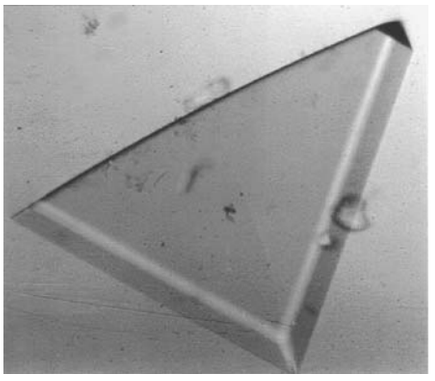

Fig. 24.4.5.1. Crystal of recombinant rat liver glutathione *S*-transferase (Ji *et al.*, 1994) grown from optimized conditions based on the data in BMCD entries M0P3 and C13R.

absence or presence did not affect the crystallization. Crystals of the recombinant enzyme grew within 5 to 10 days (Fig. 24.4.5.1).

24.4.6. Crystallization screens

With the introduction of the fast screen by Jancarik & Kim (1991), almost all attempts to crystallize a protein begin with experiments based on a screen of one form or another. The first screen was based on the ideas put forth by Carter & Carter (1979) in their discussion of the use of incomplete factorial experiments to limit the search, the experience of the investigators themselves, and the experience of others. A number of screens have been developed and even commercialized (*e.g.* Cudney *et al.*, 1994). The first screens were quite general and applicable to a wide range of biological macromolecules, but fast screens based on specific classes of molecules such as RNA soon developed (Scott *et al.*, 1995).

The BMCD is an ideal tool for facilitating the development of screens for general or specific classes of macromolecules. For example, if it were desired to produce a screen for endonucleases, a quick search of the PDB would provide the information in Table 24.4.6.1. An examination of the crystallization conditions of the endonucleases reveals that crystals are grown using a protein concentration ranging from 2.5 to 12.9 mg ml^{-1}, ammonium sulfate, sodium phosphate, or polyethylene glycol 400 to 8000 as precipitants at 4 to 20 °C between pH 4.5 and 8.3. A variety of buffers and standard biochemical additives are also used. From an examination of these parameters, a small subset of the crystallization experiments comprising an endonuclease screen could be developed.

24.4.7. A general crystallization procedure

The use of the BMCD has been incorporated into more general procedures required for the crystallization of a biological macromolecule that has never been crystallized (Gilliland, 1988; Gilliland & Bickham, 1990; Gilliland *et al.*, 1994, 1996). One such general procedure is shown in Fig. 24.4.7.1. The example below illustrates how the data in the BMCD were used to develop this general procedure for soluble proteins. The BMCD can be used to develop analogous procedures for other classes of biological macromolecules. Briefly, in this procedure the purified biological macromolecule is concentrated (if possible) to 10 to 25 mg ml^{-1} and dialysed into 0.005 to 0.025 *M* buffer at a neutral pH or at a pH required to maintain solubility of the biopolymer. Other stabilizing agents such as EDTA and/or dithiothreitol may be included at low concentrations to stabilize the biological macromolecule during the crystallization trials.

Once the protein has been prepared, commercial or customized fast screens are carried out using vapour-diffusion experiments. If crystals are obtained, X-ray diffraction studies are initiated, but frequently small or poor-quality crystals are observed. Experiments that systematically vary the crystallization parameters (pH, ionic strength, temperature *etc.*) are then carried out. Micro- or macroseeding may also be required to optimize crystal growth (McPherson, 1982, 1999).

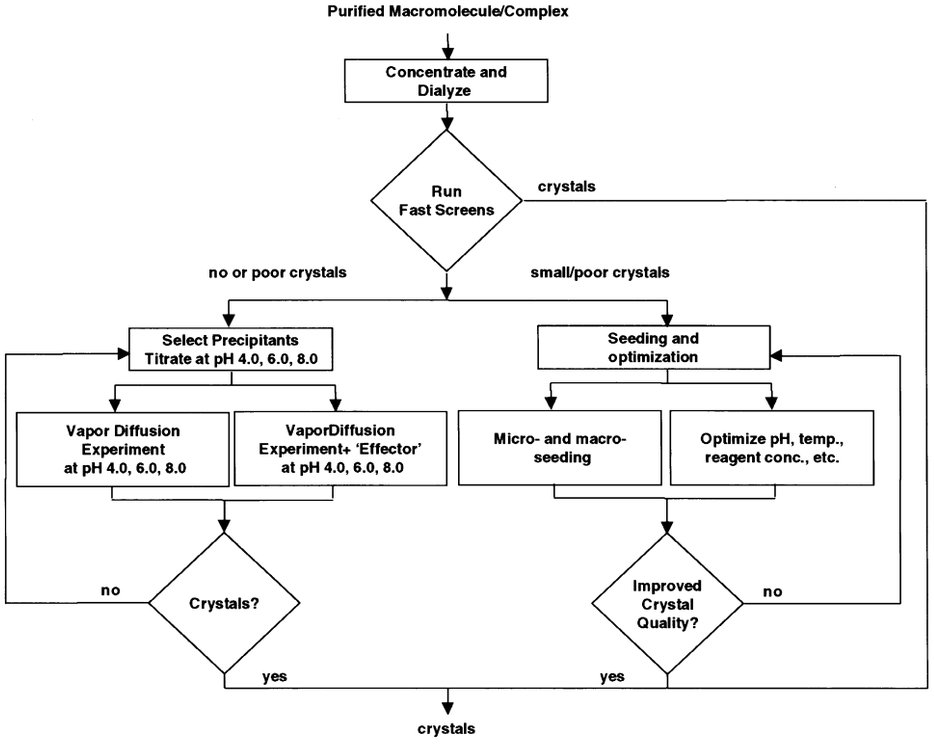

Fig. 24.4.7.1. A general crystallization strategy based on the data contained in the BMCD.

If the fast screens produce no crystals, a more systematic approach can be undertaken that is based on the data contained in the BMCD. An analysis of the BMCD data reveals that out of the large number of reagents used as precipitating agents, a small set accounts for the majority of the crystals observed. The pH range for all crystals is quite large, but most proteins crystallize between pH 3.0 and 9.0. Even though temperature can be an important factor, crystallization experiments are usually set up at room (~20 °C) or cold-room (6 °C) temperatures. Protein concentration varies quite markedly, but it appears that investigators typically use >10 mg ml^{-1}.

After examining the data in the BMCD, the precipitating agents, ammonium sulfate, polyethylene glycol 8000, 2-methyl-2,4-pentanediol and sodium–potassium phosphate might be selected for the initial crystallization attempts, and experiments might be restricted to a pH range of 3.0 to

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