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An Experiment Regarding Crystallization of Soluble Proteins in the Presence of β-Octyl Glucoside*

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Twenty-one soluble proteins, five tRNAs, and three protein-nucleic acid complexes were studied in a systematic manner with regard to their crystallization behavior from polyethylene glycol and ammonium sulfate solutions in the presence of 0 to 1.5% β-octyl glucoside. Our observations suggest that this neutral detergent does influence in a very positive way the growth characteristics of the macromolecules included in this experiment. In general, more reproducible and rapid growth was noted with an increased number of large individual crystals at the expense of microcrystals. In several cases, new crystal forms were discovered. Selected x-ray diffraction analyses imply that crystals grown in the presence of β-octyl glucoside diffract as well or better than those grown in its absence. In addition, a screen of two proteins grown in the presence of 14 different common detergents suggested that a general detergent effect may be beneficial for the growth of crystals of biological macromolecules.

An impediment of the successful crystallization of some proteins is likely to be microheterogeneity arising from the formation of different states of aggregation during the crystallization process. This is particularly true because the time required to grow crystals is often weeks or even months and because the concentration of protein required to obtain crystals is generally very high by most standards, *i.e.* 10 to 50 mg/ml. Both of these contribute to both specific and nonspecific aggregate formation of even those proteins which do not normally demonstrate such behavior under dilute conditions or under physiological circumstances. In some cases, such aggregation can be eliminated by imposing a reasonably high ionic strength. Presumably, this is effective because it disrupts electrostatic interactions by which some molecules aggregate. We have observed, however, that in most cases this is not particularly effective. We suspect that for the majority of proteins aggregation is primarily mediated, even if imperceptibly, by hydrophobic contacts between molecules.

It was our contention that if hydrophobic interactions between molecules could be reduced, and these are usually nonspecific and without stringent geometrical constraints, then ionic and electrostatic interactions might be made to predominate between molecules, or at least be enhanced. Ionic bonds are more directional in character since an array of charges in space demands a very specific array of complementary counterions with which to form an interface. Such complementation must occur when proteins crystallize, or at least when they crystallize in an ordered manner permissive of x-ray diffraction analysis. From a practical standpoint, this means finding ways to diminish nonspecific hydrophobic interactions and encouraging electrostatic interactions.

The problem, we believe, is similar to that which has recently been overcome in several instances by those working on the crystallization of proteins extracted from membranes. These are proteins that are highly hydrophobic in nature and demonstrate severe aggregation in the absence of detergent molecules. The successful crystallization of the protein porin (1) from the plasma membrane of *Escherichia coli* and the reaction center complex of proteins from photosynthetic bacteria (2) showed unequivocally that proteins could be crystallized under otherwise conventional conditions if maintained in the presence of a neutral detergent such as β-octyl glucoside. Crystallization further demonstrated that the structure of these proteins was not randomized or appreciably disordered by the presence of the detergent. The experiments we describe below were inspired by the work with membrane proteins. They were intended to test the effect of the neutral detergent, β-octyl glucoside, on the crystallization of a broad range of soluble proteins and determine whether by modifying the hydrophobic interactions between the individual molecules a helpful, interesting, or deleterious effect was produced. In particular, we were interested in determining whether the inclusion of β-octyl glucoside in the crystallization samples produced crystals of the same or different habit, crystals that diffracted to higher or lower resolution, crystallizations that were more or less reproducible, crystallizations that yielded smaller or larger crystals, and to see whether crystals could be produced from proteins that previously had not been crystallized. Because quantitation is difficult when applied to the problem of crystal growth and crystal quality, it is not entirely straightforward to accurately evaluate success or failure. Therefore, we concede at the start some subjective process in the assessment of our results. We believe, nevertheless, that the experiments we describe definitely imply a usefulness of neutral detergents such as β-octyl glucoside in macromolecular crystallization and encourage challenge of our observations by other investigators conducting analogous trials with diverse protein molecules.

MATERIALS AND METHODS

In the crystallization trials, 21 proteins, 5 transfer RNAs, and 3 protein-nucleic acid complexes were investigated in a parallel manner. The technique employed was vapor diffusion in plastic boxes with eight samples in each box (3, 4). For all macromolecules, the initial concentrations of β-octyl glucoside were 0.0%, 0.125%, 0.50%, and 0.75% and equilibration was such that the final concentrations were

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0.0%, 0.25%, 1.00%, and 1.50%, respectively. The microdroplets contained in all cases 5 μl of protein solutions at concentrations that varied from 6 to 50 mg/ml, 5 μl of β-octyl glucoside, and 10 μl of the reservoir used in the particular box that the trials were being made. Thus, the initial volume of all trial droplets was 20 μl. The reservoirs used with each protein were 5%, 9%, 14%, and 20% polyethylene glycol (PEG[1]) 4000. In a number of cases where the protein was known to crystallize from ammonium sulfate solutions, the trials were repeated with reservoirs of 40%, 45%, 50%, 60%, and 70% saturated ammonium sulfate. The crystallization trials were followed over a period of 7 months by examination of the individual samples with a dissecting microscope.

