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Screening and Optimization Strategies for Macromolecular Crystal Growth

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Abstract

Today the determination of successful crystallization conditions for a particular macromolecule remains a highly empirical process. Sparse-matrix and grid-screening procedures are rapid and economical means to determine preliminary crystallization conditions. During optimization the variable set (pH, precipitant type and precipitant concentration) utilized in these procedures is screened in an attempt to determine appropriate conditions for the nucleation and growth of single crystals suitable for X-ray diffraction analysis. Unfortunately, in many cases this strategy will not produce single crystals suitable for X-ray diffraction analysis. We have explored, in an empirical sense, other tools for use during optimization. First, a new screening protocol is evaluated which employs less classical precipitating agents. Second, a set of 24 electrostatic crosslinking agents are evaluated for their ability to promote crystallization. Third, a panel of more than 30 detergents are evaluated for their ability to prevent sample aggregation and influence crystal growth.

1. Introduction

In spite of recent innovations and an expanded base of experience, the growth of single crystals of macromolecules generally remains an empirical and frequently tedious process. The variable set over which successful crystallization conditions must be sought is vast while the quantity of macromolecule available may be extremely limited (McPherson, 1990). Nonetheless, the number of macromolecules crystallized during the past decade has increased at a near exponential rate (Gilliland & Bickham, 1990). A major contribution to this increased success has been the development of novel screening protocols and optimization strategies which have provided investigators with an expanded portfolio of effective crystallization tools (Carter & Carter, 1979; Jancarik & Kim, 1991; McPherson, 1992; Samudzi & Fivash, 1992; Stura, Nemerow & Wilson, 1992; Weber, 1990). In addition, the increasing number of investigators engaged in the crystallization of macromolecules has further enhanced the database of successful crystallization conditions and approaches.

The process of crystallizing macromolecules can be divided into three discrete stages. These are (1) screening for useful crystallization conditions or leads; (2) optimization of one or more initial condition to produce single crystals suitable for X-ray diffraction analysis; and (3) reproducible production of single crystals for X-ray data collections. The first stage, locating successful crystallization conditions, is essentially the search of an extensive matrix of parameters to identify an initial combination of variables which produce crystals. In less successful cases they may yield only solubility information which must in turn be used to design subsequent crystallization trials. An effective screening technique often termed 'grid screening' evaluates the variance of pH and precipitant concentration for different kinds of precipitants to promote crystal growth (Weber, 1990; McPherson, 1990). Typically, such a crystallization trial utilizes a four by six grid to screen pH (example 4, 5, 6, 7, 8, 9) *versus* a concentration range of a single precipitant which may be a salt, polymer or organic solvent. Based upon microscopic examination, generally, of vapor-diffusion crystallization trials, a more refined screen can then be set up and searched in the neighborhood of the initial, promising condition. The advantage of the 'grid-screening' technique is the ease and rapidity in creating and interpreting the crystallization trials as a consequence of the limited range of variables. The

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principal disadvantage of this approach is that an exhaustive search of potential crystallization conditions by varying pH *versus* precipitant type and concentration would consume a large amount of macromolecule. An effective compromise to such an exhaustive search is to evaluate ranges of pH, precipitant type and precipitate concentration which have yielded high rates of success in other crystallization investigations. Based primarily upon personal experience and the contents of the NIST/CARB Biological Macromolecule Crystallization Database (BMCD), grid-screening strategies using polyethylene glycol (M_r 400 to 20 000; 6000 being the most frequently used PEG), ammonium sulfate and 2-methyl-2,4-pentanediol (MPD) have proven very successful in providing initial crystallization conditions for a wide variety of proteins (Samudzi & Fivash, 1992; McPherson, 1990; Weber, 1990).

More recently, approaches for expanding the ranges of salts, polymers, organic solvents and pH included in a screen and at the same time conserving macromolecule sample, have appeared (Carter & Carter, 1979; Jancarik & Kim, 1991; McPherson, 1992; Samudzi & Fivash, 1992; Stura *et al.*, 1992; Weber, 1990). These have seen great success in identifying initial crystallization conditions for a wide variety of macromolecules including proteins, viruses, peptides and nucleic acids. These strategies, termed 'incomplete-factorial' and 'sparse-matrix' screens are designed to evaluate a large number of pH and precipitant combinations with a limited amount of macromolecule sample. Here we describe a general approach for developing a novel 'sparse-matrix' screen, specifically that presented in Fig. 1.

2. Development of the screen

A focus of our research is to produce crystals of as many different macromolecules, under as many different conditions, as possible, and then to apply a variety of phyical techniques to delineate the essential characteristics of the growth process. Therefore, in developing this novel 'sparse-matrix' screen our objective was to expand the current set of successful, initial screening conditions by utilizing hanging- or sitting-drop vapor-diffusion crystallization in a single 24-well plate. Another intention was to employ a novel set of precipitants, or combination of precipitants, in order to escape the limitation of the salts and polymeric precipitants one typically uses. In so doing we hoped to uncover new additional leads for further optimization. With a more diverse set of leads in hand, the opportunities for successful optimization or the discovery of different crystal forms might be enhanced.

