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Cryocrystallography of Ribosomal Particles

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Abstract

Crystals suitable for X-ray study have been prepared from biochemically active ribosome particles or their complexes with tRNA and polypeptide chains. At ambient temperature the useful lifetime of these crystals under synchrotron irradiation is limited to a few minutes. However, upon cooling to cryogenic temperatures around 85 K, the original resolution limit (up to 4·5 Å) can be recorded and radiation damage is virtually eliminated. Hence it has become possible to collect a complete data set from one single crystal. Crystals were cooled as rapidly as possible, either in a cold gas stream, or by immersion in liquid propane. Before cooling crystals were transferred either to an inert hydrocarbon environment, or to solutions similar to the crystallizing ones but with a higher viscosity. In several cases soaking in a cryosolvent was required. Crystallographic data were collected with intense synchrotron radiation. Full data sets have been measured for native and derivatized crystals of 50S ribosomal subunits from *H. marismortui* as well as from their complexes with tRNA and nascent polypeptide chains, from the wild type and a mutant of 50S subunits from *B. stearothermophilus*, and from crystals of native and derivatized 30S ribosomal subunits from *T. thermophilus*.

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Introduction

One of the major questions in biochemistry still awaiting clarification relates to the detailed molecular mechanism of protein biosynthesis. Ribosomes are the

Table 1. *Two- and three-dimensional crystals of ribosomal particles*

Source	Crystal form	Cell dimensions (Å), determined by Electron microscopy	X-ray crystallography	Resolution (Å)	Comments*
70S *E. coli*	A†	340 × 340 × 590; 120°; $P6_3$			
70S *B. stearothermophilus*	2D†,AS†	190 × 420; 91°; *P*2		40	
30S *T. thermophilus*	M†		407 × 407 × 170; $P42_12$	8·5	*N,H*
50S *H. marismortoui*	1,P†	310 × 350; 105°			
	2,P	148 × 186; 95°	147 × 181; 97°	13	
	3‡,P	170 × 180; 75°	210 × 300 × 581; $C222_1$	4·5	*N,H*
50S *B. stearothermophilus*	1,A	130 × 254; 95°			
	2,A	156 × 288; 97°			
	3,A	260 × 288; 105°			
	4,A	405 × 405 × 256; 120°			
	5,A	213 × 235 × 315; 120°			
	6‡§,A	330 × 670 × 850; 90°	360 × 680 × 920; $P2_12_12$	18	*N*
	7‡§,P		294 × 542 × 712; *F*222	11	*N,H*
	2D,A	145 × 311; 89°; *P*2		35	
	2D,AS	148 × 360; 109°; *P*1		25	

* *N,H* = crystallographic data collected from (*N*) native and (*H*) derivatized crystals.

† All forms are of three-dimensional crystals, unless marked by 2D. A, M, P, AS = crystals were grown by vapor diffusion from low-molecular-weight alcohols (A), MPD (M), polyethylene glycol (P), or ammonium sulfate (AS).

‡ Same form and parameters for crystals of a complex of 50S subunits + tRNA molecule and a segment (18–20 mers) of a nascent polypeptide chain.

§ Same form and parameters for crystals of large ribosomal subunits of a mutant (−L11 protein) of the same source.

cell organelles where genetic information is expressed in the construction of polypeptide chains. During this process they bind several compounds in a specific manner: the messenger RNA containing the genetic code, the various tRNA molecules carrying the required amino acids, the initiation, elongation and translation factors, energy-rich molecules, and several ions. The function, and the chemical, physical and biological properties of ribosomes have been studied extensively, and reviewed in Chambliss, Craven, Davies, Davies, Kahan & Nomura (1980), Wittman (1982, 1983) and Hardesty & Kramer (1986). It is clear that in order to understand the process of protein synthesis it is necessary to establish the three-dimensional structure of ribosomes.

The ribosomes themselves are complex assemblies composed of several strands of RNA and a large number of different proteins arranged into two independent subunits of unequal size: the large one, which for bacterial ribosomes is often referred to as 50S, from sedimentation behavior, and the small one, the 30S. These associate upon initiation of protein synthesis. A typical bacterial ribosome contains 32 different proteins and two RNA chains in the large and 21 proteins and one RNA chain in the small subunit. The molecular weights of bacterial ribosomes are $2{\cdot}3 \times 10^6$ for the assembled particles, $1{\cdot}45 \times 10^6$ for the large subunit, and $0{\cdot}85 \times 10^6$ for the small subunit. There is no internal symmetry that could be utilized in structure determination.

In a program aimed at the unraveling of their three-dimensional structure we have initiated crystallographic studies of ribosomal particles. Despite their enormous size, inherent flexibility, and tendency to disintegrate, it has been possible to grow several types of crystals of intact ribosomal particles or their complexes with other components involved in biosynthesis of proteins [see Table 1, Yonath & Wittmann (1988) and Yonath, Frolow *et al.* (1988)]. So far all crystals have been obtained from functionally active particles, and the crystalline material has retained its integrity and biological activity for long periods. All crystals found useful for diffraction studies were grown under conditions not very different from natural ones. The crystals are extremely fragile, and have required development of special techniques for initial manipulation, mounting in different orientations for X-ray studies and soaking in heavy-atom solutions.