In addition to these trials, 12 boxes containing the protein concanavalin B were set up over the range of 35 to 42% saturated ammonium sulfate, where it was known to crystallize, for the purpose of evaluating reproducibility, habit, and average crystal size. In those cases where a protein-nucleic acid complex was involved, the initial droplet size was 22 μl, reflecting the addition of enough oligonucleotide stock solution to achieve a stoichiometric ratio of five oligomers per protein molecule. The oligonucleotides, $d(pA)_4$ and $d(pT)_4$, were purchased from Collaborative Research of Waltham, MA.

All crystallization trials were carried out at 23 °C although the temperature probably varied over a range of 6 degrees about this point. The PEG 4000 was purchased from Baker and was not purified further before its use. All concentrations of PEG given here are percentage w/w. The stock solutions of PEG used in reservoirs and samples contained 0.1% sodium azide as an inhibitor of microbial growth. The glass plates, Corning 7220, used to support the crystal samples were siliconized with Prosil.

Light microscope photographs were made with an Olympus BH microscope fitted with an Olympus OM-2 camera with either 5 times or 10 power objective lenses. The film was Panatonic X and the exposures were made in the automatic mode. A microfocusing screen was employed.

Those crystals that were examined by x-ray diffraction photography were mounted in quartz capillaries in the conventional manner (4) with a small quantity of mother liquor and sealed from the air with beeswax. For photography, Buerger Precession cameras with a crystal to film distance of 75 mm were used and the precession angles were generally 12°. The exposure times varied, depending on the crystal, from 10 to 18 h. The x-ray source was a GX20 Elliott rotating anode x-ray generator operated at 35 kV and 35 mA with a focal spot size of 200 μ^2. X-ray diffraction data were collected from the crystals of concanavalin B using an Enraf-Nonius automated diffractometer with omega scans. These data sets were generally limited to 5.0 Å resolution. The data were corrected and processed as described elsewhere (5).

The macromolecules and macromolecular complexes used in this study were as follows.

Horse serum albumin was prepared according to the method of McMeekin (6) and twice recrystallized from 45% saturated ammonium sulfate before use. The stock solution was 50 mg/ml.

Egg albumin (ovalbumin) was prepared according to the method of Hopkins and Pinkus (7) and three times recrystallized from 45% saturated ammonium sulfate prior to use. The stock solution was 40 mg/ml.

Hen egg white lysozyme was purchased from Calbiochem. The stock solution was 20 mg/ml.

Bovine ribonuclease B was purchased from Sigma. The stock solution was 20 mg/ml.

Bovine ribonuclease A was purchased from Sigma. The stock solution was 20 mg/ml.

The lectin from *Abrus precatorius* (rosary pea) was prepared from the seed according to the procedure of McPherson and Rich (8). The stock solution was 12 mg/ml.

The base plate protein from bacteriophage P22 was made and provided by Dr. Peter Bergett, University of Texas. The stock solution was 25 mg/ml.

Alkaline form fructose-1,6-diphosphate from turkey liver was prepared by the procedure of McPherson *et al.* (9) and once crystallized from 45% saturated ammonium sulfate. The stock solution was 12 mg/ml.

Neutral form of fructose-1,6-diphosphatase from rabbit liver was provided by Dr. Bernard Horecker, Hoffman-LaRoche Co. The stock solution was 12 mg/ml.

[1] The abbreviation used is: PEG, polyethylene glycol.

α_1-Acid glycoprotein from human serum was provided by Dr. Brian Halsall, University of Cincinnati. The stock solution was 20 mg/ml.

Catalase from sheep liver was prepared by the authors according to the procedure of Burkey and McPherson (10) and once crystallized. The stock solution was 8 mg/ml.

Cystathionine synthetase from *E. coli* was made and provided by Steve and Libby Holbrook, University of California, Berkeley. The stock solution was 20 mg/ml.

An unknown crystalline protein from pineapple stem was prepared by ammonium sulfate fractionation of pineapple stem extracts by an unpublished procedure. The protein was crystallized once from 45% saturated ammonium sulfate. The stock solution was 8 mg/ml.

The α subunit of bovine luteinizing hormone was provided by Professor John Pierce, University of California, Los Angeles. The stock solution was 20 mg/ml.

The α subunit of bovine thyroid-stimulating hormone was provided by Professor John Pierce, University of California, Los Angeles. The stock solution was 20 mg/ml.

α-Amylase from *Bacillus subtilis* was purchased from Sigma and recrystallized according to the procedure of McPherson and Rich (11). The stock solution was 30 mg/ml.

α-Amylase from pig pancreas was prepared and recrystallized according to the procedure of McPherson and Rich (12). The stock solution was 36 mg/ml.