The determination of a suitable pH range was based upon a review of the NIST/CARB BMCD (Gilliland & Bickham, 1990). We selected a pH range between 4 and 9 reflecting the observation that more than 90% of all macromolecules reported crystallized were within this pH range. The decision to use Good's (Good *et al.*, 1966) series of buffers was based upon our objective of minimizing the occurence of inorganic crystals as phosphate, carbonate and borate salts when combined with ions possibly used during screening and optimization procedures. A comprehensive panel of precipitants (salts, polymers and organic solvents) were selected from the NIST/CARB BMCD and from the literature (McPherson, 1990, 1992; Weber, 1990). For each precipitant a suitable concentration range was selected based again upon a review of the NIST/CARB BMCD and the literature. Weight was given to entries in the NIST/CARB BMCD which had been successful in the crystallization of multiple macromolecules. Combinations of precipitants and pH were then generated based upon a further analysis of the NIST/CARB BMCD but clearly biased by our own personal experience and inclinations.

The initial list of precipitant and pH combinations was comprised of more than 250 unique solutions. Those which closely approximated one another by having very similar precipitant types and concentration, as well as similar pH's were consolidated. Those solutions which resembled or appeared in published screening procedures (McPherson, 1990, 1992; Jancarik & Kim, 1991; Stura *et al.*, 1992) were eliminated. This process pared the list of screening solutions to slightly more than 50. During formulation several solutions were removed from the

1. 30%(*v*/*v*) MPD, 0.5 *M* ammonium sulfate, 0.1 *M* Hepes pH 7.5
2. 5%(*v*/*v*) MPD, 10%(*w*/*v*) PEG 6000, 0.1 *M* Hepes pH 7.5
3. 20%(*v*/*v*) Jeffamine M-600, 0.1 *M* Hepes pH 7.5
4. 2.0 *M* Sodium chloride, 0.1 *M* Na acetate pH 4.5
5. 30%(*v*/*v*) MPD, 0.2 *M* sodium chloride, 0.1 *M* Na acetate pH 4.5
6. 1.6 *M* Magnesium sulfate, 0.1 *M* MES pH 6.5
7. 2.0 *M* Sodium chloride, 0.2 *M* K/Na tartrate, 0.1 *M* MES pH 6.5
8. 2.0 *M* Sodium chloride, 0.2 *M* Na/K phosphate, 0.1 *M* MES pH 6.5
9. 2.0 *M* Ammonium sulfate, 0.2 *M* K/Na tartrate, 0.1 *M* Na citrate pH 5.6
10. 1.0 *M* Lithium sulfate, 0.5 *M* ammonium sulfate, 0.1 *M* Na citrate pH 5.6
11. 30%(*v*/*v*) MPD, 0.2 *M* Na/K phosphate, 0.1 *M* Na citrate pH 5.6
12. 1.6 *M* Ammonium sulfate, 0.1 *M* sodium chloride, 0.1 *M* Hepes pH 7.5
13. 2 *M* Ammonium formate, 0.1 *M* Hepes pH 7.0
14. 12%(*w*/*v*) PEG 20000, 0.1 *M* MES pH 6.0
15. 1.6 *M* Ammonium sulfate, 10% dioxane, 0.1 *M* MES pH 6.0
16. 15%(*v*/*v*) Jeffamine M-600, 0.1 *M* MES pH 6.0
17. 8% Polyethyleneimine, 0.5 *M* sodium chloride, 0.1 *M* Na citrate pH 5.0
18. 40% Ethanol, 0.1 *M* Na citrate pH 5.0
19. 10% Jeffamine M-600, 0.8 *M* ammonium sulfate, 0.1 *M* Na citrate pH 5.0
20. 20% Jeffamine T-403, 0.8 *M* ammonium sulfate, 0.1 *M* Na citrate pH 5.0
21. 20% Ethanol, 0.1 *M* Tris pH 8.0
22. 40%(*w*/*v*) Hexanediol, 0.2 *M* magnesium chloride, 0.1 *M* Tris pH 8.0
23. 25%(*v*/*v*) *tert*-Butanol, 0.1 *M* Tris pH 8.0
24. 2.0 *M* Sodium chloride, 0.2 *M* lithium sulfate, 0.1 *M* Tris pH 8.0

Fig. 1. Novel matrix screening solutions.

screen when it was noted that there was a failure to form stable homogeneous solutions for more than 30 d. The final screen contained 32 unique solutions. Based upon the success of crystallization trials with a library of more than 20 unique macromolecules, the final composition of the screen was reduced to 24. Thus, the screen was conveniently formatted for use in a 24-well plate that consumed approximately 1 mg of macromolecule. This screen was then evaluated against the same library of 20 unique macromolecules which includes two peptides and a virus (Fig. 2). Of the 20 macromolecules screened with the protocol, 14 produced crystals. In most cases crystals were produced under several conditions included in the screen.

We have used this screen to grow crystals which have been crystallized previously, and those which have not. For those macromolecules where previous crystallization conditions have been known, this screen identified new conditions to consider for optimization. In several cases better crystals were obtained than had previously been grown using other screening approaches. In some instances different crystal forms were obtained from some of the proteins investigated as well as differences in the frequency of nuclei, overall morphology and and crystal quality. This screen was not intended to replace the use of the sparse-matrix screen which we commonly use (Jancarik & Kim, 1991), but rather it extends the number of screening solutions which can be applied to macromolecules where the sparse-matrix screen fails. In addition, this screen provides an even greater number of leads from which the best candidates for optimization can be selected.

