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Cloning, purification, crystallization and preliminary X-ray analysis of DOS heme domain, a new heme oxygen sensor in *Escherichia coli*

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The heme-containing PAS domain of the direct oxygen-sensor protein (DOS_H), a *bona fide* oxygen-sensor protein, has been cloned from *Escherichia coli* strain K12 and successfully purified. The oxidized form of this protein was crystallized by the hanging-drop method with a PEG 8000-based precipitant. Preliminary X-ray diffraction studies of the PAS-domain crystal show that it belongs to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 46.1, b = 68.1, c = 82.6 Å. A complete diffraction data set was collected to 1.9 Å for MAD phasing. The electron-density map shows two molecules in an asymmetric unit and a unique six-coordination of the heme iron.

1. Introduction

PAS-domain[1] heme oxygen sensors are a recently discovered class of heme proteins (Gilles-Gonzalez *et al.*, 1994; Rodgers, 1999; Chan, 2001). To date, three proteins have been identified in this class: one direct oxygen sensor (DOS) from *Escherichia coli* and two FixL proteins. The FixL proteins have been identified and characterized from field-crop symbionts in the family of *Rhizobia*, one from *Sinorhizobium meliloti* (Gilles-Gonzalez *et al.*, 1991; Lois *et al.*, 1993; Gilles-Gonzalez & Gonzalez, 1993) and one from *Bradyrhizobium japonicum* (Anthamatten & Hennecke, 1991). These two proteins are both part of a two-component sensing/signaling system that involves another protein, FixJ, as a transcription regulator for *nif* and *fix* genes (David *et al.*, 1988; Gilles-Gonzalez & Gonzalez, 1993). The two FixL proteins are multi-domain proteins containing a heme oxygen-sensing domain and an enzymatic kinase domain. Crystal structures of the heme domains from *S. meliloti* and *B. japonicum* illustrate the PAS structural motif, which is unlike any previous heme-binding domain (Gong *et al.*, 1998, 2000; Miyatake *et al.*, 2000).

The DOS protein from *E. coli* was identified from sequence comparisons of the FixL PAS heme domain with other genome sequences (Delgado-Nixon *et al.*, 2000). Initial biochemical studies on DOS have established that this protein is a *bona fide* oxygen sensor in *E. coli*. For example, it shows discrimination between CO binding and O_2 binding. Its physiological function is likely to be as a sensor for controlling the enterobacterial switch between aerobic and anaerobic metabolism. Like the FixL proteins, DOS is a multidomain protein with a sensing domain that is similar to the PAS heme domain and a signaling domain with sequence similarity to phosphodiesterase (Delgado-Nixon *et al.*, 2000).

Unlike the FixL PAS heme domains, absorption and NMR spectra indicate that the DOS heme domain (DOS_H) is six-coordinate in both the ferric heme and ferrous heme oxidation states (Delgado-Nixon *et al.*, 2000; our unpublished data). It is imperative, therefore, to structurally characterize DOS in order to understand and interpret existing ligand-binding dynamics data as well as kinetics and spectroscopic studies that are now in progress.

We have used PCR technology to clone the gene for DOS from *E. coli* strain K12 and to produce a truncation of the full-length DOS gene containing only the heme domain (DOS_H). The protein DOS_H was expressed, purified and its stable core was subsequently crystallized using methods described in this communication.

2. Experimental procedure

2.1. Construction of the expression plasmid and DOS_H expression

Genetic material for the DOS heme domain was obtained by PCR amplification of codons 1–147 of the *yddU* (Blattner *et al.*, 1997; Delgado-Nixon *et al.*, 2000) gene of wild-type *E. coli* strain K12. This DOS gene was

[1] Period, Arnts and Simple-minded domain (*Drosophila Period* gene, the AHR nuclear translocator and *Drosophila Single-minded* locus).

subcloned into a pET24(a)+ vector, which was used to transform BL21 (DE3) *E. coli*. DOS_H was expressed using 0.1 *M* IPTG in *E. coli* cells grown in LB medium containing kanamyacin and 0.5% dextrose at 310 K with 200 rev min^{-1} agitation for 5 h.