The diffracting power of crystalline ribosome particles is so low that virtually all diffraction studies have been carried out with synchroton radiation (Bartels, Weber, Weinstein, Wittmann & Yonath, 1988). A common feature in the diffraction patterns is a rapid drop in intensity around 18 Å resolution. In the early stages it led to the belief that this represented the best resolution attainable (Yonath, Bartunik, Bartels & Wittmann, 1984; Yonath, Saper, Makowski *et al.*, 1986; Yonath, Saper, Frolow, Makowski & Wittmann, 1986). Only later, when we were able to grow larger crystals, and had access to improved diffraction facilities, did we find that diffraction to 4·5 Å was attainable for 50S subunits from *Halobacterium marismortui* (Makowski, Frolow, Saper, Shoham, Wittmann & Yonath, 1987).

Initially the diffraction measurements were performed with the crystal close to ambient temperature (269–292 K). Under these conditions radiation damage was so rapid and severe that all intensities beyond about 18–20 Å disappeared after only a few minutes of irradiation. In an attempt to obtain a complete data set, over 260 individual crystals were mounted and exposed on an oscillation camera. In order to conserve precious

higher resolution reflections, no alignment photos were taken. In spite of this, only one data exposure could be obtained from each crystal. Additional difficulties arise from the generally wide mosaic spread (up to 3°) of these crystals. With the long axis (Table 1) oriented nearly parallel to the X-ray beam, the oscillation ranges must be kept small in order to avoid overlap of reflections, and for many exposures not a single reflection could be recorded in full, rendering proper integration of intensities virtually impossible. The problems described here constitute nearly insurmountable difficulties in data collection, and prospects for further progress without significant changes in the procedures were slim.

Cryocrystallography has been used successfully for many years in the structure determination of low-molecular-weight compounds. During the last two decades there have been several attempts to extend this technique to structural studies of biological macromolecules. Such studies have been reported already in the 1960's and 1970's (Low, Chen, Berger, Singman & Pletcher, 1966; Haas, 1968; Haas & Rossmann, 1970; Marsh & Petsko, 1975; Petsko 1975; Petsko, Douzou & Hoa, 1975; Parak, Moessbauer, Hoppe, Thomanek & Bade, 1976; Singh, Bode & Huber, 1980; Walter, Steigemann, Singh, Bartunik, Bode & Huber, 1982; Hartmann, Parak, Steigemann, Petsko, Ringe Ponzi & Frauenfelder, 1982; Drew, Samson & Dickerson, 1982; Dewan & Tilton, 1987; Hope, 1988). However, owing to a variety of problems, mostly technical, cryocrystallography of biological macromolecules saw little use.

At the time the impasse in our data collection had been reached, a new procedure for biological cryocrystallography had been developed (Hope, 1988). Furthermore, results from low-temperature experiments on a number of different biocrystals (Teeter & Hope, 1986, Hope, 1988; Eisenstein, Hope, Haran, Frolow, Shakked & Rabinovich, 1988; H. Hope, F. Frolow & J. L. Sussman, private communication; L. Joshua-Tor & J. L. Sussman, private communication), suggested that application of cryotechniques could provide a solution to the most crucial problem in data collection, the rapid decay of the diffraction patterns. Cryocrystallographic experiments on ribosome crystals were therefore initiated. The results of these experiments are described in this paper.

Experimental procedures

(1) *Preparation and crystallization of ribosomal particles*

Ribosomal particles were isolated and crystallized according to Yonath & Wittmann (1988), Gewitz *et al.* (1987) and Glotz *et al.* (1987). The crystals were kept in stabilizing or transfer solutions that contained the crystallization mixtures in somewhat higher concentrations than used during crystallization (Yonath, Frolow *et al.*, 1988).

(2) *The precooling treatment and cooling procedures*

Two methods were employed in the handling and cooling of the crystals. The first, 'method 1', in which crystals are immersed in hydrocarbons, has been described in detail by Hope (1988). In 'method 2', which is essentially an extension of the first one, hydrophilic viscose materials were used for protecting the crystals. This method is described in detail in this paper.

All crystals of ribosomal particles obtained so far were either thin plates or needles, with minimum dimension commonly in the range 0·01–0·05 mm, and only rarely exceeding 0·10 mm. The crystals are flexible, easily deformed, and cannot be mounted on a standard glass mounting fiber. In the early experiments on crystals of 50S ribosomal subunits from *Halobacterium marismortui* we employed a straight microspatula instead, constructed as described by Hope (1988), and illustrated in Fig. 1(*a*). This resulted in the platelike crystals always being oriented with their short dimension (corresponding to the longest axis in the unit cell) perpendicular to the goniometer rotation axis, in the end giving rise to severe overlap problems. To circumvent these, spatulas with the blade oriented at different angles to the mounting fiber were constructed (Fig. 1*b*). The most useful orientation is perpendicular to the fiber.

Later a second generation of spatulas, the 'double-layer' or 'sandwich type', was developed (Fig. 1*c*). This construction provides extra protection from drying, and from bending stress that results from surface tension effects. These spatulas are made by gluing two parallel pieces of extremely thin glass to the mounting fiber, separating the pieces with a small dab of glue. The crystals were placed between the two layers and it was found that when the distance between the glass plates matched the thickness of the crystal, the quality of the diffraction pattern was optimized.

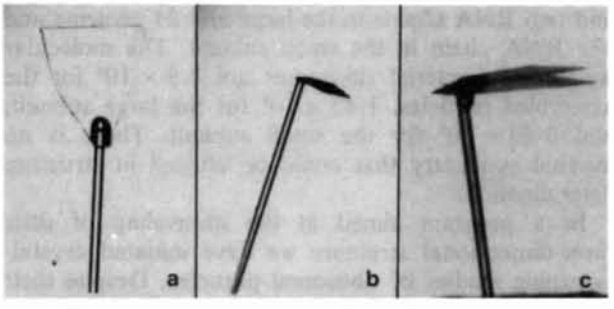

Fig. 1. Three types of glass spatulas constructed for mounting crystals: (*a*) straight and (*b*) perpendicular single-layered spatulas, and (*c*) a double-layered ('sandwich') spatula.