3. Optimization strategies

Successes in initial screening protocols may range from microcrystals to small single crystals, and include a wide variety of both favorable and unfavorable habits, as well as occasional single crystals suitable for X-ray analysis. In most instances, the next step in producing single crystals is optimization of the preliminary set of crystallization conditions. Although the variables for optimization are extensive (McPherson, 1990) a systematic optimization plan can be formulated. In most cases one can further optimize conditions by incrementing precipitant and/or macromolecule concentration, making slight pH adjustments, or evaluating different temperatures between 277 and 300 K. If these procedures fail then one must look to other variables which influence crystal growth and to the unique characteristics of the macromolecule.

1.	Satellite tobacco mosaic virus	1, 2, 5, 7, 8, 10, 11, 12, 14, 16, 17, 18, 23, 24
2.	Lysozyme	4, 7, 11, 16, 17, 18
3.	Thaumatin	7, 9
4.	Ribonuclease A	9
5.	DPDPE	3, 17, 19, 20
6.	Xanthine oxidase	16
7.	Yeast Phe tRNA	24
8.	Edestin	2, 24
9.	Catalase	2
10.	Leupeptin	9, 19
11.	Leutenizing hormone beta	14
12.	Lactate dehydrogenase	2, 14
13.	Papain	20
14.	Hemoglobin	4

Other macromolecules screened but not crystallized include peanut peroxidase, ribonuclease B, α-amylase, aldolase, leutenizing hormone alpha, and glyceraldehyde-3-phosphate dehydrogenase. All initial macromolecule concentrations were 20 mg ml $^{-1}$.

Fig. 2. Macromolecules crystallized using the novel matrix screen.

3.1. *Electrostatic crosslinkers as additives*

We have evaluated, though by no means exhaustively, two classes of additives which may have value for optimization of crystallization conditions. Here we would like to share our somewhat limited experience with these additives since our results in some cases indicate that these compounds may be useful for increasing the probability of obtaining high-quality crystals.

Ionic and electrostatic interactions between macromolecules certainly play a role in the crystallization of macromolecules. The exterior surfaces of macromolecules expose an array of unique functional groups and these form favorable interactions with other macromolecules as they condense under conditions of supersaturation. We were interested in seeing if manipulation of intermolecular interactions by addition of small charged polymers would alter and perhaps enhance the crystallization of macromolecules. To test our hypothesis we selected a group of low-molecular-weight (<500), short-chained, branched and unbranched polymers with carboxyl and amino functional groups which would be expected to engage in electrostatic interactions with macromolecules through non-covalent bonds. Our initial collection of 24 reagents, which we term electrostatic crosslinking agents (linkers), is illustrated in Fig. 3. This collection of molecules samples varying lengths of dicarboxylic and diamine alkyl compounds with both similar and dissimilar charges at either end of the chain, as well as along the chain in some instances. Stock solutions ranging from 0.01 to 1 *M* for each compound were made in water, titrated to neutrality, and sterile filtered using a 0.2 μm filter. We evaluated the influence of these compounds on macromolecular crystallization using the panel of 20 macromolecules which included a variety of proteins, two peptides and a virus. Sitting-drop vapor-diffusion crystallization trials were performed at room temperature with Cryschem plates

(Charles Supper Company, Natick, MA) or Costar (No. 3424) plates with Micro-Bridges (Crystal Microsystems, England). In the experiments, drops of a macromolecule solution were comprised of 10 μl of macromolecule, 5 μl of an electrostatic crosslinking agent and 5 μl of an appropriate precipitant. Drops were equilibrated against 700 μl of an appropriate reservoir precipitant. Plates were sealed with clear tape (Manco No. 07307). All experiments were conducted at room temperature (296 K). Each trial was performed in duplicate. Controls for each macromolecule with the appropriate precipitant substituting water in place of the linker were run in parallel and a second control using the linkers in the presence of the macromolecule but without precipitant in either drop or reservoir were also run for each macromolecule. No crystals were observed to grow in the presence of a linker without a precipitant in the drop and reservoir. The following are the observations we recorded for a selection of the macromolecules evaluated.

(*a*) Lactate dehydrogenase (LDH) was crystallized in 12%(*w*/*v*) polyethylene glycol 20 000, 0.1 *M* 2-morpholinoethanesulfonic acid (MES) pH 6.0. In the absence of linker we typically observe a shower of small polyhedral crystals. Crystals grown in the presence of linkers 4, 6 and 17 were slightly larger, fewer in number and had improved surface features. Crystals grown in linker 13 showed a marked improvement. In the control, more than 150 crystals are typically observed, approximately 0.1 mm in length and less than 0.1 mm in width and height. The crystals grown in the presence of linker 13 were fewer in number, typically ten crystals per drop, two to three times as long, and with increased breadth. No crystals were grown in other linker solutions.

(*b*) Crystals of satellite tobacco mosaic virus (STMV) were grown in the presence of 10–16% saturated ammonium sulfate over a range of pH levels. Crystals grown in linker 20 (Fig. 4*a*, control is 4*b*) demonstrated the best morphology while also being fewer in number and larger than the control. Smaller crystals were grown in the presence of linker 16 and fewer, larger crystals with morphology as good or better than the control crystals were grown in linkers 4, 6, 7, 11, 12, 14, 15 and 19. No crystals were grown in linkers 3, 5, 19, 22 and 23.

(*c*) Hemoglobin crystals were grown in 30% polyethylene glycol 8000, 0.2 *M* ammonium sulfate, 0.1 *M* sodium cacodylate pH 6.5. The control crystals were very thin blades dispersed in a microcrystalline precipitate. The crystals were virtually transparent. In linkers 2, 12 and 22, single well defined crystals with a deep red color were grown (Fig. 4*c*, control is 4*d*). Fewer nucleation sites were observed using these linkers. The remaining linkers yielded crystals similar to the control. Crystals were grown in the presence of all linkers.