Canavalin, the major seed storage protein of jack bean (*Canavalis ensiformis*), was prepared according to the procedure of Sumner and Howell (13) and three times recrystallized according to the procedure of McPherson and Spencer (14). The stock solution was 45 mg/ml.

Concanavalin B from jack bean (*C. ensiformis*) was prepared according to the procedure of Sumner (15) and twice recrystallized as described by Morrison *et al.* (16). The stock solution was 7 mg/ml.

Gene 5 DNA unwinding protein from fd bacteriophage-infected *E. coli* was made according to the method of Alberts *et al.* (17). The stock solution was 14 mg/ml.

An extracellular serine protease from *Penicillium cyclopium* was prepared from culture filtrate (18). The stock solution was 8 mg/ml.

Transfer RNAs cysteine, valine 1, phenylalanine, arginine, and lysine were made and provided by Susan Ribiero and Professor Brian Reid, University of Washington, Seattle, WA. The stock solutions were 8 to 14 mg/ml.

Complexes of bovine ribonuclease A with $d(pA)_4$ and with $d(pT)_4$ at a stoichiometric ratio of 5:1 DNA oligomer to protein were prepared as described by Brayer and McPherson (19). The stock solutions were 20 mg/ml.

The complex of gene 5 DNA unwinding protein (20) with $d(pA)_4$ at a stoichiometric ratio of 4:1 DNA oligomer to protein was made in 20 mM Tris at pH 7.4. The stock solution was 13 mg/ml.

Crystallization of the gene 5 DNA unwinding protein from fd bacteriophage and canavalin from jack bean was also attempted in the presence of a range of different detergents in common laboratory use in order to evaluate their effects. The detergents surveyed were Triton X-405, X-100, X-165, and N-101, Nonidet-40, Brij 35, and Sarcosyl. These were added to the protein solutions so that their final concentrations were 0.3%. In addition, the detergents Triton X-45 and X-35, Tween 20, Spar 85, Brij 58, and Lubrol 10X were also added in separate but identical trials, but their final concentration was 2.5% saturation with the detergent.

RESULTS

The results of the trials were somewhat complicated to evaluate because we were interested in knowing not only whether crystals appeared but also additional facts regarding their growth. We will state here some observations we feel are manifest and which have fairly straightforward implications. We will also give some qualitative observations regarding crystal growth in the presence of β-octyl glucoside about which we feel reasonably confident.

Crystals were observed in at least one sample and generally at least several, and in some cases all, of 16 of the 21 proteins investigated. They appeared over the entire range of β-octyl glucoside and PEG 4000 concentrations used in this study.

For the growth of three protein crystals, we attempted to quantitate the results in terms of frequency of growth and size for those crystals suitable for x-ray diffraction. The three

proteins we followed in this way were ones in which the amount of available material was not limiting and were proteins with which we had worked extensively and therefore knew the optimal growth conditions. These three proteins were concanavalin B, pancreatic α-amylase, and rabbit liver fructose-1,6-diphosphatase. We define here as a crystalline shower a sample of 10 μl or less which contains more than 10 crystals, clusters of crystals that are not individually useful, or samples that have crystals of an average estimated maximum dimension of less than 0.2 mm.

From 30 parallel trials (30 in the presence and 30 in the absence of β-octyl glucoside) using concanavalin B and a concentration of β-octyl glucoside of 0.25%, the following results were obtained. In the absence of β-octyl glucoside showers or clusters of crystals occurred in 22 out of 30 samples while in the presence of β-octyl glucoside, the occurrence was 8 out of 30. Crystals of some type were observed in all trials. The average diameter (length is not a relevant consideration with concanavalin B crystals) of crystals in the 22 useful samples containing β-octyl glucoside was 0.45 mm while the average size for the crystals in the eight useful samples grown in the absence of β-octyl glucoside was 0.22 mm. We observed that crystals generally appeared first in the samples not containing β-octyl glucoside but that the time required to reach maximum size was the same within 2 to 4 days and was on average about 12 days. Thus, the per cent of successful trials in the presence of the detergent was 73% *versus* 27% for those lacking the detergent.

For 20 parallel samples of pancreatic α-amylase, the frequency of crystal appearance was 8 in 20 in the absence of β-octyl glucoside and 18 in 20 in its presence. In the absence of β-octyl glucoside, 5 of the 8 crystalline samples contained crystals of the smallest dimension, greater than 0.2 mm, while in the presence of the detergent, 14 of the 18 samples contained such crystals. Therefore, useful samples in the absence and presence of β-octyl glucoside were 25% and 70%, respectively.

Twenty parallel trials were conducted on rabbit liver fructose-1,6-diphosphatase. Of these, crystals appeared in 14 out of 20 in the absence of β-octyl glucoside and 17 out of 20 in the presence. Of the 14 obtained in the absence of β-octyl glucoside, 6 contained one or more crystals of the smallest dimension greater than 0.2 mm, while in the presence of the detergent, 12 of 17 contained such crystals. Thus, the percentage of trials producing useful crystals (greater than the 0.2-mm smallest dimension) was 30% and 60% in the absence and presence of β-octyl glucoside, respectively.