In the first experiments, performed on crystals of 50S ribosomal subunits from *H. marismortui,* the oil protection method (method 1) of Hope (1988) was used. Later we found that crystals of other types did not tolerate this treatment, but could be immersed in 'cooling solutions' that mimic the original stabilizing or growth solutions, but modified to afford increased viscosity by addition of polyethylene glycol of a high molecular weight. In this way we could avoid the hazardous transfer of the crystals from their hydrophilic growth solution into a totally hydrophobic environment, which often damaged the crystals. These crystals were best cooled by plunging into an appropriate coolant such as liquid propane (Parak *et al.,* 1976). For the transfer of the cooled crystals from the propane bath (volume about 5 ml), an apparatus allowing transfer from the propane to the cold stream without exposure to the atmosphere was constructed. Fig. 2 is an illustration of this device. In some cases it was also necessary to add a cryosolvent (Petsko, 1975), in order to prevent destruction of the crystal from internal ice formation.

We found that it was necessary to remove all excess liquid before cooling, so that the crystals would not be damaged by freezing of the solvent. After the crystals had been lifted from their stabilizing solution they were blotted virtually dry with filter paper, and then immediately plunged into liquid propane. The sandwich spatulas are particularly useful in these cases, since the crystals are better protected from drying than when the single-layer spatulas are used. We have seen several cases of unprotected crystals being destroyed from dehydration in less than 2 s.

Whichever cooling method was used, turbidity within the cooled droplet indicated water crystallization or drying-out of the crystal and was invariably associated with poor resolution and/or split reflections. When the frozen crystal and its surrounding solution remained completely clear and transparent in the cold stream we could expect usable data.

(3) *Cryocrystallographic experiments*

All data collection was carried out with film methods on rotation cameras at three synchrotron installations (SSRL, DESY and CHESS). Preliminary experiments were carried out also by using a rotating-anode source together with an area detector or a rotation camera. In no case were capillaries used for sample protection. In all experiments the sample temperature was controlled with a stream of cold, dry nitrogen gas flowing over the crystal. The following instruments were used:

(*a*) An Enraf–Nonius low-temperature attachment, modified for improved stability and lower temperature

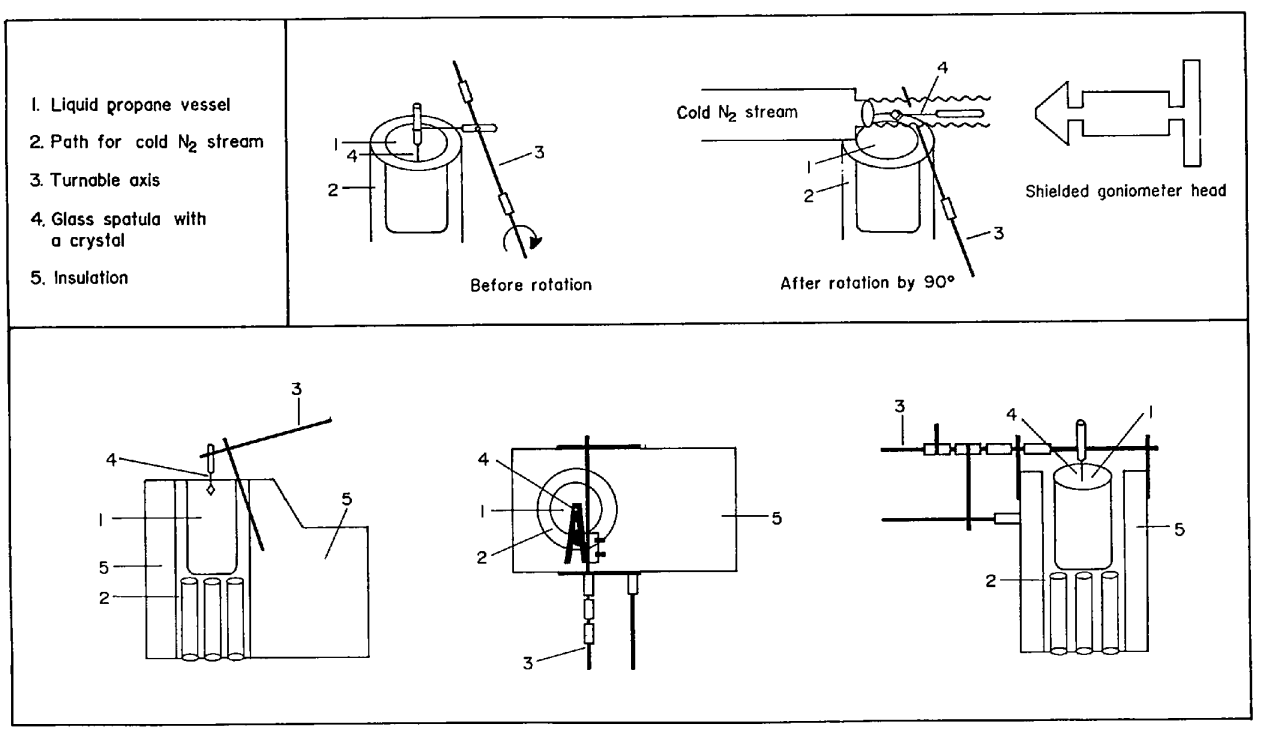

Fig. 2. The device used for cooling crystals (on spatulas) in liquid propane and transferring them to a cold stream collinear with the rotation axis of the crystal. This device was used for mounting crystals on rotation cameras at stations A1/CHESS, X11 and X31/DESY as well on the Rigaku AFC5-R rotating-anode diffractometer (at the Weizmann Institute). Frosting of the goniometer head was prevented with an electrically heated shield.

