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Crystallization and preliminary X-ray characterization of the acylphosphatase-like domain from the *Escherichia coli* hydrogenase maturation factor HypF

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Maturation of prokaryotic hydrogenase involves several protein factors, among which is the accessory protein HypF, which hosts the consensus sequence of acylphosphatases and a sequence motif common to proteins catalyzing *O*-carbamoylations. The specific functions of HypF are largely unknown, although it has been observed that CN^- and CO ligands at the hydrogenase Ni,Fe active centre originate from carbamoylphosphate. The HypF N-terminal domain (91 residues, acylphosphatase-like domain) has been crystallized in two different crystal forms belonging to the orthorhombic $P2_12_12_1$ space group (unit-cell parameters $a = 35.5$, $b = 59.8$, $c = 87.6$ Å) and to the rhombohedral space group $R32$ (unit-cell parameters $a = b = 58.1$, $c = 155.6$ Å in the hexagonal setting).

1. Introduction

HypF is one of six protein factors encoded by the six *hyp* genes of *Escherichia coli*. These factors participate, together with a specific endopeptidase, in the maturation of prokaryotic [Ni,Fe]-hydrogenases, which are key enzymes in hydrogen metabolism (Friedrich & Schwartz, 1993; Casalot & Rousset, 2001). HypF is a large multidomain protein (MW 82 kDa) whose N-terminal 91-residue domain shares a striking sequence similarity to *E. coli* and human acylphosphatases (about 50 and 22% amino-acid identities, respectively), both of which belong to a widespread family of soluble enzymes that catalyse the hydrolysis of carboxylphosphate bonds (Stefani *et al.*, 1997). The primary structure homology between acylphosphatases and the HypF N-terminal domain extends to conservation of the family sequence consensus pattern and of the acylphosphatase catalytic residues (see Fig. 1). These findings, together with the recently reported results indicating that *hypF* genes undergo duplication and that one of the copies is truncated at the N-terminal domain (Wolf *et al.*, 1998), suggest a possible route for the appearance of the acylphosphatase gene in the prokaryotic genomes.

The specific function(s) performed by each Hyp factor in hydrogenase maturation remains largely unknown, with the exception of some recent information on the role played by HypF. It has been shown that in addition to the acylphosphatase-like N-terminal domain, HypF contains a second domain displaying the consensus sequence of enzymes catalyzing *O*-carbamoylations and two zinc-finger motifs similar to those found in the DnaJ chaperone. These findings have been further stressed by the observation that the CN^- and CO ligands in the hydrogenase active-site metal centre originate from carbamoylphosphate (Paschos *et al.*, 2001), a known acylphosphatase substrate. A likely scenario has therefore been depicted in which the HypF 'chaperone domain' interacts with the hydrogenase large-subunit precursor, followed by synthesis and transfer of the CN^- and CO ligands to the enzyme active site by the acylphosphatase and

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                        ~ ~~~~~~ ~~
HypF          -MAKNT-SCGVQLRIRGKVQGVGFRPFVWQLAQQLNLHGDVCNDGDG-VEVRLREDP--- 54
Ecoli         -MSK---VC-IIAWVYGRVQGVGFRYTTQYEAKRLGLTGYAKNLDDGSVEVVACGEEGQV 55
Bovine        SMAEGDTLISVDYEIFGKVQGVFFRKYTQAEGKKLGLVGWVQNTDQGTVQGQLQGPASKV 60
               *              * **** **         * * *   *   * *

HypF          ETFLVQLYQHCPPLARIDS--VEREPFIWSQLPTEFTIR- 91
Ecoli         EKLMQWLKSGGPRSARVER--VLSEPHHPSGELTDFRIR- 92
Bovine        RHMQEWLETKGSPKSHIDRASFHNEKVIVKLDYTDFQIVK 100
                    *                 *       *   *
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Figure 1
Amino-acid sequence alignment of the HypF N-terminal domain *versus* acylphosphatases from *E. coli* (Ecoli) and from bovine erythrocyte. Active-site residues recognized in acylphosphatase enzymes are boxed in black and the acylphosphatase sequence consensus pattern is highlighted by ~; residues conserved in the three sequences are marked by asterisks.

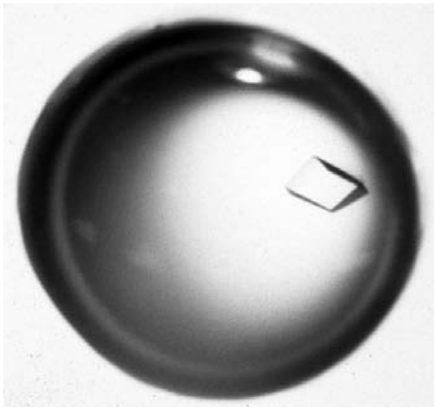

Figure 2
A photograph of the acylphosphatase-like HypF N-terminal domain crystals grown from ammonium sulfate. The maximum dimension of the crystal shown is approximately 0.2 mm.

carbamoyltransferase domains (Casalot & Rousset, 2001).