Crystals appeared in samples of cysteine, phenylalanine, and lysine transfer RNA, but not in the other three.

All three of the protein-DNA oligomer complexes yielded crystals.

A sampling of crystals actually grown in this experiment are shown in Fig. 1. The crystals demonstrate a variety of habits and sizes. In general, all of the crystals shown here, with some interesting exceptions to be described below, are of the same morphologies as those grown in the absence of β-octyl glucoside but otherwise under the same conditions. In this experiment, only cystathionine synthetase and the base plate protein of bacteriophage P22 had never been crystallized by us in any form. These proteins also did not crystallize in this study in the presence of β-octyl glucoside. In addition, we did not obtain crystals of horse serum albumin, ovalbumin, or lysozyme, even though these are among the easiest proteins to crystallize. We noted however, that the two albumins have traditionally been grown from solutions of ammonium sulfate and the experiment was therefore repeated with ammonium sulfate for these three cases as well as cystathionine synthetase and the P22 base plate protein. In the presence of β-octyl glucoside, no crystals again appeared. Concanavalin B, however, which we routinely grow from 38% saturated ammonium sulfate, grew from both PEG 4000 and the solutions of ammonium sulfate at all concentrations and, as will be described below, with improved features.

Although most crystals grown in the presence of β-octyl glucoside had a morphology no different than observed in its absence, there were two notable exceptions and they were the gene 5 DNA unwinding protein from bacteriophage fd seen in Fig. 2 and α-amylase from *B. subtilis* seen in Fig. 3. X-ray examination of the new form of gene 5 protein crystal shows it to have a unit cell distinctly different from that which grows in the absence of β-octyl glucoside.

Collection of x-ray diffraction data from eight crystals of concanavalin B grown in the presence of β-octyl glucoside and investigation of these crystals for heavy atom derivative formation showed the following. The native unit cell is $P6_1$ with $a = b = 80.9$ Å and $c = 100.9$ Å and that of the crystals grown in the presence of the β-octyl glucoside is again $P6_1$ with $a = b = 81.3$ and $c = 102.0$ Å with estimated errors of no more than 0.02 Å on any cell edge. Thus, in the presence of the detergent, the unit cell expands by about 1% on *c* and about 0.5% on *a* and *b*. Three-dimensional diffraction data (5.0 Å) from an otherwise native concanavalin B crystal grown in the presence of the detergent scales to the native data with a conventional *R* factor of less than 0.04 on */F/*. Thus they are, within the error of the measurements, isomorphous. Of the eight crystals into which heavy atom compounds were diffused. 5.0-Å difference Fourier analyses and subsequent refinement (16) showed that those treated with $KAu(CN)_2$ and $AgNO_3$ both formed useful and very isomorphous derivatives in the presence of the β-octyl glucoside.

The crystals of the gene 5 protein seen in Fig. 2*B* grown in the presence of β-octyl glucoside were shown to have space group $C222_1$ with $a = 68.4$ Å, $b = 86.6$ Å, and $c = 105.0$ Å. These have not been previously observed or reported in spite of nearly 5 years of effort on our part to grow crystals different from the monoclinic C2 crystals used for the protein's structure determination (20). These crystals have an entire dimer of the protein (2 × 9,800 Da) in a highly hydrated asymmetric unit with a mass to volume ratio V_m of 3.9 Da/Å^3.

In those trials where only the gene 5 protein and canavalin were set up for crystallization in the presence of the 14 detergents named above, every sample produced crystals. While this is not so unusual in the case of canavalin which crystallizes quite readily anyway, we have never in the past 10 years observed such a success rate with the gene 5 protein. It generally gives no better than about a 25% yield even in the most successful attempts. Furthermore, contrary to our expectations, the crystals in every one of these trials were of the native monoclinic variety and none were of that type grown in the presence of the β-octyl glucoside. The canavalin crystals were of the same habit as that usually observed in the absence of detergent under otherwise identical conditions and the same as those grown in the presence of β-octyl glucoside.

X-ray diffraction photographs from a crystal of the complex between ribonuclease A and $d(pA)_4$ shown in Fig. 4, grown in the presence of β-octyl glucoside showed it to be isomorphous and indistinguishable from crystals grown in the absence of β-octyl glucoside (19).