(around 85 K), with decreased liquid-nitrogen consumption. In this apparatus the cold gas stream is produced by boiling liquid nitrogen with a heating element; the gas is directed at the crystal through glass Dewar pipes. The modifications include an electrically heated teflon nozzle of diameter of 8–9 mm.

(*b*) A homemade apparatus mounted on the Xentronics area detector at the Max-Planck Research Unit in Hamburg (Fig. 3) with operating principles similar to those of the equipment in (*a*).

(*c*) The low-temperature apparatus constructed and installed at station A1 at CHESS/Cornell University. In this instrument the cold stream is produced by passing dry nitrogen gas through a liquid-nitrogen heat exchanger, and directed at the crystal *via* flexible steel Dewar tubing.

The cold gas stream was either directed at an angle of about 40° from the rotation axis of the crystal, or perpendicular to (Fig. 3) or collinear with this axis. In the latter case frosting of the goniometer head was prevented with an electrically heated shield (Fig. 2).

Results

In the crystallographic experiments carried out at cryotemperature there was virtually no radiation damage observed even after hours in the synchrotron X-ray beam (Fig. 4). In favorable cases a complete dataset could be collected from one crystal. Crystals from different sources were treated differently. The choice of precooling treatment and cooling method was largely dictated by the properties of the crystals. The systems studied are described in this section, together with preliminary summaries of data obtained.

(1) 50*S subunit from Halobacterium marismortui*

These crystals were the first to be subjected to low-temperature treatment. The crystals were

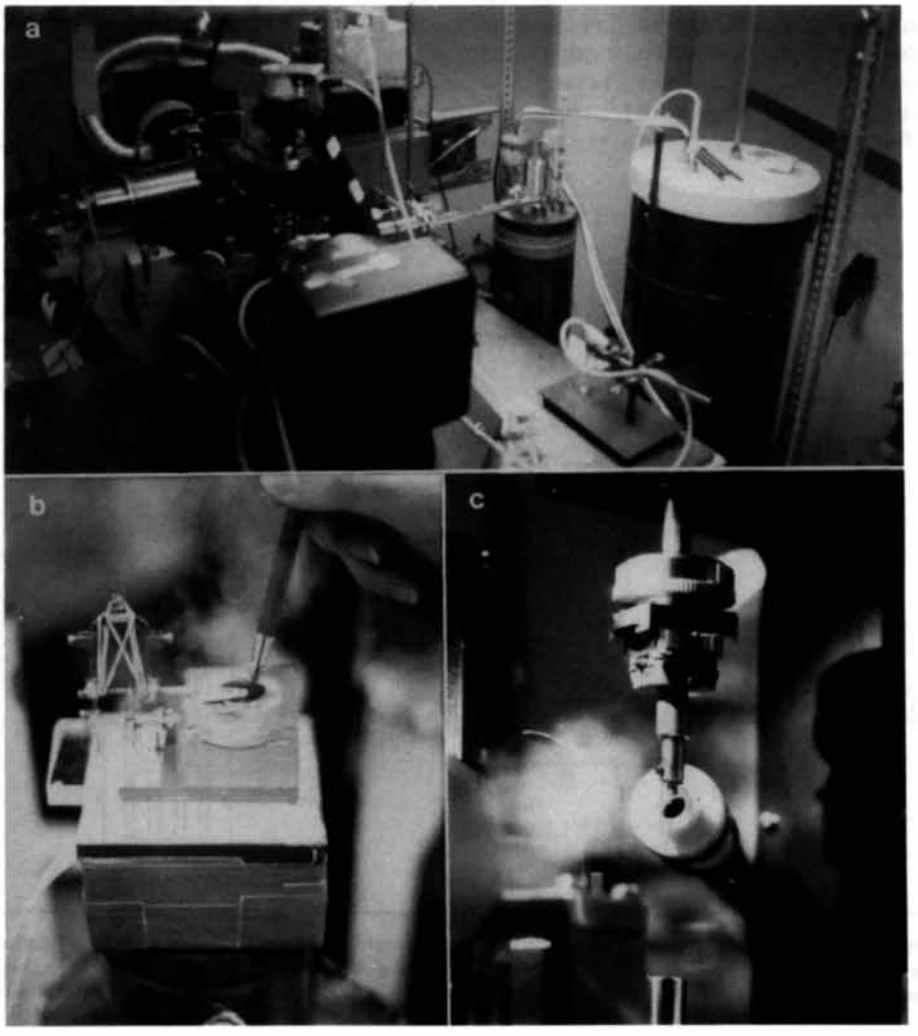

Fig. 3. (*a*) The cryotemperature apparatus mounted on the Xentronics–Nicolet area detector at the Max-Planck Research Unit in Hamburg. The cold nitrogen-gas stream is perpendicular to the rotation axis of the crystal. (*b*) The device used for cooling crystals (on spatulas) in liquid propane and transferring them to the cold-stream path of the apparatus shown in (*a*). (*c*) A crystal mounted on the Xentronics area detector, bathed in a cold nitrogen stream.

grown from solutions of high salt concentrations, containing 5–7% polyethylene glycol (PEG, Makowski *et al.*, 1987). The crystals were transferred to a hydrocarbon environment and the adhering growth solution was removed mechanically. The crystals were then flash cooled in the cold stream. Both straight and preferably perpendicular spatulas were used. With very few exceptions these crystals are thin, very fragile plates (Fig. 5). The manipulations involved in transfer to the hydrophobic oil environment and the removal of the hydrophilic growth solvent, resulted in the loss of a large number of crystals, particularly in the early stages of our experiments. Later we found that these crystals could also be treated successfully by method 2. In the latter case, the cooling solution contained the original components as well as 20% ethylene glycol.

