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Improving the diffraction quality of MTCP-1 crystals by post-crystallization soaking

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Significant improvement in the resolution and quality of the X-ray diffraction of crystals of MTCP-1 protein was observed on post-crystallization soaking. The MTCP-1 crystals grown from 1.5 *M* ammonium sulfate diffracted to only 3.0 Å resolution with some disorder in the diffraction. After post-crystallization soaking in a solution containing 2.0 *M* ammonium sulfate, the disorder was eliminated and diffraction extended to better than 2.0 Å resolution. Both native and selenomethionine-enriched crystals demonstrated better diffraction after soaking for several months. This simple technique may be useful to improve the diffraction quality of protein crystals generally.

1. Introduction

Determination of the crystal structure of proteins is often limited by the quality of the diffraction. Imperfect crystal lattices can result in low-resolution diffraction, high mosaicity and diffuse scattering. These defects are common for protein crystals and can prevent the solution of the structure. Methods of increasing the size of protein crystals have been reported, such as macroseeding (Thaller *et al.*, 1985) or crystallization from a larger volume (Fox & Karplus, 1993). The larger crystals generally show more intense diffraction. Crystal growth in microgravity can improve the morphology and diffraction limit of protein crystals (DeLucas *et al.*, 1989). However, twinning was not resolved by growth in microgravity (Esposito *et al.*, 1998). Recently, two methods have been reported that can improve the diffraction quality and resolution limit of protein crystals: annealing after flash freezing (Harp *et al.*, 1998; Yeh & Hol, 1998) and controlled dehydration (Esnouf *et al.*, 1998). Here, we describe a simple new method that was used to improve the diffraction limit and quality of MTCP-1 protein crystals.

MTCP-1 is a 107-residue protein that is involved in lymphoid proliferation and T-cell malignancies (Stern *et al.*, 1993; Thick *et al.*, 1996). Recently, the crystal structure of MTCP-1 was solved by our group using MAD phasing and refined to an *R* factor of 0.21 at 2.0 Å resolution (Fu *et al.*, 1998). The determination of the crystal structure of MTCP-1 was originally limited by the poor diffraction to about 3.0 Å resolution with streaky spots. However, a great improvement in the quality and resolution of the diffraction was obtained by soaking the crystals in an artificial buffer solution. In order to confirm this effect, controlled soaking tests were conducted on crystals of both native MTCP-1 and selenomethionine enriched protein (Se-Met MTCP-1).

2. Experimental methods

Human recombinant MTCP-1 protein was expressed and purified as described previously (Du Bois *et al.*, 1998). The selenomethionyl MTCP-1 protein was also prepared for MAD phasing using the same procedure; however, all buffers were degassed and 1.0 m*M* β-mercaptoethanol was included (Fu *et al.*, 1998). The purified MTCP-1 was dialyzed into 50 m*M* Tris pH 7.8 and concentrated to 5.0 mg ml^{-1} for crystallization. Crystals were grown from hanging drops by the vapor-diffusion method. The well solution was 1.5 *M* ammonium sulfate, 50 m*M* Tris pH 7.8. The hanging drops have a 1:1 ratio of protein to well solution. The crystals grew to a size of 0.25 × 0.25 × 0.5 mm at room temperature in two weeks. The Se-Met MTCP-1 was crystallized under the same conditions, except for the addition of 1.0 m*M* β-mercaptoethanol. For post-crystallization soaking, large crystals were transferred to a solution of 2.0 *M* ammonium sulfate in the Tris buffer and soaked for 1–5 months before measuring the diffraction.

X-ray diffraction data were collected on an R-AXIS IIC imaging-plate detector mounted on a Rigaku RU200 rotating-anode X-ray generator with a monochromator. Each crystal was mounted in a capillary. The crystal-to-detector distance was 100 mm and 2.0° oscillation frames were collected at room temperature with an exposure time of 1 h. Both native MTCP-1 and Se-Met MTCP-1 crystals diffracted to about 3.0 Å resolution in the absence of post-crystallization soaking. For

Table 1
Unit-cell dimensions and space group of the native MTCP-1 and Se-Met MTCP-1 crystals before and after soaking.

The highest resolution (Res), completeness (Comp), multiplicity (Mult) and R_{sym} are shown for the soaked crystals.

Crystal	Space group	Unit-cell dimensions (Å)	Res (Å)	Comp (%)	Mult	R_{sym}	Volume (Å^3)
Native MTCP-1	*P*6222	$a = b = 62.408$, $c = 86.903$	3.0	—	—	—	293120
Soaked native MTCP-1	*P*6222	$a = b = 62.660$, $c = 85.959$	2.0	99.6	4.0	0.058	292282
Se-Met MTCP-1	*P*6222	$a = b = 62.315$, $c = 87.049$	3.0	—	—	—	292738
Soaked Se-Met MTCP-1	*P*6222	$a = b = 62.536$, $c = 86.162$	2.0	97.6	4.5	0.060	291824

the control experiment only six oscillation frames were collected in order to verify the space group and determine the unit-cell parameters while reducing radiation damage. The same crystals were transferred to the artificial solution for soaking. After post-crystallization soaking, the crystals diffracted to 2.0 Å resolution, and complete diffraction data were collected and processed with the program *DENZO* (Otwinowski & Minor, 1997).