2.2. Isolation and purification of DOS_H

Cells were harvested by centrifugation and lysed using lysozyme at room temperature. Cells were disrupted with a French Press (SLM-Aminco) and by sonication.

The lysate was acidified to pH 6.5 with 1 *M* acetic acid and applied to a DEAE-Sepharose column (60 × 2.5 cm) equilibrated with 0.05 *M* Tris–HCl pH 6.5. DOS_H was eluted using a NaCl concentration gradient. Combined fractions were concentrated using an Amicon YM10 membrane. This concentrated sample was further purified by gel filtration on a G-75 Sephadex column (80 × 2.0 cm) using 0.1 *M* potassium phosphate buffer pH 6.8. Purity of the protein was confirmed by SDS–PAGE, mass spectrometry and N-terminal amino-acid sequencing. The sample was crystallized after natural mass loss, which was followed by mass spectrometry.

2.3. UV–visible spectroscopy

UV–visible spectroscopy measurements of DOS_H were carried out in 0.1 *M* potassium phosphate buffer pH 6.5 with a dual-path GBC Cintra Spectrophotometer (Fig. 1). Solid sodium dithionite was added to one aliquot from the crystallizing solution to form reduced (ferrous, Fe^{2+}) deoxy-DOS_H. A second sample was similarly treated with dithionite and then exposed to a stream of purified oxygen gas to form reduced (Fe^{2+}) oxy-DOS_H.

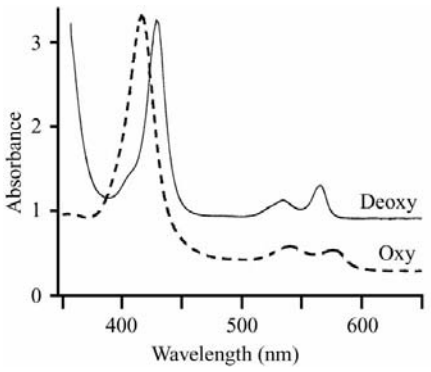

Figure 1
Optical absorption spectra of oxy-DOS_H (dotted line) and deoxy-DOS_H (solid line) taken at 294 K, 0.1 *M* potassium phosphate buffer pH 6.5. Peak maxima are given in Table 2.

2.4. Crystallization

Crystallization trials were performed using the hanging-drop vapor-diffusion method at two temperatures (277 and 293 K). Initially, red whisk-shaped crystals appeared from several sparse-matrix screen conditions at 293 K. From the initial results, one condition containing the best shaped crystals was refined to yield type I crystals. These type I crystals were obtained by mixing 1.5 µl of protein (10 mg ml^{-1}, 100 m*M* NaCl, 10 m*M* Tris–HCl pH 7.5) with an equal volume of reservoir solution containing 15%(*w*/*v*) PEG 8000, 200 m*M* ammonium sulfate and 100 m*M* sodium acetate pH 4.5. Crystals grew to dimensions of 0.3 × 0.1 × 0.1 mm after a week at 293 K. During further optimization, type II crystals with dimensions of 0.1 × 0.05 × 0.05 mm were obtained (Fig. 2) from 20%(*w*/*v*) PEG 8000, 200 m*M* sodium tartarate and 100 m*M* sodium acetate pH 4.5. The type I crystals were often produced as multiple crystals and their edges were not sharp, even though their dimensions were larger than those of the type II crystals.

2.5. X-ray data collection

The crystals were transferred into the same reservoir buffer containing in addition 20%(*v*/*v*) glycerol as a stabilizer. A crystal was picked up with a CryoLoop (0.3 mm diameter; Hampton Research) and immediately frozen in a nitrogen stream at 100 K (Oxford Cryosystems Cryostream). Diffraction data from both type I and type II crystals were collected on an R-AXIS IIc imaging plate using a Rigaku rotating-anode X-ray generator (Cu *Kα*, 5.4 kW) equipped with mirror optics (Z. Otwinowski & G. Johnson, Yale University; as marketed by Rigaku, Texas, USA). Crystals were positioned and rotated based on a data-collection strategy from the *R-AXIS IIc* v2.1 software. Intensities were integrated and scaled using the *HKL* program suite (Otwinowski & Minor, 1997). The crystals belonged to space group $P2_12_12_1$, with unit-cell parameters a = 53.8, b = 58.9, c = 92.0 Å for the type I crystal and a = 46.1, b = 68.1, c = 82.6 Å for the type II crystal. The quality of the diffraction data was better with type II crystals than with type I. The type I crystals were highly mosaic. Therefore, structure determination was only performed with type II crystals.