In the course of these experiments, seven proteins were crystallized in the presence of β-octyl glucoside and in some cases also in its absence that have not previously been re-

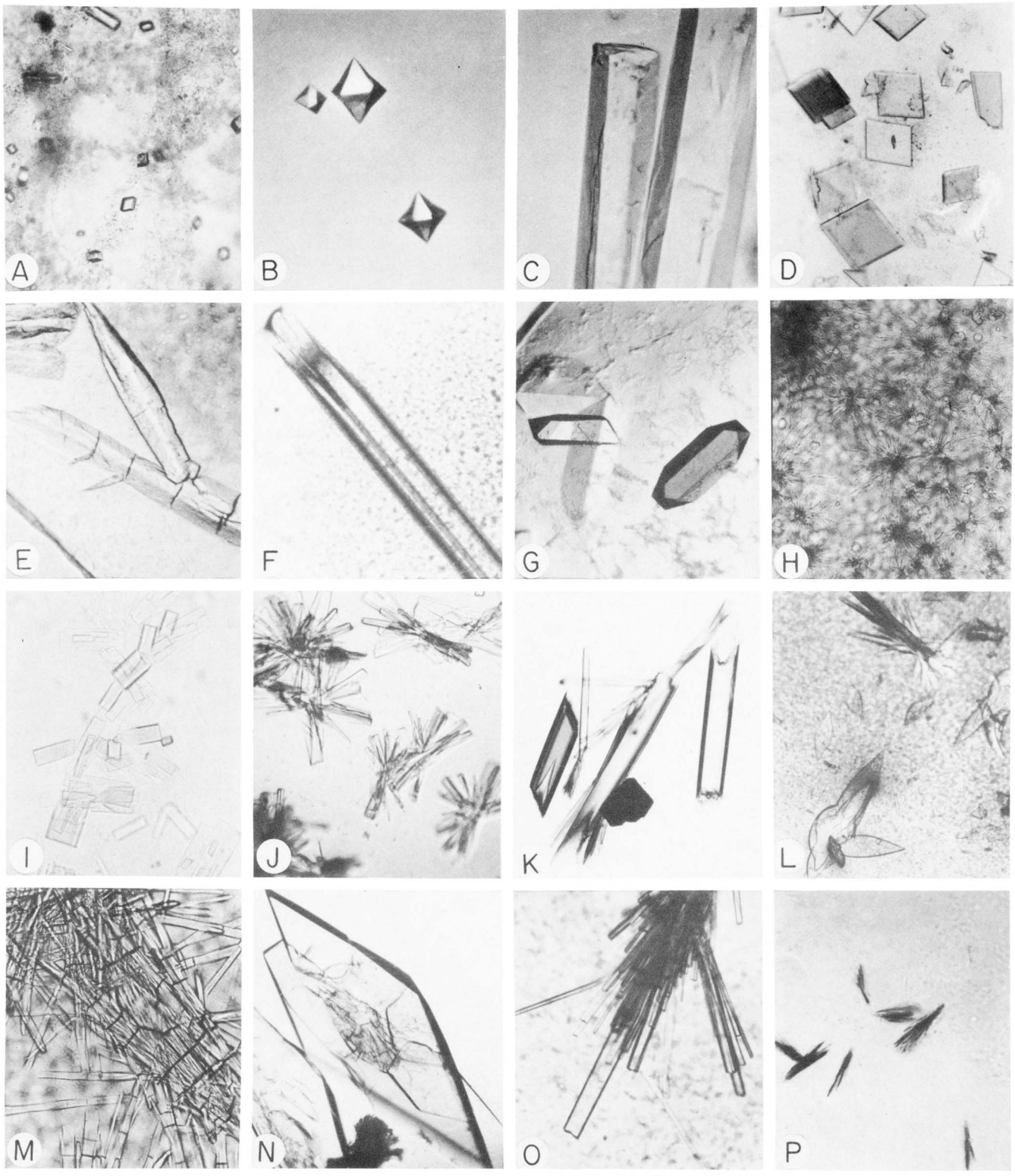

FIG. 1. **A sampling of crystals grown during the course of this investigation.** All photographs were made with a low power light microscope. From *upper left* to *lower right*, the crystals are of alkaline form fructose-1,6-diphosphatase from turkey liver (*A*), canavalin from jack bean (*B*), ribonuclease A (*C*), serine protease from *Penicillium cyclopium* (*D*), *E. coli* phenylalanine tRNA (*E*), neutral form fructose-1,6-diphosphatase from rabbit liver (*F*), pig pancreas α-amylase (*G*), lysine tRNA (*H*), sheep liver catalase (*I*), α subunit of thyroid-stimulating hormone (*J*), concanavalin B from jack bean (*K*), gene 5 DNA unwinding protein plus $d(pA)_4$ (*L*), cysteine tRNA (*M*), ribonuclease B (*N*), lectin from *Abrus precatorius* (O), protein of unknown function from pineapple stem (*P*).

