I3C Phasing Kit

HAMPTON RESEARCH
Solutions for Crystal Growth

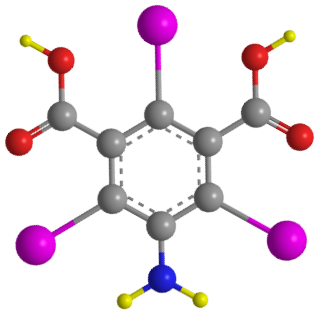
Applications

Produce heavy atom derivative of biological macromolecules for phasing.

Kit Contents

- 12 aliquots of I3C (280 mg each)
- 12 aliquots of 2.0 M Lithium hydroxide (650 µl each)

Figure 1
I3C Structure



Specifications

Property Test	Results
Name	I3C
Synonym	5-Amino-2,4,6-triiodoisophthalic acid
Formula	$C_8H_4I_3NO_4$
M_r	558.84
CAS Number	[35453-19-1]
EC Number	252-575-4
Appearance (Starting Material)	White to Pale Yellow Powder
Solubility	1.0 M in 2.0 M LiOH

Property Test	Results
Name	Lithium hydroxide
Synonym	None
Formula	LiOH
M_r	23.95
CAS Number	[1310-65-2]
EC Number	215-183-4
Appearance (Starting Material)	White Chunks

Storage

- Store at room temperature.
- Protect from light.
- Best if used within 12 months of receipt.

Discussion

I3C (see Figure 1 on left side of page 1) can be used for heavy atom derivatization of biological macromolecules for subsequent single wavelength anomalous dispersion (SAD) or single isomorphous replacement plus anomalous scattering (SIRAS).

The two carboxylic acid groups and one amino group of I3C can interact by way of hydrogen bonds with both the backbone and side chains of proteins. This can result in a relatively high occupancy of the bound ligand I3C. The three iodine atoms per I3C molecule provide for a strong anomalous signal.

The three iodine atoms in I3C form an equilateral triangle with 6.0 Å side lengths. These triangular structures can readily be identified in the anomalous electron density map.

Soaking with I3C

Soaking macromolecular crystals with I3C allows the I3C to diffuse into and through the solvent channels of the crystal and provide I3C access to the macromolecules in the crystal lattice.

1. Add 500 µl of 2.0 M Lithium hydroxide to a single vial of I3C, mix well and then centrifuge to prepare 1.0 M I3C supernatant. Lithium hydroxide is added to I3C to fully deprotonate the I3C carboxyl groups.

Note: I3C has been tested with several counter ions, but lithium seems to give the highest solubility. Lithium salts sometimes have an effect on sample solubility. If your sample doesn't tolerate lithium salts, you can use a lower concentration of I3C with NaOH or KOH instead.

2. Prepare 10 milliliters or more of a stabilizing solution for the crystal. The stabilizing solution is a mixture of sample and crystallization reagent in which the crystal will not dissolve nor continue to grow, but is a solution which will support the stability of the crystal. Some empirical experimentation will be required to determine the reagent composition of the stabilizing solution. The stabilizing solution will be a reagent composition somewhere between that of the reservoir used to obtain the crystal and that of the drop at the initial mixing stage. A solution closely approaching that of the drop from which the crystal is removed is a good starting point for the stabilizing solution.

3. The soaking solution is a mixture of the I3C reagent and the stabilizing solution. Begin with a soaking solution that contains 0.5 M I3C in the presence of stabilizing solution.

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Example: Let's say it was determined that 0.1 M Buffer, 2.0 M Salt was a suitable stabilization solution and that we have a 1.0 M Buffer stock and a 4.0 M Salt stock. To prepare 1.0 ml the soaking solution one would combine the following:

- 500 µl 1.0 M I3C
- 100 µl 1.0 M Buffer
- 200 µl 4.0 M Salt
- 200 µl Deionized water

4. Pipette an appropriate volume of stabilizing solution (no I3C) into the reagent well of the crystallization plate.

5. Pipette an appropriate drop volume of soaking solution into the drop of the crystallization plate.

6. Transfer the crystal into the drop well containing the soaking solution. A CryoLoop can be used for the transfer. Seal the experiment.

7. After 5 minutes observe the crystal for changes such as cracks or other signs of degradation. If no degradation is present, proceed with data collection at either room or cryogenic temperature. If using cryogenic temperature, pursue cryoprotection methods before data collection. Note: Fragile crystals can be soaked for as little as 10 seconds.

8. If crystal degradation is present following soaking, repeat the soaking procedure using a soaking solution with a more dilute I3C concentration. Appropriate I3C concentrations in the soaking solution are between 0.05 to 0.5 M.

9. In addition to the soaking procedure outlined in steps 1-8, one may also perform soaking by adding the I3C reagent directly to the crystallization drop. Final I3C concentrations in the drop should be between 0.05 - 0.5 M. Try to add less than 25% of the drop volume. Adding too much volume of I3C reagent directly to the drop can dilute the drop, reducing the relative supersaturation of the drop and dissolve the crystal.

Cocrystallization with I3C

I3C can be added to the crystallization reagent to allow for incorporation of I3C into the crystal lattice during crystallization.

1. Add 500 µl of 2.0 M Lithium hydroxide to a single vial of I3C, mix well and then centrifuge to prepare 1.0 M I3C supernatant. Lithium hydroxide is added to I3C to full deprotonate the I3C carboxyl groups.

Note: I3C has been tested with several counter ions, but lithium seems to give the highest solubility. Lithium salts sometimes have an effect on sample solubility. If your sample doesn't tolerate lithium salts, you can use a lower concentration of I3C with NaOH or KOH instead.

2. Add I3C reagent to the crystallization reagent such that the final I3C concentration is 10 mM.

Example: If the crystallization plate reagent well volume is 1 milliliter, add 10 µl of 1.0 M I3C to 990 µl of crystallization to generate a 10 mM I3C concentration.

Note: It is recommended the final I3C concentration in the crystallization reagent exceed the sample concentration by a factor of 2 or more.

Example: If the sample concentration is 20 mM, the I3C concentration in the crystallization reagent should be 40 mM or higher.

3. Add the crystallization reagent containing I3C from the reagent well to the drop well and combine with the sample.

4. Seal the experiment.

Note: As an option, I3C reagent may be added directly to the drop. IC3 does not need to be present in the reagent well. For example, one could generate a drop using 3 µl of sample plus 3 µl of crystallization reagent and 0.5 µl of 0.5 M I3C for a final I3c concentration of approximately 40 mM.

Phasing

The anomalous signal of the iodine atoms in I3C is utilized for phasing with the single-wavelength anomalous dispersion (SAD) method. Data collection utilizing in house or synchrotron sources is appropriate for I3C.

Standard crystallography heavy atom search programs such as SHELXD may be used to search for and identify the I3C heavy atom and the I3C may be visualized as a triangle of three iodine atoms, positioned 6.0 Å apart.

References

1. A magic triangle for experimental phasing of macromolecules. Beck et al. Acta Cryst. D64, 1179, 2008.
2. Structure determination of the cancer-associated Mycoplasma hyorhinis protein Mhp37. Sippel et al. Acta Cryst. D64, 1172, 2008.
3. 5-Amino-2,4,6 triiodo-isophthalic acid monohydrate. Beck et al. Acta Cryst. E64:1286, 2008.

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