RESEARCH NOTE

Cadmium SAD phasing at CuKα wavelength

[version 1; peer review: 2 approved]

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Abstract

Single-wavelength anomalous diffraction (SAD) is the most common method for *de novo* elucidation of macromolecular structures by X-ray crystallography. It requires an anomalous scatterer in a crystal to calculate phases. A recent study by Panneerselvam *et al*. emphasized the utility of cadmium ions for SAD phasing at the standard synchrotron wavelength of 1 Å. Here we show that cadmium is also useful for phasing of crystals collected in-house with CuKα radiation. Using a crystal of single-domain antibody as an experimental model, we demonstrate how cadmium SAD can be conveniently employed to solve a CuKα dataset. We then discuss the factors which make this method generally applicable.

Keywords

Protein crystallography, experimental phasing, single-wavelength anomalous diffraction, cadmium ions, Cd-SAD

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 21 Jan 2019	✓ view	✓ view

1. **Thomas E. Edwards**, Bainbridge Island, Bainbridge Island, USA

2. **Igor Melnikov**, European Synchrotron Radiation Facility (ESRF), Grenoble, France

Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the Mathematical, Physical, and Computational Sciences collection.

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Author roles: Eliseev IE: Investigation, Writing – Original Draft Preparation, Writing – Review & Editing; **Yudenko AN**: Investigation; **Ukrainskaya VM**: Investigation; **Chakchir OB**: Funding Acquisition, Supervision

Competing interests: No competing interests were disclosed.

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Introduction

Elucidation of atomic structures of macromolecules by X-ray crystallography requires knowledge of the phases of measured reflections. Nowadays this phase problem is most often solved by molecular replacement (MR), a computational technique which utilizes the known structure of a homologous molecule to estimate phases. However, in the case of *de novo* structure elucidation when an appropriate homologous structure is unavailable, phases should be determined experimentally. This is predominantly achieved by analyzing anomalous scattering produced either by atoms naturally occurring in the molecule, or intentionally introduced into crystal during growth or soaking. The two phasing methods exploiting the anomalous scattering, multiwavelength anomalous diffraction (MAD) and single-wavelength anomalous diffraction (SAD), were reviewed by Hendrickson[1]. Synchrotron radiation with tunable wavelength allows achieving the absorption edges of all elements with Z≥20 to maximize anomalous signal, thus making these methods remarkably versatile.

On the contrary, the choice of anomalous scatterer is minimal when data are to be collected in-house using a laboratory X-ray generator, most often equipped with a copper anode (λ=1.5418 Å, CuKα). Indeed, in some cases, even weak anomalous signal of sulfur (f''=0.56e^- at CuKα) can be used for phasing, as demonstrated in pioneering SAD work on crambin[2]. Similarly, zinc (f''=0.68e^- at CuKα) was proposed to be useful for in-house SAD experiments[3]. Perhaps the most impressive result came from the structural genomics project, where iodine ion soaks were systematically used for *de novo* SAD phasing of datasets collected with CuKα radiation[4]. Iodine has a strong anomalous scattering (f''=6.9e^- at CuKα), high solubility, and binds multiple hydrophobic sites or positively charged residues on protein surface. Iodine SAD appeared remarkably efficient for phasing the crystals of membrane proteins which possess patches of positively charged residues at the hydrophobic-hydrophilic interface, providing many binding sites for anions[5].

Another attractive opportunity is to use cadmium ions, which have a great anomalous signal (f''=4.7e^- at CuKα) comparable to that of iodine, promote crystal growth[6], and can substitute other divalent cations in metal-binding proteins. Despite all these advantages and its use in the very early SAD works[7], Cd is rarely used in the phasing of protein crystals. Recently, a paper emphasizing the utility of cadmium ions for experimental phasing at the standard synchrotron wavelength of 1 Å was published[8]. In this short research note, we show how Cd-SAD can also be conveniently used for phasing datasets collected using CuKα radiation.

