

Using Halides for Phasing Macromolecular Structures

HAMPTON
RESEARCH

Solutions for Crystal Growth

Crystal Growth 101

Preliminary research shows that bromide (as well as iodide) can diffuse into protein crystals when soaked with the appropriate solution and can successfully be used for phasing. Halide soaked crystals can be used for MAD, as well as multiple or single isomorphous replacement with anomalous scattering or for single anomalous diffraction. The procedure has been termed "Halide Cryosoaking" by Dauter and Dauter¹. In simplest terms, the procedure involves dipping the crystal for a short period of time into a cryoprotectant solution that contains a significant concentration of halide salt. Although no single recipe will suffice for all proteins since each crystal has a unique crystallization recipe and will require different cryoprotectant cocktails, there are some general suggestions to follow at this time. First, there are currently more successful examples using bromide than iodide. The soak time is approximately 10 to 20 seconds. Longer soaks have not led to more incorporation of halide ions for the examples to date. The concentration range of sodium bromide for soaking is approximately 0.25 to 1 M. Higher concentrations of halide ions may lead to more sites with higher occupancies and increased phasing power.

Factors influencing the success of the procedure include the resolution and quality of the X-ray diffraction data, crystal symmetry, packing density, and pseudo-symmetric arrangements of molecules.

Tips for a successful "Halide Cryosoak" include:

1. Initially, preserve the formulation of the crystallization reagent used to grow the crystal as well as the formulation for a successful cryosoak and then add the halide salt. In other words, leave everything constant and add the halide salt.
2. If the crystallization reagent contains salt, try substituting the halide salt, especially if the salt is sodium chloride.
3. High concentrations of the halide salt can serve as a cryoprotectant without the addition of other traditional cryoprotectants (glycerol, MPD, sucrose).
4. Experiment with soak conditions. Vary the concentration of the original reagents, the concentration of the halide salt, and the soak time.

Examples of successful crystallization reagents optimized for halide cryosoaking

Original Condition

1 M Ammonium sulfate, 5 mM guanidine, 10% glycerol, 0.1 M sodium citrate pH 3.3²

1.4 M Lithium sulfate, 0.1 M Tris pH 7.5³

1 M Sodium chloride, 0.1 M sodium acetate pH 4.7²

50% MPD, 0.1 M Sodium acetate pH 5.4²

12% PEG 4000, 0.1 M citrate, 1 M sodium chloride, 10 mM calcium chloride, pH 6.0²

10% ammonium sulfate, 0.1 M Tris HCl pH 7.4²

Halide Cryosoak Condition

1 M Ammonium sulfate, 5 mM guanidine, 5 mM guanidine, 18% glycerol, 0.1 M sodium citrate pH 3.3², 1 M sodium bromide³

1.2 M Lithium sulfate, 0.1 M Tris pH 7.5, 1 M sodium bromide, 14% glycerol³

0.1 M Sodium acetate pH 4.7, 1 M sodium bromide, 30% glycerol³

50% MPD, 0.1 M Sodium acetate pH 5.4, 1 M sodium bromide²

12% PEG 4000, 10 mM citrate, 10 mM citrate, 10 mM calcium chloride, 25% glycerol, 1 M sodium bromide²

10% Ammonium sulfate, 0.1 M Tris HCl pH 7.4, 25% glycerol, 1 M sodium bromide²

References

1. Entering a new phase: Using solvent halide ions in protein structure determination. Dauter, Z. & Dauter, M., Structure, Vol 9, R21-26, Feb 2001.
2. Novel approach to phasing proteins: derivatization by short cryo-soaking with halides. Dauter, Z., Dauter, M., & Rajashankar, K.R. Acta Cryst. (2000) D56, 232-237.
3. Practical experience with the use of halides for phasing macromolecular structures: a powerful tool for structural genomics. Dauter, Z., Li, M. and Wlodawer, A.

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