These crystals are orthorhombic, space group $C222_1$, with cell dimensions (at 85 K) of $a = 210$, $b = 300$, $c = 581$ Å, and with one particle in the asymmetric unit. In the 277 K study mentioned in the *Introduction*, no crystal had been oriented so as to show orthorhombic symmetry. Previously reported unit-cell data (Makowski *et al.*, 1987) were determined for the corresponding primitive cell.

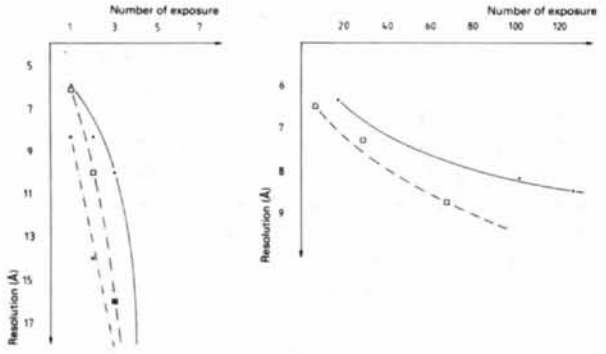

Fig. 4. Decrease in resolution with time for crystals of 50S ribosomal subunits from *H. marismortui* when irradiated at 269–293 K (left) and at cryotemperature (right). The different symbols correspond to individual crystals.

The direction of the long axis of the crystals is perpendicular to the crystal plates. After they have been lifted from the oil drop the crystals tend to float under the surface of the droplet on the microspatula. In that way, the use of a perpendicular spatula allows crystals to be oriented with the long axis nearly coinciding with the camera spindle axis, permitting relatively large oscillation ranges (3–5°) for medium-resolution data. Before we had learned to utilize the perpendicular mounting, full sets of diffraction data had been collected to 8 Å with parallel spatulas. To the extent that overlap did not prevent it, partially recorded reflections could be evaluated and scaled with the aid of data collected from a crystal in the perpendicular mounting to 12 Å (K. S. Bartels, H. Hansen & Z. Berkovitch-Yellin, in preparation).

As shown in Table 1, data have also been collected from crystals of a complex of these subunits with tRNA(Phe) and a short (18–20 mers) chain of the nascent polypeptide (poly-Phe), and from crystals soaked in three heavy-atom clusters, tetrakis(acetoxymercury)methane (TAMM, Richmond, Finch, Rushton, Rhodes & Klug, 1984), undecagold (Fig. 6 and in Yonath, Glotz *et al.*, 1988) and tetrairidium (W. Jahn, unpublished).

(2) 30*S subunit from Thermus thermophilus*

Crystals with typical dimensions of $0{\cdot}6 \times 0{\cdot}1 \times 0{\cdot}05$ mm were grown from solutions containing 15% of 2-methyl-2,4-pentanediol (MPD, see Table 1 and Glotz *et al.*, 1987). When these crystals, with their mother liquor, were introduced into an oil drop, liquid–liquid phase separation of the mother liquor was observed. Upon cooling the crystals turned opaque, and lost their diffracting power. However, following brief soaking in stabilizing solutions containing about 30% MPD, the crystals could be cooled by immersion in liquid propane without damage. The crystals are tetragonal, space group $P42_12$, cell dimensions: $a = b = 407$, $c = 171$ Å. At best they diffract to about 8·5 Å resolution. A representative diffraction pattern is shown in Fig. 7. A

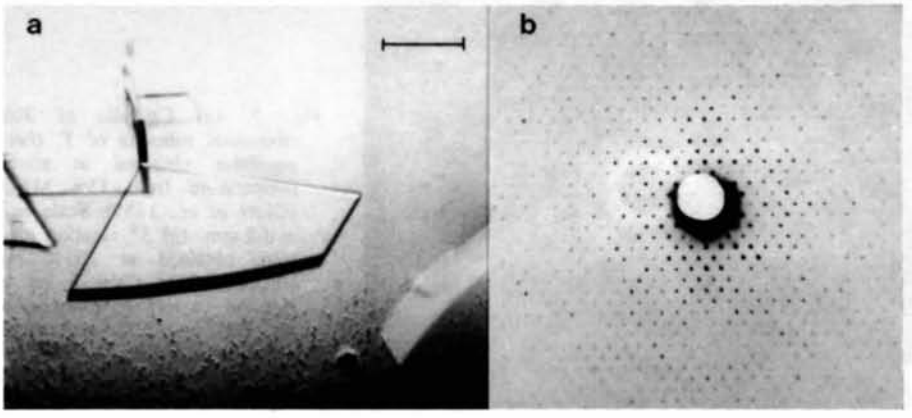

Fig. 5. (*a*) Crystals of 50S ribosomal subunits from *H. marismortui* obtained by seeding in crystallization drop containing 5% polyethylene glycol, 1·2*M* KCl, 0·5*M* NH_4Cl and 0·03*M* $MgCl_2$, at pH = 5·6, equilibrated with 7% polyethylene glycol, 1·7*M* KCl, 0·5*M* NH_4Cl and 0·03*M* $MgCl_2$, pH = 5·6. Scale bar = 0·2 mm. (*b*) 1° rotation pattern obtained at 85 K on station VII/I at SSRL, crystal-to-film distance = 135 mm, collimator = 0·3 mm, wavelength = 1·54 Å, exposure time = 2 min.

complete diffraction data set to 15 Å was collected; in addition, a tetrairidium heavy-atom derivative has been prepared, and a data set to 18 Å recorded.