To assess the structural and functional relationships between classical acylphosphatases and the HypF acylphosphatase-like domain, we have cloned, purified and crystallized the N-terminal domain of HypF from *E. coli*, the preliminary crystallographic characterization of which is reported here.

2. Materials and methods

2.1. Cloning, expression and purification

Genomic DNA for the HypF N-terminal domain was isolated from *E. coli* DH5 cells by Genomic Prep (Pharmacia). Two DNA fragments corresponding to residues 990–1263 of the complete *hypf* gene were amplified by PCR; the resulting fragments were digested with *Bam*HI and *Eco*RI, ligated into pGEX-2T downstream and in frame with glutathione S-transferase and entirely sequenced as previously reported (Chiti *et al.*, 2001). Protein expression in the *E. coli* DH5 cells and subsequent purification were carried out as previously described (Modesti *et al.*, 1995). Protein purity and quality were checked by SDS–PAGE, ES-MS and amino-acid analysis.

2.2. Crystallization and preliminary diffraction analysis

Two different crystal forms of the acylphosphatase-like HypF N-terminal domain were grown, both using vapour-diffusion techniques. In the first case, crystals were isolated by equilibration of a 600 µl reservoir solution containing 30% PEG 4000, 100 m*M* sodium acetate pH 5.5 against 2 µl droplets composed of 1 µl of the protein solution (8 mg ml^{-1}, containing 10% glycerol) and 1 µl of the reservoir solution. Thin plate crystals (0.2 × 0.05 × 0.01 mm) grew in about one week at 277 K. X-ray diffraction data collected at DESY/EMBL beamline BW7A (Hamburg, Germany) to a resolution of 2.4 Å allowed the assignment of this crystal form to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 35.5, b = 59.8, c = 87.6 Å, $\alpha = \beta = \gamma = 90^\circ$ and two molecules per asymmetric unit. A second crystal form was obtained by equilibration against 600 µl of reservoir solution containing 1.5 *M* ammonium sulfate, 100 m*M* Tris buffer pH 8.5, 12% glycerol (Hampton Research Crystal Screen II, well 42). The droplets contained 1 µl of the reservoir solution and 1 µl of protein solution (8 mg ml^{-1}, containing 10% glycerol). These conditions led to large rhombohedral crystals (0.3 × 0.1 × 0.1 mm) after 48 h at 294 K (see Fig. 2). X-ray diffraction data were collected to 1.65 Å resolution in-house at the Cu $K\alpha$ wavelength using a rotating-anode generator (Rigaku RU-H3R) and a MAR 345 imaging-plate detector and subsequently at DESY/EMBL beamline BW7B (to a resolution of 1.25 Å). The second crystal form belongs to the rhombohedral space group $R32$, with unit-cell parameters $a = b = 58.1$, $c = 155.6$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$ (in the hexagonal setting) and one molecule per asymmetric unit. The relevant data-collection statistics are reported in Table 1. All data were reduced, integrated and scaled using the *HKL* suite (Otwinowski & Minor, 1997).

Several structure-solution attempts *via* molecular-replacement methods (Navaza, 1994; Kissinger *et al.*, 1999) based on the bovine erythrocyte acylphosphatase as search model (PDB code 2acy; Thunnisen *et al.*, 1997; 34% amino-acid identity) were unsuccessful. Structure solution will therefore be achieved through isomorphous replacement methods; a search for suitable heavy-atom derivative(s) is in progress.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

	Orthorhombic crystal form	Rhombohedral crystal form
Beamline	DESY/EMBL BW7A	DESY/EMBL BW7B
Wavelength (Å)	0.9762	0.8443
Resolution range (Å)	25–2.40 (2.44–2.40)	25–1.25 (1.29–1.25)
Completeness (%)	75.7 (76.4)	92.9 (93.0)
Total reflections collected	54665	499640
Unique reflections	7809	28517
Redundancy	7.0	17.5
$R_{\rm merge}$ (%)	8.8 (17.4)	5.1 (13.0)
$\langle I/\sigma(I)\rangle$	6.17 (3.5)	16.33 (3.1)
Unit-cell parameters (Å, °)	a = 35.5, b = 59.8, c = 87.6, $\alpha = \beta = \gamma = 90$	a = 58.1, b = 58.1, c = 155.6, $\alpha = \beta = 90$, $\gamma = 120$
Space group	$P2_12_12_1$	$R32$
Molecules in asymmetric unit	2	1
$V_{\rm M}$ (Å^3 Da^{-1})	2.32	2.52
Solvent content (%)	47.1	51.3

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