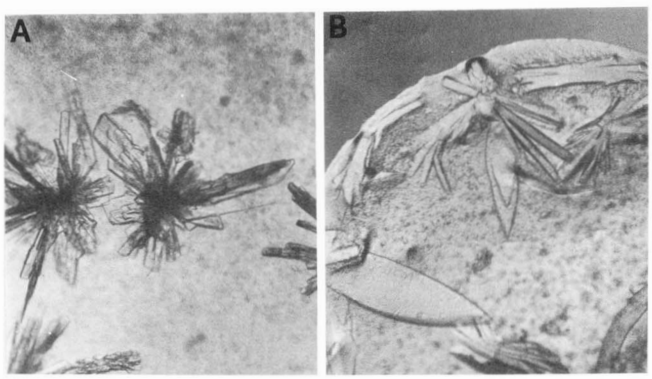

FIG. 2. In *A* is seen the common monoclinic habit crystals of the gene 5 DNA unwinding protein grown from PEG 4000 in the absence of β-octyl glucoside. They are of space group C2. In *B* is seen the form of crystals produced from the protein grown from PEG 4000 in the presence of the neutral detergent. These crystals are of space group $C222_1$ and have a highly hydrated unit cell distinctly different than the monoclinic crystals used for the structure determination of the native protein.

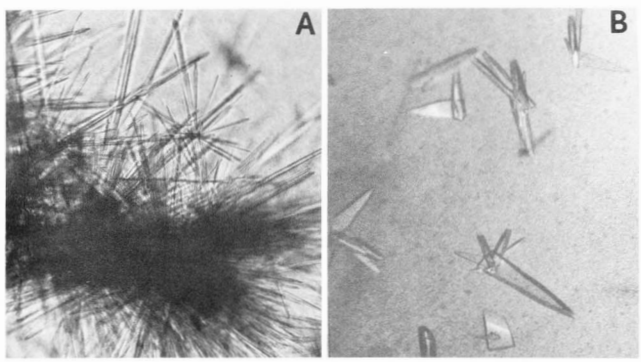

FIG. 3. In *A* are seen masses of needle crystals that are the typical habit of *B. subtilis* α-amylase. These crystals are unsuitable for x-ray diffraction due to their morphology. In *B* are seen crystals of the same protein grown from the identical medium of PEG 4000 in water but in the presence of 1.5% β-octyl glucoside. This change in habit is the first observed in over 12 years of dealing with this protein.

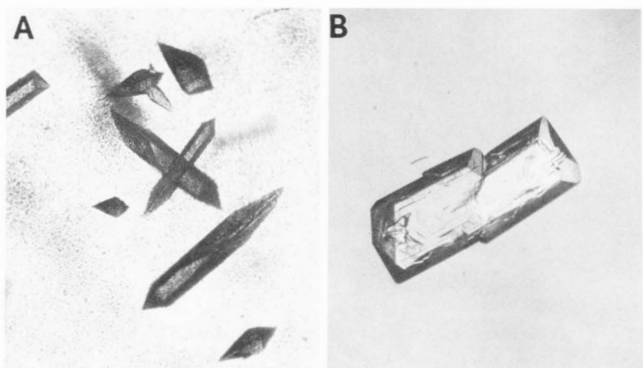

FIG. 4. In *A* are crystals of RNase A + $d(pA)_4$ and in *B* crystals of RNase + $d(pT)_4$, both grown from 20% PEG 4000 in the presence of 1.0% β-octyl glucoside. These crystals were shown by x-ray diffraction to be isomorphous with similar crystals grown in the absence of β-octyl glucoside.

ported in the literature. Their unit cell parameters, diffracting properties, and important characteristics are given in Table I. The data presented here suggest that crystals grown in the presence of β-octyl glucoside diffract at least as well as those grown in the absence of the detergent.

CONCLUSIONS

Our conclusions from the experiments and results presented above as well as from qualitative but comprehensive observations of the trials are as follows.

The observation that virtually all of the proteins previously crystallized from PEG 4000 and several of those obtained from ammonium sulfate solutions such as concanavalin B were readily crystallized from PEG in the presence of β-octyl glucoside shows that the detergent does not inhibit the growth of protein crystals from PEG solutions. The crystallization further shows that the proteins were not significantly disordered by exposure to the compound. Nearly all of the proteins investigated crystallized with higher frequency and greater reproducibility in the presence of the detergent than in its absence. The success rate in several specific cases such as pancreatic α-amylase, neutral form fructose-1,6-diphosphatase, and ribonucleases A and B, was significantly higher.

In general, the protein crystals we obtained were of perceptibly larger size and accompanied by fewer satellite crystals than normally observed with this particular set of proteins. In particular, the ribonuclease A and B crystals, concanavalin B, and *Abrus precatorius* lectin crystals were substantially larger on average. In the presence of β-octyl glucoside, we seldom observed showers of microcrystals and far more single or isolated crystals appeared.

