

JBS Magic Triangle Phasing Kit

Cat. No.	Amount
PK-104	1 Kit

For *in vitro* use only.
Quality guaranteed for 12 months.
Store at room temperature in the dark.

Application

Heavy atom derivatization of biological macromolecules for anomalous and/or isomorphous phasing methods.

Kit contents

- 6 pre-weighted solid aliquots of I3C (33 mg each)
- 6 aliquots of lithium hydroxide solution (60 µl each)

Specifications

Magic Triangle

Name: I3C
Synonym: 5-Amino-2,4,6-triiodoisophthalic acid
Formula: $C_8H_4I_3NO_4$
MW: 558.84 g/mol
Appearance: pale yellow powder
Solubility: ~ 1 M in 2 M LiOH

Solvent

Name: Lithium hydroxide
Formula: LiOH
Conc.: 2 M aqueous solution

Features

The phasing kit **JBS Magic Triangle** has been developed in co-operation with Tobias Beck (tobiasbeck@gmail.com) in the research group of Prof. George M. Sheldrick, Georg August University of Göttingen.

The researchers in the Sheldrick group have successfully demonstrated that I3C can be utilized for heavy-atom derivatization of biological macromolecules. Experimental phases for several

proteins have been derived using single-wavelength anomalous dispersion (SAD) or single isomorphous replacement plus anomalous scattering (SIRAS) methods [1].

The three-dimensional structure of I3C is shown in Figure 1. The three iodine atoms form an equilateral triangle (side length 6.0 Å) which can be readily identified in the anomalous electron density map.

The amino group and the two carboxyl groups of I3C are capable of forming hydrogen bonds to side chains and the main chain of the protein under investigation [1].

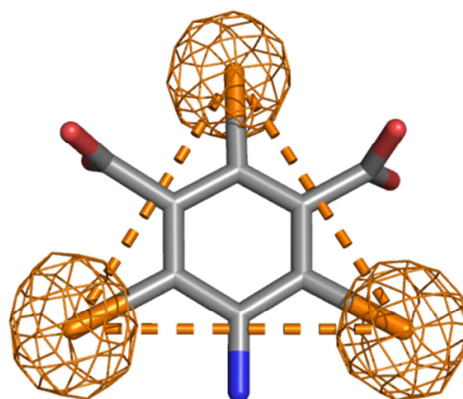


Fig. 1: I3C – The Magic Triangle: 5-amino-2,4,6-triiodoisophthalic acid in thaumatin. Anomalous electron density is shown for iodine atoms at 4σ [1]. The picture is by courtesy of Tobias Beck, Georg August University of Göttingen.

Usage

I3C is incorporated into proteins by:

- Soaking protein crystals with a solution containing I3C
- **or**
- Adding I3C directly to the crystallization drop for co-crystallization.

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Soaking

For soaking experiments, protein crystals are transferred into a stabilizing solution drop containing the crystallization solution together with the heavy atom compound. Make sure that the stabilizing solution contains all the components of the precipitant solution in which the protein was crystallized, otherwise the crystal could start to suffer upon soaking. Concentration of I3C in the final soaking solution and soaking time is dependent on the protein under investigation. However, it is usually recommended to use a high concentration of the heavy atom compound in conjunction with a short soaking time. Concentrations of I3C in soak solutions have been in the range of 40 mM to 500 mM. If crystal degradation like cracking or dissolution occurs, decreasing compound concentration and extending soaking incubation time, along with a gradient soak, may be advised. Crystal cross-linking is also helpful to stabilize crystal lattice when the soaking approach is applied [2].

- (1) Add 50 μ l of the LiOH solution to the pre-weighted solid aliquot of I3C and mix well in order to prepare a 1 M stock solution of I3C.
- (2) Prepare a **stabilizing solution**, oriented on your crystallization condition, with a volume of e.g. 10 ml.

Please Note: Soaking is usually performed in a stabilizing solution, wherein the protein crystal is stable. The concentration of each component from the stabilizing solution has to be determined experimentally. As starting point we recommend the same FINAL concentrations (after addition of the heavy atom compound) as the reservoir solution where crystallization occurred. Hypotonic shocks (when concentration of stabilizing solution is lower than crystallization solution) can lead to irreversible crystal damage/dissolution while mild hypertonic shocks can cause crystal dehydration, often used to improve diffraction data [3, 4].