(*d*) Lysozyme was crystallized in 6.5%(*w*/*v*) sodium chloride, 0.1 *M* sodium actetate pH 4.2. In the presence of linkers 1, 3–12, 14, 15, 17, 18 and 19 we observed increased nucleation, as well as crystals being interpenetrated and twinned more than in the control. No crystals appeared in linker 2. In linker 13 thin plates grew (a different morphology to the control). In linker 16 a rhombic habit along with a tetragonal blade form were noted. In linker 20 there was less nucleation than for the control and in linker 23 we observed half the number of nucleation sites with crystals approximately twice the size of those in the control sample.

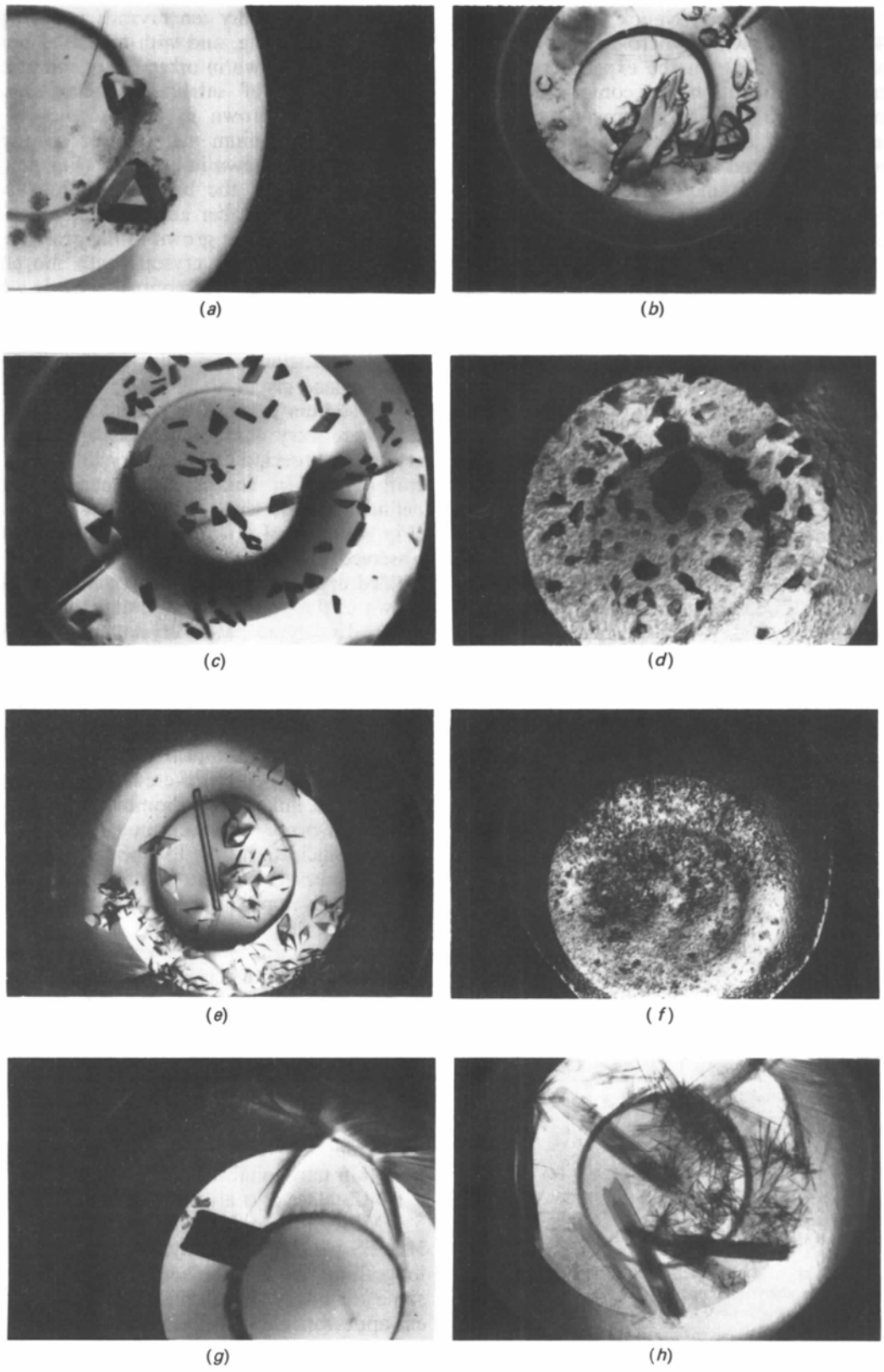
(*e*) Thaumatin crystals were grown from of 1 *M* potassium, sodium tartrate, 0.1 *M* *N*-(2-acetamido)-iminodiacetic acid (ADA) pH 6.5. Crystals similar to the control were observed in linkers 1, 4, 9, 11, 17, 21, 23 and 12. No crystals were observed in linkers 5 and 19. Fewer nucleation sites were observed in linkers 2, 3, 6, 7, 8, 10, 13, 14, 15, 16, 18, 20 and 22. Linker 12 contained two crystal forms, the typical tetragonal bipyramid and a previously unobserved long thin tetragonal blade (Fig. 4*e*, control is 4*f*).