Methods

As an experimental model for in-house cadmium SAD, we used a crystal of an anti-ErbB3 single-domain antibody BCD090-M2, which we recently studied[9]. The details of protein purification, characterization, and structural analysis are given in the paper[9]. Briefly, the protein was expressed in *E. coli SHuffle* cells as a SUMO fusion, purified by immobilized metal affinity chromatography, cleaved by TEV protease, and then polished by an additional step of high-resolution cation-exchange chromatography. The antibody was crystallized by hanging-drop vapor diffusion in two different forms: in a space group C2 without divalent cations (PDB accession number: 6EZW) and in P1 with two cadmium ions per unit cell (PDB accession number: 6F0D)[9]. Crystals of both types diffracted below 2 Å. The data were collected on a Kappa Apex II diffractometer (Bruker AXS) using CuKα radiation generated by a IμS microfocus X-ray tube. Both structures were solved by molecular replacement in Phenix software suite v. 1.11[10]. The dataset with cadmium (6F0D) with unmerged Friedel pairs was used for SAD analysis. For experimental phasing, we used a standard protocol employing *SHELXC/D/E* programs[11] through *HKL2MAP* v. 0.4 graphical interface[12]. Data were processed with *SHELXC* v. 2016/1, anomalous substructure was solved by *SHELXD* v. 2013/2 and phasing and density modification were done by *SHELXE* v. 2018/2. The automatic model building and refinement were done in Phenix v. 1.14[10], and manual refinement was done in *Coot* v. 0.8.9.1[13]. Figures were prepared with *PyMOL*.

Results and discussion

The phasing of protein crystals by SAD starts from finding the positions of an anomalous substructure, which is usually done by direct methods. First, the dataset was processed with *SHELXC*, and the statistical analysis of the anomalous signal is shown in Figure 1A and Table 1. The use of kappa goniometer for data collection allowed achieving high completeness (96.4%) and multiplicity (5.9) of anomalous pair measurements. The signal-to-noise ratio defined as $\langle d''/\sigma(d'')\rangle$ and the correlation coefficient $CC_{1/2}$ indicate that useful anomalous signal is present almost in the whole resolution range. For further substructure solution, we implied a rather conservative high-resolution cut-off of 2.4 Å corresponding to $CC_{1/2}$ (anom.) ~ 0.3.

The anomalous substructure was immediately solved by *SHELXD* as judged by high correlation coefficients (combined figure of merit = 55.6%), high occupancies of the two cadmium sites (1.00, 0.99), and the rapid drop in occupancy of the next site (0.17). The positions of Cd ions corresponded to the largest off-origin peak of the anomalous Patterson function at (0.58, 0.02, 0.03). The solution was used in *SHELXE* for phasing, electron density modification, and chain tracing. This yielded electron density maps with high contrast, and the solutions for original and inverted substructure were indistinguishable due to centrosymmetry (Figure 1B). As discussed previously[14], centrosymmetric anomalous sites in SAD can impede interpretation of electron density maps, because the resulting map is a superposition of the true electron density with its negative mirror-image. However, in our case the major portion of the protein chain (87%) was traced after density modification. This incomplete model was further improved in *phenix.autobuild*, and then refined manually in *Coot* and *phenix.refine* giving final R_{work}/R_{free} of 17.8/21.0%.

In this particular case, structure determination by in-house Cd-SAD was almost as straightforward as an automated molecular replacement. The causes of this simplicity were the relatively small protein size, high completeness and multiplicity of the

