OASIS and Xe phasing: potential in high-throughput crystallography

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The CCP4 supported program *OASIS* has been tested using one-wavelength anomalous scattering data collected from a xenon derivative of the lobster apocrustacyanin A1 protein [Cianci *et al.* (2001). The Xe atoms were located by the program *SAPI* and the absolute configuration was determined by the program ABS. The electron density map after *OASIS* and density modification clearly revealed the solvent boundary and the $C\alpha$ trace. The test demonstrated that, by exploiting the anomalous signal at single wavelength, OASIS can be used to determine phases at moderate (~2.3 Å) macromolecular crystallographic resolution for a medium-size protein (3500 non-H atoms in the asymmetric unit). As the xenon derivatives can be obtained from native protein crystals using commercially available equipment in a relatively short time frame (a few hours), the method described in this paper may provide a good alternative to MAD or MIR phasing, in particular when high-throughput is desirable.

Keywords: OASIS; one-wavelength anomalous scattering; xenon derivative.

1. Introduction

In view of the mounting evidence that one-wavelength anomalous scattering (OAS or otherwise known as SAD) may be sufficient to solve protein structures (Hao, 2000), the OASIS program (Hao et al, 2000) was written to determine phases using one-wavelength anomalous scattering data instead of using additional multiwavelength diffraction data (MAD). This is of particular importance when protein crystals are sensitive to X-ray irradiation or the absorption edges of the anomalous scatterers, such as xenon and sulfur, are difficult to access. A number of minor changes in the new version of OASIS to be released by CCP4 include new keywords to allow resolution and sigma cutoff. The upper limit on the number of reflections has been increased to 150,000 (from 90,000).

In preparing samples for MAD or OAS phasing, the most favored approach is the incorporation of selenium into protein using seleno-methionine during the expression of the protein. However, this is only successful when the gene encoding the particular protein is known and an expression is established and when the substitution does not affect crystalline order. In cases where seleno-methionine substitution is not plausible an attractive method for preparing samples is to incorporate xenon gas into the crystal. Xenon is known to bind to hydrophobic pockets within proteins at modest pressure. The one-wavelength anomalous scattering data (courtesy of Dr Rizkallah and Professor Helliwell, see Table 1 for details) collected from a xenon derivative of the lobster apocrustacyanin A1 protein (Cianci *et al.*, 2001) was used to test the possibility of *ab initio* phasing.

Table 1
OAS data

Values in parentheses refer to the highest resolution shell. The abbreviation a.s.u. stands for asymmetric unit.

Space group	$P2_12_12_1$
Unit cell	a = 41.11 Å
	b = 79.81 Å
	c = 109.86 Å
Non-H atoms in a.s.u.	3505
Number of Xe sites in a.s.u.	3 major + 1 minor
Source	Daresbury SRS Station 7.2
Wavelength	λ = 2.045 Å
f'' (in electrons)	11.5
Resolution	64.5-2.3 Å
Unique reflections	16723
Completeness	99.7%
Redundancy	7.1
$I/\sigma(I)$	23.4 (12.3)
R_{sym}	7.3 (14.5)%

2. Locating the xenon sites

The Se anomalous scatterers for both structures were located by the conventional direct-methods program *SAPI* (Fan *et al.*, 1990; http://staff.chess.cornell.edu/~hao/sapi/sapi.html) using magnitudes of anomalous differences,

$$|\Delta F(H)| = ||F(H)| - |F(H)||$$

for reflections within 3.0 Å. The solution was selected by a default run of the program. The largest 416 normalized structure factors E's were used in tangent formula phase refinement. The resultant electron density map produced a group of 3 highest peaks; there was a clear gap between this group and other peaks in terms of peak height. A Karle-recycle refinement (an option in SAPI) of these three sites yielded an additional minor site. The absolute configuration of these sites was determined by the program ABS (http://staff.chess.cornell.edu/~hao/abs/abs.html) based on the P_s-function method (Woolfson & Yao, 1994). These Xe sites agreed well with the published sites (Cianci *et al,* 2001) and formed the basis for the next phasing step.

3. OASIS and DM phasing

The *ab initio* phasing of the OAS data was implemented in the computer program *OASIS* (Hao *et al.*, 2000). All Friedel pairs (including centric reflections) were evaluated using *OASIS*. The script that was used to run OASIS is shown below:

```
#oasis.com
oasis HKLIN xea1_19_trn.mtz HKLOUT xea1_oasis.mtz << eof
TITLE DIRECT PHASING OF xea1 xenon OAS DATA
HCO XE 12
FIT
```

```
RES 2.3
LCE 7
ANO XE 11.5
POS XE    -0.62360 -0.52335 -0.50331  1  0.318
      XE    -0.87037 -0.55929 -0.99017  2  0.419
      XE    -0.65256 -0.76443 -0.87725  3  0.268
      XE     0.47078  0.20858  0.15206  4  0.080
LABIN F1=F SIGF1=SIGF F2=DANO SIGF2=SIGDANO
LABOUT F1=F SIGF1=SIGF PHI=PHIdp W=Wdp
END
eof
```

Density modification using the CCP4 program *DM* (Collaborative Computational Project, Number 4, 1994) was then applied to the resulting phase sets. Phase error analysis and figures of merit before and after *DM* are given in Table 2. The electron density maps after *OASIS* and density modification clearly revealed the solvent boundary. The $C\alpha$ trace was clearly visible but there were a number of places where the electron density was broken. A correlation coefficient between the *OASIS* + *DM* phased map and the final refined structure was 0.57.

Table 2
Phase error analysis and figure of merit

Reflections were sorted in descending order of F_{obs} and cumulated into groups. Phase errors were calculated against the refined models (Cianci *et al.*, 2001, PDB reference 1h91) weighted by F_{obs}.

	Phase errors (°)	
Number of reflections	***OASIS***	***OASIS* + *DM***
3000	58.5	44.3
6000	59.5	47.9
9000	60.0	49.6
12000	60.9	51.2
15000	61.7	52.3
16723	62.1	52.9
Mean figure of merit	0.49	0.71

4. Discussion

Here we demonstrate that, by exploiting the anomalous signal at single wavelength, OASIS can be used to determine phases at moderate (~2.3 Å) macromolecular crystallographic resolution for a medium-size protein. The total CPU time consumed by SAPI, ABS and OASIS was about 3 minutes on an Alpha XP10000 workstation. As the xenon derivatives can be obtained from native protein crystals using commercially available equipment in a relatively short time frame (a few hours), the method described in this paper may provide an attractive alternative to MAD or MIR phasing, in particular when high-throughput is desirable.

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