(*f*) Catalase crystals were grown in the presence of 5% MPD, 10% polyethylene glycol 6000 and 0.1 *M* HEPES pH 7.5. Typically, we observed six to eight long thin, but wide, blades in a single drop, with some secondary growth of needles. Crystals of different appearance, but with no improvement in quality, were observed in linkers 1, 3, 7, 10, 11, 13, 14, 16 and 19. No crystals were grown in the presence of linkers 2, 5 and 20. Crystals similar to the control were

1. Dextran sulfate (1.5 mg ml $^{-1}$)
2. 6-Aminocaproic acid (1.0 *M*)
3. 1,5-Hexanediamine (1.0 *M*)
4. 1,10-Decanedicarboxylic acid (100% saturated)
5. 1,8-Diaminooctane (1.0 *M*)
6. 1,12-Dodecanedioic acid (100% saturated)
7. Spermine tetrahydrochloride (0.01 *M*)
8. Cadaverine (0.5 *M*)
9. *trans*-2-Dodecenedioic acid (100% saturated)
10. Spermine (0.2 *M*)
11. Azelaic acid (100% saturated)
12. Poly-L-aspartic acid (0.01 *M*)
13. Poly-DL-lysine hydrobromide (0.01 *M*)
14. Spermidine (1.0 *M*)
15. Poly-L-glutamic acid (0.01 *M*)
16. Poly-L-lysine (0.01 *M*)
17. Hexadecanoic acid (0.4 *M*)
18. Hexaglycine (0.01 *M*)
19. 1,7-Diaminoheptane (1.0 *M*)
20. Glycylglycine (1.0 *M*)
21. Triglycine (0.3 *M*)
22. Tetraglycine (0.02 *M*)
23. 1,10-Diaminodecane (100% saturated)
24. Glycylglycine (0.3 *M*), triglycine (0.1 *M*), tetraglycine (0.007 *M*) mixture

All stock solutions are unbuffered, titrated to pH 7.0 using HCl or NaOH. Concentrations listed are stock values prior to dilution in droplet.

Fig. 3. Electrostatic crosslinking agents.



(a) (b) (c) (d) (e) (f) (g) (h)

Fig. 4. Crystals in the presence of electrostatic crosslinking agents.

grown in linkers 6, 9, 11, 15, 17, 21 and 22. Fewer and slightly larger, thicker crystals were grown in the presence of linkers 4, 8, 12, 18 and 23 (Fig. 4*g*, control is 4*h*). A different crystal form, small and short, and numerous tetragonal bipyramids appeared in the presence of linkers 3, 14 and 19.

(*g*) Valine tRNA crystals were grown in the presence of 30% PEG 4000, 0.1 *M* sodium acetate pH 4.6, 0.2 *M* ammonium acetate. These were a shower of needles. In the presence of linkers 4, 9, 17 and 23 the needles were fewer and slightly larger but of a size still not suitable for diffraction analysis.

(*h*) Crystals of 3-phosphoglycerate kinase were grown in the presence of 30% PEG 4000, 0.1 *M* Tris–HCl pH 8.5, 0.2 *M* sodium acetate. These crystals are in general small, of poor quality and have a needle morphology. In the presence of linkers 17 and 22, however, fewer needles of greater size were observed. Nonetheless, these crystals were not of sufficient quality for diffraction analysis.

(*i*) Leupeptin, a small peptide, was crystallized in the presence of ammonium sulfate as long thin blades. Fewer thicker blades were observed in the presence of linker 16.

(*j*) Edestin was crystallized in the presence of 0.5 *M* ammonium phosphate, 0.05 *M* Na HEPES pH 5.0. These crystals have a hexagonal morphology with irregular surface features. The best crystals, suitable for diffraction analysis, were grown in the presence of linker 6.

Although this survey of macromolecules is limited we consistently noted several features that we feel deserve attention. In all cases where macromolecules were grown in the presence of linkers, we observed at least one linker that demonstrated a perceptible improvement in the crystallization. This improvement was either an increase in size, a reduction in the number of nucleation sites, or an improvement in crystal quality. In the cases of LDH, STMV, hemoglobin, lysozyme, thaumatin, catalase and edestin this effect was obvious, suggesting that we might profitably pursue the use of such linkers as a variable for optimization of crystal growth.

The change of crystal form with thaumatin, lysozyme and catalase in the presence of certain linkers implies, as well, that the use of similar linkers might provide a useful approach for the modification of crystal habit.

The inclusion of electrostatic crosslinking agents may serve as a useful extension of the broadly empirical approach to obtaining useful crystals. We currently use the methodology described in Fig. 3 during optimization after we have limited the conditions with regards to precipitant type, concentration and pH. Such a screen typically requires less than 1 mg of macromolecule. Hopefully, the identification of other even more effective linkers may be useful for producing different crystal forms and increase the number of available paths for optimization of crystal growth. In terms of mechanism, it appears to us that short flexible charged polymers could act as multivalent tethers where one end of the linker would interact with a charged surface group of one macromolecule while a second charged group on the same tether could interact with another. Such favorable interactions might influence both pre- and post-nucleation events, altering the kinetics of association leading to crystal growth, and lending enhanced stability to crystals once they have matured.

