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Predispensed Gradient Matrices – a New Rapid Method of Finding Crystallization Conditions

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Abstract

Predispensed gradient matrices allow the boundary between precipitate and clear solution to be located very rapidly for a particular protein and precipitant. In many cases crystals grow in the trials which were used to identify this boundary. The method involves dispensing a series of between 10 and 72 microbatch trials in which some parameter, such as precipitant concentration, is gradually changed. (Protein is not dispensed at this stage.) Protein is then added to selected trials using a predetermined algorithm, which takes into account the level of precipitation caused by previous additions. Thirteen crystal forms were obtained using the method with eight proteins and eight precipitants. Six forms were prisms or plates with maximum dimensions above 400 μm.

Introduction

Automatic dispensing methods allow the introduction of powerful new routines for both screening and optimization. In particular, the microbatch method (Chayen, Shaw Stewart, Maeder & Blow, 1990; Chayen, Shaw Stewart & Blow, 1992) is automated, uses small amounts of protein and allows easy addition of extra material. Predispensed gradient matrices (PGM) use these features of microbatch for rapid location of the boundary between precipitate and clear solution for a particular protein and precipitant. In many cases crystals grow in the trials used to identify this boundary.

For greatest resolution, trials are carried out in two or more stages, where a coarsely spaced series is followed by successively finer spaced series as illustrated in the phase diagrams of Fig. 1.

The method must be carried out using the microbatch technique, crystallizing under light paraffin oil. The oil prevents evaporation, and facilitates the addition of protein.

Methods

The major features of predispensed gradient matrices are as follows:

(1) A series of trials is dispensed in which some parameter, such as precipitant concentration, is gradually changed. (Protein is not dispensed at this stage.) Each series comprises between 10 and 72 wells.

(2) Protein is added to selected trials using a predetermined algorithm, which takes into account the level of precipitation caused by previous additions. (Protein is, therefore, not wasted on regions far from the precipitation point.)

(3) For greatest resolution, a coarsely spaced series of trials is carried out first, and the result 'fine tuned' by setting up a finer spaced series using the information obtained.

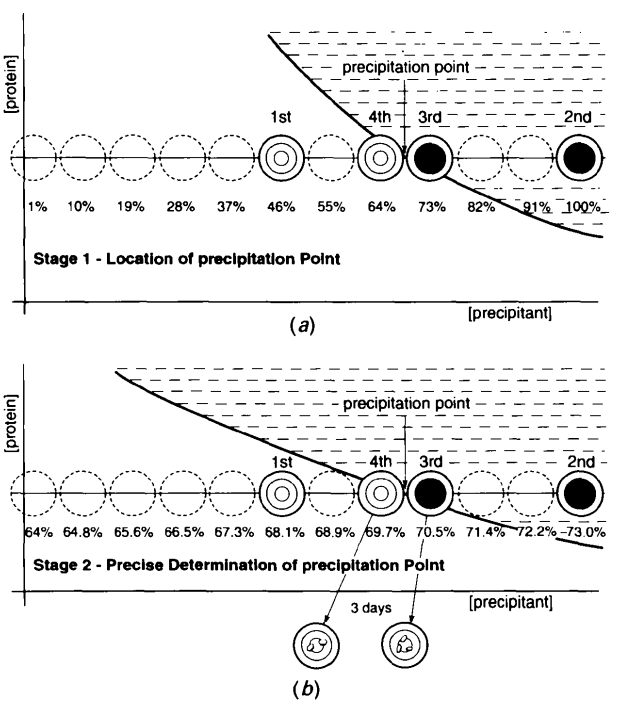

Fig. 1. (*a*) An example of the first stage of the PGM method. A series of trials is dispensed in which some parameter, in this case the concentration of potassium citrate precipitant, is gradually changed, here from 1 to 100%. Protein is not dispensed with the other ingredients. The protein is added by hand to the middle well (marked 1st), using a standard 2 μl micro-pipette. Because no precipitate was formed, protein was now added to the highest trial (2nd). This produced heavy precipitate, so protein was added to the middle intervening well (3rd). This produced light precipitate, and the final addition (4th) located the precipitation point under these conditions to between 64 and 73%. (*b*) In the second stage another series, covering the region between 64 and 73%, was dispensed. By a similar procedure, the precipitation point was located to between 69.7 and 70.5%. Three days later, large crystals formed in the trials lying just above and below the precipitation point.

Generally, several series are dispensed side-by-side on a plate to form a matrix, where each series corresponds to a different precipitant (or protein).

In cases where protein is in short supply, two variations on the method can reduce protein consumption:

(1) Initially 0.25 μl of protein can be added to a trial without stirring. If a heavy precipitate is observed the result is recorded, and no further protein is added to this well.

(2) Instead of performing two rounds of dilution, a gradient covering a whole plate (72 wells) is used. This reduces repetition of trials.

By using these two variations with the microbatch method, where around 1 μl of protein is required per trial, each precipitant was thoroughly screened with around 5 μl of protein - typically 50–200 μg. 2 μl of precipitant solution were dispensed into each well using the automatic protein crystallization system IMPAX (Douglas Instruments, London), and 1 μl of protein was added manually with a micropipette. Trials were carried out in the polystyrene Terazaki tissue-culture plates that are routinely used for microbatch crystallization (15 μl well volume; see Chayen *et al.* 1992).

The optimal algorithm for adding protein to trials has not yet been determined, but the following is currently used:

(1) Add protein to the middle well of a series.

(2) If the well is clear, add protein to the highest concentration trial in the series. If the well gives precipitate, add protein to the lowest concentration trial. (Within two additions it is determined whether or not a series covers the appropriate range.)

(3) Proceed by adding protein to the middle point of the range indicated, carrying out a series of 'binary chops' until the trial that gives very light precipitate is identified.

(4) 'Bracket in' the well identified by adding protein to the two neighbouring wells if this has not already been done.

Results of initial trials

During the initial trials of the method, the following proteins were used: carboxypeptidase G2, GROEL, dehydroquinate synthase, *Clostridium* glucose isomerase, trypsin, lysozyme, concanavalin A and thaumatin. The precipitation point was found for each protein with the following precipitants: ammonium sulfate, jeffamine, lithium chloride, potassium citrate, sodium potassium phosphate, PEG 4000, sodium formate, and two mixtures of these precipitants. Once the method was developed, each protein took under 2 h to test with all ten solutions. The total dispensing time using the automatic dispensing system (Chayen *et al.*, 1990) was 40 h.

All proteins were crystallized except thaumatin and GROEL. At least 13 different crystal forms were obtained. Six forms that were plates or prisms had maximum dimensions above 400 μm.

Discussion - in searching for the precipitation point you are likely to find crystals

It was generally found that the best concentration of precipitant for crystallization was close the precipitation point. Where crystals appeared, approximately half were in the precipitation point well (the lowest well with light precipitate) and half were in the next (lower concentration) well. An exception was found in the case of carboxypeptidase G2 and jeffamine, where the concentration was well below the precipitation point, but crystals were obtained over a wider range of concentrations.

Because a variety of precipitants are used at various concentrations without careful planning, there is a good chance of generating leads for further investigation. In this respect the approach is similar to the sparse-matrix method (Jancarik & Kim, 1991). However, because the PGM method results in a greater density of trials around the precipitation point, there is a good chance of obtaining good quality crystals straight away.

Overall, the PGM method was found to be a very rapid and economic method of obtaining crystals of previously uncrystallized proteins.

References

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