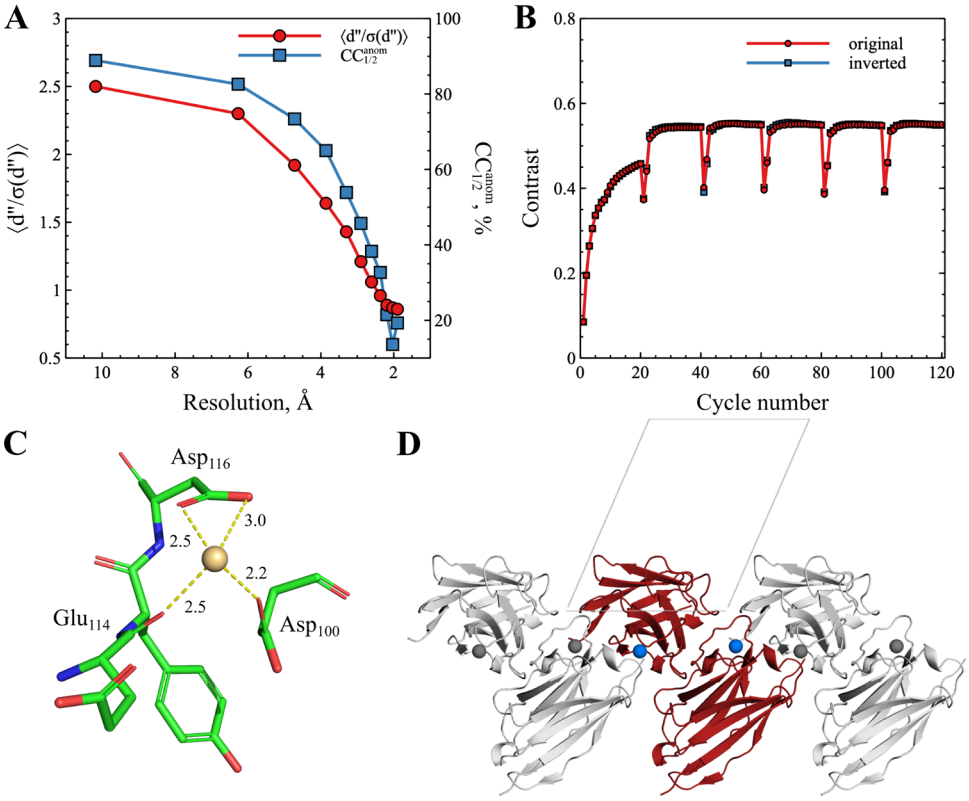

Figure 1. Cadmium SAD phasing of the dataset collected at CuKα wavelength. The crystal of the single-domain antibody BCD090-M2 with cadmium ions was used as an experimental model for in-house Cd-SAD. (**A**) Strength of the anomalous signal represented by ⟨d″/σ(d″)⟩ and $CC_{1/2}$ as a function of resolution. (**B**) Electron density modification in SHELXE as monitored by an increase in map contrast; solutions with original and inverted anomalous substructure give indistinguishable contrast due to centrosymmetry. (**C**) Cadmium ion binding site. (**D**) Schematic representation of the crystal unit cell.

Table 1. Cadmium SAD phasing and model building results. Values in parentheses are for the highest resolution shell.

Parameter	Value
Dataset statistics (6F0D)	
Space group	P1
Unit cell: a b c (Å) α β γ (°)	35.77 41.53 46.49 89.99 67.92 76.06
Resolution range, Å	32.34–1.90 (1.94–1.90)
Reflections: total / unique	246903 (6236) / 18859 (1265)
Completeness (all), %	100.0 (100.0)
Completeness (anom.), %	96.4 (89.9)
Multiplicity	13.1 (4.9)
Multiplicity (anom.)	5.9 (2.4)
Mean I/σ(I)	14.9 (2.2)
SHELXD	
Resolution range, Å	32.34–2.4
CFOM	55.61
CC_{all}/CC_{weak}	32.40 / 23.21
No. of sites	2/2
SHELXE	
No. of residues built	222 / 256 (87%)
CC	43.57
phenix.autobuild	
No. of residues built	245 / 256 (96%)
CC	0.80
Refinement	
No. of residues built	256/256 (100%)
CC	0.90
R_{work} / R_{free}, %	17.8 / 21.0

CFOM, combined figure of merit; CC, correlation coefficient.

anomalous data, and the small number of high-occupancy cadmium sites. Furthermore, the recent theoretical study gives the following simple dependency for expected anomalous signal $\langle S_{ano} \rangle \sim (N_{refl}/n_{sites})^{1/2}$, where N_{refl} is the number of independent reflections and n_{sites} is the number of anomalous scatterers[15]. Our case with maximum N_{refl} due to the lowest symmetry (P1) and only 2 anomalous sites appears virtually optimal for SAD. The high metal-binding affinity of cadmium sites was achieved through coordination with carbonyl oxygen of Glu_{114}, and carboxylic groups of Asp_{100} and Asp_{116} (Figure 1C). By bridging these residues to the N-terminal Gly residue of the neighboring molecule, cadmium ions effectively defined crystal contacts (Figure 1D). Data associated with this study are available on OSF[16].