3.2. *Detergents as additives*

Detergents are a crystallization additive tool that has been widely and successfully used to produce and improve the quality of crystals of macromolecules, particularly where aggregation is a serious problem (McPherson *et al.*, 1986*a*,*b*; Reiss-Husson, 1992). β-Octylglucoside, especially, has been found to be a useful additive for the crystallization of a number of proteins and nucleic acids (Dock *et al.*, 1984; McPherson *et al.*, 1986*a*,*b*). The inclusion of β-octylglucoside can have a positive, and in the case of membrane proteins, a striking effect on the parameters of crystal growth.

For the optimization of crystal growth of a variety of macromolecules we have frequently utilized β-octylglucoside in the mother liquor where observations have suggested that specific and/or non-specific heterogeneity due to aggregation may be a problem. This occurs with particular frequency in the case of hydrophobic or lipophilic proteins. Unfortunately, the use of β-octylglucoside is not an ultimate solution of this problem and the question remains whether other detergents might be of greater effectiveness.

With the development of membrane protein biochemistry there has been an attendant increased availability of non-ionic detergents. These are often used for the isolation and purification of membrane proteins. There has also been an increased diversity of detergents applied to the crystallization of membrane proteins. It seems to follow that one should evaluate detergents as one would classes of salts, polymers or organic solvents for the crystallization of macromolecules.

To test this idea we evaluated a panel of 32 detergents (Fig. 5) against the library of 20 macromolecules for their influence on crystal growth. Detergents were formulated at 10%(*w*/*v*) (lyophilized detergents) or (*v*/*v*) (solution detergent) stocks and stored at 277 K for short periods (30 d) and at 203 K in the long term (6 months to 1 year). The experiment trials were set up and evaluated within one week. This was done to minimize the effect of

decomposition, as some detergents are unstable and degrade within 30–60 d. It is our experience that detergent stocks stored at 277 K are generally stable for about 30 d but after that should be discarded. We typically formulate small volumes of working stocks, flood the tube with nitrogen and store frozen aliquots at 203 K. Using this procedure the solutions appear stable for extended periods of up to at least one year. Final detergent concentrations in the drops of mother liquor were 0.14%(*w*/*v*) unless specified otherwise. Solutions were always dispensed in the following order: protein first, followed by detergent and finally precipitant from the reservoir. It should also be noted that detergents were not added to the reservoir.

(*a*) Lysozyme was crystallized from 6.5% sodium chloride, 0.1 *M* sodium acetate pH 4.2. The best crystals of lysozyme were obtained using the detergents 5, 8, 9, 12, 13, 14 and 15. A long square rod, different from the typical polyhedral crystal form, appeared in detergent 9. Crystals grown in the presence of other detergents had generally poorer morphologies, often demonstrated excessive nucleation and showed interpenetration among crystals.

(*b*) Thaumatin was crystallized in 1.0 *M* potassium, sodium tartrate, 0.1 *M* ADA pH 6.5. The best crystals were obtained in detergents 9, 14, 19, 21, 22, 23, 24, 26, 27, 28, 29, 30, 31 and 32. Of these, detergent 30 (Fig. 6*a*) yielded perfectly formed tetragonal bipyramids on a more consistent basis than any other detergent as well as for controls. Excessive nucleation, and twinned, interpenetrating crystals were observed in the remaining detergents (Fig. 6*b*).

(*c*) LDH was crystallized in 12%(*w*/*v*) polyethyleneglycol 20 000, 0.1 *M* MES pH 6.0. Good crystals were grown in detergents 6, 7, 8, 12, 13, 14, 17, 18, 19, 20, 21, 24, 31 and 32. Reduced nucleation and crystals having improved surface features developed in detergent 23 (Fig. 6*c*). Crystals grown in the remaining detergents showed no improvement and, in general, were of poorer quality, again showing excessive nucleation as well as the formation of amorphous precipitate (Fig. 6*d*).

(*d*) Catalase crystals were grown in 5% MPD, 10% PEG 6000, 0.1 *M* Na HEPES pH 7.5. No improvements were observed in any of the crystals as a consequence of the inclusion of detergents. Most of the crystals demonstrated a deterioration in quality (3, 5, 6, 9, 11, 12, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32) while the remaining crystals were similar to the control – long thin blades.

(*e*) Papain was crystallized in the presence of 1.6 *M* ammonium sulfate, 10% dioxane, 0.1 *M* MES pH 6.0. Under these conditions we typically observe clusters of small thin plates and some larger, individual, thin plates with rhombic faces. These same crystal forms were observed in the presence of detergents 14, 16, 21, 26 and 30. Some deterioration was observed in detergents 1–12, 13, 15, 17, 18, 19, 20, 22, 24, 28 and 29. In the presence of detergents 23, 25 and 28, however, many single thin blades of unique habit were observed. This is the first observation of this crystal form, at least in this precipitant system.

(*f*) 3-Phosphoglycerate kinase, ficin, edestin, hemoglobin and protease crystals showed no improvement when grown in the presence of the detergents. It was observed that for all but a few of the detergents tested with these proteins, the crystals were identical to those in control samples. In only a few cases was there any degradation in the quality of the crystals.