Table 1
Summary of crystallographic data collection and processing.

Values in parentheses are for the highest 0.1 Å resolution shell.

	λ_1	λ_2	λ_3
Space group	$P2_12_12_1$		
Unit-cell parameters (Å)	a = 46.1, b = 68.1, c = 82.6		
Asymmetric unit	2 molecules		
Resolution (Å)	2.1	1.9	2.1
Wavelength (Å)	1.738	1.033	1.741
Total reflections	120932	160792	101446
Unique reflections	18529	25182	16222
Completeness (%)	99.6 (98.3)	99.3 (91.8)	99.6 (99.5)
R_{merge}†	0.033 (0.090)	0.034 (0.121)	0.044 (0.149)
R_{ano}‡	0.066	0.033	0.055

† $R_{merge} = \sum_h \sum_i |\langle F_h\rangle - F_{hi}|)/\sum_h F_h$, where $\langle F_h\rangle$ is the mean structure-factor magnitude of i observations of symmetry-related reflections with Bragg index h. ‡ $R_{ano} = (\sum ||F^+_{PH}| - |F^-_{PH}||)/\sum(|F^+_{PH}| + |F^-_{PH}|)/2$.

2.5.1. Multiple-wavelength anomalous diffraction (MAD) data collection. A MAD data set was collected at the Stanford Synchotron Radiation Laboratory (SSRL) beamline 9-2 synchrotron-radiation source using the BLUE-ICE interface in order to obtain initial phase information. Three wavelengths, 1.738 Å (7132.5 eV, peak), 1.741 Å (7121.4 eV, edge) and 1.033 Å (12000 eV, remote), were chosen after a fluorescence scan revealed the sharp absorption edge of the heme iron. The diffraction data were collected at 100 K with a crystal-to-detector distance of 100 mm. The crystal diffracted to 1.9 Å with an R_{merge} of 0.034 (Table 1).

3. Results and discussion

Delgado-Nixon and coworkers proposed that both DOS and the FixL proteins belong to a heme oxygen-sensing subgroup of the PAS-domain superfamily. They based this upon sequence similarities (Delgado-Nixon *et al.*, 2000) and the published FixL crystal structures (Gong *et al.*, 1998, 2000; Miyatake *et al.*, 2000). They found that DOS and FixL

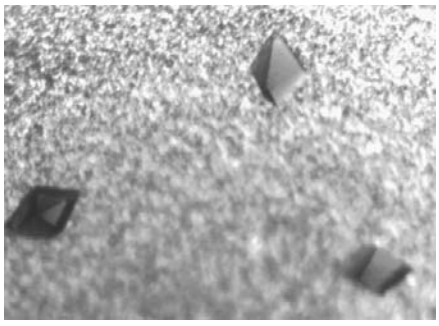

Figure 2
Crystal pictures of type II crystal of DOS_H. These were grown at 293 K. After one week of growth at 293 K, crystals grew to dimensions of 0.1 × 0.05 × 0.05 mm.

Table 2
Comparison of absorption properties for different reduced-heme truncated DOS heme domains.

Wavelengths of absorption maxima are given in nanometres at 294 K, 0.1 *M* potassium phosphate buffer pH 6.8.

	Wavelengths of maxima (nm) for recombinant preparations	
Protein form	Delgado-Nixon (2000)	This work (2002)
Oxy-DOS	579	577
	540	540
	418	416
Deoxy-DOS	563	564
	532	532
	427	427

sequences were only 25% identical and 60% similar. We have carried out this work in order to determine whether this third example of the oxygen-sensing protein subgroup also contains a PAS heme domain and, if so, to determine how structurally similar it is to the FixL heme domains. Our recombinant truncated DOS_H shares highly similar UV–visible spectra with the recombinant form reported by Delgado-Nixon and coworkers, as shown in Fig. 1 and Table 2. These figures also indicate that whereas we have crystallized the oxidized form (Fe^{3+}), the reduced form of our truncated heme-domain preparation maintains full oxygen-binding functionality. This heme domain is obtained with the heme in the reduced (Fe^{2+}) oxygenated form (Fig. 1) immediately following lysis and extraction from *E. coli*. It maintains this form through isolation from the DEAE column, but is finally collected with the heme in the oxidized (Fe^{3+}) form at the end of the isolation. Therefore, oxidation of the heme iron takes place during the course of our preparation. It is this ferric form that was crystallized. UV–visible spectroscopy of collected crystals confirmed that the heme domain remained in the oxidized form.