- (3) Prepare 2 μ l of the soaking solution on a cover slide by mixing the **stabilizing solution (without I3C)** with the **stock solution of I3C** at the desired ratio, e.g. for a 500 mM I3C

soaking solution, mix 1 μ l I3C (1 M) + 1 μ l stabilizing solution. **Remember that in this case your stabilizing solution should be 2x the desired final concentration.**

- (4) Transfer the crystal into the soaking solution using a loop, or a MicroMount™ and place the cover slide on the top of a well containing the original crystallization condition.
- (5) Observe the crystal under a microscope to check for degradation. If no degradation occurs after about 3–5 minutes, proceed with usual crystal mounting for X-ray data collection.

It may be advised to test the soaking conditions with a low quality crystal and if no problems occur then proceed with a similar but high quality crystal.

Co-crystallization

Small molecules such as I3C may also be added to the crystallization drop to allow for incorporation in the crystal lattice during crystallization [1]. For co-crystallization, centrifugation of the I3C solution is recommended. When setting up crystallization drops by mixing a small volume of the reservoir solution with protein solution, I3C is added to the reservoir well before the drops are set up.

- (1) Add 50 μ l of the LiOH solution to the pre-weighted solid aliquot of I3C and mix well in order to prepare a 1 M stock solution of I3C.
- (2) Transfer the I3C stock solution into a small vial and centrifuge this solution.
- (3) Add I3C stock solution to the reservoir well. The final concentration of I3C in the crystallization drop should be about 10 mM (exceeding the protein concentration by at least twofold). For example, for a plate with 500 μ l reservoir wells, pipette 5 μ l I3C into one reservoir well. Add buffer and precipitant stock solutions (according to previous crystallization trials), fill with water to a final volume of 500 μ l and mix well. For the drop, use 1-2 μ l of the reservoir solution and 1-2 μ l protein solution for a 2-4 μ l drop,

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respectively. Seal the plate and set it for crystallization.

The I3C solution can also be added directly to the crystallization drop prior to crystallization. Dilution of the stock solution may then be necessary.

Phasing

The anomalous signal of the iodine atoms is exploited for phasing with either SAD or SIRAS data collection approaches. The signal strength is sufficient even at in-house Cu-K α sources. Heavy atom search can be carried out with any program used for heavy atom location.

The I3C triangle consisting of three iodine atoms is easily identified in the heavy atom substructure. Even at lower resolution, the iodine positions can be resolved since the iodine atoms are 6.0 Å apart. Successful identification of the triangle may point to a correct solution of the heavy atom positions.

Refinement

Restraints for I3C may be downloaded from <http://shelx.uni-ac.gwdg.de/tbeck> (restraints available for REFMAC and SHELX, also a model file in PDB format). These restraints have been derived from the crystal structure of I3C [5].

They may also be generated by the PRODRG server [6].

Safety Information

Although I3C has a lower toxicity than other traditional heavy atom formulations, wear safety glasses, lab coat and gloves when working with I3C. Please also refer to the material safety data sheet.

References

- [1] Beck *et al.* (2008) A magic triangle for experimental phasing of macromolecules. *Acta Cryst.* **D64**:1179.
- [2] Begona *et al.* (2005) Post-crystallization treatments for improving diffraction quality of protein crystals. *Acta Cryst.* **D61**:1173.
- [3] Lopez-Jaramillo *et al.* (2002) Soaking: the effect of osmotic shock on tetragonal lysozyme crystals. *Acta Cryst.* **D58**:209.
- [4] Garman *et al.* (2003) Heavy-atom derivatization. *Acta Cryst.* **D59**:1903.
- [5] Beck *et al.* (2008) 5-Amino-2,4,6 triiodo-isophthalic acid monohydrate. *Acta Cryst.* **E64**:1286.
- [6] <http://davapc1.bioch.dundee.ac.uk/programs/prodrq>

Selected Literature Citations

- Benjdia *et al.* (2012) Structural insights into recognition and repair of UV-DNA damage by Spore Photoproduct Lyase, a radical SAM enzyme. *Nucleic Acids Research* **40**:9308.