

24. CRYSTALLOGRAPHIC DATABASES

24.4.4. BMCD implementation – web interface

The BMCD is a web-accessible resource available to the crystallographic community through the website at <http://www.bmcd.nist.gov:8080/bmcd/bmcd.html>. The current version of the BMCD includes 3547 crystal entries from 2526 biological macromolecules. The web interface provides an easy mechanism for browsing through the data contained in the BMCD. The user can examine the complete list of macromolecule names and tabulations of the number of macromolecules and crystal forms for each source, prosthetic group, space group, chemical addition and crystallization method. In addition, the listing of complete references is available along with a set of general references concerning all aspects of crystal growth.

The web interface offers a number of ways to query the database. For example, the results of precomputed queries are available through the tabulations of chemical additives, space groups, crystallization methods and prosthetic groups mentioned above. The tables provide links to lists of macromolecules and crystal forms that match the database query for these parameters. Allowing the user to enter specific parameter values that must be matched provides another method of searching the database. Examples of this include querying for macromolecules based on their molecular weight. A user may also search for crystal forms of macromolecules that crystallize at a particular temperature, macromolecule concentration and pH. A range of values or a single value of one or all of the parameters may be used to limit the search. For references, queries for specific authors, keywords and journal information are allowed.

24.4.5. Reproducing published crystallization procedures

The BMCD contains the information needed to reproduce the crystallization conditions for a biological macromolecule reported in the literature. This is an activity performed by many laboratories engaged in protein engineering, rational drug design, protein stability and other studies of proteins whose structures have been previously determined. The crystallization of sequence variants, chemically modified derivatives, or ligand–biological macromolecule complexes can sometimes be considered problems that fit into this category. Usually, the reported crystallization conditions of the native macromolecule are the starting points for initiating the crystallization trials. The crystallization of the biological macromolecule may be simple to reproduce, but differences in the isolation and purification procedures, reagents, and crystallization

methodology of different laboratories can dramatically influence the results. The crystallization conditions in the database should be considered a good starting point for the search or optimization that will require experiments that vary pH, macromolecule and reagent concentrations, and temperature, along with the crystallization method.

The crystals for one of the isozymes of glutathione S-transferase of rat liver grown from conditions reported in the literature (Sesay *et al.*, 1987) are used to illustrate these points. The original crystallization conditions were for an enzyme isolated from rat liver (entries MOP3 and C13R). However, the enzyme used in the crystallization trials was cloned and expressed in *Escherichia coli*. The crystals of the natural enzyme were grown in 3 to 5 days from vapour-diffusion experiments at 4 °C, with droplets containing a protein concentration of 11.3 mg ml⁻¹, 0.46% β-octylglucoside, 30–37% saturated ammonium sulfate and 0.1 M phosphate buffer, pH 6.9 equilibrated against well solutions containing 60–74% ammonium sulfate.

The recombinant enzyme required an optimization of these conditions to produce large single crystals (Ji *et al.*, 1994). The recombinant protein crystallized best at 4.0 °C, with droplets containing a protein concentration of 12 mg ml⁻¹, 0.2% β-octylglucoside, 20–25% saturated ammonium sulfate, 1 mM EDTA and 0.025 M TrisHCl, pH 8.0 equilibrated against well solutions containing 40–50% ammonium sulfate. Both crystallization protocols required the presence of 1 mM (9*R*,10*R*)-9-*S*-glutathionyl-10-hydroxy-9,10-dihydrophenanthrene, a product inhibitor. The recombinant enzyme, β-octylglucoside and ammonium concentrations were adjusted. The pH was varied, with the largest crystals being found at pH 8.0. Thus, TrisHCl was substituted for the phosphate buffer. EDTA was also included as an additive; its

Macromolecule -- subtilisin BPN' : prodomain (M1MT)

Molecule Name : subtilisin BPN' : prodomain

Alias Names :

Genus: Bacillus

Species: amyliloquefaciens

Common Name: recombinant

EC Number : 3.4.21.14

Catalytic Reaction : hydrolysis of proteins and peptide amides

Total Molecular Weight: 36500 Total No. Subunits: 2

1. Subunit Molecular Weight: 28000; Subunit Number: 1
2. Subunit Molecular Weight: 8500; Subunit Number: 1

Prosthetic Group :

Remarks : During biosynthesis of subtilisin, the 77 amino acid prodomain is autocatalytically cleaved.

Total Number of Crystal Entries: 2

1. Crystal C2CJ: A=71.9, B=96.7, C=48.9, alpha=90, beta=90, gamma=90
2. Crystal C2CK: A=74.1, B=77.85, C=57.65, alpha=90, beta=90, gamma=90

Fig. 24.4.3.1. A representative example of a biological macromolecule, subtilisin BPN' : prodomain, entry M1MT in the BMCD.

Crystallization Data for Molecule - subtilisin BPN' : prodomain

Crystal Data (C2CK).

Molecular Name: subtilisin BPN' : prodomain.

Unit cell dimension						
A	B	C	Alpha	beta	gamma	Z
74.1	77.85	57.65	90	90	90	4

Space group:

P2<1>2<1>2 - Orthorhombic

Crystal Density: 0

Diffraction limit (Angstroms): 2

Diffraction life time (Hours): 0

Crystal dimensions in mm: 0.1 x 0.5 x 0.5

Crystallization Data.

Method(s) used for the crystallization: vapor diffusion in hanging drops

Macromolecule concentration range	0	0	mg/ml
Temperature range	0	0	° C
pH value	4.6	0	
Crystal growth time	0	0	Day

Chemical additions in the reservoir:

Chemical Additive	Range	Unit
ammonium sulfate	2	0 M
Sodium acetate	0.1	0 M

Note: In the above tables, 0 indicates that no data is available.

Reference for Crystal - C2CK.

Reference (R2NE).

Reference Title: "The prosegment-subtilisin BPN' complex: crystal structure of a specific 'foldase'."

Author Names: Gallagher, T; Gilliland, GL; Wang, L; Bryan, P;

Reference Source: Structure 3, 907- 914, 1995.

Reference also for: Crystal C2CJ |

Other Information Related to the Crystal.

Protein Data Bank: 1SPB

NDB:

Fig. 24.4.3.2. A representative example of a crystal entry C2CK for the subtilisin BPN' : prodomain, entry M1MT in the BMCD.