

IN-HOUSE MEASUREMENT OF THE ANOMALOUS SIGNAL OF SULFUR AND ITS USE FOR PHASING

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Recent developments in X-ray instrumentation, like high intensity sources, cryo devices and new detectors now make it possible to measure reflection intensities precisely enough so that even the anomalous signal of weak scatterers such as sulfur, present in most proteins, can be employed for phase determination. This eliminates the need to prepare selenomethionine or other derivatives. The anomalous signal of sulfur at Cu K α wavelength is 0.5 electrons, which, although on the verge of measurability, under optimal conditions can be successfully exploited to give sufficient phase information. In-house sources, especially if equipped with a goniometer (rather than a single rotation axis) enabling a much higher redundancy to be obtained, even have advantages over synchrotron sources for phasing: it is possible to measure for a longer time without significant radiation damage. For phasing purposes the highest possible resolution is not required, often a high-resolution synchrotron dataset at shorter wavelength may be used for phase extension and structure refinement. Using the knowledge accumulated from several test cases, the key steps in data acquisition will be presented and analyzed from the point of view of success in subsequent phasing. Selected examples will illustrate the solution of test and unknown structures using SHELXD and SHELXE. In favorable cases i.e. very high resolution or high solvent content, SAD phasing based on the anomalous scattering of sulfur alone can give very high quality maps.

Keywords: SAD PHASING ANOMALOUS SCATTERING SHELX

A NEW CLASS OF LANTHANIDE COMPLEXES TO OBTAIN HIGH PHASING-POWER HEAVY-ATOM DERIVATIVES FOR HIGH-THROUGHPUT MACROMOLECULAR CRYSTALLOGRAPHY

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The current emphasis on high-throughput crystallography leads to develop heavy-atom preparation methods that are more reliable and less disruptive than traditional heavy-atom soaking. Seven tested gadolinium complexes are found to be excellent candidates to obtain heavy-atom derivatives in macromolecular crystallography. These highly soluble lanthanide complexes can be easily introduced at high concentration (100 mM or higher) in protein crystals either by soaking or by co-crystallisation, without changing significantly the crystallisation conditions, as was already demonstrated for gd-hpd3a derivative crystals of hen egg-white lysozyme [girard et al., acta cryst. (2002), d58, 1-9]. These complexes allow to revisit the preparation of heavy-atom derivatives and thus, combined to the single-wavelength anomalous dispersion (sad) method, are of special interest for high-throughput macromolecular crystallography.

Using this new class of heavy-atom derivative crystals, de novo phasing by the SAD method has been carried out on several proteins of known structures as well as of unknown structures. Diffraction data have been collected either with a laboratory source making use of the high anomalous signal ($f' = 12 e^-$) of gadolinium for CuK α radiation, or using synchrotron radiation at the white line in the gadolinium L_{III} absorption edge ($\lambda = 1.711 \text{ \AA}$, $f' = 28 e^-$). Using Gd-HPDO3A, one of these gadolinium complexes, we have determined the structure of a chimeric form of OTCase from *P. aeruginosa* and *E. Coli*, which is a dodecameric protein of 450 kDa. This is to our knowledge the largest protein structure determined by the SAD method.

Keywords: LANTHANIDE COMPLEXES MACROMOLECULAR CRYSTALLOGRAPHY SAD

EXTRACTING WEAK SAS SIGNAL BY USING 3D EXPERIMENTAL ERROR CORRECTION MODELS WITH FREE-R TYPE TESTS

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SAS (single-wavelength anomalous scattering) phasing has proved to be a useful method for solving macromolecular structures. Clean and high quality anomalous signal is the key for success of this method especially when using the weak anomalous signal from light atoms such as phosphorus, sulfur, and chlorine. In x-ray diffraction experiments, reflection intensities are not only determined by the crystal itself, but also affected by many other non-intrinsic factors. For macromolecular crystals, these could include absorption, radiation damage, x-ray beam stability, detector defects and other systematic errors. In practice, most of the factors can only be corrected through computer-aided data processing during scaling. Thus, the correction model used in scaling is critical for retrieving the weak anomalous signal from these light atoms. In this study, an sas data set recorded from Zn-free insulin crystal is scaled using different error correction models. Each scaled data set is then used to derive the sulfur partial structure from the anomalous signal. The results show that the 3-d error-correction model (Fu et al., 2000) yields a better anomalous signal than other models that are currently employed in widely-used computer programs for scaling.

Reference:

Fu, Z.-Q. et al: Program and Abstracts, ACA Annual Meeting, St. Paul, MN, USA, July 21-28 (2000), P066.

Keywords: ERROR CORRECTION, DATA PROCESSING, SAS PHASING,

DATA COLLECTION STRATEGIES FOR FAST STRUCTURE DETERMINATION

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Multi-wavelength Anomalous Dispersion (MAD) phasing is a widely used and successful method to solve macromolecular structures. MAD data collection on at least three different wavelengths, optimizing both anomalous (Bijvoet) and dispersive differences, is the approach taken in a majority of experiments. However, it has been shown that it is also possible to solve structures using only two wavelengths (1) or even just one (2).

In an effort to investigate how much it is possible to reduce the experiment time and exposure to the sample, several MAD data sets have been collected from a variety of crystal samples. Then, the minimum requirements for MAD or SAD (Single-wavelength Anomalous Dispersion) phasing in terms of data completeness, redundancy and number of wavelengths were determined for each case.

The results from these experiments show that, paradoxically, single wavelength experiments may require more data and total exposure time than two-wavelength MAD ones, because in the cases studied two-wavelength-MAD phasing consistently required less redundancy and completeness of the data. The results also suggest that, once enough data to solve the structure are collected, the quality of the maps and phases after density modification does not significantly improve by using more data. This implies that two-wavelength MAD might be the preferred data collection method for high throughput projects and when radiation damage needs to be minimized.

References

1. González, A., Pédelacq, J. D., Solà, M., Gomis-Rùth, F. X., Coll, M., Samama, J. P. & Benini, S. (1999). Acta Cryst. D55, 1449-1458.
2. Dauter, Z., Dauter, M. & Dodson E. (2002). Acta Cryst. D58, 494-506.

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