Halides for Phasing

HAMPTON RESEARCH
Solutions for Crystal Growth

Bromide and iodide can diffuse into protein crystals when soaked with the appropriate solution and can successfully be used for phasing. Bromide soaked crystals can be used for multiwavelength anomalous diffraction (MAD), or single-wavelength anomalous diffraction (SAD). Iodides can be used for SAD or multiple isomorphous replacement (MIRAS). The procedure has been termed "Halide Cryosoaking" by Dauter and Dauter.[1] 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. First, there are currently more successful examples using bromide than iodide. The soak time is approximately 10 to 20 seconds. Longer soak times sometimes degraded crystal diffraction or led to crystalline phase transition, but other times extended the resolution limit of the diffraction. 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	Halide Cryosoak Condition
1.0 M Ammonium sulfate, 5 mM Guanidine, 10% Glycerol, 0.1 M Sodium citrate pH 3.32[3]	1.0 M Ammonium sulfate, 5 mM guanidine, 18% Glycerol, 0.1 M Sodium citrate pH 3.32, 1.0 M Sodium bromide[3]
1.4 M Lithium sulfate, 0.1 M Tris pH 7.5[3]	1.2 M Lithium sulfate, 0.1 M Tris pH 7.5, 1.0 M Sodium bromide, 14% glycerol[3]
1.0 M Sodium chloride, 0.1 M Sodium acetate pH 4.7[2]	0.1 M Sodium acetate pH 4.7, 1.0 M Sodium bromide, 30% glycerol[2]
50% MPD, 0.1 M Sodium acetate pH 5.4[2]	50% MPD, 0.1 M Sodium acetate pH 5.4, 1.0 M Sodium bromide[2]
12% PEG 4,000, 0.1 M Citrate, 1.0 M Sodium chloride, 10 mM Calcium chloride, pH 6.0[2]	12% PEG 4,000, 10 mM Citrate, 10 mM Calcium chloride, 25% Glycerol, 1 M sodium bromide[2]
10% Ammonium sulfate, 0.1 M TRIS hydrochloride pH 7.4[2]	10% Ammonium sulfate, 0.1 M TRIS hydrochloride pH 7.4, 25% Glycerol, 1.0 M Sodium bromide[2]

References

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