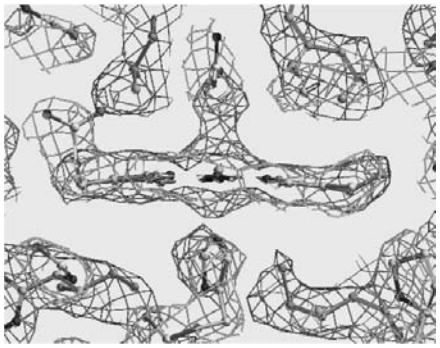

Figure 3
Heme area of the DOS_H model structure, drawn from the initial density map from MAD phasing. This picture was generated using *O* (Jones *et al.*, 1991).

Heme iron position, phase calculation and parameter refinement were performed with the automated phasing program *SOLVE* using data in the resolution range 50–3 Å, followed by maximum-likelihood density modification as implemented in *RESOLVE* (Terwilliger & Berendzen, 1999; Terwilliger, 2000). Two heme irons were found in one asymmetric unit. Initial MAD map around the iron clearly showed a heme electron density (Fig. 3). Model building and refinement are currently under way.

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References

Anthamatten, D. & Hennecke, H. (1991). *Mol. Gen. Genet.* **225**, 38–48.
Blattner, F. R., Plunkett, G. III, Bloch, C. A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J. D., Rode, C. K., Mayher, G. F., Gregor, J., Davis, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D. J., Mau, B. & Shao, Y. (1997). *Science*, **277**, 1453–1474.
Chan, M. K. (2001). *Curr. Opin. Chem. Biol.* **5**, 216–222.
David, M., Daveran, M.-L., Batut, J., Dedieu, A, Domergue, O., Ghai, J., Hertig, C., Boistard, P. & Kahn, D. (1988). *Cell*, **54**, 671–683.
Delgado-Nixon, V. M., Gonzalez, G. & Gilles-Gonzalez, M. A. (2000). *Biochemistry*, **39**, 2685–2691.
Gilles-Gonzalez, M. A., Ditta, G. S. & Helinski, D. R. (1991). *Nature (London)*, **350**, 170–172.
Gilles-Gonzalez, M. A. & Gonzalez, G. (1993). *J. Biol. Chem.* **268**, 16293–16297.
Gilles-Gonzalez, M. A., Gonzalez, G., Perutz, M. F., Kiger, L., Marden, M. C. & Poyart, C. (1994). *Biochemistry*, **34**, 8067–8073.
Gong, W., Hao, B. & Chan, M. K. (2000). *Biochemistry*, **39**, 3955–3962.
Gong, W., Hao, B., Mansy, S. S., Gonzalez, G., Gilles-Gonzalez, M. A. & Chan, M. K. (1998). *Proc. Natl Acad. Sci. USA*, **95**, 15177–15182.
Jones, T. A., Zou, J. Y., Cowan, S. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.
Lois, A. F., Ditta, G. S. & Helinski, D. R. (1993). *J. Bacteriol.* **175**, 1103–1109.
Miyatake, H., Mukai, M., Park, S.-Y., Adachi, S.-I., Tamura, K., Nakamura, H., Nakamura, K., Tsuchiya, T., Iizuka, T. & Shiro, Y. (2000). *J. Mol. Biol.* **301**, 415–431.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Rodgers, K. (1999). *Curr. Opin. Chem. Biol.* **3**, 158–167.
Terwilliger, T. C. (2000). *Acta Cryst.* D**56**, 965–972.
Terwilliger, T. C. & Berendzen, J. (1999). *Acta Cryst.* D**55**, 849–861.