(3) *50S subunit from Bacillus stearothermophilus*

These ribosomal particles are of extraordinary interest. They have been well characterized chemically. Furthermore we were able to develop procedures to detach several proteins from the core particles in a controlled manner by use of biochemical and genetic methods (Yonath, Saper, Frolow *et al.*, 1986; Gewitz *et al.*, 1987). The depleted ribosomes can be reconstituted, and regain biological activity by addition of these proteins, either in their native form, or modified by binding a heavy-atom cluster to them.

The first crystals from this source were grown at 277 K, using low-molecular-weight alcohols as precipitants (Table 1 and in Yonath et al., 1984; Yonath, Saper, Makowski *et al.*, 1986; Yonath, Saper, Frolow *et al.*, 1986). Very large, well formed crystals, up to 2 mm in length, could be grown directly in X-ray capillaries, and some useful data could be collected. However, since the precipitants are volatile, we were unable to handle or soak the crystals. Subsequently, we have learned to grow crystals by use of nonvolatile materials. At present we can produce at room temperature well ordered crystals from solutions with low concentrations of PEG and of the ions essential for the integrity and biological activity of these particles (Müssig *et al.*, 1988). These grow as flat needles, with dimensions up to 0·05 × 0·2 × 0·37 mm (Fig. 8).

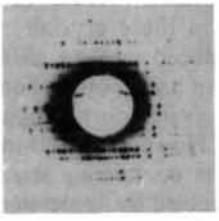

Fig. 6. Superposition of the central parts of rotation photographs of a native and an undecagold derivated crystal, slightly displaced against each other. Conditions as in Fig. 5.

These crystals diffracted well at 277 K, but showed the usual rapid radiation damage. It was found that they could not be cooled by either method without a dramatic loss of the resolution accompanied by smearing of the diffraction patterns (Fig. 9), unless they were first soaked in a cryosolvent, in this case stabilizing solution with 10–20% ethylene glycol. Crystals treated in this manner produced reasonable diffraction patterns when cooled by either method 1 or method 2. Sharper reflections and higher resolution were obtained from crystals treated by method 2, when 20–30% PEG (of molecular weight 40 000) was included in their cooling solution. Treated in this manner these crystals diffract to 11–12 Å in a moderately intense synchrotron beam, about the same as for fresh crystals at room temperature (Fig. 9). The crystals are orthorhombic, of space group $F222$, with $a = 294$, $b = 542$, $c = 712$ Å. Complete diffraction data sets were collected from crystals of native and mutated (lacking protein BL11) particles from this source (Müssig *et al.*, 1988).

Discussion

We have demonstrated that cryocrystallographic methods applied to crystals of ribosome particles provide an extremely valuable enhancement to data-aquisition techniques. The most dramatic change observed in going from ambient to cryogenic temperatures (around 85 K) is the virtual elimination of decay attributable to radiation damage. Under favorable conditions a complete data set can be obtained from one single crystal. The case of crystals of 50S ribosomal subunits from *H. marismortui* is illuminating. One crystal was exposed for many hours over a three-day period without showing significant signs of damage. However, when this crystal was allowed to warm up at

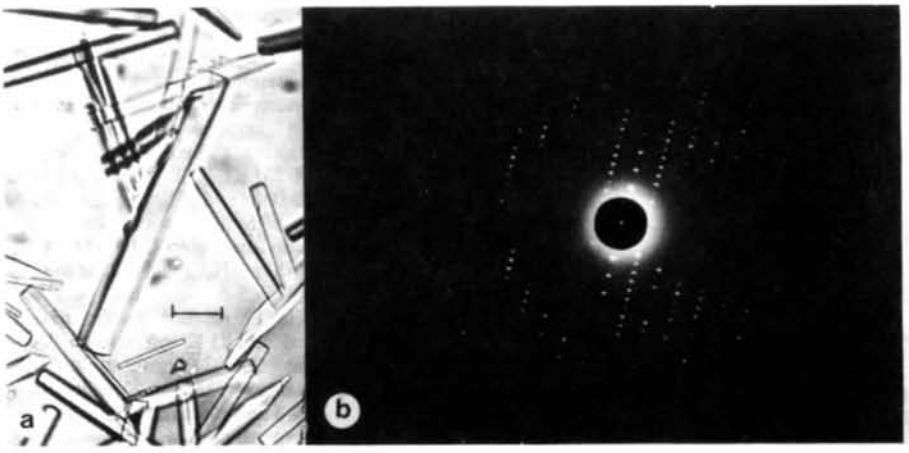

Fig. 7. (*a*) Crystals of 30S ribosomal subunits of *T. thermophilus* obtained at room temperature from 15% MPD (Glotz *et al.*, 1987). Scale bar = 0·2 mm. (*b*) 3° rotation pattern, obtained at 100 K on station A1 at CHESS, on a rotation camera equipped with an He path. Crystal-to-film distance = 330 mm, collimator = 0·3 mm, wavelength = 1·558 Å, exposure time = 10 min.

the end of data collection, it suddenly decomposed completely, leaving only a black mass. We interpret this to show that production of free radicals responsible for radiation damage continued at the low temperature, but that the radicals were trapped in place as long as the crystal remained cold, and only did their damage when the temperature had risen enough to allow diffusion to proceed rapidly.