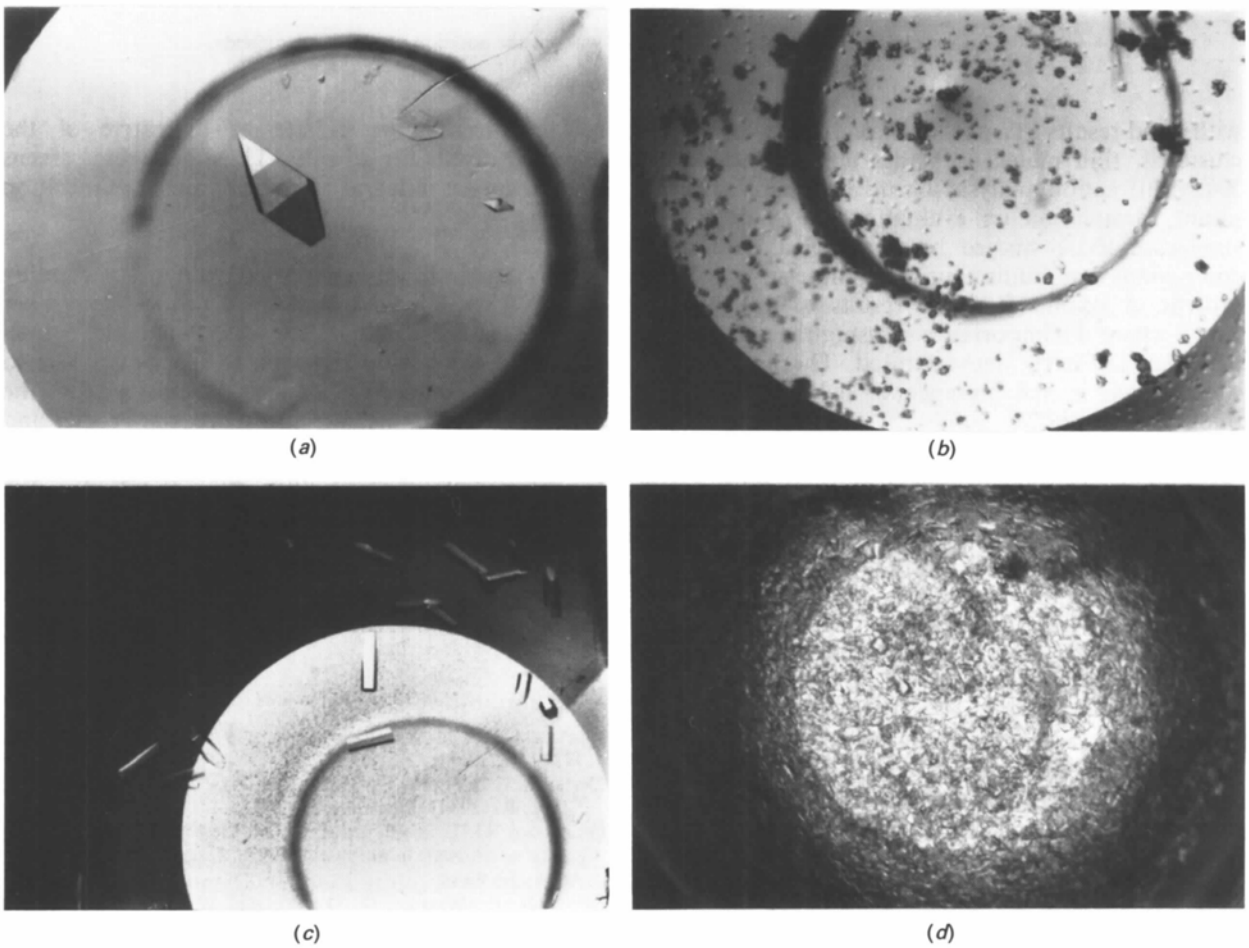
(*g*) We have been working with vitamin D binding protein (VDBP) which is purified from pooled human plasma and comes to us in lyophilized form. This protein has been crystallized previously (Koszelak, McPherson, Bouillon & Van Baelen, 1985) but subsequent preparations have proved futile for crystal growth. Current samples of the protein form heavy brown precipitates or oils in virtually every precipitant we have investigated. The addition of 0.01–1% β-octylglucoside seemed to have no effect on the formation of either precipitate or oil. Because the material is limited, we did not have the means to test all 32 detergents against all available precipitants. To obviate the problem we used the

1. Triton X-45
2. Triton N-111
3. Triton N-60
4. Triton N-101
5. Triton X-200
6. Sigma Triton X-114
7. Sigma Triton X-100
8. Sigma Nonident P-40
9. Triton X-405
10. Triton X-102
11. BASF Pluronic L-62
12. BASF Pluronic L-64
13. BASF Tetronic 901
14. Lonza Barlox 105
15. Continental Conco XA-L
16. β-Octylthioglucoside
17. Isotridecylpoly(ethylene glycol ether)$_n$
18. Triton X-100 (Boehringer)
19. Triton X-114 (Boehringer)
20. Thesit
21. *n*-Octylglucoside
22. *N*-Dodecyl-1-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate
23. Mega-8
24. *n*-Dodecylmaltoside
25. CHAPS
26. CHAPSO
27. Octylmethylammonioacetate
28. Dimethyloctylphosphine oxide
29. Dimethyloctylammoniopropylsulfate
30. Methyloctylsulfide
31. *N*,*N*-Dimethyloctylamine oxide
32. Dodecyl-D-maltoside

Fig. 5. Detergent screening solutions.

