User Guide

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, 2].

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].

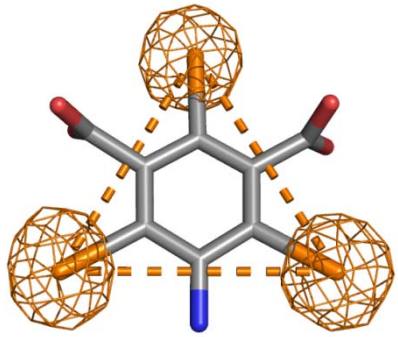

Fig. 1: I3C – The Magic Triangle: 5-amino-2,4,6-triiodoisophthalic acid in thaumatin. Anomalous electron density is shown for iodine atoms at 4σ [1]. The picture is by courtesy of Tobias Beck, Georg August University of Göttingen.

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.

Soaking

For soaking experiments, protein crystals are transferred into a stabilizing solution that contains the heavy atom compound, but also buffer and precipitant, usually at a higher concentration than the crystallization condition. Concentration of I3C in the final soaking solution and soaking time depend 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 occurs, lower concentration and longer soaking times, along with a gradient soak, may be advised [1].

JBS Magic Triangle

Phasing Kit

(1) Add 50 µl 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 composition of the stabilizing solution has to be determined experimentally. The stabilization solution usually has a somewhat higher concentration of precipitant (and buffer) than the original crystallization condition.

(3) Prepare the **soaking solution**. This solution should contain I3C in a concentration of 40 – 500 mM and the same buffer and precipitant concentration as your stabilizing solution. We recommend to start with a concentration of I3C of 500 mM. For example, if your stabilizing solution contains 0.1 M precipitant and 20 mM buffer, fill 5 µl of 1 M precipitant stock solution, 1 µl of 1 M buffer stock solution, 25 µl I3C stock solution (1 M) in a small vial, add 19 µl water and mix well.

(4) Fill one reservoir well of a sitting-drop plate with the **stabilizing solution (without I3C)**, e.g. 500 µl for a regular 24 well sitting-drop plate.

(5) Fill the protein well with the **soaking solution (containing I3C)**, e.g. 5 µl for a regular 24 well sitting-drop plate.

(6) Transfer the crystal into the soaking solution in the protein well using a loop, a MicroMount™ or a pipette. Seal the well during soaking.

(7) Observe the crystal under a microscope to check for degradation. If no degradation occurs after about 3–5 minutes, continue with cryo-protection or data collection (room temperature). If degradation occurs, dilute the soaking solution with your stabilizing solution. Soak again and also try different soaking times.

→ A small aliquot of the I3C stock solution can also be transferred directly into the crystallization drop for soaking [2].

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 µl 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 µl reservoir wells, pipette 10 µl 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 µl and mix well. For the drop, use 2 µl of the reservoir solution and 2 µl protein solution for a 4 µl drop. 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 the single-wavelength anomalous dispersion (SAD) method. The signal strength is sufficient even at in-house Cu-Kα 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.

JBS Magic Triangle

Phasing Kit

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 [3].

They may also be generated by the PRODRG server [4].

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.* D**64**, 1179.

[2] Sippel *et al.* (2008) Structure determination of the cancer-associated *Mycoplasma hyorhinis* protein Mh-p37. *Acta Cryst.* D**64**, 1172.

[3] Beck *et al.* (2008). 5-Amino-2,4,6 triiodo-isophthalic acid monohydrate. *Acta Cryst.* E**64**:1286.

[4] http://davapc1.bioch.dundee.ac.uk/programs/prodrg