electronic reprint

Journal of
Applied Crystallography
ISSN 0021-8898

A simple technique to control macromolecular crystal nucleation efficiently using a standard vapour-diffusion setup

Luca Jovine

Journal of
Applied Crystallography
ISSN 0021-8898

Received 3 March 2000
Accepted 4 April 2000

A simple technique to control macromolecular crystal nucleation efficiently using a standard vapour-diffusion setup

Luca Jovine†

Structural Studies Division, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England. Correspondence e-mail: jovinl02@doc.mssm.edu

Excessive nucleation often leads to a large number of small macromolecular crystals that are not useful for X-ray diffraction analysis. Crystals with dimensions suitable for data collection can be reproducibly obtained by releasing, for a discrete amount of time, the vapour pressure in both hanging- and sitting-drop experiments, set up at lower precipitant concentrations than those required for crystallization.

1. Introduction

Although use of synchrotron radiation can allow diffraction data collection from even very small crystals (Perrakis *et al.*, 1999), in-house X-ray experiments still depend on the availability of macromolecular crystals of reasonably large dimensions. Indeed, converting showers of microcrystals into a few large specimens can be in many cases a serious obstacle to successful crystallographic structure determination (McPherson, 1995). Moreover, excessive crystal nucleation and growth rates often result in poor diffraction quality, which cannot be overcome by using a more powerful X-ray source.

Several different approaches to the solution of this problem have been described. Further purification and/or recrystallization can yield larger and better quality crystals if the macromolecule to be crystallized is heterogeneous (Lorber & Giegé, 1999; Giegé *et al.*, 1994). On the other hand, when nucleation kinetics cause the problem, possible solutions are: addition of small amounts of co-solvents, such as glycerol, to the crystallization solutions (Scott *et al.*, 1995; C. Oubridge, personal communication); growth of the crystals in a gel matrix (Robert *et al.*, 1999); separation of the nucleation and growth stages by seeding (Stura, 1999) or by shifting the temperature at which crystallization trials are exposed (Blow *et al.*, 1994); the use of a fine sampling of pH limits (McPherson, 1995). More recently, the use of oil layers over the reservoir of vapour-diffusion experiments was shown to be effective in reducing the number of crystals while increasing their size (Chayen, 1997, 1998).

Serious problems caused by excessive nucleation were encountered during crystallization of a 43-nucleotide RNA construct (43 RNA) encompassing the conserved domain IV of *E. coli* 4.5S RNA (Jovine *et al.*, 2000). The RNA crystallized within a few hours in the presence of relatively high concentrations of Mg^{2+} ions (120–170 m*M*), with seemingly identical sitting drops set up in parallel, giving a random number of crystals ranging from 0 to >200. Because of the very low reproducibility of the experiments, large crystals could only rarely be obtained, essentially precluding the solution of the structure. Since application of the techniques mentioned above was unsuccessful (Jovine, 1998), a new method to control nucleation was devised in order to obtain reproducibly large crystals suitable for X-ray diffraction studies.

2. Experimental procedure

The protocol takes advantage of the vapour diffusion setup and of the sensitivity of crystal nucleation to even millimolar differences of precipitant concentration.

The first step of the technique consists of setting up crystallizations at precipitant concentrations below the minimum required for nucleation, so that, after complete equilibration, no crystals are observed in the drops. In the case of the 43 RNA crystals, a 10 m*M* decrease in magnesium concentration from that experimentally determined to be necessary for nucleation of a given batch of material was applied.

In the second step, nucleation is started by release of vapour diffusion pressure. This is achieved by opening the cover slide over each crystallization experiment for a precise amount of time, empirically determined as 1 min 40 s to 2 min in the case of the 43 RNA crystals. This increases the evaporation of water from the drop and leads to a minor and temporary increase of precipitant concentration, usually just enough to promote a single nucleation event before the system returns to equilibrium, in which state no additional nuclei can be formed. If no nucleation is observed within a reasonable period of time, the procedure can be repeated until crystals start to appear.

3. Conclusions

Using this method, 43 RNA crystals of approximate dimensions 1.20 × 0.25 × 0.07 mm, diffracting beyond 3.0 Å resolution, can be grown routinely. The procedure is simple and requires little 'hands-on' time; furthermore, it has the advantage that it can be iteratively repeated until crystals are obtained in all drops of a crystallization tray. It is expected that it could be most useful in other cases in which nucleation is highly affected by very small differences in precipitant concentration and crystal growth is fast. Nevertheless, it can also be used to improve less sensitive systems (L. Jovine, unpublished results). Nucleic acid crystals often show very fast nucleation and growth rates (W. G. Scott, personal communication; L. Jovine,

† Present address: Department of Biochemistry and Molecular Biology, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029-6574, USA.

unpublished results); therefore, this method could be particularly useful in the expanding field of large RNA crystallography.

References

Blow, D. M., Chayen, N. E., Lloyd, L. F. & Saridakis, E. (1994). *Protein Sci.* **3**, 1638–1643.

Chayen, N. E. (1997). *Structure*, **5**, 1269–1274.

Chayen, N. E. (1998). *Acta Cryst.* D**54**, 8–15.

Giegé, R., Lorber, B. & Théobald-Dietrich, A. (1994). *Acta Cryst.* D**50**, 339–350.

Jovine, L. (1998). PhD thesis, University of Cambridge, England.

Jovine, L., Hainzl, T., Oubridge, C. & Nagai, K. (2000). *Acta Cryst.* D**56**. In the press.

Lorber, B. & Giegé, R. (1999). *Crystallization of Nucleic Acids and Proteins*, 2nd ed., edited by A. Ducruix & R. Giegé, pp. 17–43. Oxford: IRL Press at Oxford University Press.

McPherson, A. (1995). *J. Appl. Cryst.* **28**, 362–365.

Perrakis, A., Cipriani, F., Castagna, J. C., Claustre, L., Burghammer, M., Riekel, C. & Cusack, S. (1999). *Acta Cryst.* D**55**, 1765–1770.

Robert, M.-C., Vidal, O., Garcia-Ruiz, J.-M. & Otalora, F. (1999). *Crystallization of Nucleic Acids and Proteins*, 2nd ed., edited by A. Ducruix & R. Giegé, pp. 149–175. Oxford: IRL Press at Oxford University Press.

Scott, W. G., Finch, J. T., Grenfell, R., Fogg, J., Smith, T., Gait, M. J. & Klug, A. (1995). *J. Mol. Biol.* **250**, 327–332.

Stura, E. A. (1999). *Crystallization of Nucleic Acids and Proteins*, 2nd ed., edited by A. Ducruix & R. Giegé, pp. 177–208. Oxford: IRL Press at Oxford University Press.