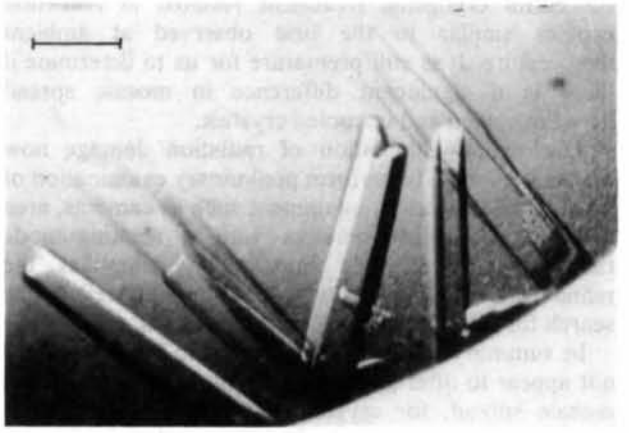

Fig. 8. Crystals of 50S ribosomal subunits from *B. stearothermophilus* grown at room temperature from 2·5% polyethylene glycol (molecular weight 8000), 0·2–0·6*M* KCl, 0·1*M* $MgCl_2$, 0·2*M* $(NH_4)_2SO_4$, at pH = 6·6. Scale bar = 0·2 mm.

It is difficult to assess quantitatively the effect of cooling on properties such as resolution, mosaic spread or overall data quality because (*a*) the same crystal could not be exposed both at room and cryo temperatures due to the irreversibility of the effects of radiation damage; and (*b*) there is a considerable hetereogeneity among individual crystals even when grown and examined under the same conditions. We note also that at room temperature different specimens differ considerably in resolution. Typically, for crystals of 50S ribosomal subunits from *H. marismortui*, initial resolution may vary from 4·5 to 15 Å on a high-intensity synchrotron beam (Fig. 10).

We have never observed cryotemperature diffraction patterns better in resolution than the best obtained at room temperature. This may be due to the inherent flexibility of ribosomal particles. With a resolution limit around 5–6 Å one would expect rather high B values, perhaps of the order of 50 to 100 $Å^2$. Assuming the temperature dependence of B to be of the form $B_T = B_0 + bT$ (Hope, 1988), lowering the temperature is not expected to improve the resolution much, since B_0, 'zero-point' B, must represent most of the contribution to B_T (the value of B at temperature T), at any temperature. This is to say that if the limit of resolution arises from conformational heterogeneity, the resolution is not improved on cooling. On the contrary, it appears that the various steps in the cooling procedure with their associated thermal, osmotic and mechanical stresses lead to a deterioration of crystal ordering.

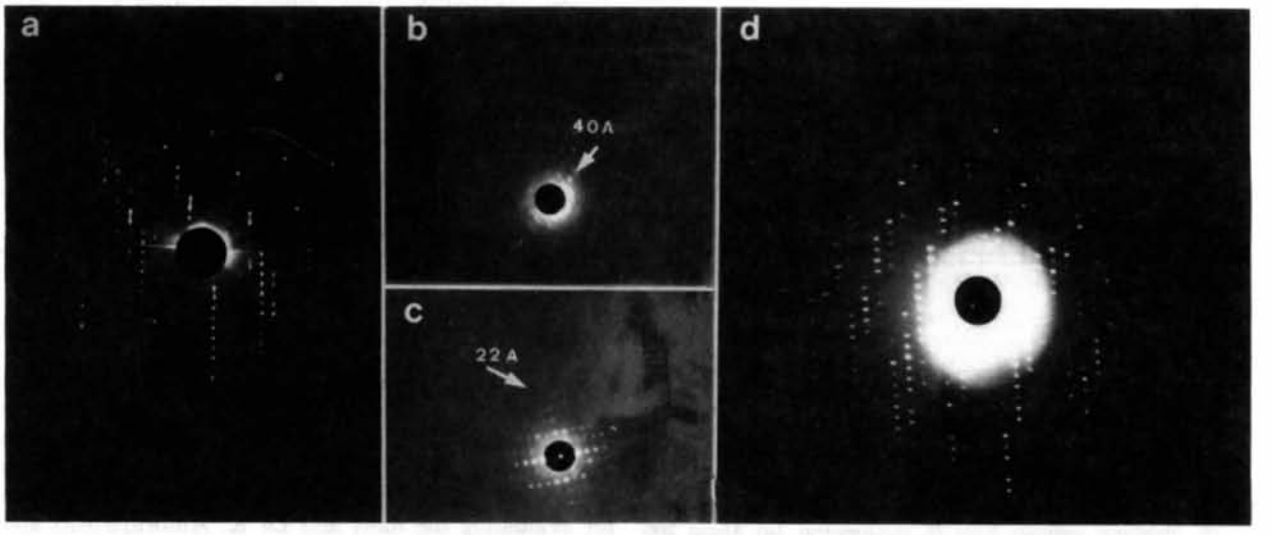

Fig. 9. (*a*) A 'still' photograph, obtained at 292 K from crystals similar to those shown in Fig. 8 mounted in 'traditional' X-ray capillaries on station VII/I at SSRL. The beam path (crystal-to-film distance 240 mm) was partially filled with helium. Diameter of the collimator = 0·3 mm, wavelength = 1·542 Å, exposure time = 5 min. (*b*) 'Still' diffraction patterns, obtained at 90 K, from crystals shown in Fig. 8 immersed in an inert hydrocarbon (Parapol 950, Exxon). (*c*) Immersed as in (*b*) but with 20% ethylene glycol. (*d*) Immersed in a solution containing the growth medium and 30% ethylene glycol and 20% polyethylene glycol of molecular weight 40000. The patterns [shown in (*b*), (*c*) and (*d*)] were obtained on station A1 at CHESS, under the conditions described for Fig. 7(*b*).