Conclusion

In conclusion, we suggest that cadmium SAD can be generally applied for the phasing of protein crystals collected in-house using CuKα radiation. We see the following advantages of this approach: (1) cadmium has a great anomalous signal (f''=4.7e^- at CuKα); (2) cadmium ions frequently promote crystal growth and can substitute other divalent cations; (3) cadmium binding sites are complementary to that of iodine, another strong anomalous scatterer, and therefore Cd-SAD can be useful in cases where I-SAD does not work.

Data availability

Data for this study, including unmerged experimental intensities, structure factors and final atomic coordinates after refinement, are available on OSF. DOI: https://doi.org/10.17605/OSF.IO/KYH6D[16].

Grant information

This work is funded by the Ministry of Science and Higher Education of the Russian Federation (contract 14.577.21.0217, unique identifier RFMEFI57716X0217) and co-funded by CJSC Biocad.

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http://www.doi.org/10.17605/OSF.IO/KYH6D

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Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 01 April 2019

https://doi.org/10.5256/f1000research.19346.r45676

✔ **Igor Melnikov**
European Synchrotron Radiation Facility (ESRF), Grenoble, France

In this experiment report the authors present their work on derivatising crystals of a single-domain antibody protein with cadmium ions and subsequent solving the structure via SAD. They demonstrate that home-lab X-ray crystallographic equipment is capable of solving crystal structures via SAD at CuKalpha X-ray energy. The authors then conclude by discussing the result and suggesting the method for use in home-lab source diffraction experiments. To sum up, I find the study to be clearly presented and well prepared.

Additional notes:

- To my point of view, the data were collected competently to reach higher multiplicity (which is indispensable for SAD phasing) for P1 space group by exploiting kappa goniometer.
- The workflow for structure solution via Cd-SAD presented in this article seems to be relatively straightforward even considering the problem with centrosymmetric Cd sites (easily resolved by chain tracing).
- "The phasing of protein crystals by SAD starts from finding the positions of an anomalous substructure, which is usually done by direct methods." - In fact, the process involves Patterson search coupled with direct methods-based calculations[1] namely dual-space refinement.
- I might suggest adding a few words on how the authors introduced cadmium ions into the crystal (I see that it was co-crystallisation as reported in their previous article).

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Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Macromolecular crystallography, structural biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 18 February 2019

https://doi.org/10.5256/f1000research.19346.r43346

Thomas E. Edwards
Bainbridge Island, Bainbridge Island, WA, 98110, USA

Eliseev et al. demonstrate the ability to use cadmium divalent cations, X-ray data collected on an in house X-ray source (Cu Kalpha), and single wavelength anomalous dispersion (SAD) phasing to determine the structure of a protein, in this case the anti-ErbB3 single domain antibody (sdAb). They originally solved the structure by molecular replacement (MR) and reported it in a previous publication, but here they demonstrate the applicability of Cd-SAD to this data set to determine the structure. By our examination of the literature, at least two other structures have been solved by Cd-SAD but both of these structures were solved using data collected at a synchrotron source at a longer wavelength (PDB IDs 2X7K and 5AM6). This particular application provides an excellent alternative method to others presented in the literature and referenced in this paper.
A couple of notes and comments:

1. The authors may help the reader understand the number of electron possible for phasing by referring the reader to the UW X-ray anomalous scattering web page (http://skuld.bmsc.washington.edu/scatter/) designed by Ethan Merritt.
2. The authors do an excellent job of data collection on their in house instrument by using the

kappa goniometer to increase completeness and enhance the anomalous signal.
3. The authors should also site a second paper by Terwilliger et al. from the same issue as reference 15 but with pages 359-374[1] which provides additional information on experimental design and execution.
4. We performed an overnight soak at 5 mM CdCl2, collected a high resolution data set in house on a Cu Kalpha source, and obtained sufficient anomalous signal to solve the structure by Cd-SAD. This provided us with good confidence that this method should be generally applicable.

Overall, the current paper by Eliseev provides a good alternative approach to experimental structure determination, complementing previously reported techniques.

References

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Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: X-ray crystallography, small molecular and antibody therapeutic development, de novo phasing.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.