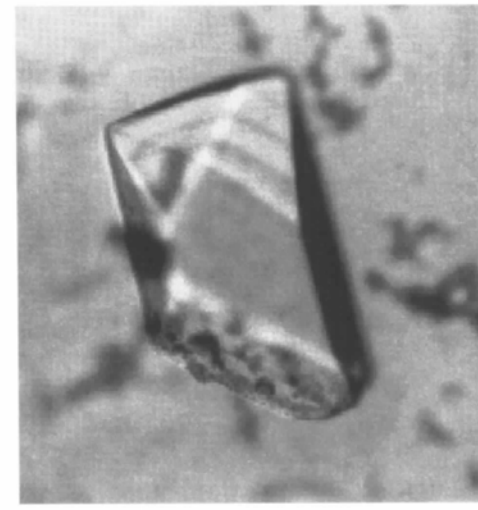

Fig. 1. An orthorhombic crystal of PHO4(63) dimer–UASp2(17) complex. The crystal was obtained by hanging-drop vapor diffusion as described in the text.

complex were grown when the 15 μl drops containing 0.4 m*M* protein, 0.2 m*M* DNA, 1%(*w*/*v*) PEG 6000, 20 m*M* sodium citrate buffer (pH 3.6) were equilibrated with a 500 μl reservoir solution of 1%(*w*/*v*) PEG 6000 and 20 m*M* sodium citrate buffer (pH 3.6). Typical dimensions of the crystals were 1 × 0.4 × 0.2 mm (Fig. 1).

## 3. Results

Three-dimensional data sets to 3.0 Å were collected by a Rigaku R-AXIS IIc on a rotating-anode generator operated at 40 kV, 100 mA with Cu $K\alpha$ radiation. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell dimensions $a = 53.51, b = 68.30, c = 108.77$ Å. A total of 15397 independent measurements were merged to obtain 6156 unique reflections which constitute 70.9% of theoretically possible data. The $R_{merge}$ value based on intensity data ($1.0\sigma$ cutoff) was 7.22%. The present crystal diffracts better and more stable than the previous crystals (Hakoshima *et al.*, 1993). Assuming one protein dimer–DNA complex in the asymmetric unit, the packing density ($V_m$) of the crystal (Matthews, 1968) is evaluated to be 4.2 $Å^3\,Da^{-1}$, which indicates that the solvent content is high (~71%). The structural determination is in progress, using the multiple isomorphous replacement with the introduction of 5-iodo deoxyuridine into the DNA oligomer and the initial map shows the crystal contains one complex in the asymmetric unit.

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