The variations in the cooling technique described were developed to minimize these stresses. It is our experience that all manipulations, from the moment the crystal is removed from its stabilizing solution until it has reached cryotemperature, must be carried out as quickly as possible. In particular when the oil method (method 1) is not used, drying of the crystal on the spatula on its way from the solution to the refrigerant must be avoided, at the same time the crystal should be surrounded by as little solvent as possible to attain maximum rate of cooling with no crystallization of the mother liquor.

Especially when method 1 was used, a large fraction of the crystals shows unacceptably poor resolution when examined after cooling. Therefore, with this method it was often necessary to examine over two dozen crystals before we found one that justified data collection. We do not yet have sufficient data to determine to what extent the manipulations they undergo in preparation for cooling, or the cooling process itself, lead to reduced resolution. In general, use of method 2 results in fewer crystal losses and does not require as extensive searches for acceptable diffraction patterns as needed for method 1. We assume that the damage from oil immersion is in part caused by the introduction of the crystal to a hydrophobic environment, and in part by differences in the shrinkage coefficients of the crystals and their hydrocarbon coating. Of course, the severe losses, and the effort in searching for a suitable specimen are more than outweighed by the practically infinite lifetime afforded by the cooling. In spite of the problems experienced in the handling of the crystals, several cooled crystals of 50S ribosomal subunits from *H. marismortui* were found to diffract to about 4·5 Å, similar to the best resolution seen from fresh crystals at 277 K.

In general the mosaic spread appeared similar at the two temperatures. However, for some crystals, mostly those treated by method 1, a smearing of higher-resolution reflections was seen. In general, the most successful cryogenic treatment resulted in reflection profiles similar to the best observed at ambient temperature. It is still premature for us to determine if there is a significant difference in mosaic spread between cooled and uncooled crystals.

The virtual elimination of radiation damage now makes it possible to perform preliminary examination of crystals on laboratory equipment such as cameras, area detectors or diffractometers with a rotating-anode radiation source. This substantially simplifies the refinement of crystal-growth conditions as well as the search for suitable isomorphous derivatives.

In summary, although the cryogenic treatment does not appear to offer any improvement in resolution or in mosaic spread, for crystals of ribosomal particles it provides the only practical route to data collection found so far. Considering the wide variation in intensity distribution from crystal to crystal, the ability to collect a complete data set from one crystal becomes the major advantage of this method.

The methods we use are under constant review, resulting in steady, evolutionary improvement. Currently our experience is too limited to permit a thorough comparison of data collected at cryotemperature under different cooling conditions. Neither can we now address the intriguing question of how the cooling influences the resulting structure.

Although it remains to be seen whether data collected from our crystals at cryotemperature have the potential to yield an interpretable electron density map, it is of course our hope that they do contain the key to the determination of the three-dimensional structure of ribosomes.

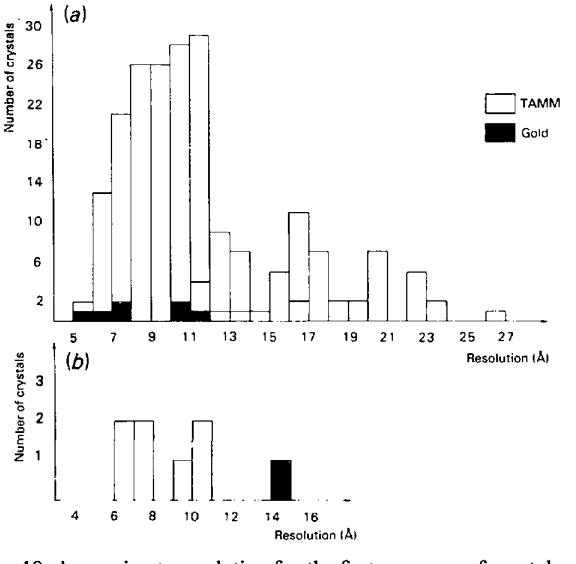

Fig. 10. Approximate resolution for the first exposure of crystals of 50S ribosomal subunits from *H. marismortui*. (*a*) About 200 crystals, investigated at station X11/EMBL/DESY at 269–292 K were included. Shading indicates heavy-atom-derivative test crystals (soaked in TAMM and in undecagold cluster). (*b*) Measured from diffraction patterns of eight crystals (including an undecagold derivative), investigated at station VII/1 at SSRL under conditions described for Fig. 5(*b*).

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SHORT COMMUNICATIONS

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Acta Cryst. (1989). B**45**, 199

Commensurately modulated structure of thiourea at 170 K. Erratum.

By S. TANISAKI, H. MASHIYAMA and K. HASEBE, *Department of Physics, Yamaguchi University, Yamaguchi* 753, *Japan*

(*Received* 13 *September* 1988; *accepted* 19 *October* 1988)

Abstract

In *Experimental at* 153 K on page 443 of the paper by Tanisaki, Mashiyama & Hasebe [*Acta Cryst.* (1988), B**44**, 441–445], the second sentence should read 'The least-squares calculation converged at $R = 0{\cdot}047$, $wR = 0{\cdot}043$ with $S = 0{\cdot}38$, $(\Delta/\sigma)_{max} = 0{\cdot}51$ and $\Delta\rho = 0{\cdot}28$ e Å^{-3} for 1299 independent reflections with $0 \le h \le 11$, $0 \le k \le 15$ and $0 \le l \le 17$ ($2\theta_{max} = 92°$).' In addition, the atomic parameters given in Table 3 should be modified within their e.s.d.'s. The deposited data have been revised.

All relevant information is given in the *Abstract*.

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