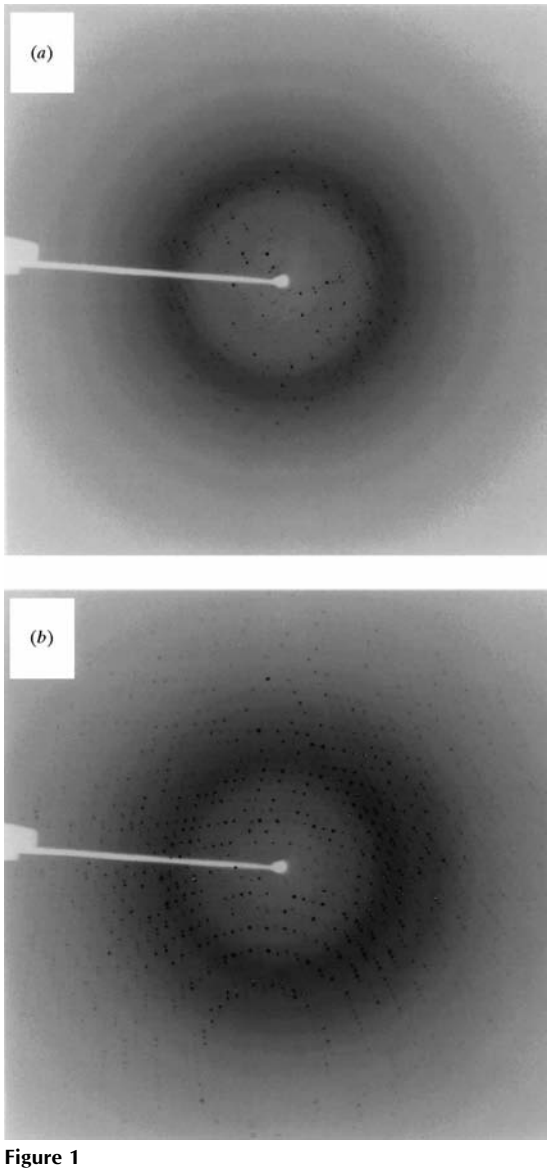

Figure 1
Diffraction from MTCP-1 crystal is shown in a 2.0° oscillation frame. (*a*) The crystal before soaking showed diffraction to 3.0 Å resolution. (*b*) After soaking the same crystal, the diffraction extended to 2.0 Å resolution.

3. Results

Both native MTCP-1 and Se-Met MTCP-1 crystals diffracted to about 3.0 Å. However, the diffraction showed some streaky spots and diffuse scattering, which suggested lattice defects in the crystals. Some crystals showed possible twinning. When searching for heavy-atom derivatives of the native crystals, it was found that crystals after soaking had much stronger diffraction, up to 1.9 Å resolution, and all the spots were clearly formed without streaking. However, this was not because of the heavy-atom compound. Both the native MTCP-1 crystals and Se-Met MTCP-1 crystals demonstrated the same effect after soaking without any heavy-atom compound. All the native MTCP-1 and Se-Met MTCP-1 crystals tested after soaking demonstrated improved diffraction, while crystals without soaking did not.

These results strongly suggested that the improved diffraction was a consequence of soaking the crystals. However, it was still possible that the crystals selected for soaking happened to be much better, even before soaking. Therefore, control tests were conducted as follows. (i) Crystals were mounted for diffraction testing; (ii) the crystals were removed from the X-ray diffraction system after six frames of diffraction data were recorded; (iii) the crystals were transferred to the soaking solution and left undisturbed for 1–5 months and (iv) the soaked crystals were remounted and complete diffraction data were collected. Significant improvement in both diffraction quality and resolution is shown by comparison of the diffraction from a crystal tested before and after soaking (Fig. 1). The soaking time needed for this improved diffraction varied between 1 and 5 months. The unit-cell parameters and space group of the tested crystals are listed in Table 1 before and after soaking. The cell parameters change slightly on soaking, the *a* and *b* axes increasing by about 0.2 Å and the *c* axes decreasing by about 0.9 Å for both crystals. These changes lead to a very small decrease in unit-cell volume of 0.3% on soaking. Complete diffraction data were collected to 2.0 Å resolution with an R_{sym} of 6% after soaking both native and Se-Met MTCP-1 crystals.

4. Discussion

A significant improvement in both the diffraction quality and resolution was observed for MTCP-1 crystals after post-crystallization soaking. This very simple procedure has not yet been reported for crystallization of other proteins. Therefore, it is not clear whether this phenomenon is generally useful, nor what takes place during the soaking. In general, the diffraction quality and resolution of crystals are determined by the molecular packing or correlation between the molecules, as well as the molecular building blocks. Defects in lattice periodicity and packing, which exist to a greater or lesser degree in all crystals, will affect the diffraction. For protein crystals, the loose packing of molecules in the unit cell and the large volume of disordered solvent generally results in weak diffraction with high diffuse scattering. The loose packing of molecules arises from the flexible surface residues of proteins. Mutation of residues on the protein surface can produce a different lattice type and altered packing (Jelsch *et al.*, 1998). However, both the original and soaked MTCP-1 crystals have the same lattice type and space group. Also, dehydration of some protein crystals can improve the diffraction (Esnouf *et al.*, 1998).

The crystal lattice usually shrinks on dehydration, which leads to more extensive packing contacts between molecules in the lattice and hence improved diffraction. We observed no significant shrinking of the crystal lattice after soaking and, most importantly, there was no streaking owing to lattice defects as was observed for the unsoaked crystals. The lattice shrinking alone is unlikely to remove such defects. Therefore, we propose another mechanism. The improvement in diffraction quality and resolution may arise from rearrangement of the surface residues during soaking, similar to the annealing of small-molecule crystals. Annealing of protein crystals after flash freezing (Harp *et al.*, 1998; Yeh & Hol, 1998) may improve the diffraction by a similar mechanism. Analysis of protein lattice contacts suggested that crystal growth depends on random interactions (Carugo & Argos, 1997). During crystallization, some surface residues may not pack optimally or may form inappropriate interactions. Soaking for a long time in a stabilizing solution may enable the surface residues to rearrange and form better packing interactions, leading to the greatly improved diffraction observed.

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