DynaPro-801 dynamic light scattering instrument (Protein Solutions, Charlottesville, West Virginia, USA) to evaluate whether the sample was mono- or poly-dispersed. 200 μl samples of 2 mg ml^{-1} VDBP were prepared and filtered with a 0.1 μm filter. Prior to the analysis in the presence of detergent the sample appeared to be fairly mono-dispersed, but actually had an approximate molecular weight of 800 000. The monomer of VDBP has a molecular weight of 40 000. Thus it was clear that the protein was present as a large aggregate in solution. We then repeated our light-scattering measurements using 200 μl of VDBP in the presence of 0.2%(*v*/*v*) detergent, evaluating each of the detergents listed in Fig. 6. Large aggregates were observed in the presence of β-octylglucoside as well as all other surfactants, except for detergents 20 and 30. In the presence of the latter two detergents the analysis clearly showed the protein to correspond approximately to the size of a monomer. Using these two detergents we have begun screening a variety of precipitants to identify crystallization conditions. We have yet to reproduce crystals of VDBP, but we have observed a significant decrease in the number of samples which yield precipitate or oils, and of those samples which do precipitate, this typically occurs after 3–7 d rather than immediately as was previously observed. We plan to continue our crystallization trials using these two detergents and VDBP. We have recently initiated a similar set of experiments where we have applied these detergents to six different intact monoclonal antibodies and Fab fragments, and have again noted a significant reduction in the number of samples yielding precipitants or oils. We are optimistic that future experiments will support the value of detergents in the crystallization of antibodies.

An impressive result using the detergent screen was obtained in collaboration with Dr Karl Weisgraber at the Gladstone Institute (San Francisco, CA). This laboratory has been working on the crystallization of apolipoprotein CI for some time



(*a*) (*b*) (*c*) (*d*)

Fig. 6. Crystals in the presence of detergents.

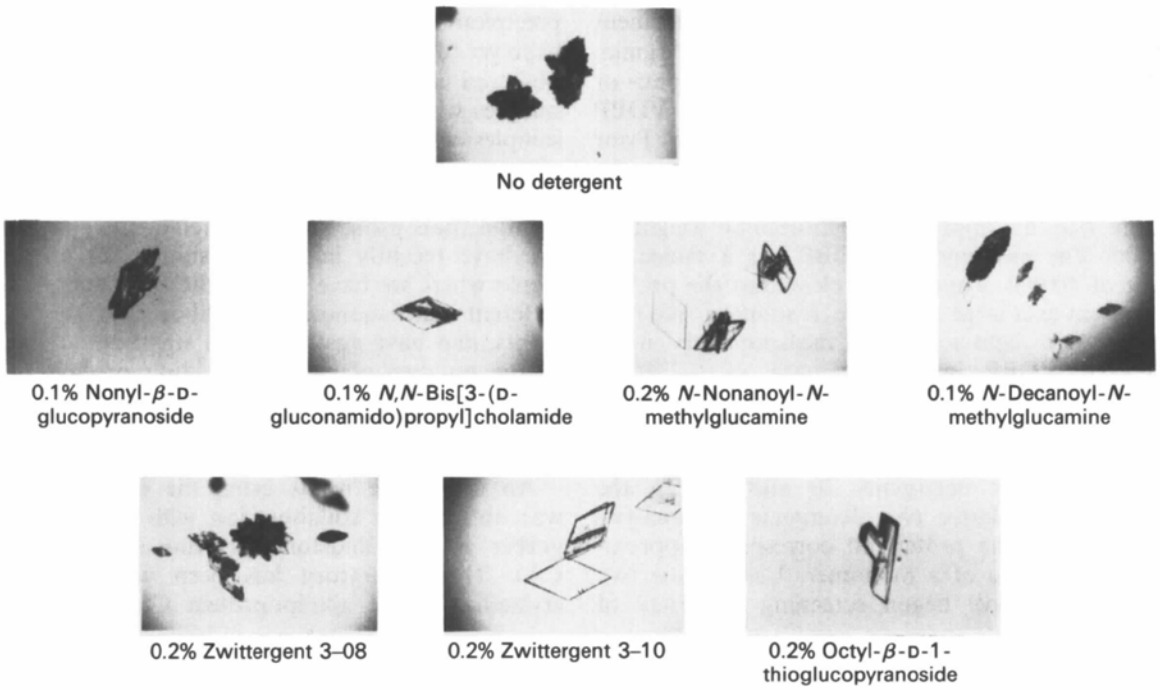


Fig. 7. Crystals of apolipoprotein C1 with and without the addition of various detergents.

with good results except that the crystals were always clustered, thin plates or blades and unsuitable for X-ray diffraction analysis. Using an optimized precipitant formulation an evaluation of 18 detergents similar to those studied here as well as additional compounds was studied for their influence on crystal growth. A summary of the results is illustrated in Fig. 7 where the importance of using the appropriate detergent is clearly demonstrated. The best crystals were grown in the presence of 0.2% octyl-β-D-1-thioglucopyranoside. Single crystals, although thin, were grown in the presence of 0.2% Zwittergent 3–10 while small disordered clusters were grown in 0.2% Zwittergent 3–08. It is interesting to note the reduction in chain length of the detergent could result in such a drastic change in crystal quality.

Although the evaluation of protein crystallization in the presence of detergents in our laboratory was strictly limited, and the selection of detergents incomplete, we feel our observations support other evidence (Reiss-Husson, 1992) suggesting the value of a detergent screen for increased improvements, particularly where aggregation is suspected to be a complication. The screen we describe is simple and rapid to perform and it requires only about 1 mg of macromolecule for 32–48 detergents. An important point, we feel, is that use of a single detergent is inadequate and that a variety should be investigated. Evaluating the role of a single detergent in preventing aggregation would be analogous to accepting the role of ammonium sulfate as indicative of the expected results for all salts. Thus, detergent screens are now an integral part of our optimization strategy.

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