In four cases, the gene 5 DNA unwinding protein, ribonucleases A and B, and *B. subtilis* α-amylase, a crystal form was obtained that was distinctly different from that grown under otherwise identical conditions. While this may not be particularly meaningful in the cases of RNases A and B which are noted for their polymorphic crystal growth (21), it is certainly significant in the case of the gene 5 protein and *B. subtilis* α-amylase. Figs. 2*A* and 3*A* show the common form of these two protein crystals. Since these crystals were first reported (19, 11), 9 and 12 years ago, respectively, we have made concerted efforts to obtain different crystal habits or different unit cells. *B. subtilis* α-amylase has never been amenable to x-ray diffraction analysis because of its intractable propensity to form needle crystals. The native monoclinic gene 5 crystals, because of their dense packing motif, have proven extremely difficult to approach with physiological ligands designed to complex with the protein. Thus, relevant complexes of the protein with nucleic acid have been difficult to obtain.

In this experiment, we reproducibly obtained crystals of *B. subtilis* α-amylase of a habit we believe suitable for diffraction analysis: they are shown in Fig. 3*B*. In addition, we can now grow a new, highly hydrated form of the gene 5 protein, a form never previously observed in the absence of β-octyl glucoside, that can and probably does (see below) form complexes in the crystal with deoxyoligonucleotides. These results suggest that inclusion of β-octyl glucoside or the manipulation of its concentration in crystallization samples may provide a means of improving the habit of an otherwise difficult crystal, of obtaining new crystal forms with possible advantages over previous forms, and conceivably obtaining crystals of proteins not previously crystallized at all.

Our results suggest that the effects of β-octyl glucoside are more beneficial with crystallization from PEG than from salt solutions. The exception to this in our experiments was concanavalin B whose crystallization properties were definitely and significantly improved by the inclusion of the detergent. Horse serum albumin, ovalbumin, and several other proteins that we know to be crystallizable from ammonium sulfate yielded no crystals at all from salt solutions in the presence of the concentrations of β-octyl glucoside employed in these experiments. Because our range of experimental variables was

TABLE I

Unit cell parameters and crystal properties of new crystal forms

Protein and source	Space group	Cell dimensions	Cell volume	Volume/mass (V_m)	Molecules/asymmetric unit and weight of molecule	Resolution of diffraction
		Å	Å^3	Da/Å^3		Å
Canavalin (jack bean)	$P2_13$	$a = b = c = 178$	5.64×10^6	3.13	1 trimer (150,000)	>2.5
Serine protease (*Penicillium cyclopium*)	$P2_12_12_1$	$a = 59.12$, $b = 62.33$, $c = 70.62$	2.56×10^5	2.00	1 monomer (33,000)	>2.5
Ribonuclease B (bovine)	C2	$a = 101.8$, $b = 33.36$, $c = 70.62$, $\beta = 90.4$	2.50×10^5	2.31	2 monomers (13,500)	>2.2
Ribonuclease A[a] (bovine)	$P2_12_12_1$	$a = 46.4$, $b = 73.8$, $c = 44.6$	1.45×10^5	2.70	1 monomer (13,500)	>2.2
Fructose-1,6-diphosphatase (chicken liver)	$P2_12_12$	$a = 107.2$, $b = 144.4$, $c = 45.1$	6.93×10^5	2.41	2 monomers (32,000)	>2.8
Ribonuclease A plus $d(pT)_4$ (bovine)	$P2_12_12_1$	$a = 44.57$, $b = 75.69$, $c = 43.16$	1.45×10^5	2.70[b]	1 monomer (13,500)	2.0
Gene 5 DNA binding protein[a] (fd bacteriophage)	$C222_1$	$a = 68.4$, $b = 86.6$, $c = 105.0$	6.22×10^5	3.89	2 monomers (9,800)	3.0

[a] *Obtained only in the presence of β-octyl glucoside.*
[b] Assumes only the protein component of the crystal.

limited for practical considerations, it is possible that other results, perhaps much more favorable than our own, would be obtained by a more diligent search for optimal conditions of crystal growth from ammonium sulfate solutions. Certainly, the results of Garavito and Rosenbusch (1) and Michel and Oesterhelt (2) show clearly that protein crystallization from salt solutions in the presence of β-octyl glucoside is feasible and in their cases yielded outstanding results.

The appearance of crystals of one form or another in three of the five samples of tRNA shows that, as is the case with proteins, β-octyl glucoside does not inhibit, and in fact also appears to significantly enhance the growth of tRNA crystals. We had not previously, except for the *E. coli* phenylalanine tRNA, obtained any crystals of these tRNAs. The phenylalanine crystals that we had previously obtained were grown only after optimization of buffer, metal ion, Mg^{2+}, pH, and spermine levels. What makes the appearance of the tRNA crystals here particularly significant is that no effort whatever was made to refine growth conditions. The crystals appeared in the absence of any added Mg^{2+}, metal ions, polyamines, or even adjustment of pH. The samples contained only PEG, distilled water, tRNA, and β-octyl glucoside. We believe that if there were some effort made to optimize and refine growth parameters in the presence of the detergent, crystals suitable for x-ray diffraction analysis might be grown.

The growth of crystals of protein-nucleic acid complexes, RNase + $d(pA)_4$, RNase + $d(pT)_4$ seen in Fig. 4, and gene 5 protein + $d(pA)_4$ in Fig. 1 demonstrate that protein-ligand formation is not reduced or affected by the presence of the concentrations of β-octyl glucoside employed in these trials. In addition, growth of crystals of these complexes is not inhibited but appears again to be enhanced by the presence of β-octyl glucoside. That the complexes are not different in the presence of the detergent is demonstrated by the isomorphism of the RNase + $d(pA)_4$ and RNase + $d(pT)_4$ crystals with those grown in the absence of β-octyl glucoside. While we have not yet shown whether the crystals of gene 5 protein grown in the presence of $d(pA)_4$ contain $d(pA)_4$, the results with the RNase + deoxyoligomers suggest that complex formation is not affected. Since we know that under the same conditions in the absence of β-octyl glucoside the gene 5 + $d(pA)_4$, complex does crystallize, we believe that the crystals seen in Fig. 1*I* are of the protein plus nucleic acid. If so, it is interesting that they have the same habit as the native gene 5 protein crystals grown in the presence of the detergent, Fig. 2*B*. This is not the case in the absence of β-octyl glucoside.

In the one case studied carefully, that of concanavalin B, a maximum of 1% change was consistently produced in a unit cell dimension by the inclusion of β-octyl glucoside in the mother liquor. The concanavalin B crystals grown in the presence of β-octyl glucoside diffracted as strongly and to as high or higher a resolution than the crystals grown in its absence. Careful quantitation was not, however, carried out and the improved diffraction properties we observed may only have been a consequence of the greater average size of the detergent-grown crystals rather than any real increase in crystalline order.

Heavy atom derivative formation of the concanavalin B was not adversely affected although we cannot say it was enhanced. Two very isomorphous and useful derivatives, $AgNO_3$ and $KAu(CN)_2$, were obtained and are being incorporated into the MIR procedures.

The dramatically enhanced frequency of successful crystallizations of the gene 5 protein and the maintenance of a high success rate of canavalin crystallization in the presence of a broad array of detergents suggests that a general detergent effect may be an important factor in the usefulness of β-octyl glucoside. It further suggests, given the many possible detergents that remain to be surveyed and the optimal conditions for each that must be determined, that a great amount of screening remains to be done. It is possible that the inclusion of the proper detergent at the optimal concentration in crystallization samples may prove to be an important parameter in macromolecular crystal growth.

The observation that gene 5 protein in the presence of each of 14 different detergents crystallizes reproducibly in the usual C2 monoclinic unit cell but in the presence of only 0.25% β-octyl glucoside crystallizes reproducibly in the highly hydrated $C222_1$ unit cell implies that there is something unique about β-octyl glucoside. It clearly has an effect, at least upon the gene 5 protein, that is significantly different from that of the other 14 detergents, and that effect has striking consequences for crystal formation.

Six of the proteins crystallized in this experiment are glycoproteins. These are the α subunit of both luteinizing and thyroid-stimulating hormone, pancreatic α-amylase, ribonuclease B, *Abrus precatorius* lectin, and α_1-acid glycoprotein. Their success rate in the presence of β-octyl glucoside and

the other enhancements noted above were as good as the noncarbohydrate-conjugated proteins. Several of the proteins used, for example, catalase, the α-amylases, concanavalin B, the fructose-1,6-diphosphatases, and canavalin have bound metal ions. No deleterious effects were noted for those proteins. *Abrus precatorius* lectin, gene 5 protein, fructose-1,6-diphosphatases, and canavalin are all oligomers of two, four, or six subunits. They were not unfavorably affected.

Overall, it is our contention that the inclusion of β-octyl glucoside in the mother liquor, at least over the range of concentrations we utilized, had a generally positive, and, in some cases, a strikingly favorable effect on the parameters of crystal growth. A broader range of concentrations, a greater range of other crystallization parameters along with β-octyl glucoside, and a more extensive set of proteins need to be explored. Our results with other detergents suggest that the effects are not limited to β-octyl glucoside and that other detergents might be profitably considered as well. Certainly the inclusion of β-octyl glucoside or some detergent in crystallization samples is an important parameter that should be included along with other considerations. This is particularly true if an improvement in crystal properties is the objective. Although we were not able to crystallize a protein in the presence of β-octyl glucoside that previously had not been crystallized, our choice of such proteins was limited and they were poorly represented in this experiment. Thus, it may well be that a detergent could have a decisive influence and provide the difference between growing and not obtaining crystals. Certainly this was the case with the membrane proteins.

We have no definitive evidence that the improvements or changes in crystal growth properties we observed in this experiment are due to elimination or reduction of microaggregation states which was our original point of departure. The results we have obtained, however, are, we believe, consistent with that hypothesis and we are planning additional experiments to further evaluate its likelihood.

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